

MestReNova Manual

© 2024 MESTRELAB RESEARCH
Last Revision: 5th Nov 2024

MestReNova 15.1.0

---

by MESTRELAB RESEARCH

This is the manual of MestReNova 15.1.0

MestReNova

© 2024 MESTRELAB RESEARCH

All rights reserved. No parts of this work may be reproduced in any form or by any means - graphic, electronic, or mechanical, including photocopying, recording, taping, or information storage and retrieval systems - without the written permission of the publisher.

Products that are referred to in this document may be either trademarks and/or registered trademarks of the respective owners. The publisher and the author make no claim to these trademarks.

While every precaution has been taken in the preparation of this document, the publisher and the author assume no responsibility for errors or omissions, or for damages resulting from the use of information contained in this document or from the use of programs and source code that may accompany it. In no event shall the publisher and the author be liable for any loss of profit or any other commercial damage caused or alleged to have been caused directly or indirectly by this document.

Table of Contents

Part I Introduction 11

1 Formats supported ... 12

2 System Requirements ... 13

Windows System Requirements ... 13

Mac OS X System Requirements ... 13

Linux System Requirements ... 13

Part II Installation Guide 14

Part III Mnova 15 19

Part IV Fast Visual Guide to process routine 1D-NMR datasets 21

Part V Fast Visual Guide to process routine 2D-NMR datasets 27

Part VI The Mnova interface 33

1 Multipage Software ... 34

Working on a Single Page ... 38

Graphics, Annotations and Signatures ... 41

2 Data Browser ... 44

3 Intelligent NMR Clipboard ... 47

4 Properties ... 51

5 Preferences ... 61

6 Ribbon Customization ... 71

7 View Ribbon ... 73

Zooming ... 73

Full View ... 79

Create Expansions ... 81

Increasing and Decreasing Intensity ... 81

How to measure coupling constants? The Crosshair Tool ... 82

Multiple Crosshair for 2D analysis ... 82

Cutting Spectra ... 86

Blind Regions ... 88

3D Spectrum viewer ... 90

Tables ... 101

Shortcuts ... 112

Audit Trail ... 113

Layout Templates ... 114

8 Undo & Redo ... 122

© 2024 MESTRELAB RESEARCH

Part VII Processing Basics 123

1 1D NMR Processing Tour ... 125
2 2D NMR Processing Tour ... 130
3 Fourier Transform ... 138
- Group Delay ... 141
- FID Shift ... 143
- Diagonal Suppresion ... 144
- Signal Suppresion ... 146
- Apodization ... 148
- Truncation ... 161
- Frequency Shift ... 162
- Zero Filling and Linear Prediction ... 163
- Drift Correction ... 166
- The FT Command ... 167
- NUS Processing ... 168

4 Phase Correction with Mnova ... 169
5 Baseline Correction with Mnova ... 179
6 Symmetrization ... 188
7 Tilt 45 ... 189
8 Reducing t1 Noise ... 190
9 Covariance NMR ... 191
10 Normalization ... 201
11 Binning ... 202
12 Compression ... 203
13 VOI Compression ... 204
14 Smoothing ... 205
15 Denoise ... 207
16 Resolution Booster ... 209
17 ElPulpo Resolution Enhancement ... 211
18 Reference Deconvolution ... 214
19 Inverting, Transposing and Reversing Spectra ... 217
20 Processing Templates ... 218

Part VIII Analysis Tools 221

1 Chemical Shift Referencing ... 222
- Absolute Reference of x-Nuclei experiments ... 225
- Reference of 2D-NMR spectra with the 1D external trace ... 228

2 Peak Picking ... 229
- Peak Picking Options ... 234
  - Impurities Auto detection ... 239
- GSD (Global Spectral Deconvolution) ... 241
  - What else can I do with GSD? ... 249
- Peaks Table and Reports ... 251
- Peak Picking Properties ... 259

© 2024 MESTRELAB RESEARCH

3 Integration ... 260
Integrals Table ... 272
Integral Properties ... 274
4 Multiplets analysis ... 277
Multiplet Manager ... 281
Using GSD for multiplets analysis ... 287
Setting multiplets ... 290
How to resolve overlapped multiplets? ... 292
Multiplets Table and Reports ... 295
Multiplet Analysis options ... 306
Multiplets Properties ... 307
Importing spectral data from literature to synthesize a spectrum ... 312
5 How to carry out assignments? ... 315
Assignments Table ... 333
Transfering assignments through datasets ... 339
Assisted assignment by using the predictions ... 342
Copy assignments from one molecule to another ... 344
Assignments Properties ... 345
Assigning Estradiol - Practical Case ... 349
6 TD Quantitation ... 363
TD Quantitation panel ... 366
7 Digital JC ... 367
8 Arithmetic ... 368
9 13C/HSQC Molecular Search ... 372
10 Line Fitting (Deconvolution) ... 375
Line Fitting Properties ... 391
Part IX Stacked NMR Processing 393
1 How to apply a peak picking or an integration analysis in stacked mode? ... 407
2 Setup Arrayed Spectra ... 409
3 Table of stacked items ... 411
4 Stacked Options and Properties ... 418
5 Data Analysis Quick Start Guide ... 422
Data Analysis ... 424
Data Analysis panel ... 436
Data Analysis Properties ... 444
T1 Analysis ... 444
6 DOSY/ROSY Transform ... 446
7 Reference Alignment ... 466
8 Align Spectra ... 467
Part X Mnova Verify 477
1 Single Mode ... 479
2 Verification Results panel ... 484
3 Options ... 491

© 2024 MESTRELAB RESEARCH

4 Troubleshooting a negative result ... 491
5 Rank potential molecular candidates ... 494
6 Verification in batch mode ... 498

Part XI Molecular Structure 507

1 Compounds Table ... 515
2 SDF Browser ... 518

Part XII Molecules Editor 520

1 IUPAC Name ... 541
2 3D Molecule ... 543

Part XIII Prediction 551

1 Mestrelab Predictor ... 553
2 Modgraph NMR Predict Desktop ... 554
Running predictions ... 555
Automatic Assignments ... 567
Predict & Highlight ... 568
Predict & Compare ... 569
Predict & Verify ... 574
Prediction Database ... 578
3D Molecule Viewer ... 585
Prediction Options and Properties ... 586
3 NMRPredict Server-Based ... 588
Drill down to full 13C databases ... 592
4 Spin Simulation ... 593
Spin Simulation panel ... 603

Part XIV MSChrom 607

1 First Steps with the MSChrom Plugin ... 612
2 Working with the MSChrom plugin ... 618
Importing Empower, Chromeleon and OpenLab2 formats ... 620
Mass Tools ... 626
Spectrum Selection Mode ... 626
New Mass chromatogram ... 634
MS Browser ... 636
Chromatograms Subtraction ... 649
Synchronize ... 654
Plots Visibility ... 655
Relative Masses Display ... 657
Superimpose Chromatograms ... 658
Chromatography ... 660
Mass Analysis ... 664
Peak Detection (Integration) ... 665
Blind regions ... 672
Charge State Deconvolution ... 675
Peak Purity ... 677
Elemental Composition ... 680

© 2024 MESTRELAB RESEARCH

Relative Elemental Composition ... 684
Molecular Match ... 685
Assignments ... 691
MS Spectrum Prediction ... 695
Trace Baseline Correction ... 699
Align traces retention times ... 700
Traces autoalignment ... 701
MS Scripts ... 703
Fractions ... 705
MS Tables ... 707
Context menu ... 711
Properties and layouts ... 713
Preferences ... 726

Part XV ElViS 731

1 Formats supported ... 732
2 Fast Visual Guide to process routine datasets ... 732
3 Basics Processing ... 736

Baseline Correction ... 737
Normalization ... 741
Multiplicative Scatter Correction ... 743
Smoothing ... 743
Derivative ... 744
Peak Picking ... 745
Peak Picking Properties ... 746
Integration ... 747
Processing Templates ... 750
Data Analysis ... 752
Arithmetics ... 752

Part XVI Mnova Database 754

1 Connecting to the Server ... 755
2 Create a Database ... 756
3 Saving items to Database ... 756
4 Displaying database contents ... 759

Operations ... 759
Searching in the Database ... 760
5 View Results ... 783
6 Deleting items/Records ... 799
7 User Preferences ... 800
8 Managing Databases ... 803

Create custom fields ... 808
9 Mnova DB scripts ... 814

Part XVII Scripting with Mnova 820

1 Connecting scripts with Mnova objects ... 828
2 Connecting scripts with Mnova GUI ... 836
3 Mnova processing scripts ... 837

© 2024 MESTRELAB RESEARCH

4 Mnova properties scripts ... 840
5 GUI Actions ... 841
6 Running Scripts from the command line ... 841
7 Import ... 843
8 Export ... 847
9 Report ... 849
10 NMR Tools ... 852
11 NIST Search ... 862
12 Scripts Samples ... 864
13 Python ... 878

Part XVIII Basic Chemometrics - PCA 882

Part XIX Advanced Plugins 899

1 Binding ... 900
2 IUPAC Name ... 901
3 MBioHOS ... 901
4 Mgears ... 904
5 PhysChem ... 905
6 qNMR ... 905
Concentration Workflow ... 906
Settings ... 910
Purity ... 916
Purity Calculator script ... 928
Mgears qNMR ... 931
7 Reaction Monitoring (R-M) ... 937
Importing Datasets ... 937
Selecting Regions ... 945
Practical Example ... 948
8 Screen ... 952
9 SMA ... 953
10 Stereofitter ... 954
11 Structure Elucidation ... 954

Part XX Tutorials 956

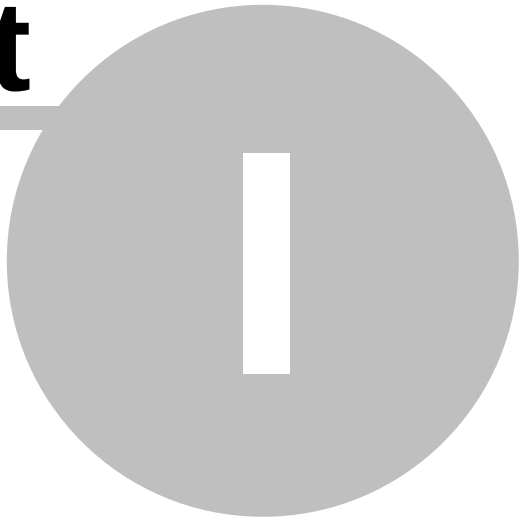
1 Importing Spectrum Parameters ... 957
2 Basics on Arrayed-NMR Data Analysis ... 966
3 Superimposed Spectra ... 981
4 How to process Varian DEPT spectra? ... 983
5 Mnova for metabonomics ... 988
6 Show Traces in 2D-NMR ... 994
7 Manual Phase Correction in 2D-NMR ... 1002

© 2024 MESTRELAB RESEARCH

8 Pasting Spectra into Reports..... 1004
9 How to predict a spectrum with NMRPredict Desktop?..... 1008

© 2024 MESTRELAB RESEARCH

Part



1 Introduction

Welcome to **MestReNova (Mnova)**. **Mnova** is the latest Nuclear Magnetic Resonance and LC/GC/MS data processing, visualization, simulation, prediction, presentation and analysis software package available on the market. This **Help Manual** introduces you to this new software, ensuring that you can use it successfully and achieve good results right from the start. We strongly suggest that you print this manual and follow it when first using **Mnova**, as this will make your introduction to the software very simple and painless, and will make sure that you quickly identify all the main functionality in the software.

Mnova is the natural evolution of our very popular application **MestReC**. However, it is more than a new version of **MestReC**. Whilst including all the NMR processing and analysis functionality present in MestReC, it is a completely new development and a completely new concept which will open up a whole new range of possibilities when storing and sharing data within organizations, changing the way in which scientists process and report NMR and LC/GC/MS data. **Mnova** has been designed to make NMR and LC/GC/MS both more convenient and more powerful, and to give the user improved results with minimum effort.

The program provides a variety of **conversion facilities** for most NMR and LC/GC/MS spectrometer and chromatogram formats and includes all the conventional processing, displaying, and plotting capabilities of an NMR program, as well as more advanced processing techniques with a very user friendly graphical interface. To read a little bit more on the main features, and familiarize yourself with the principles behind the program, follow this guide.

1.1 Formats supported

Here you can see a list of some of the main formats supported by Mnova:

NMR: Bruker Aspect 2000/3000, Bruker UXNMR/XWIN-NMR, Bruker WinNMR, GE/Nicolet, Galactic (*.spc), JEOL Alice (.als), JEOL Delta, JEOL EX/GX, JEOL Lambda (.nmfid, .nmdata, .nmf, .nmd), Magritek Prospa (*1D, *2D), Old Gemini, Nanalysis RS2D, QoneTech (*.nmr), SIMPSON, Siemens Magneton Vision, Tecmag (.tnt), Varian Gemini/VXR from VHelper, Varian VNMR, VARIAN/Chemagnetic Spinsight (data), Philips Achieva, Siemens Syngo.
Other NMR formats: ASCII (.txt), Mnova (.mnova), ZIP (.zip), MestReC (.mrc), NUTS Type 1-3, SwaN-MR, JCAMP-DX (.jcamp, .dx, .jdx, .jcm), ACD-Labs (1D), Int 32 WinNMR, LCModel ASCII, Sparky 2D

MS:
Currently supported formats on Windows
AB SCIEX Analyst
AB SCIEX Data Explorer
Advion
Agilent – ChemStation, MassHunter, Ion Trap, OpenLab CDS
Bruker Compass, Xmass
Chromeleon
Empower
JEOL – MSQ1000, FastFlight
MS CSV File
NetCDF ANDI-MS
Shimadzu LCMS
ThermoScientific Xcalibur
Varian Saturn
Waters MassLynx

© 2024 MESTRELAB RESEARCH

Waters OpenLynx Report
mzXML/mzData
PerkinElmer Files

Currently supported formats on Mac / Linux
Agilent – ChemStation, Ion Trap
Bruker Compass
JEOL – MSQ1000
MS CSV File
Waters OpenLynx Report
Waters – MassLynx (Mac only)
mzData and mzXML

ElViS: ASCII (.txt, .csv), JCAMP-DX (.jcamp, .dx, .jdx, .jcm), OPUS (.0, .1,...), PerkinElmer Block Structured (.sp), SERSBT (*.meas), Thermo Nicolet Omnic (.spa), Thermo Galactic GRAMS (.spc).

1.2 System Requirements

Before installation, please read this chapter. In this section you will find information on which hardware and software requirements must be met before you can install Mnova. In any case, it is worth noting that Mnova is a **multiplatform** software which will run alternatively on Windows, Mac OS X, Linux and Unix-like systems. From now on, multiplatform laboratories will be able to allow each researcher to work on their Operating System of choice, and to seamlessly share data and processing capabilities with other researchers working on different platforms. There is no longer a need to have three different software packages, and three different file formats, for different Operating Systems.

The minimum and recommended system requirements for the software are:

Windows
Mac OS X
Linux

1.2.1 Windows System Requirements

The *minimum* recommended configuration for Mnova installation is at least 1 GHz or faster processor, 1 GB RAM, a VGA color monitor with 800 x 600 pixels resolution, a compatible mouse and Windows 7 as operating system. However, ideal system requirements for optimum operation of the software are Pentium 1,6 GHz or higher CPU with 2 GB or more of RAM memory and Windows W10 or 11.

Mnova will not install under Windows XP or lower. The default installation requires about 1,1 GB of disk space.

If you are going to install (or uninstall) the program on a Windows system, be sure you have administrator privileges, because the installation procedure is going to install several files in your system folders. If you don't have such privileges, you won't be able to install or uninstall the program correctly.

1.2.2 Mac OS X System Requirements

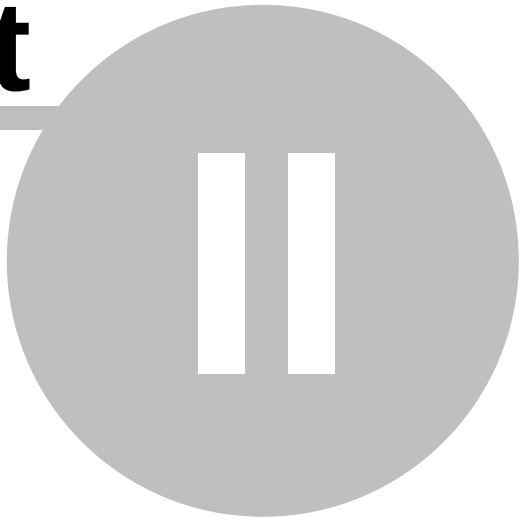
Mnova requires at least Mac OS X 10.13

1.2.3 Linux System Requirements

Mnova requires at least Pentium 1 GHz, 1 GB RAM, Video Adapter Super VGA (800 x 600) with X Library (Xlib). Please, make sure to choose the correct setup file for your Operating System (Debian, OpenSUSE, Fedora, Ubuntu, Red Hat Enterprise...)

© 2024 MESTRELAB RESEARCH

Part



2 Installation Guide

Instructions to Install and Activate an Individual Copy of Mnova:

1. If you are going to install the program on a Windows system, be sure you have administrator privileges, because the installation procedure is going to install several files in your system folders. If you don't have such privileges, you won't be able to install or uninstall the program correctly.

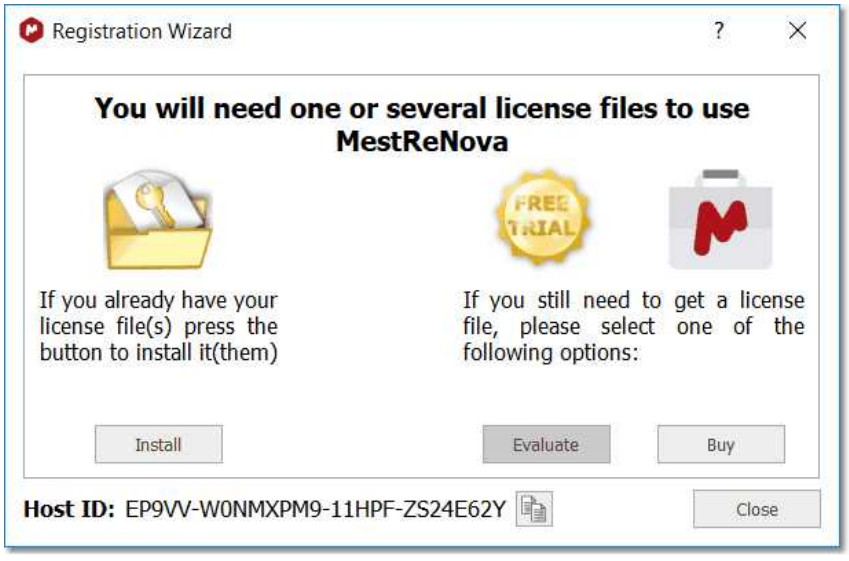
2. On each host, download the Mnova SETUP file from our website at http://mestrelab.com/download/mnova/. Then, double click on the Mnova SETUP file and follow the instructions as they appear on screen.

If you have had a version of Mnova installed previously, the installer will inform you that it is necessary to uninstall the previous version of Mnova. Make sure that this version is closed and click OK and then OK again to confirm. This will uninstall Mnova from your computer. You will get a message to inform you that uninstall was successful: click 'Continue'.

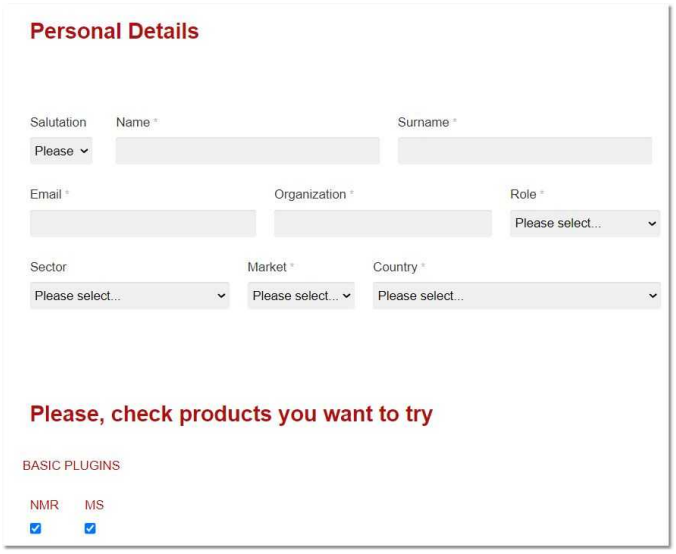
Once you have read and accepted the terms of the License Agreement, click on "**Next**" and select the destination folder where the program files will be placed in your computer. We suggest that you accept all the installation defaults. Once you have chosen the Start Menu Folder as prompted by the installer, click on **"Install"**. This will install the application on your computer. On completing the installation, it will prompt you to select **"Finish"** to run the application.

Note: if you receive a message telling that you need to enable your Microsoft NET 2.0, please check this tutorial, or uncheck the Masshunter provider during the installation.

3. Run the application on each host. The following dialog, notifying you that some licenses (for some plugins) were not found, will appear:



4a. If you want to make a trial, click on the 'Evauate' button to be redirected to a website where you will be asked for a few registration details. An illustrative example appears below.

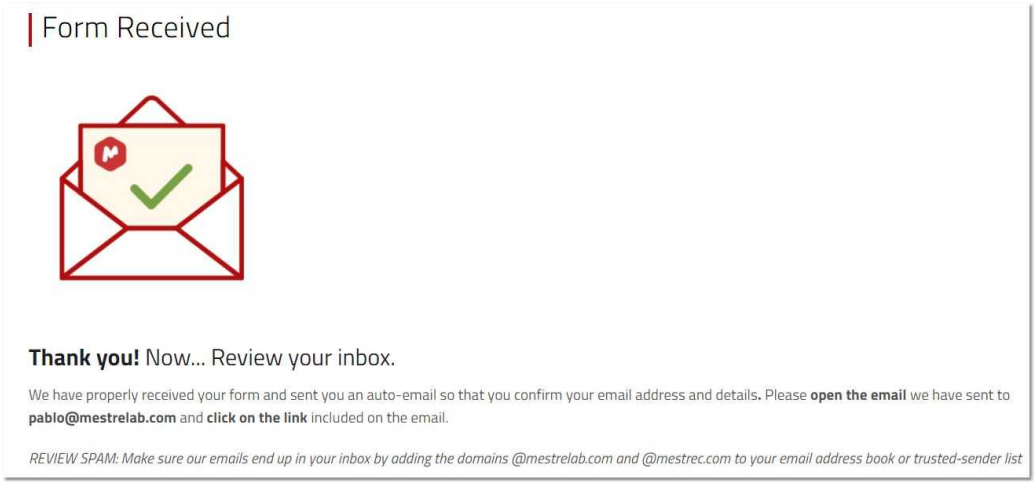


Your evaluation license will be generated after having clicked on 'Get a free evaluation license'.

4b. Alternately if you press the 'Buy' button you are again redirected to a web-page where a similar procedure occurs. However, instead of receiving a license right away your registration information and your Host ID number are automatically sent to our Sales Department by e-mail. They will handle your purchase request and, once the financial formalities are complete, you will receive your license file with installation and activation instructions by e-mail.

4c. If you have already a license file, click on the 'Install' button and go to the step 8.

5. It will display a window (Step 2/4) to inform you that you will receive an email to confirm your details



6. Once you have received the email, please click on the link to confirm that you are a human and **your email address and details are valid**. This will display a new window (Step 2) which will allow you to obtain your license file just by clicking on the 'Confirm email and download license files' button.Save the 'Evaluation License' file on your disc (Step 3).

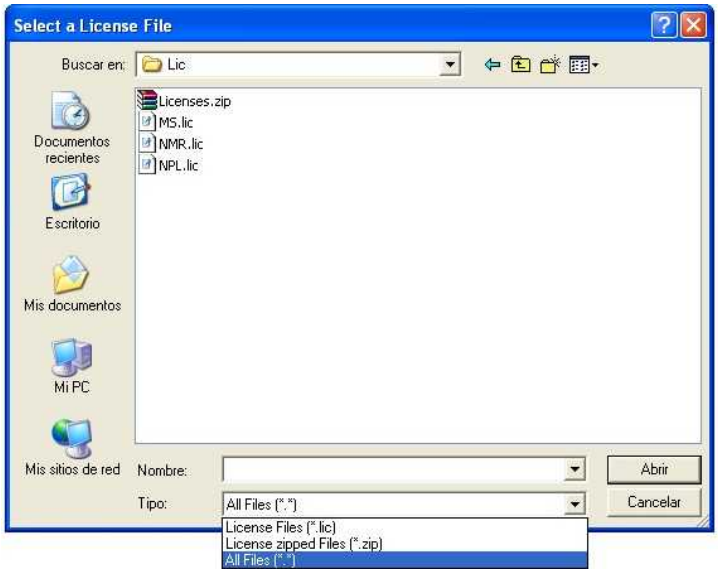
© 2024 MESTRELAB RESEARCH



7. Drag&drop the license files to Mnova in order to activate it. You can also follow the menu 'File/Help/Request Licenses' and click on 'Install' button.



9. This will open the 'Select License File' dialog (see the picture below). Navigate on your Windows browser until you find the license file (or a .zip file containing several licenses), and double click on it and select it. Now you should be able to run the application without receiving an error message dialog.



To install Mnova on **Linux**: download the Mnova SETUP file from a web address provided by us, and:

Debian, Ubuntu (*.deb): type from the command line interface: dpkg -i namefile.deb
Other (*.rpm): type from the command line interface: rpm -i namefile.rpm

To update Mnova:

Debian, Ubuntu (*.deb): type from the command line interface: dpkg -i namefile.deb
Other (*.rpm): type from the command line interface: rpm -U namefile.rpm

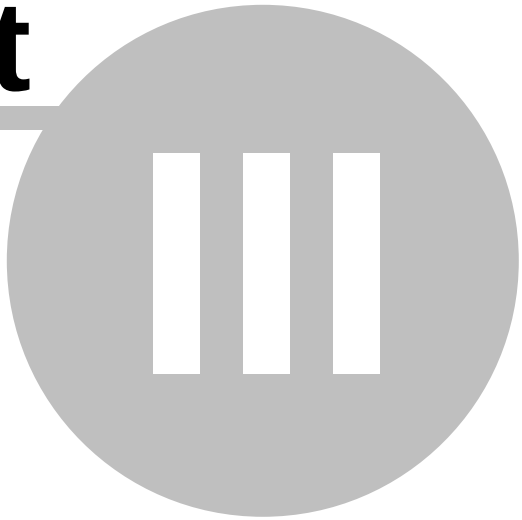
How to uninstall Mnova?

You can **uninstall Mnova** from Windows control panel or by double clicking on the installer and selecting 'Remove'
To uninstall Mnova on Mac, just move the application to the bin.
To uninstall Mnova in Linux:

Debian, Ubuntu (*.deb): type from the command line interface: dpkg -r mestrenova
Other (*.rpm): type from the command line interface: rpm -e MestReNova

© 2024 MESTRELAB RESEARCH

Part



3 Mnova 15

Welcome to **Mnova 15**

In our case, the fact that this is a *commercial release* means the following:

1. We have tested **Mnova** in-house as far as possible to make sure that we deliver to you a version which is robust, already of high quality, and which does not present any apparent bugs. However, you may find a small number of bugs which we have not yet identified. Please tell us about any you discover by e-mailing us at support@mestrelab.com

2. There are some advanced functionalities which will be included in the forthcoming commercial releases, but which are not yet in this version. The reason for this is that, in order to bring you the advantages of Mnova as soon as possible, we are bringing you the core functionality and more, while we complete some advanced and important but less-used functions. As a valued user of Mnova you will be notified the moment these additional functions become available as upgrades. As a result of this you may find some features which were present in the final release of **MestReC**, our current application, are not yet implemented into this commercial version of **Mnova**. A list of such features, together with their planned time of inclusion in the software, can be found below.

3. Although we have now released the product commercially, we are, as ever, very interested in your feedback, bug reports and implementation suggestions. Please keep this coming, as we plan for continuous and rapid improvement of the application, to make forthcoming releases even more in tune with our customer needs.

What's New? (click here to read the changelog).

Our plan is to distribute a new release approximately every four months. The functionalities you can expect to see added to the software during the intervening period are:

- Derivative Processing of Spectra
- GEN-2D NMR, Processing to Improve Resolution in the Indirect Dimension.
- Spectral Editing
- Graphical User Interface Enhancements
- New Analysis tools

We hope that you will find this version of Mnova to be one of the most complete NMR processing and analysis tools available in the market, combined with a revolutionary interface and many new, original and innovative features.

During the commercial release periods, we will welcome your **feedback**, both in the form of bug reports and also of implementation suggestions and feedback on current development. Please write with any of these to support@mestrelab.com

Thank you once again for your support of our development effort!

© 2024 MESTRELAB RESEARCH

Part

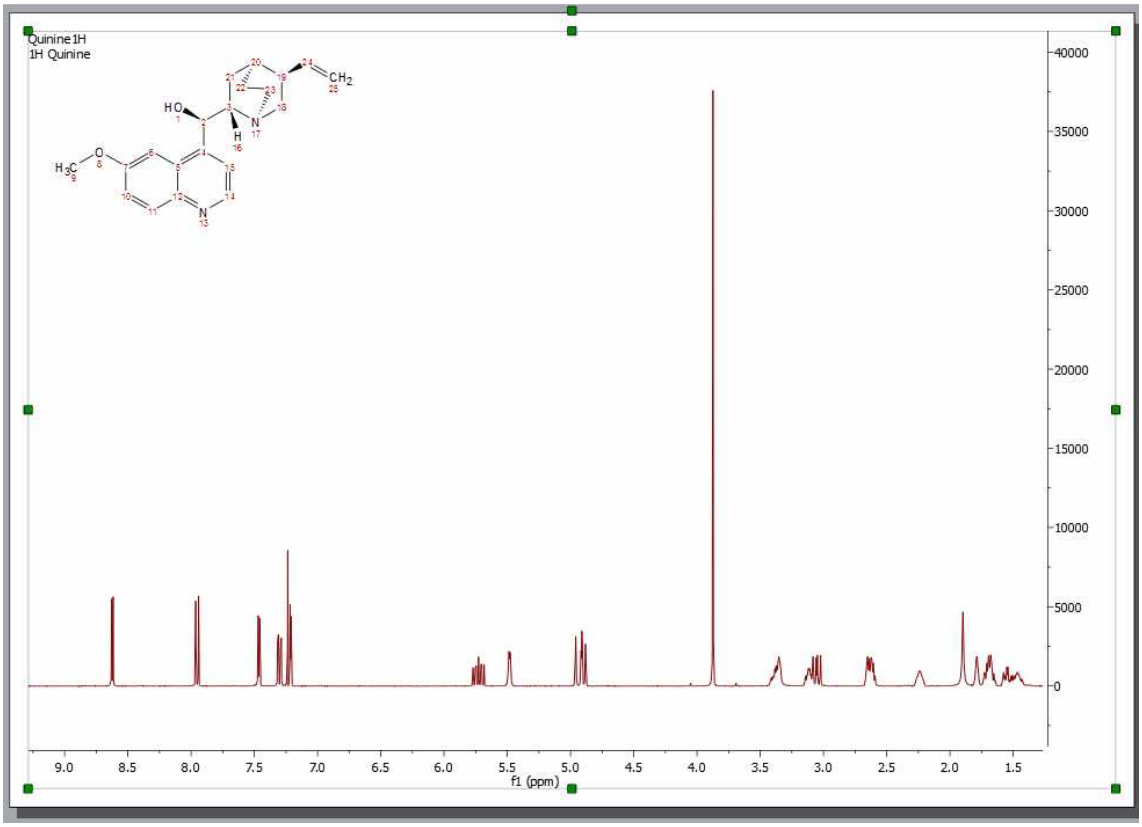


4 Fast Visual Guide to process routine 1D-NMR datasets

You will find the quinine datasets by following the menu 'File/Help/Download Example datasets'.

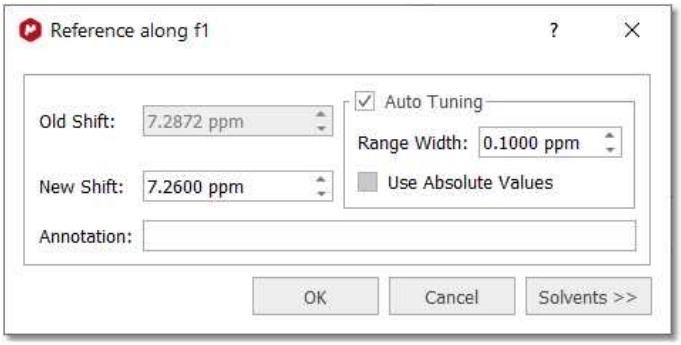
Go through the following procedure to process a routine 1D-NMR spectrum:

1. Drag and drop the folder containing the FID file into Mnova to get your spectrum fully processed. If the folder contains a .mol file, the molecular structure will be also displayed. (You can also follow the menu 'File/Open')



2. If your spectrum is not referenced, you can calibrate it by clicking on the 'Reference' button (under the analysis Ribbon) 

and then selecting the peak you want to be a reference point.

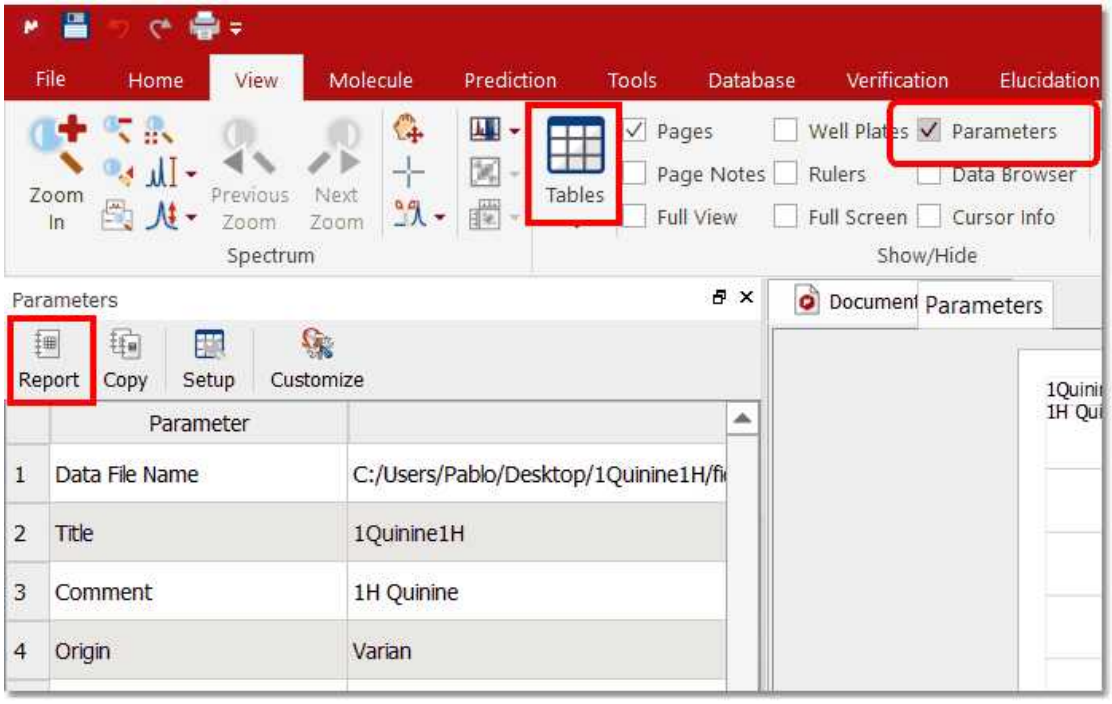


© 2024 MESTRELAB RESEARCH

3. Click on the **'Baseline Correction'** icon 

to correct the Baseline of your spectrum.

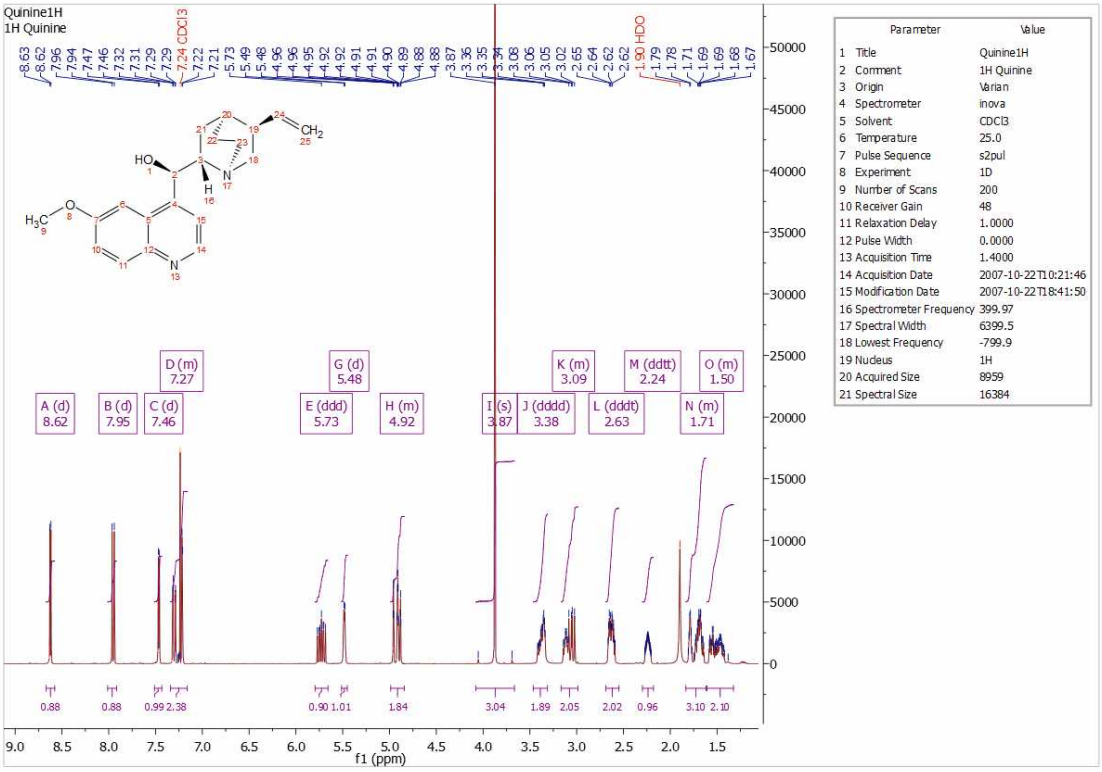
4. To paste the Parameters Table on the spectral window, just follow the menu: 'View/Tables/Parameters' and click on the **'Report'** icon. Please bear in mind that you can resize any object by just clicking and dragging on any border. In this case the spectrum has been resized in order to put the table at the right side of the page.



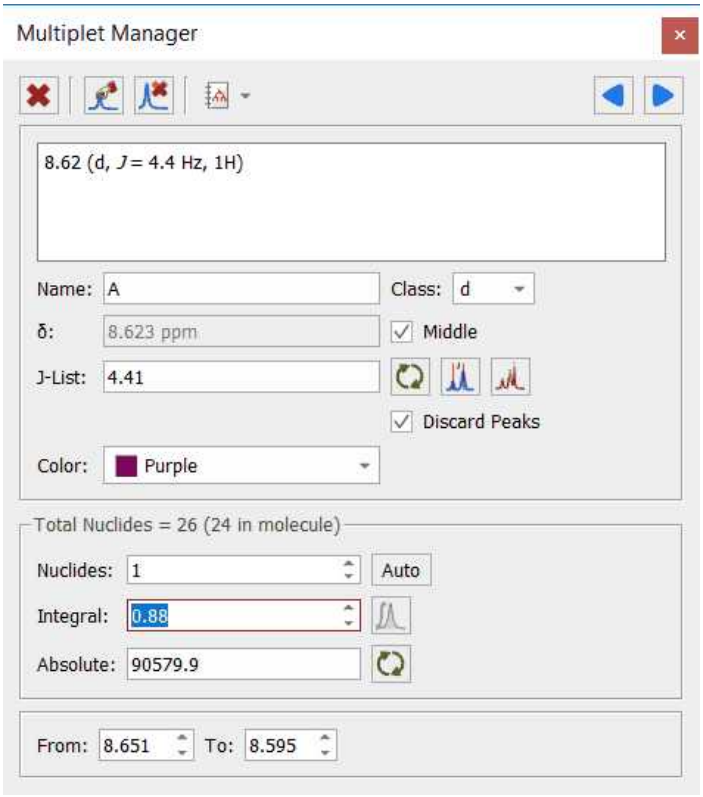
5. Apply an automatic multiplet analysis by clicking on the **'Multiplet Analysis'** icon 

. It will apply an automatic Peak Picking analysis with solvent and impurities recognition. As you can see in the example below the CDCl3 signal has been detected and its peak label appears in red.

© 2024 MESTRELAB RESEARCH

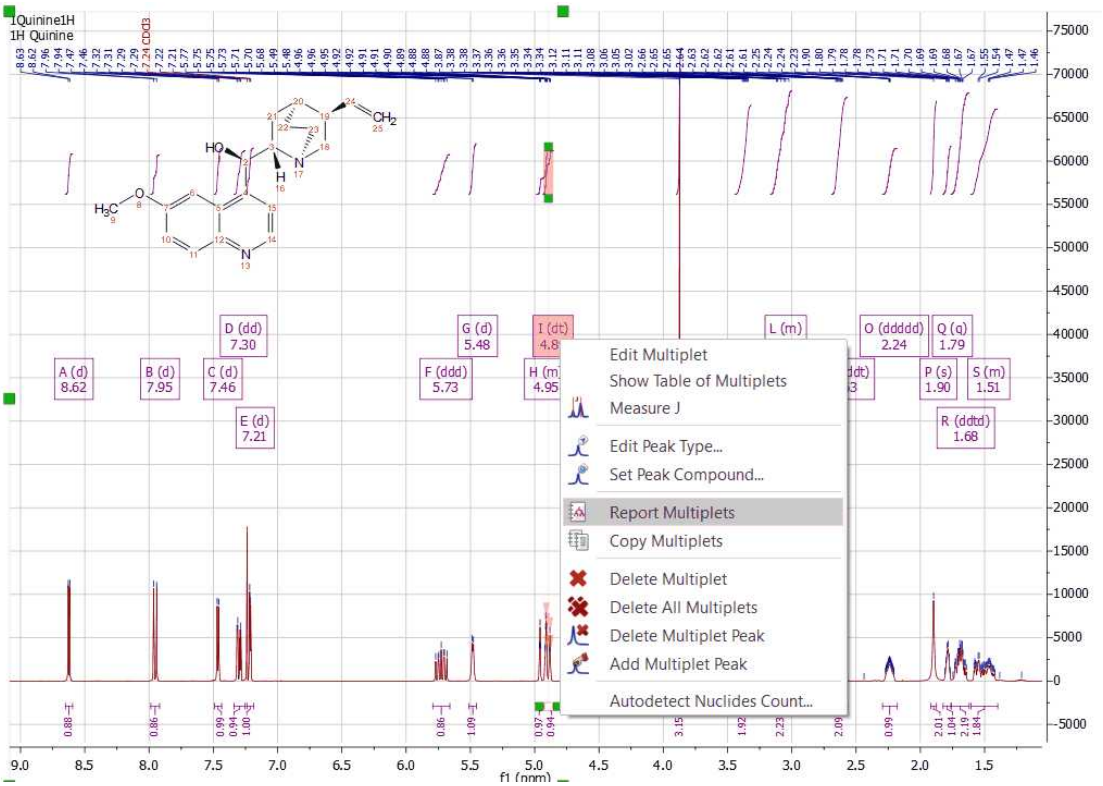


6. Double click on the multiplet box to normalize the 'multiplet integral' values:



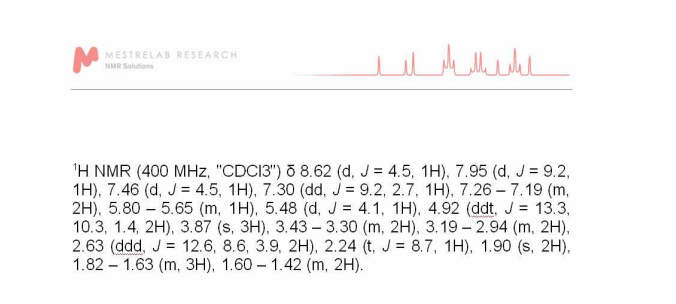
© 2024 MESTRELAB RESEARCH

7. To obtain the multiplet report list just right click on the multiplet box and select 'Report Multiplets':

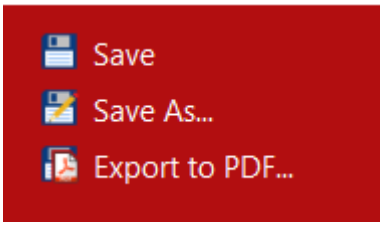


Please bear in mind that you can freely move any Mnova object just by clicking&dragging. In the example above we have moved the molecular structure and the multiplet report

8. You can copy the multiplet report to the clipboard by right clicking on the multiplet box and selecting 'Copy Multiplets' (or by selecting 'Copy Text' on the Multiplet Table) and then paste the report in another document just by using Ctrl+V (or Cmd+V in Mac).



9. Finally, you will be able to save, print or export to PDF, the document by clicking on the applicable icon under the 'file ribbon' (or on the toolbar):



Mnova documents can also be exported as CSV, JCAMP, JSON, LOGS bundle, NMReData or txt formats by following the menu 'File/Save As' and selecting the applicable format from the scroll down menu.

See also:
1D Processing Tour
Fast Visual Guide to process routine 2D-NMR experimental data

Watch a miniclip on this functionality at this link

© 2024 MESTRELAB RESEARCH

Part V

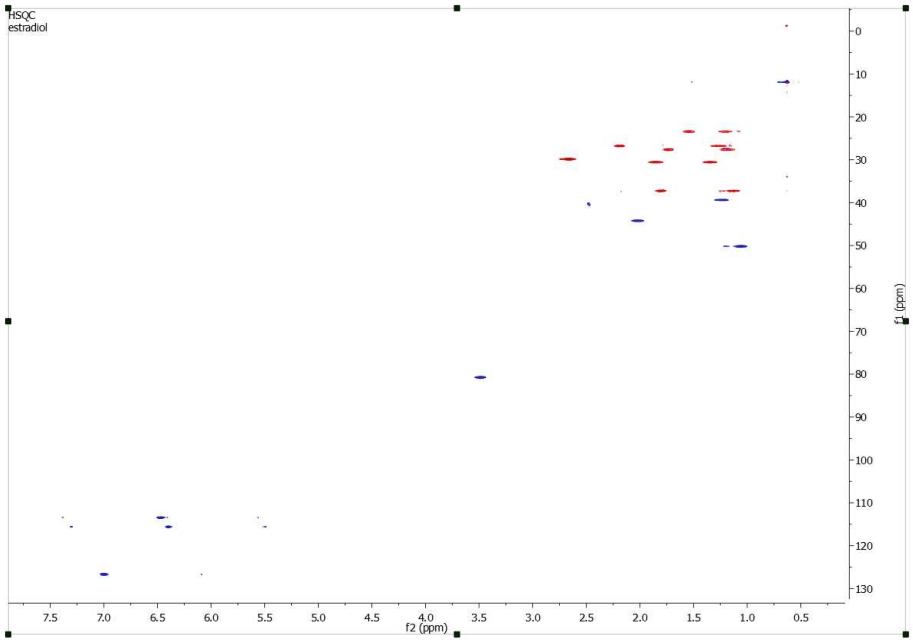
5 Fast Visual Guide to process routine 2D-NMR datasets

(Not available in Mnova Lite)
You will find the quinine datasets by following the menu 'File/Help/Download Example datasets'.

The procedure to process a **2D-NMR** spectrum is very similar with the monodimensional one:

1. Drag&drop the folder containing the spectral files or click on the **'File/Open'** button 

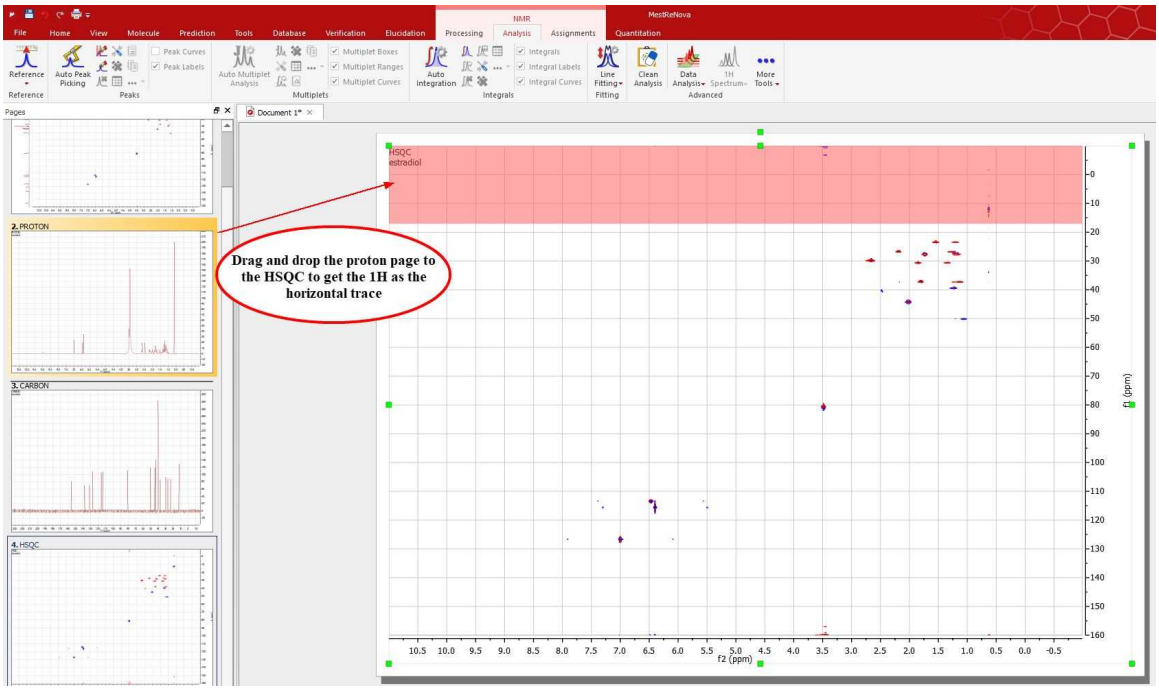
and select the FID file (or SER file in Bruker) to obtain the processed 2D-NMR spectrum. **Mnova** will select the processing functions for you (FT, Phase Correction, etc) but, of course, you may change and optimize any of them and then re-process the data if you wish.



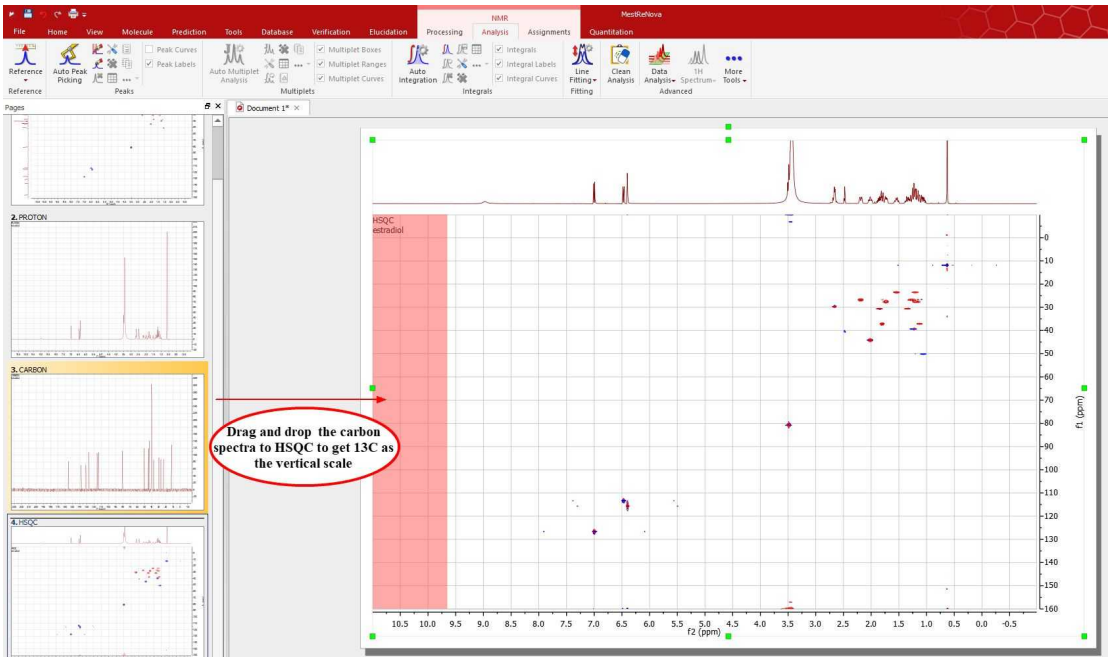
2. Mnova automatically detects the 1D spectra and use them as external traces of a 2D plot if the applicable option is checked under the 'File/Preferences/NMR/Import' menu.

If the 'Auto Attach Traces' option is not checked, you can drag and drop any currently opened 1D-NMR spectrum to a 2D NMR dataset in order to get them as traces. Here you can see how the page number 1, containing a 1H dataset is dropped to the HSQC to get the horizontal trace:

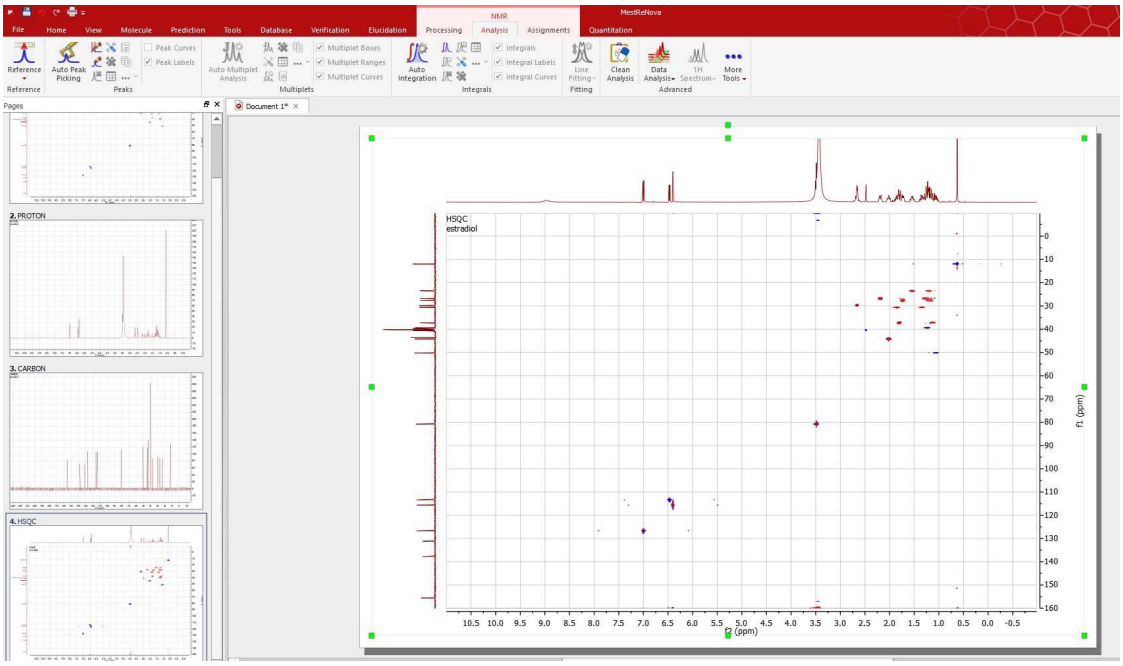
© 2024 MESTRELAB RESEARCH



The same can be done with the page 2 (containing a 13C spectrum) to get the vertical trace:

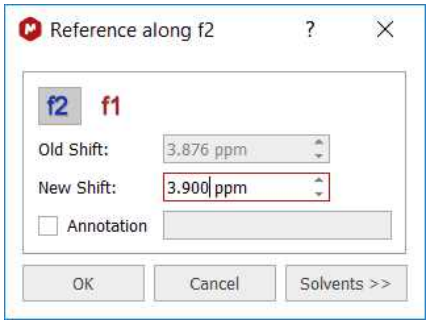


Here you can see the result:



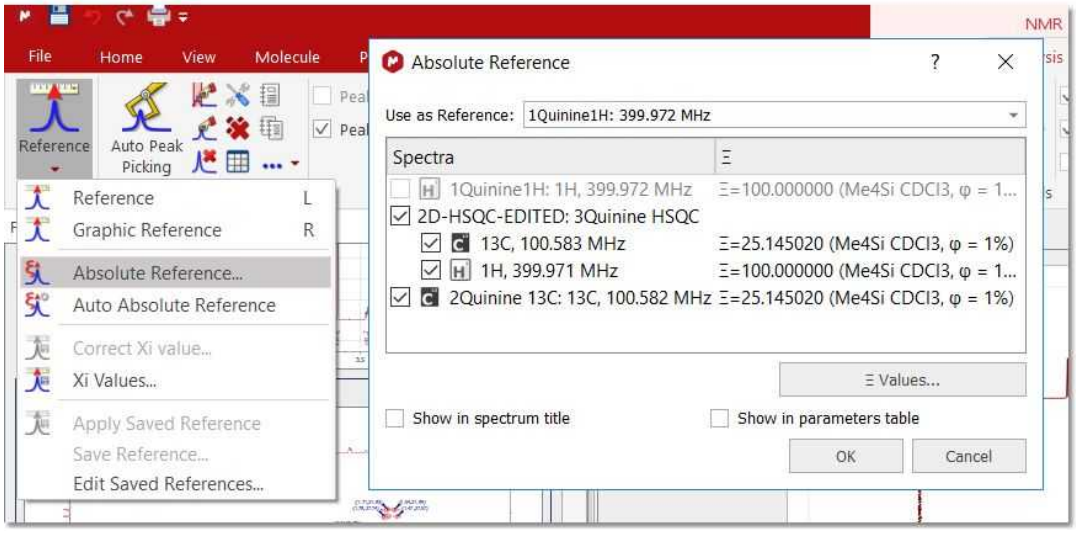
3. To calibrate your spectrum, just click on the '**Reference**' toolbar button 

and then select the peak you want to be a reference point. Finally, select the desired reference along f2 and then along f1:

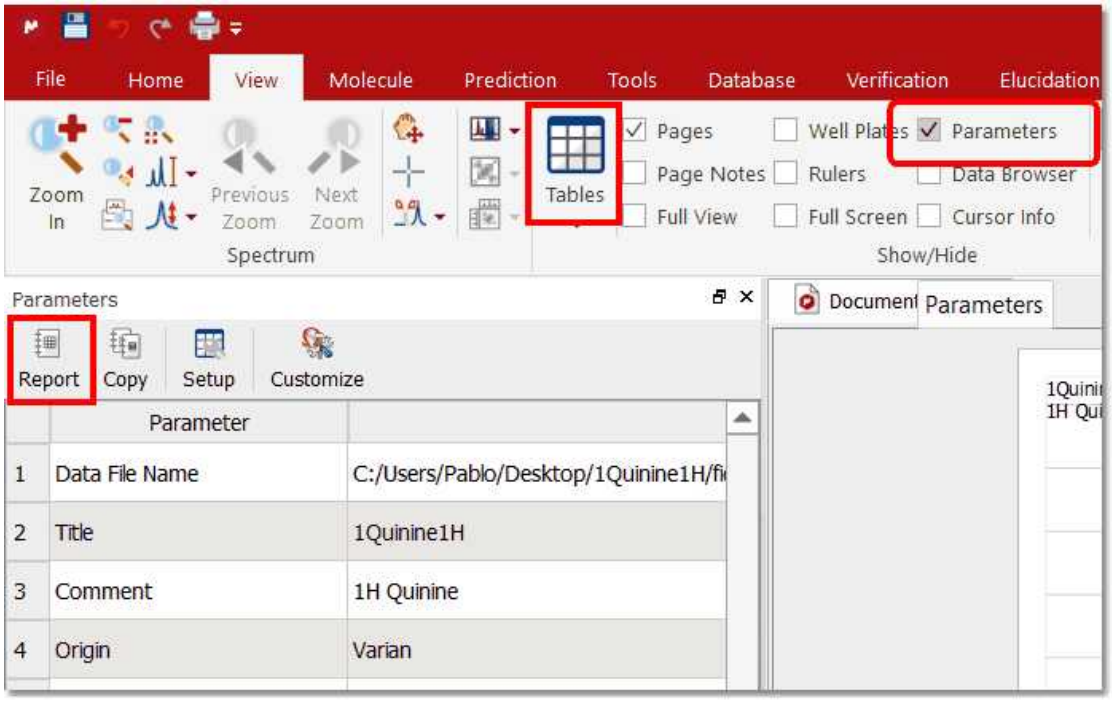


If you have your 1H and 13C NMR spectrum loaded, you can use the 'Absolute Reference' feature to reference 2D & C-13, by clicking on the 'Absolute Reference' button of the toolbar (or by following the menu 'Analysis/Reference/Absolute Reference'):

© 2024 MESTRELAB RESEARCH

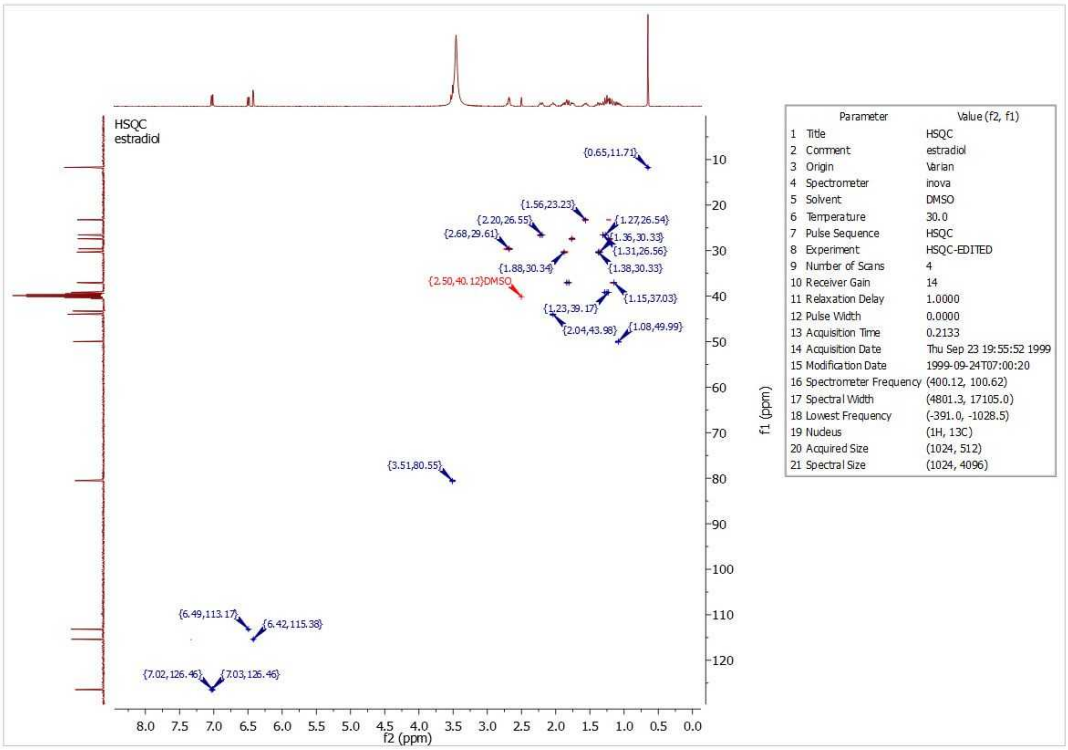


4. Click on the **'Peak Picking'** icon  to run a Peak Picking analysis and follow the menu 'View/Tables/Parameters' to display the 'Parameters Table'.



5. Then, click on the 'Report' button to paste the table on your page and move it to the right (by clicking&dragging). Finally resize the 2D spectrum to get the report below:

© 2024 MESTRELAB RESEARCH



6. Mnova documents can be exported as CSV, JCAMP, JSON, LOGS bundle, NMReData, PDF or txt formats by following the menu 'File/Save As' and selecting the applicable format from the scroll down menu.

See also:
Fast Visual Guide to process routine 1D-NMR experimental data
1D Processing Tour
2D Processing Tour
Manual Phase Correction in 2D-NMR

Watch a miniclip on this functionality at this link

© 2024 MESTRELAB RESEARCH

Part VI

6 The Mnova interface

The **Mnova** interface has been designed to minimize the learning curve for the new user, by capitalizing on familiarity and commonality with some of the most commonly used general software packages on the market. The interface consists of a main page window with a menu bar, toolbars, dialog boxes, rulers, active window tabs, etc. giving users total flexibility in the organization of their data. With **Mnova** you will only need a couple of mouse clicks to open and process your NMR data automatically. When you first open **Mnova**, you will immediately notice a few things which represent the fundamental graphical principles of the program:

- First of all, the software is **multipage**, à la **Powerpoint.**
- The **Mnova** Graphical User Interface is based on the 'What You See Is What You Get' principle (**WYSIWYG**).
- The **Mnova** graphical engine makes extensive use of the anti-aliasing technique to produce very high quality spectral plotting.
- The program includes a powerful **Undo/Redo** mechanism within a standard toolbar.

In a few minutes you will gain a good command of the most powerful and modern tools provided by Mnova, such as:

- One single command to open and process FIDs in any file format
- Powerful drawing tools with advanced text editing capabilities.
- Cutting tool to exclude uninteresting regions from the spectrum.
- Powerful scripting engine.
- Molecular viewer with assignment capabilities.
- Prediction of 1H, 13C X-nuclei NMR from chemical structures.
- And much more...

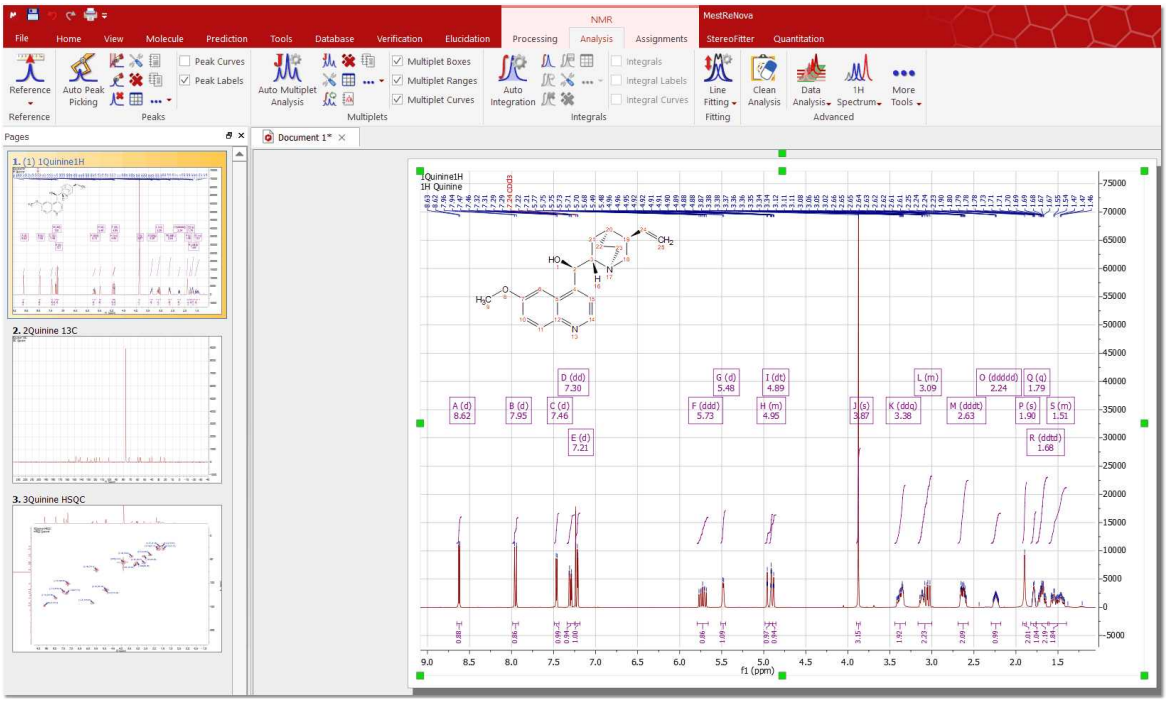
See also:
Multipage Software
Working on a single page
Zooming
Cutting
Undo & Redo
Graphics & Annotations

6.1 Multipage Software

The **Mnova interface** is designed to look and feel like **MS PowerPoint**. We think that the great majority of people in science are familiar with this presentation and reporting software, and that making our program behave in a very similar way to Powerpoint will ensure that even new users find it very easy to work with from the very start.

The first thing you will notice is that the software is **Multipage**, and incorporates a **page navigator**, which is placed, by default, as a column on the left hand side of the screen.

© 2024 MESTRELAB RESEARCH



The **Mnova document** is a **Multipage Document**. This means that *all pages within a single document will be saved as a single document*. This will allow you to keep, for example, different experiments for a certain sample (^{1}H, ^{13}C, homonuclear and heteronuclear 2D correlations) as a single, unified document.

If the Mnova document is huge and contains several pages, you can save only some of the pages, by selecting them in the page navigator and following the menu 'File/Saves as/MestReNova Pages (*.mnpag)'

If you want to merge different Mnova documents into the same one, follow the menu 'File/Open/MestReNova Pages (*.mnpag)' to select the desired documents to be merged.

The active slide is highlighted in the page navigator, and will appear in the main paper area of the document. This is the page to which all selected operations will be applied. You can choose the desired spectrum just by clicking on it on the page navigator or by following the menu 'View/Previous or Next Page'. In the same way, if you have highlighted any spectrum in the page navigator, you can move to the previous or the next spectrum by using the up or down arrow keys.

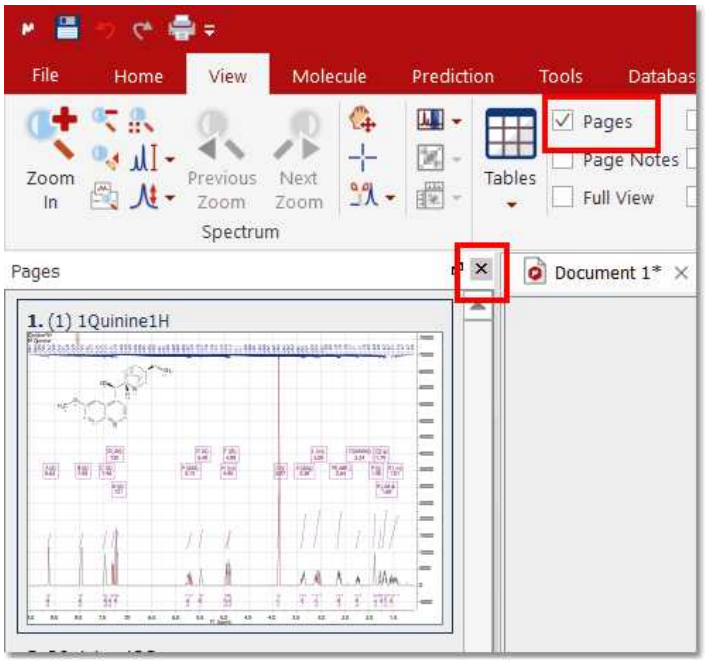
There are a number of things you can achieve by working on the page navigator:

- ✓ You can alter the **order of the pages** by left clicking on a page, dragging it and releasing the mouse to drop it at the desired position.
- ✓ You can **Create a New Page** by selecting 'Home/New Page', by right clicking on the page navigator window and selecting 'Create New Page' or by holding <Ctrl>+M (or Cmd + M in Mac).
- ✓ You can **Delete** an undesired spectrum by 'Home/Delete'' or by right clicking on the spectrum or on the page navigator and selecting 'Delete'. A selected spectrum can also be deleted using the 'Del' key.
- ✓ You can **select more than one page** by holding the <Ctrl> key (or Cmd in Mac) whilst clicking on the desired pages. When more than one page is selected, all operations (zooming, cutting, processing, etc.) will be applied to all selected pages.
- ✓ You can **copy** a page to the clipboard by pressing the <Ctrl> key (or Cmd in Mac) and then left clicking on the desired page, then dragging and releasing it whilst still holding <Ctrl> (or Cmd in Mac). Of course, a page can also be copied by selecting it and then choosing 'Home/Copy', or <Right

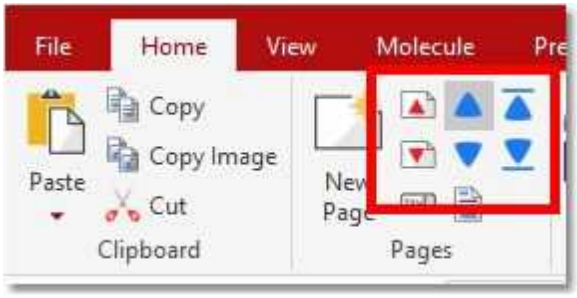
Click> and then Copy or by using the keyboard shortcut <Ctrl+C> (or Cmd+C for Mac). Any of these methods can be followed to copy one or several pages at a time, depending on what is selected when applying these operations.

✓ Conversely, a page or several pages can be pasted by using 'Edit/Paste', or <Right Click> and then Paste or by using the keyboard shortcut <Ctrl+V> (or Cmd+V for Mac).

✓ Finally, you can hide or move the page navigator, by clicking on the boxes highlighted on the picture below (or by following the menu 'View/Pages').



In order to navigate from one page to the other, you can use the page navigator or the arrows controls shown in the picture below (you will also find them at the right down corner of the spectral window):



From the 'Home/Page' ribbon, you can also move the page up and down and to the top and bottom. You could also use the applicable shortcuts:

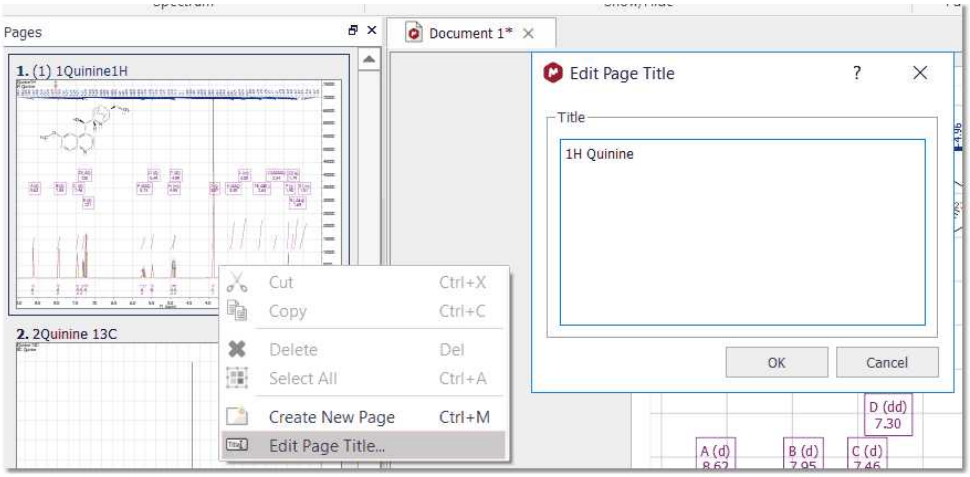
- Move Page Up: Ctrl+Shift+Up
- Move Page Down: Ctrl+Shift+Down
- Move Page to Top: Ctrl+Up
- Move Page to Bottom: Ctrl+Down

You will be able to show the page navigator by clicking on the last button of the picture below:

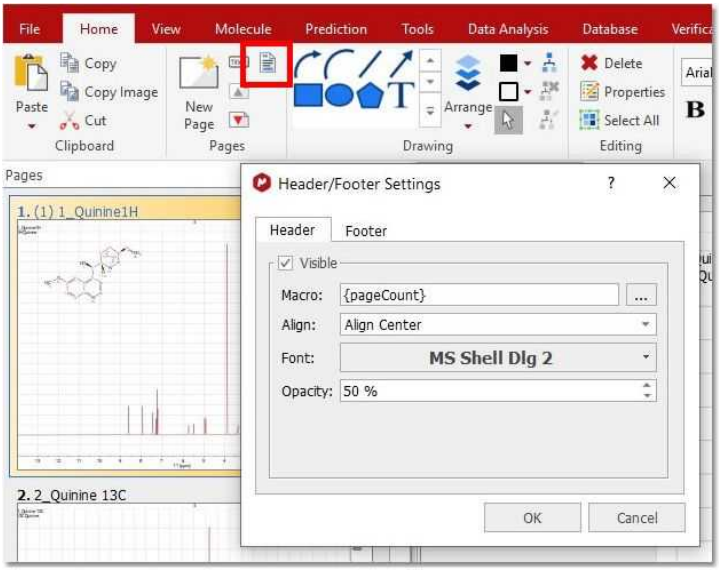
© 2024 MESTRELAB RESEARCH



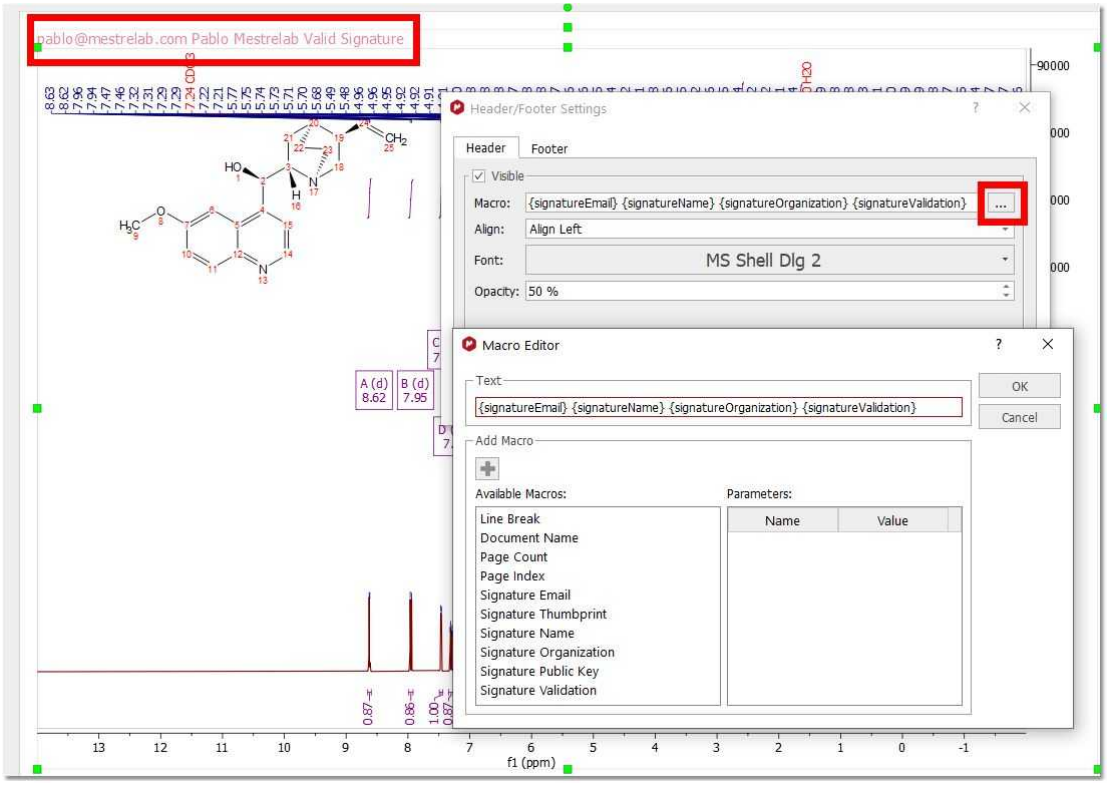
You can edit the title of the spectrum shown in the page navigator by right clicking on it and selecting 'Edit Page Title' or by following the 'Edit or Home menu':



You can include headers or footers to any page by using the applicable feature from the 'Home' ribbon:



From here, you could also include your signature:



Keep in mind that the documents are signed when saving (to a mnova file), so nothing will appear in the header until the document is saved. Also to be strict with the actual status of the signature, the signature fields will be empty too if the document has been modified since the last save. This means that if we, for instance, want to report the document as PDF including the signature header/footer, we must save the document as an mnova document first and then print to PDF the unmodified version of the document.

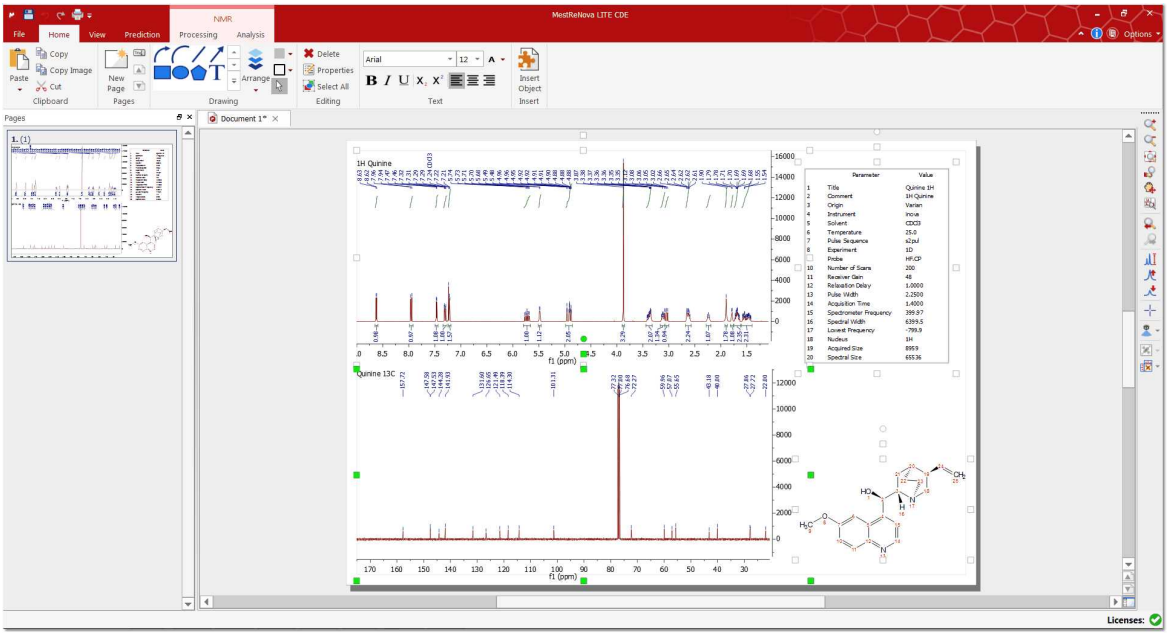
Mnova documents can also be exported as CSV, JCAMP, JSON, LOGS bundle, NMReData or txt formats by following the menu 'File/Save As' and selecting the applicable format from the scroll down menu.

6.1.1 Working on a Single Page

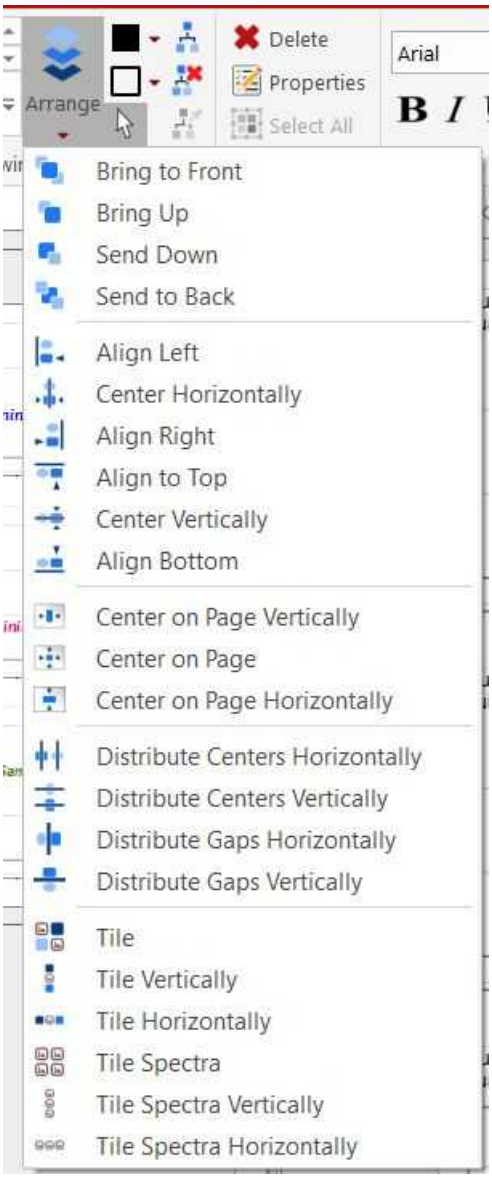
Whilst **Mnova** is a multipage document, it also allows you to have several objects (e.g. spectra) within a single page. The easiest way of placing two spectra on the same page is to simply select one of them, copy it (**'Edit/Copy'**, <Right Click> and **Copy** or <Ctrl+C> (or Cmd+C for Mac)) and then paste it directly on the desired page (Select the desired page and press 'Edit/Paste', <Right Click> and **Paste** or <Ctrl+V> (or Cmd+V in Mac)).

Note that the **active object** is highlighted with **green squares** (in the green circle in the figure below). Any **Mnova** object can be **resized** and **moved** by just clicking and dragging on the corner boxes or on the object respectively. Note also that any other objects selected but not active will be highlighted with white squares (in a blue circle in the figure below) (To select more than one object, hold the <Ctrl> (or Cmd in Mac) key while clicking on the desired objects. To select all objects on a page, just choose 'Edit/Select All' or the keyboard shortcut <Ctrl+A> (or Cmd+A in Mac).

© 2024 MESTRELAB RESEARCH



Once more than one object (spectrum or otherwise) has been placed on the same page, it can be manipulated and placed on the page by using the scroll menu shown in the figure below, accessible via the **'Arrange'** icon:



This scroll menu will allow you to **Bring to Front** or **Up**, **Send Down** or **Back**, **Align Left**, **Right**, **Top** and **Bottom**, to **Center Horizontally** or **Vertically** (applying this last operation with multiple objects selected will Superimpose them), to center the object one page, to distribute the centers and gaps and to tile the objects or the spectra. Of course, you can move the spectrum selected in a given direction using the arrow keys of your keyboard. Remember that multiple objects must have been selected for these commands to work effectively. You can also superimpose several spectra by using the 'Superimpose' feature.



Utilities

The superimpose option is very useful when attempting to identify a known compound present in a complex mixture or when comparing similar compounds.

Any operations applied to the active object will also be applied to the other selected objects (zooming, panning, cutting, processing operations, etc) as was the case with multiple pages selected.

© 2024 MESTRELAB RESEARCH

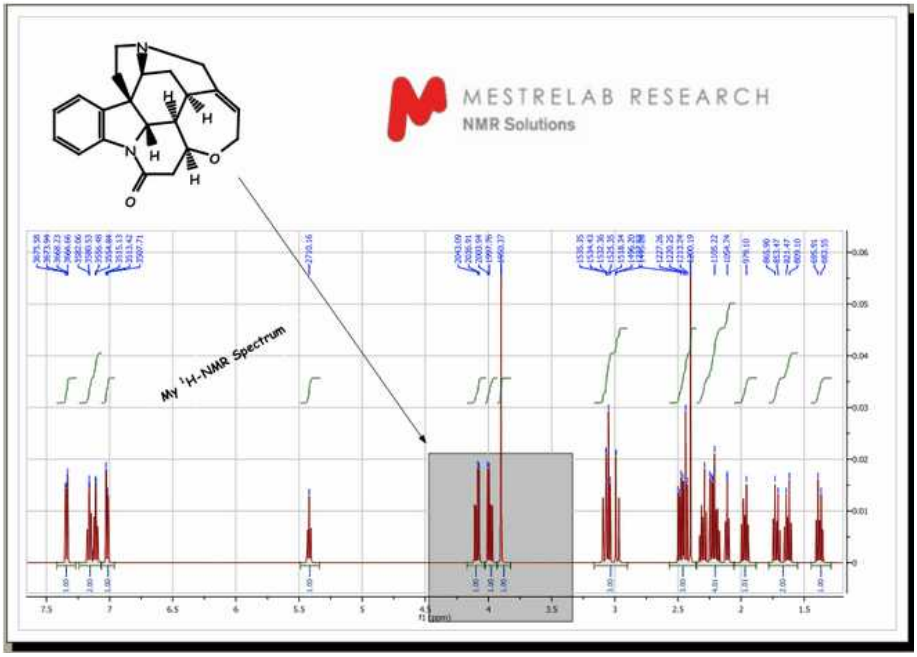
If the Mnova document is huge and contains several pages, you can save only some of the pages, by selecting them in the page navigator and following the menu 'File/Saves as/MestReNova Pages (*.mnpag)'

If you want to merge different Mnova documents into the same one, follow the menu 'File/Open/MestReNova Pages (*.mnpag)' to select the desired documents to be merged.

6.1.2 Graphics, Annotations and Signatures

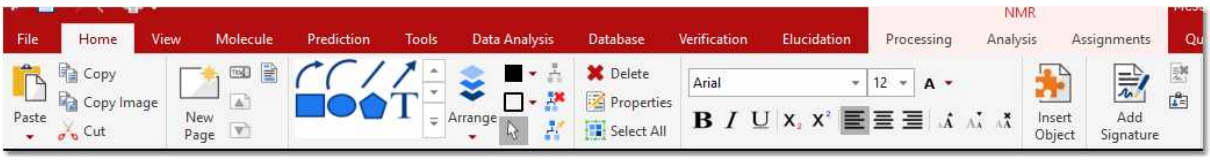
Mnova has the most powerful graphics and annotation capabilities available in any NMR software. Most of the annotations and drawing available in MS Powerpoint are available in Mnova via the graphics toolbar placed by default at the bottom of the screen, below the page navigator and the document area. The software supports Rich Text and so a wide range of fonts and font embellishments (sub- and superscript, bold, italic etc) are available.

Mnova can import objects such as **molecular structures** using 'Home/Insert Object' while **images** may be embedded using the standard Windows Copy and Paste tools.

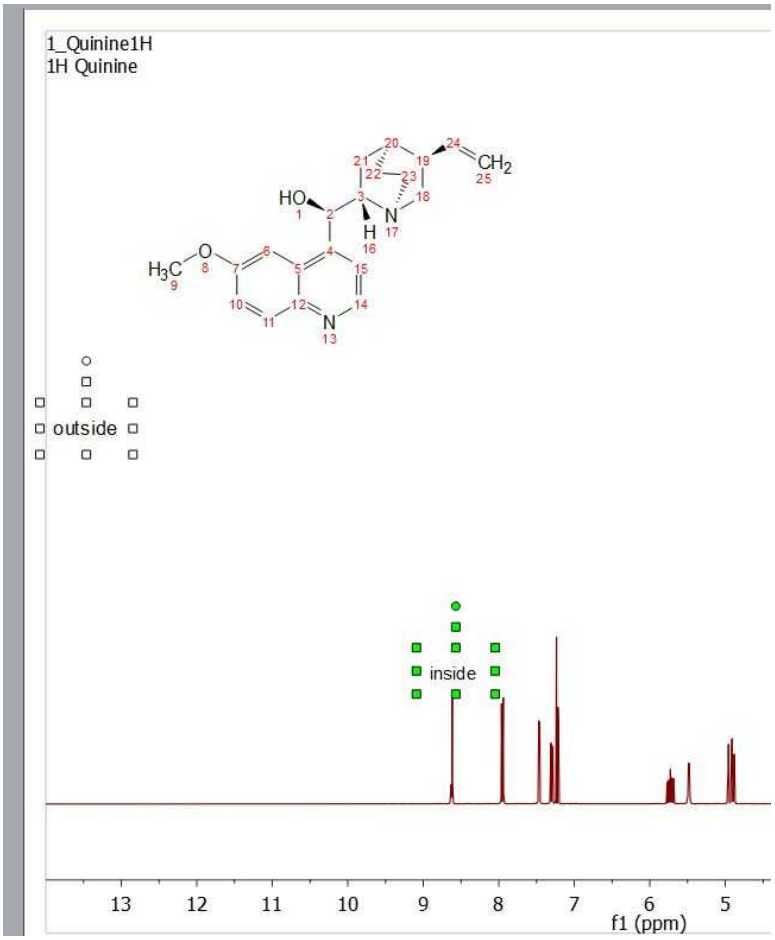


You can draw a curved arrow, line, a rectangle, an ellipse or a polygon by following the menu 'Home/Drawing' or by using the graphic toolbar, as you can see below. Once you have selected the object you want to draw, hold down the left mouse button in the spectrum display and drag the cursor over the area where the line or shape is to be drawn. Once created the line/shape may be moved, rotated and resized by selecting it and using the object's moving, sizing and rotating "tabs". You can also choose the fill color (light grey as default) as well as the colour and linewidth of the border of the object (black as default). Text may be added to the document in much the same way (T is the shortcut). The text can be aligned (right or left justified or centered) and you can select the text features **Bold**, *Italic* and Underline, sub-and superscript and the font (color, type and point size).

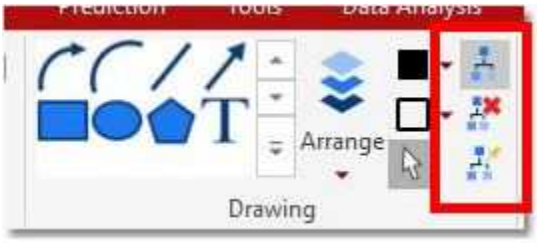
© 2024 MESTRELAB RESEARCH



Please note that if the 'text or drawing box' is **completely inside** in the spectral window (and not in contact with the scales or similar), it will be moved with the spectrum zoom in. If it is outside, or partially outside, then it will be kept when zoom in (for example):



However you could add annotations or any other object as a child item of a spectrum (or any other object). Therefore when making a transformation (move, scale or rotate) of the parent item, their coordinates get also transformed. This is also happening in the layout template document.



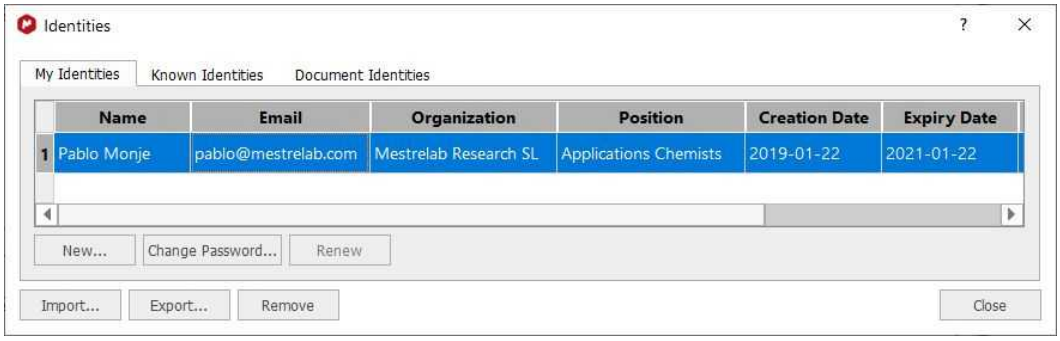
© 2024 MESTRELAB RESEARCH

The first command will allow you to set the (selected items) children of the active item. The second button will allow you to clear children items and the third button will allow you to toggle the child type.

From the annotation toolbar, you can also sign documents, so we can grant that the document was created by owner.

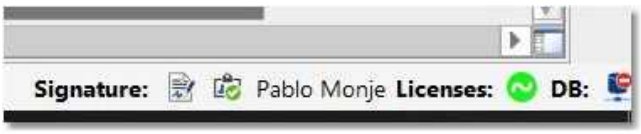


The button "Add Signature" will allow you to sign a document with an existing identity. If you don't have any, a message is shown and you get redirected to the Identities dialog (also accessible via "Show Identities" button). From here, you can add new identities, import/export/remove identities (public or private). These identities will go to "My Identities" (with private part) or to "Known Identities" tab (no private part). Documents Identities tab shows all identities used each time a document was saved.



The button "Remove Signer" allows you to remove the sign from a document so that the next time the document is saved, the sign is removed.

When loading a document it is possible to see the status of the sign in the status bar:
- first icon: current signer (available or not)
- second icon: signed document (signed or not, valid/invalid, trusted/not trusted). If you double-click it the Identities dialog is opened.

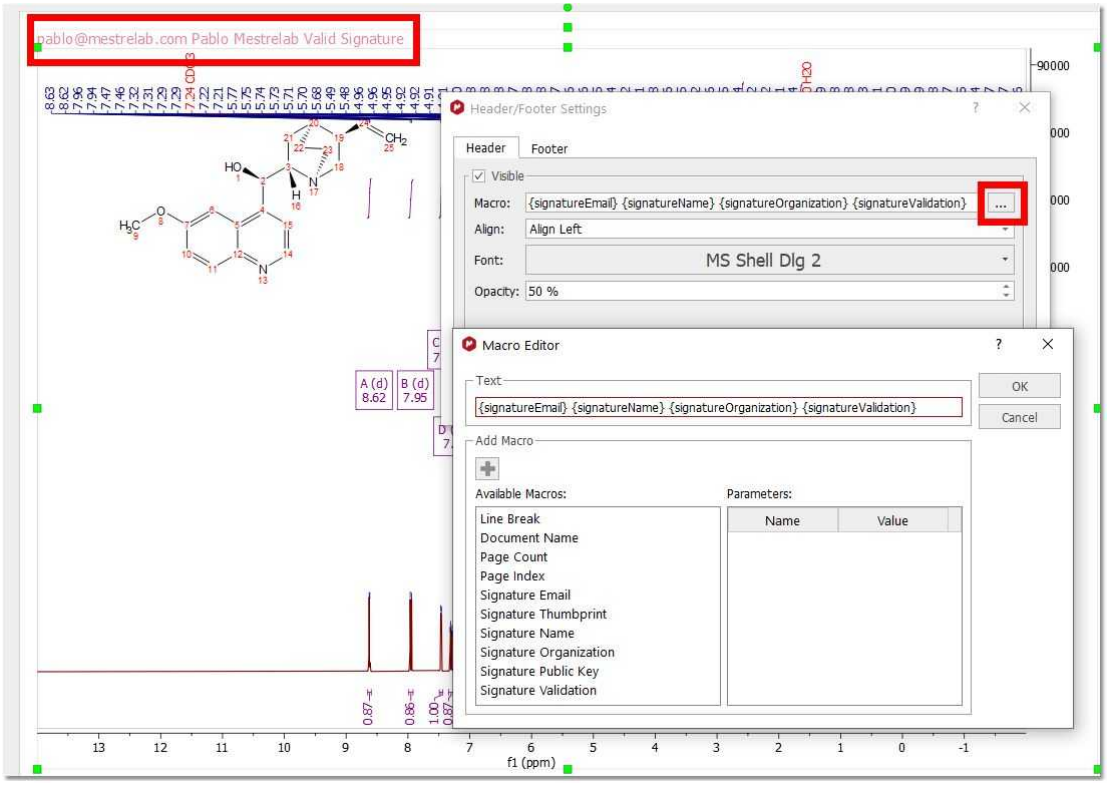


When receiving a signed document, you can see the sign in "Documents Identities", select the identity and click "Trust" to add it to the "Known Identities" list.

Mnova allows you to add several signatures, so each user can sign the document at any time.

You can display your signature as a header/footer in your Mnova document, by following the menu 'Home/Page Header/Footer'

© 2024 MESTRELAB RESEARCH

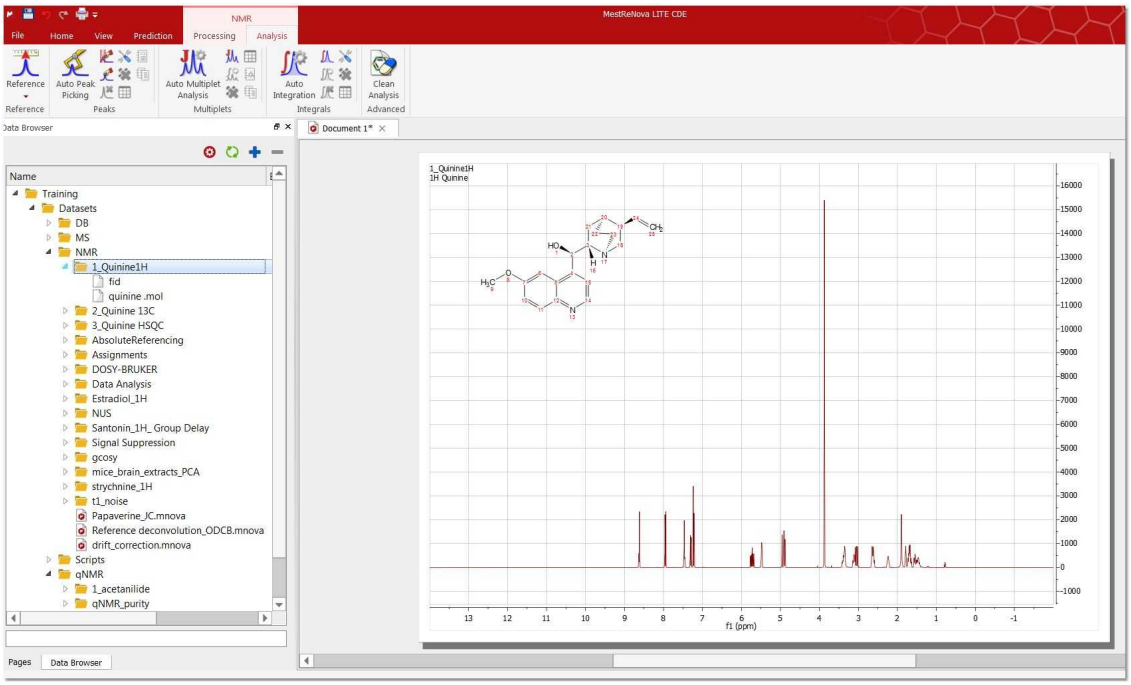


Keep in mind that the documents are signed when saving (to a mnova file), so nothing will appear in the header until the document is saved. Also to be strict with the actual status of the signature, the signature fields will be empty too if the document has been modified since the last save. This means that if we, for instance, want to report the document as PDF including the signature header/footer, we must save the document as an mnova document first and then print to PDF the unmodified version of the document.

6.2 Data Browser

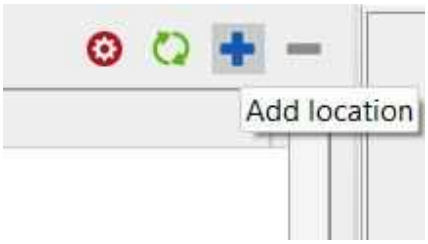
As you know, you can load datasets into Mnova just by dragging&dropping the folder(s) containing the raw data. Alternatively, you can just load the FID by following the 'File/Open' (Ctrl/Cmd +O as a shortcut) and since Mnova 9.1.0, you could also use the Data Browser to load your datasets, just by double clicking on the applicable folder (or by dragging&dropping):

© 2024 MESTRELAB RESEARCH



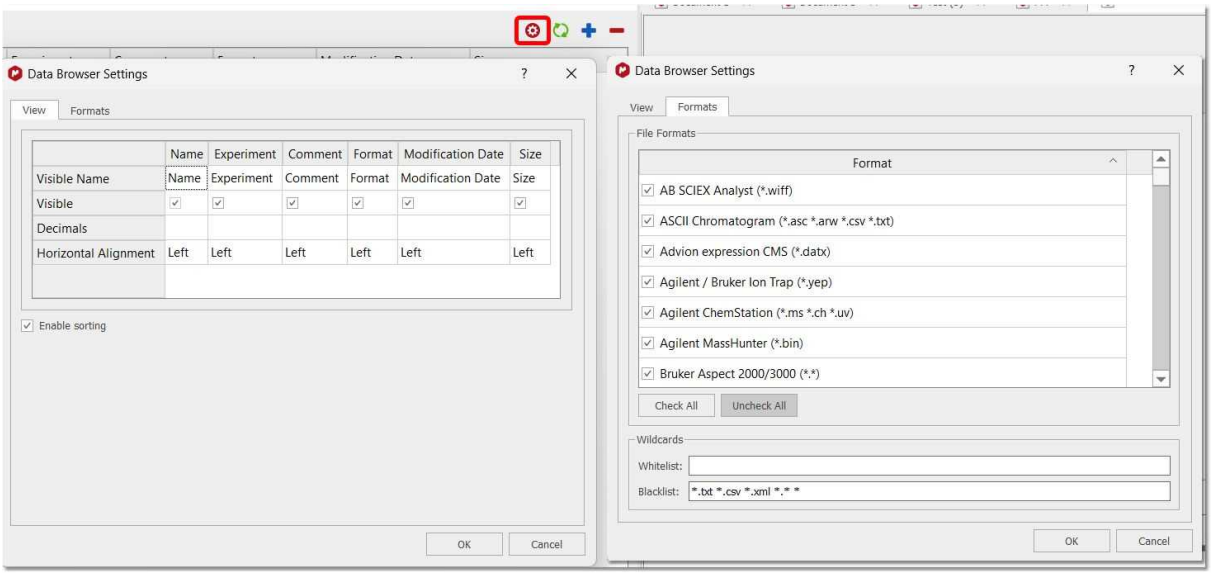
If the Data Browser is not displayed on your screen, just follow the menu 'View/Panel/Data Browser'.

You can register several data locations just by clicking on the applicable button or by dragging and dropping (into the browser) the folder containing the datasets:

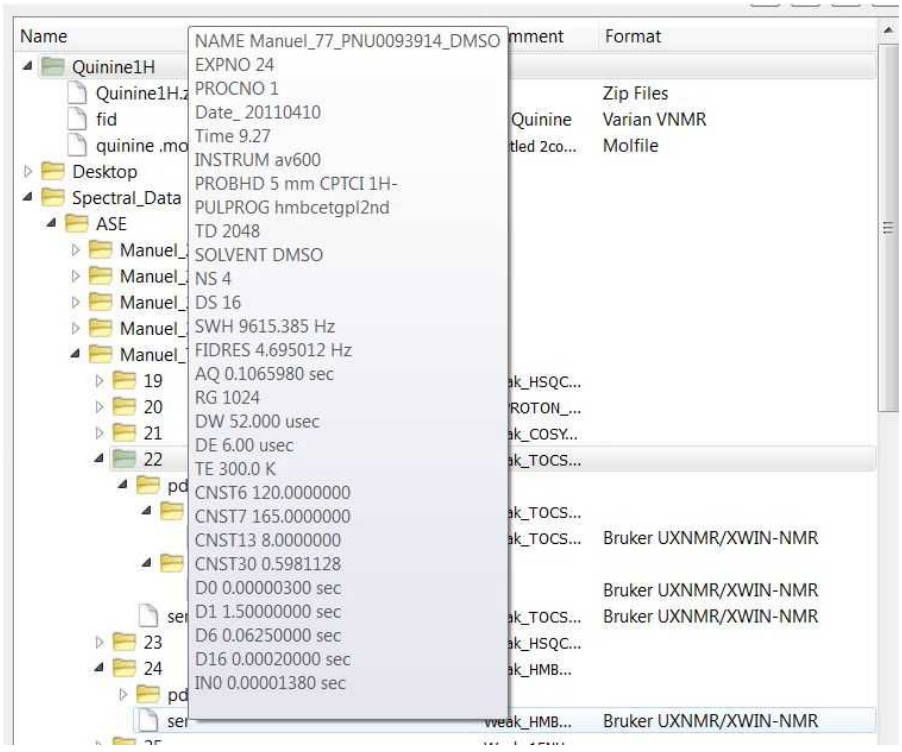


These data locations are the same as in "File/Preferences/General/Open&Save/Open Locations".

The data browser will handle all spectral and molecule formats from all vendors, but the user will be entitled to filter some of the formats (from the Formats tab) and also to decide what columns will be displayed (from the View tab):

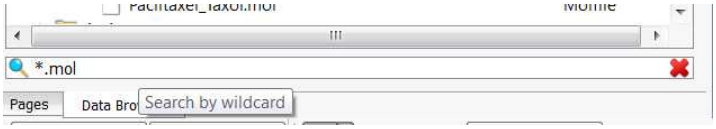


In case of Bruker datasets all openable data will be listed in the tree view: fid and all 1r's or ser and all 2rr's. The spectral titles will be shown directly in the panel and also be available as tool-tips. Hovering the cursor over the spectra folder or fid/ser file related will display some information. In case of Bruker files, the parm.txt file is shown as tooltip. In case of Varian files, the content of Comment field will be displayed.



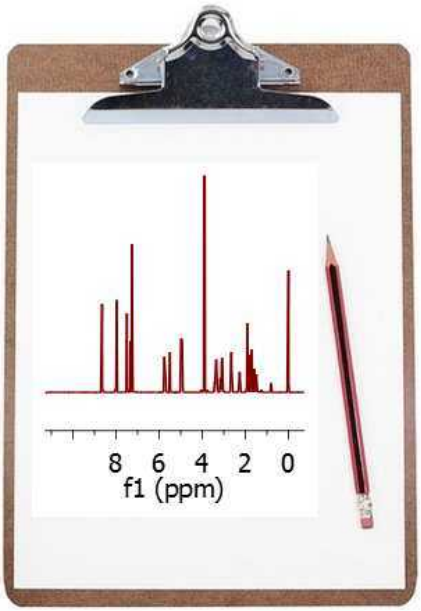
At the bottom of the Data Browser you will find a 'search feature' that will work with wildcards and strings in the same way. For example, you will get the same result by typing ".mol" or "*.mol" in the search cell, and both searches take equal time:

© 2024 MESTRELAB RESEARCH



6.3 Intelligent NMR Clipboard

The clipboard is for sure one of the most useful features in any modern Operating System and it is used for short-term data storage and/or data transfer between documents or applications, via copy and paste operations. Any modern application should support clipboard operations and, of course, **Mnova** is no exception. However, **Mnova** goes one step further compared to other applications as I will try to show in this post.

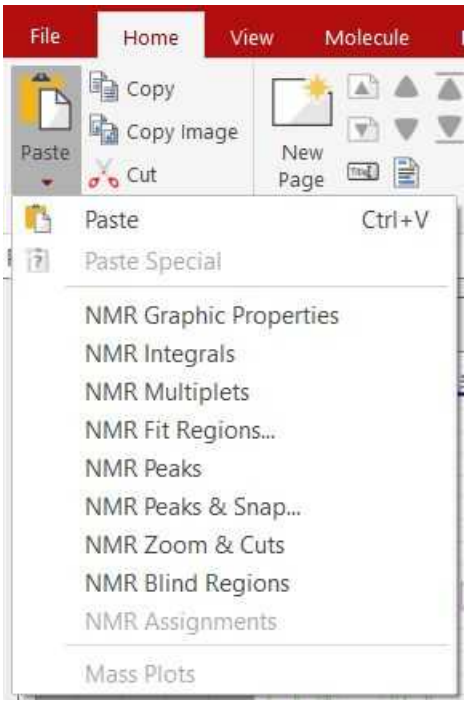


Following the same principles as standard Office applications, with **Mnova** you can copy any object (e.g. spectra, molecules, etc) into the clipboard and paste it either in **Mnova** (for example in a different page) or in any other application such as MS Office. The peculiar thing is that once a spectrum has been transferred to the clipboard (via Ctrl/Cmd+C), you can 'paste' it into another spectrum. Several examples will illustrate this feature.

Example #1

You have processed a spectrum and integrated carefully in order to quantify some signals of interest. Next you realize that you also want to integrate a different spectrum but using exactly the same integral regions as those in the first spectrum. There are several ways to do that in Mnova, but a very simple one involves copying the first spectrum into the clipboard and then selecting 'Paste Properties/NMR Integrals' from the Edit (or Home) menu. You can paste these integrals either to a single spectrum or to a bunch of selected spectra (or pages). The same can be used for the peaks

© 2024 MESTRELAB RESEARCH



The same can be done with the multiplets.

The paste option takes into account if both spectra have compatible nucleus, so if a 1H is copied, the integrals/multiplets will be pasted in another 1H but not in a 13C.

When the spectra have compatible nucleus but different dimensions, the pasting is done as:

- If the copied spectrum is a 1D and the pasted spectrum is a homonuclear 2D, the new 2D region is created by taking the 1D range for both dimensions.

- If the copied spectrum is a homonuclear 2D and the pasted spectrum is a 1D, the new 1D region is created by taking the horizontal range of the 2D region.

- If the copied spectrum is a heteronuclear 2D and the pasted spectrum is a 1D, the new 1D region is created by taking the range corresponding to the dimension with the same nucleus as the 1D spectrum. If the spectrum is a homonuclear 2D, the new 2D region is created by taking the range corresponding to the current nucleus for both dimensions.

If the copied spectrum has multiplets but no integrals, the option Paste NMR Integrals is enabled (it will copy the integration regions of the multiplets). And the same, if the copied spectrum has integrals but no multiplets, the option Paste NMR Multiplets is enabled (it will copy the integration regions).

Note: when the copied spectrum is 1D and pasted spectrum is 2D, new regions will be created with all possible combinations of the 1D regions (if N 1D regions, then N 2 2D regions). In the case of integrals; all the resulting integrals will be pasted, but in the case of multiplets only the resulting multiplets with peaks will be pasted.

Example #2

You have customized the graphical properties of a spectrum (e.g. fonts, colors, grid, etc) and you find that you want to rapidly apply the same graphical settings to another spectrum. Once more, this is very

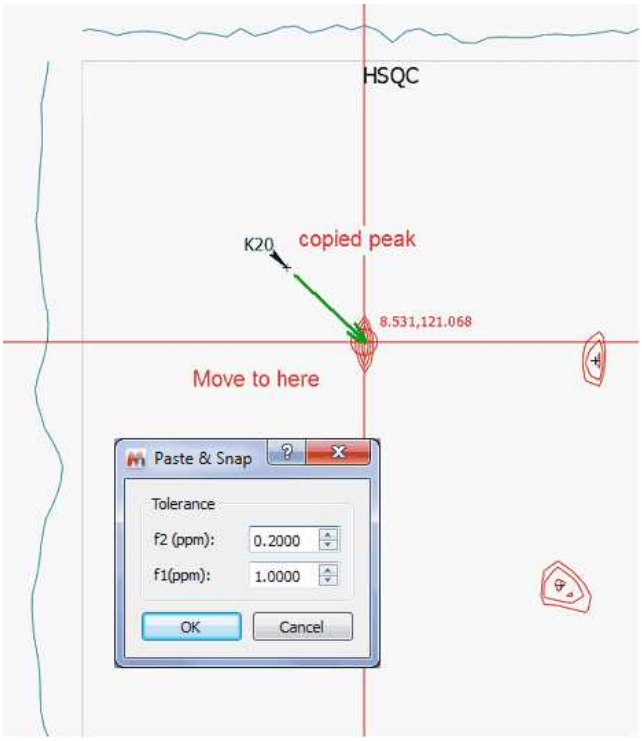
© 2024 MESTRELAB RESEARCH

easy with the clipboard: copy the first spectrum (Ctrl/Cmd+C) and then select the target spectrum and issue command ‘Paste NMR Graphic Properties’.

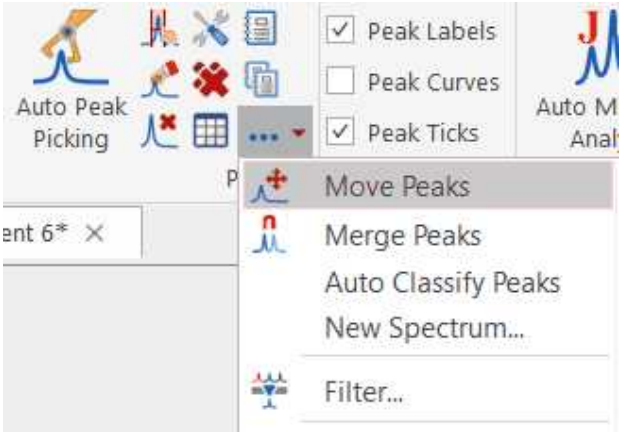
See also this blog post

Example 3 (NMR Peaks & Snap)

When copying the peaks to a new spectrum, Mnova has an option to automatically snap the peaks to its nearest peak. A peak that would be automatically picked using the current peak picking threshold. For this option, the user is able to define a chemical shift window (the maximum chemical shift changes expected in f1 and f2).



Mnova also allows the user to click-drag and move a peak label to a new location, by using the 'Move Peaks' tool under the Peak Picking menu.

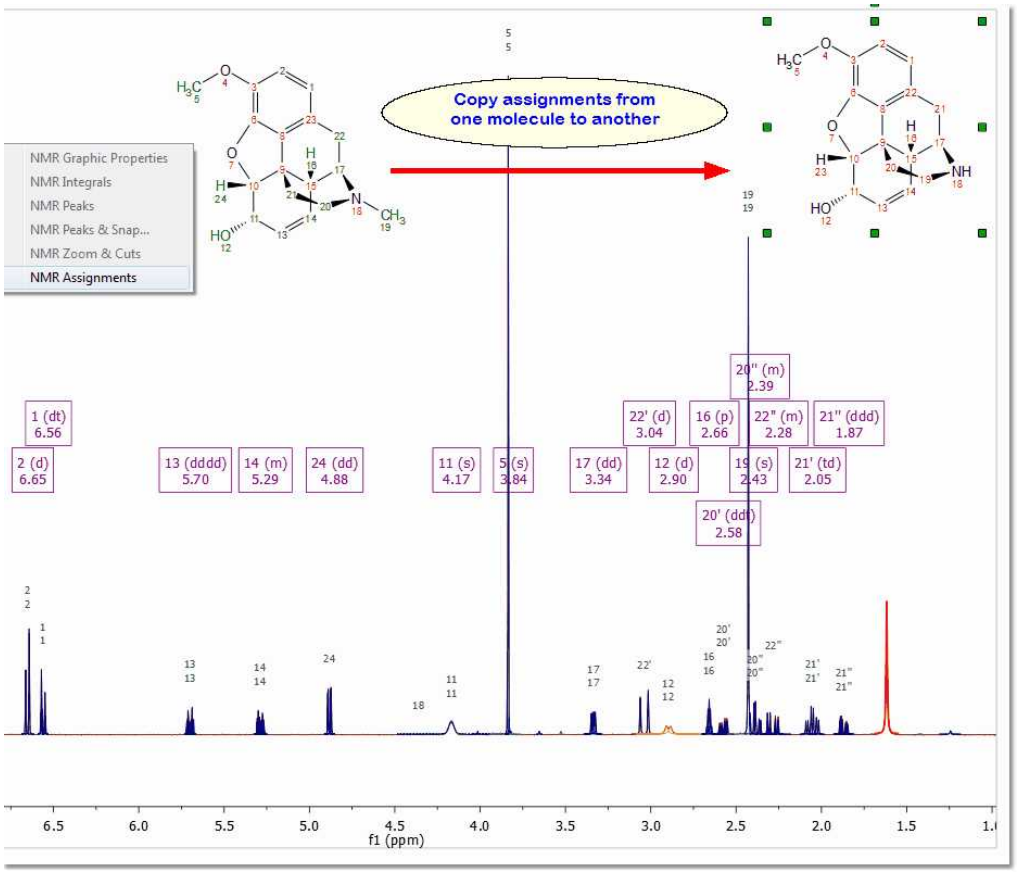


Example 4 (Zoom&Cuts)

This option will be used to copy the zoom and cuttings from one spectrum to another (or others). The same concept can be applied to blind regions

Example 5 (NMR assignments)

This feature will copy the assignments from one molecule to another. The mechanism will be: Ctrl/Cmd+C in the assigned molecule, followed by clicking on the new molecule and applying 'Edit(Home)/Paste Properties/NMR Assignments' command. Please bear in mind that the numbering of the common parts of both molecules must be the same.



Another way would be to just copy&paste the molecular structure with the assignments and make the structural changes in one of the molecules.

Example 6 (NMR Fit Regions)

This feature will be useful to paste the line fitting regions and peaks from one spectrum to another. One you have applied the Line Fitting over the first spectrum, you will only need to 'Ctrl+C' and follow the menu 'Home/Paste/NMR Fit Regions'. A new dialog box will be displayed with the options below:

© 2024 MESTRELAB RESEARCH



- The ***Regions*** option pastes only the regions and fulfill them with an automatic peak picking. The regions are bounded to the current spectrum's range.

- The ***Regions & Peaks*** option pastes the regions and their peaks, exactly as they are in the copied spectrum (same Shift, Height, Width, and L/G).

- The ***All*** option pastes the regions, peaks and locks, exactly as they are in the copied spectrum.

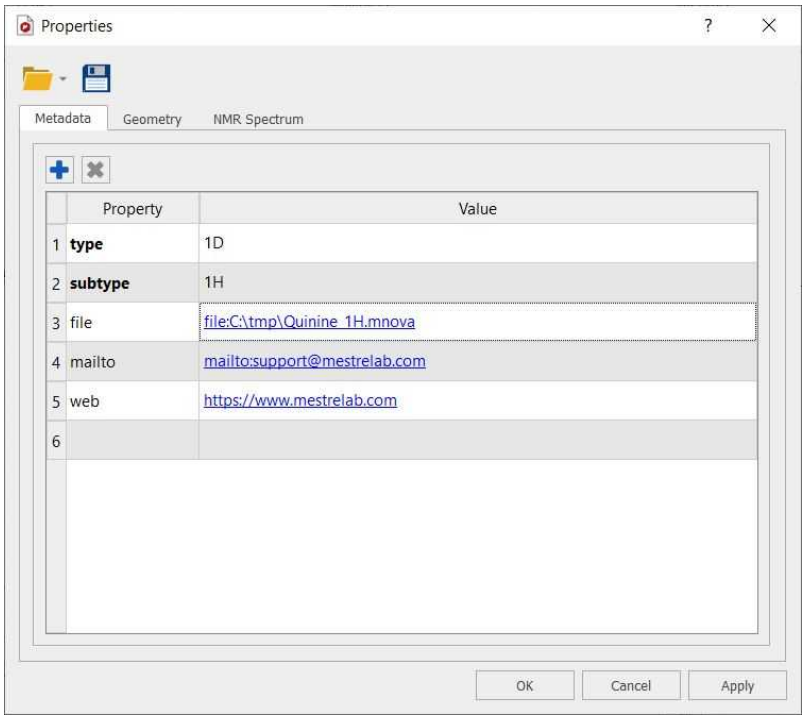
- The ***Apply Fitting*** option executes the fitting of the regions after pasting them.

6.4 Properties

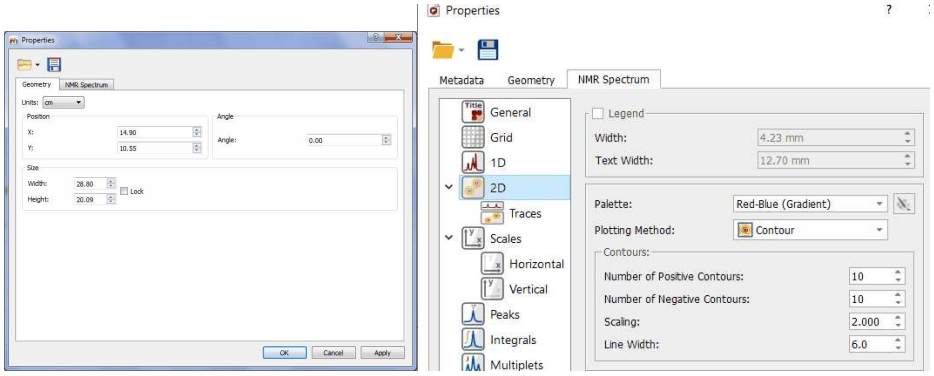
Properties: Mnova will allow the user to customize nearly all the attributes of the spectrum. The user can select the properties of the spectrum by following the menu 'Home/Properties' or by pressing the right mouse button on the spectrum and selecting '*Properties'* from the *pop-up* menu.

From the metadata tab, you can add hyperlinks to include websites, files or email address:

© 2024 MESTRELAB RESEARCH

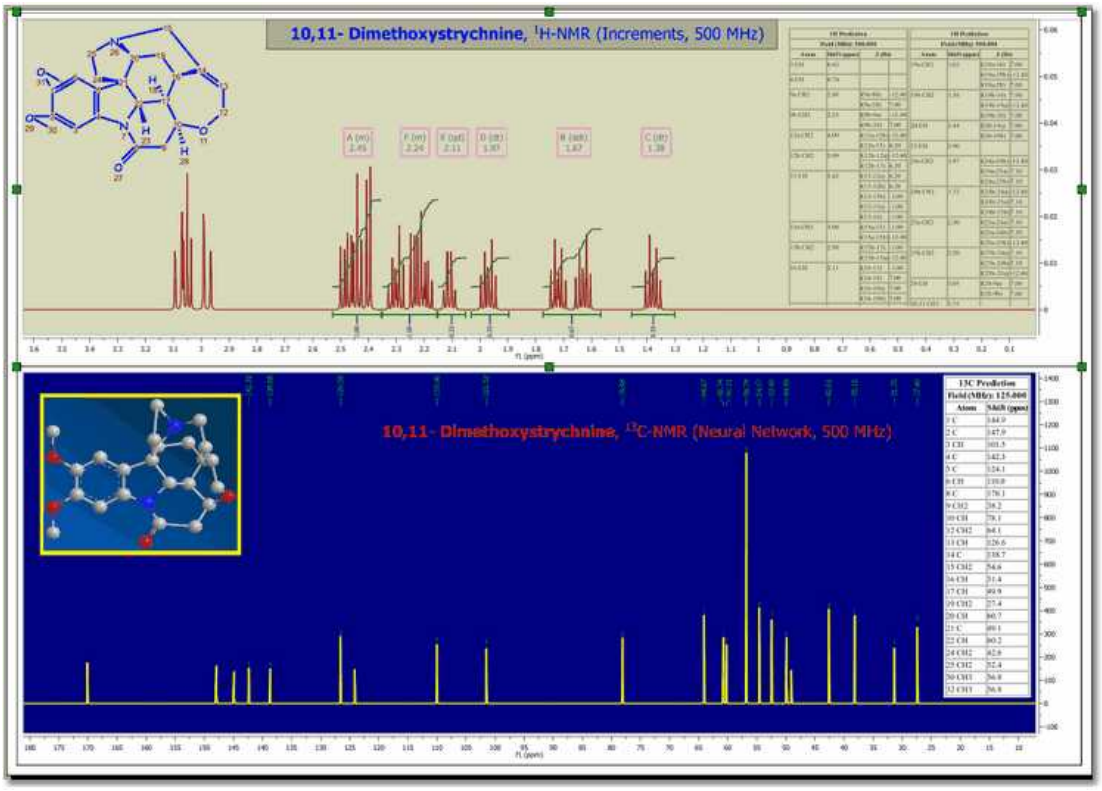


You can see in the pictures below the geometry and the Spectrum properties of a 2D NMR experiment.



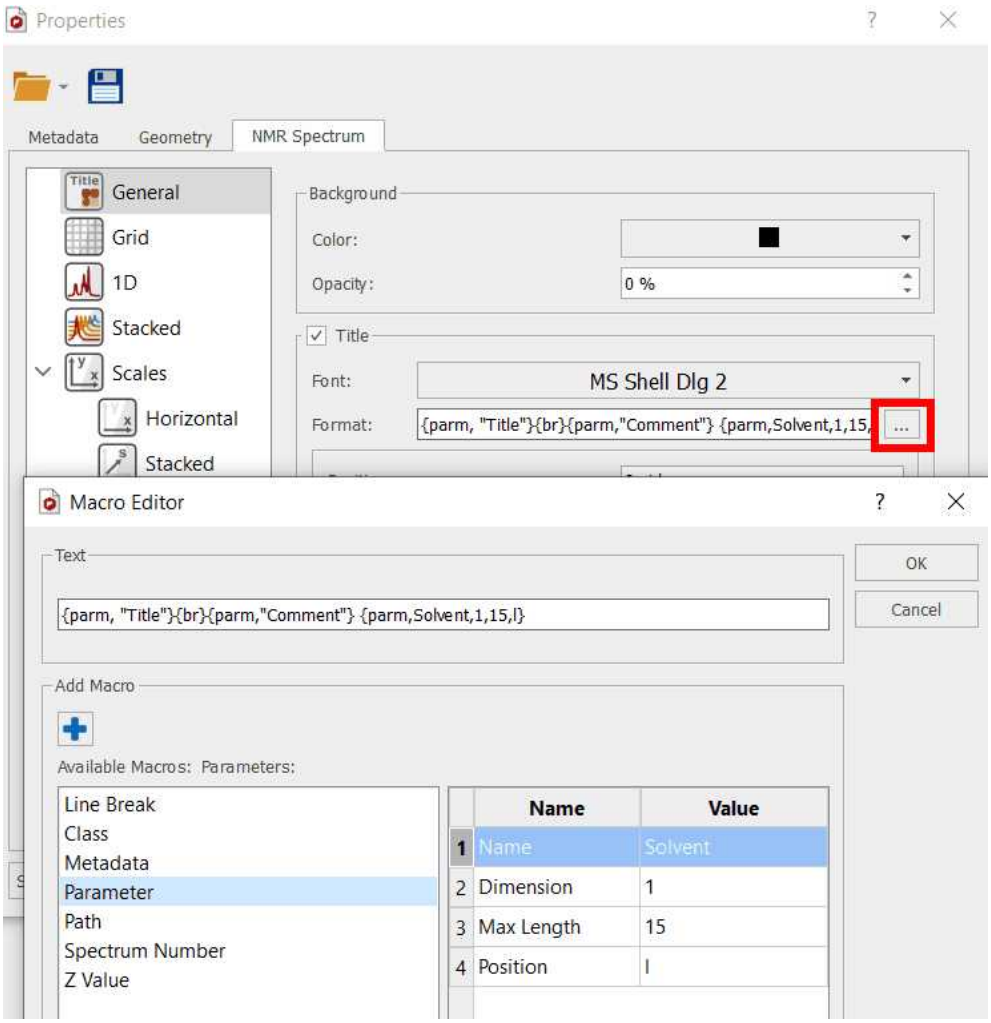
From this set of tabbed pages you can change, for instance, the spectrum's geometry (position, angle, size), the traces size, the **'Background'**, the **'Line-Style'** (line, crosses or circles), the **'Line Colour'** and the **'Line Width'**. Bear in mind that a '**Line Width'** of zero indicates a *cosmetic pen*. This means that the *pen* width is always drawn one pixel wide, independent of the transformation set on the painter. In 2D NMR spectra, you can set the legend, the number of contours, ploting method, show traces and customize the palette. The user can also set the scales' properties, change the grids, the aspect of the axes and margins or the units (ppm, Hz, points, seconds), for a more convenient representation of the spectrum. You can change the color of the spectrum background to suit your eyesight, your working hours, or your mood. Enjoy complete freedom on how to present your data! Transparency is also supported! Here you can see an example:

© 2024 MESTRELAB RESEARCH



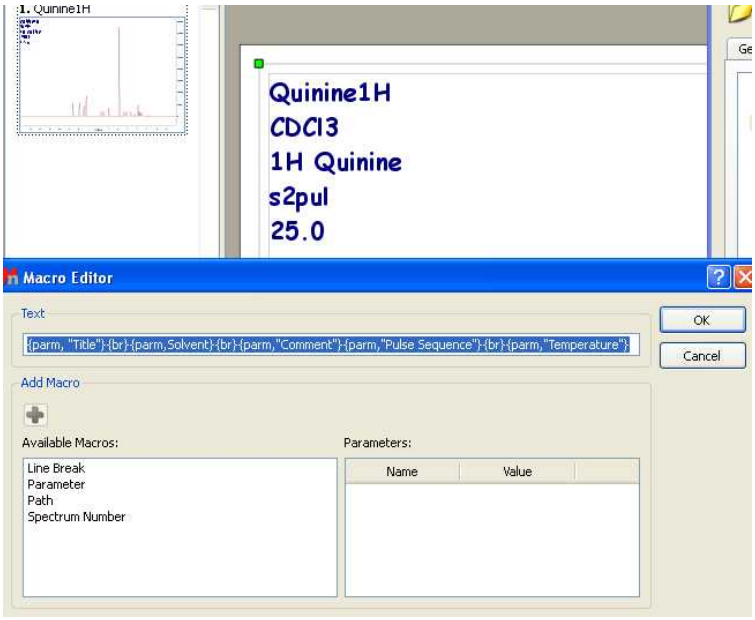
Check the 'Title' box of the 'Spectrum/Common' properties to **show the title on your spectrum**. You can select the position/alignment/offset of the title and also to customize the format by clicking on this icon . This will display a dialog box where you will be able to add 'Line Breaks', the class, the desired 'Parameters' of the Parameters Table e.g. (Title, Comment, Solvent, Owner, etc). From here you can also select the Name, Dimension, Max Length or Position. You can also add any 'Path' (with the capability to select the Level, Max Length or Position) or the 'Spectrum Number', just by selecting it on the 'Available Macros' panel and clicking on this icon  to add it to the title. Finally click on OK.

© 2024 MESTRELAB RESEARCH

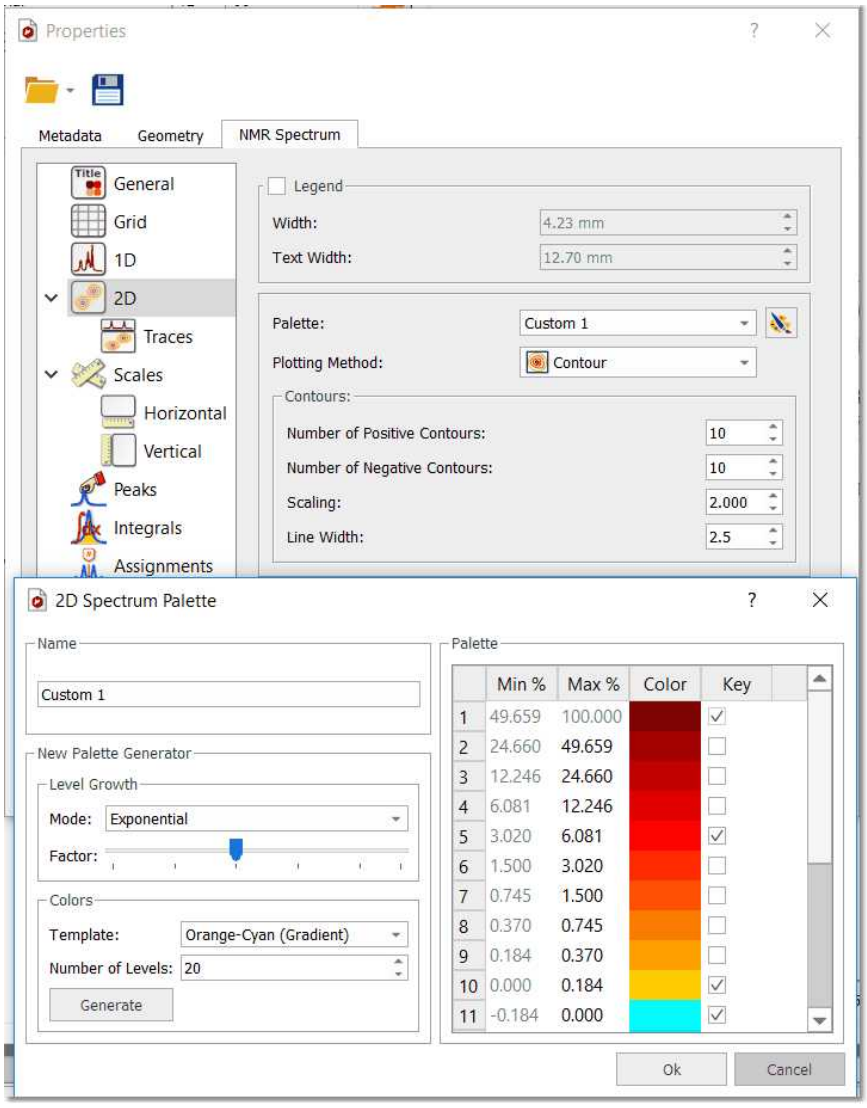


Here you can see an example where we have changed the font of the title and added several parameters (Title, Solvent, Comment, Pulse Sequence and Temperature) to the title of the spectrum:

© 2024 MESTRELAB RESEARCH



The user will be able to select the desired 2D spectrum palette and also customize his own palette by selecting for example 'Custom 1' in the 'Palette scroll down menu and then clicking on the '**Palette Editor**' button  to display the '**2D-Spectrum Palette**' dialog box, where the user will be able to customize the desired palette (Name, Level Growth, Colors, Number of Levels, etc..). You can select the number of the positive and negative contours and also the scaling (in the 'Properties' dialog box) as you can see in the picture below:



If you want to obtain monochrome printouts different with the ones that already exist in the Palette scroll down menu, please follow the procedure below to create a palette in pure black with no grayscale:

1. Run Mnova and load your 2D-NMR spectrum.

2. Double click on the spectral window to open the 'Properties dialog box'

3. Select 'Custom 1' on the 'Palette' scroll down menu

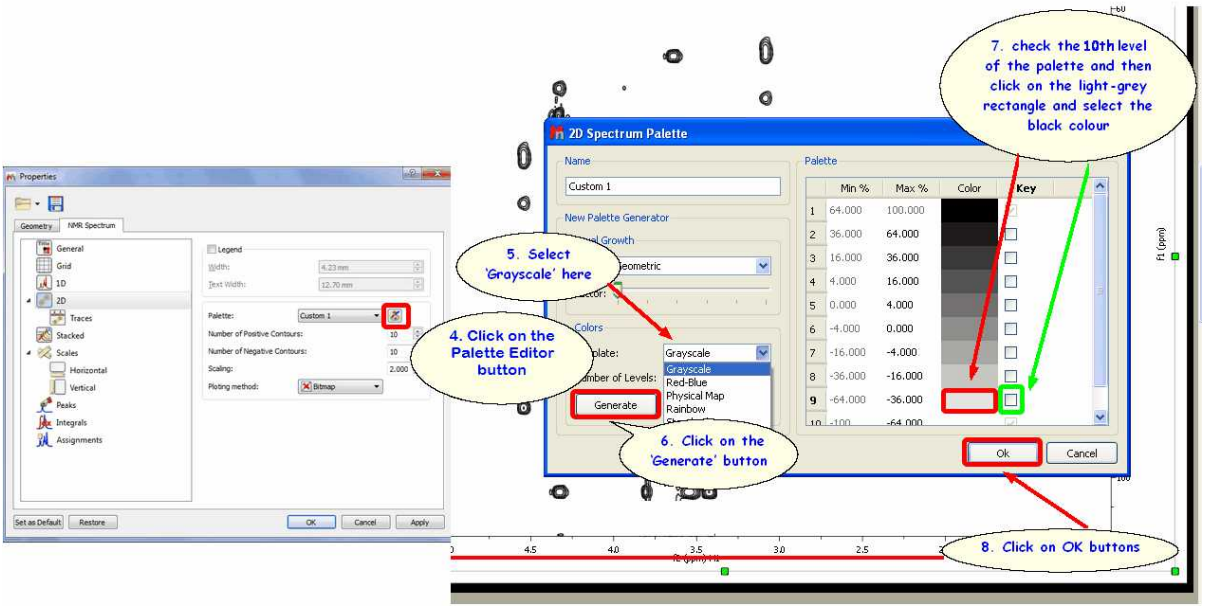
4. Click on the 'Palette Editor' button (just at the right side of the scroll down menu)

5. Select 'Grayscale' on the 'Template' colours scroll down menu

6. Click on the 'Generate' button

7a. If you have 10 levels, check the 10th level of the palette and then click on the light-grey rectangle (just at the left side of the check mark) and select the black colour. Finally click on 'OK' buttons.

© 2024 MESTRELAB RESEARCH



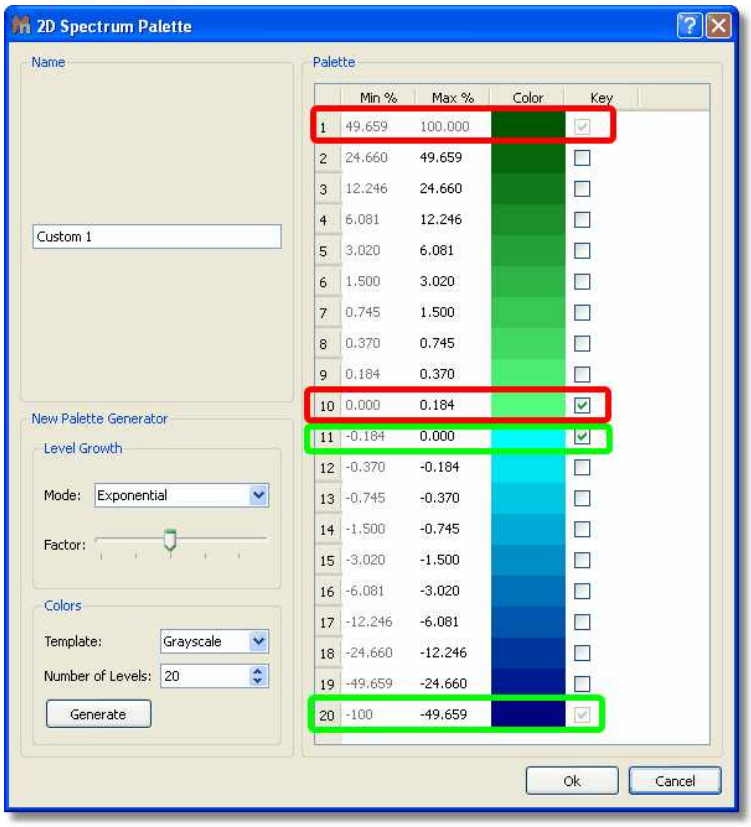
7b. If you have 20 levels, do the same with the 20th level and so on…

For further information, please take a look a this video

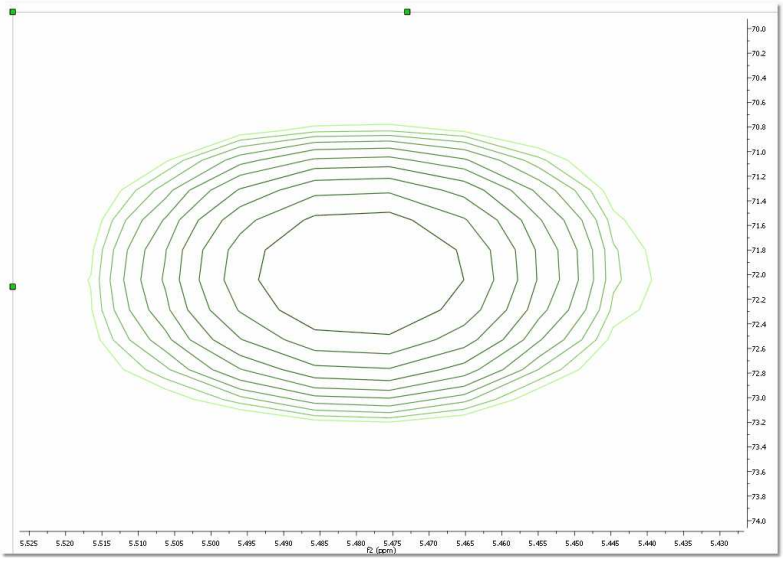
Let´s take a look at another situation: how to make all positive contours a scale of greens and all negative contours a scale of blues?

In this case, you will only need to select in the '2D Spectrum Palette' dialog box the first and the last colour of the positive peaks and the same for the negatives. For example, imagine that you have 10 positive contours, then you will need to select the colour of the contour 1 as 'dark green' (for example) and then check the applicable mark at the right side. Finally, select the colour of the contour number 10 as 'light green' and check the mark. Do the same for the negative peaks; in this case the contour number 11 will be 'light blue' and the number 20 will be 'dark blue'. Please take a look at the screenshot below for further information.

© 2024 MESTRELAB RESEARCH

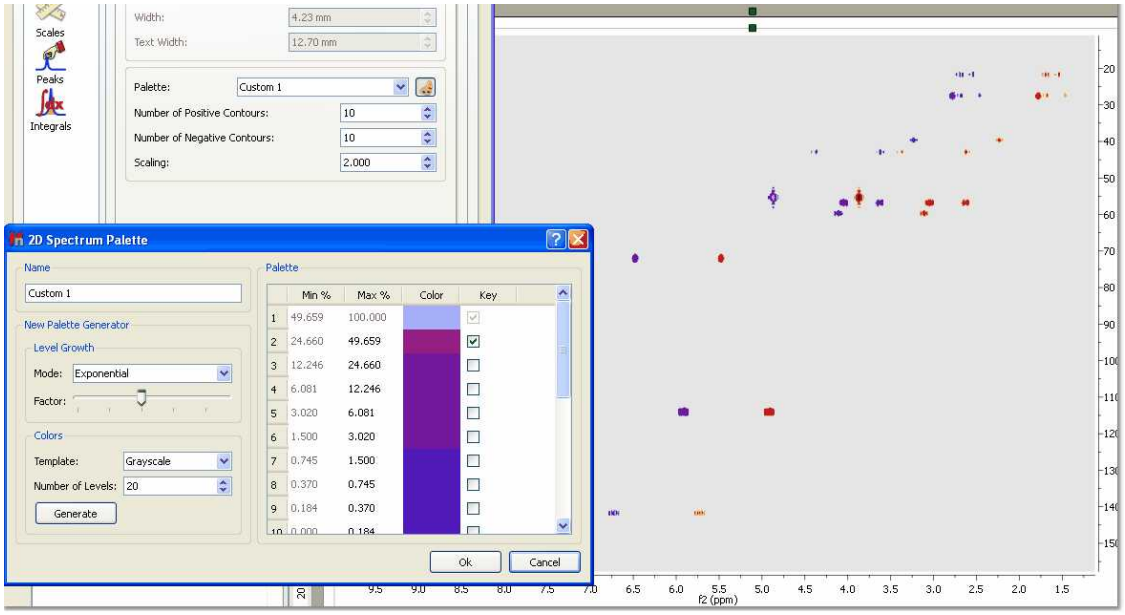


You can see below an expanded signal after having applied the resulting palette (obtained in the previous step):

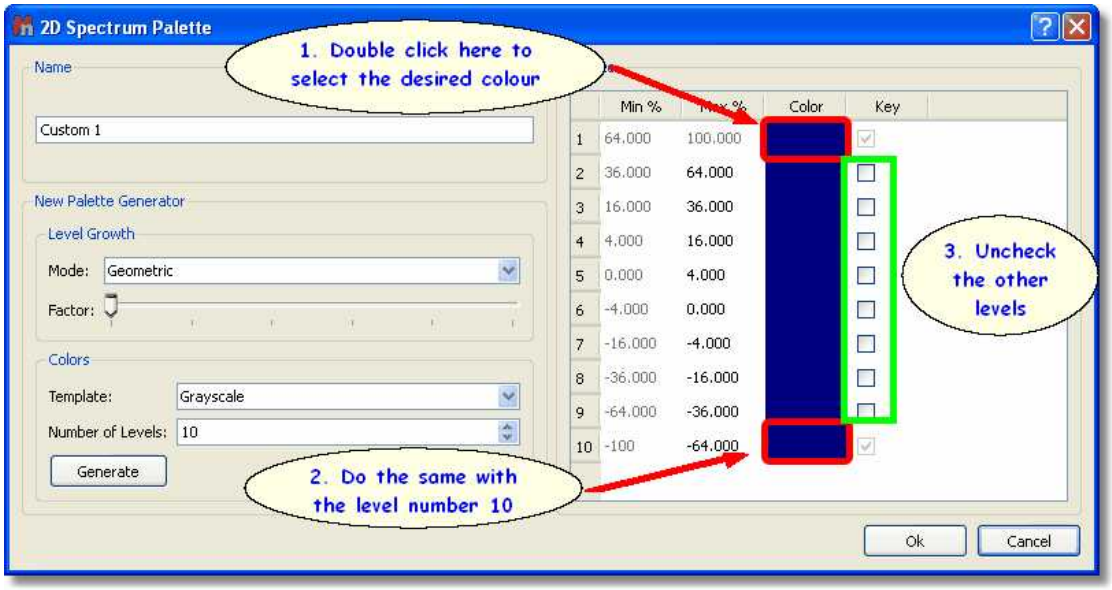


You can change the colours of the 2D overlaid spectra by following the above procedure (but changing the colours). You will find below two overlaid spectra with different colours (one red and the other purple). Please bear in mind that you can move from one spectrum to another (in the overlaid) by holding down the 'Shift' key and using the arrow keys (or the mouse wheel):

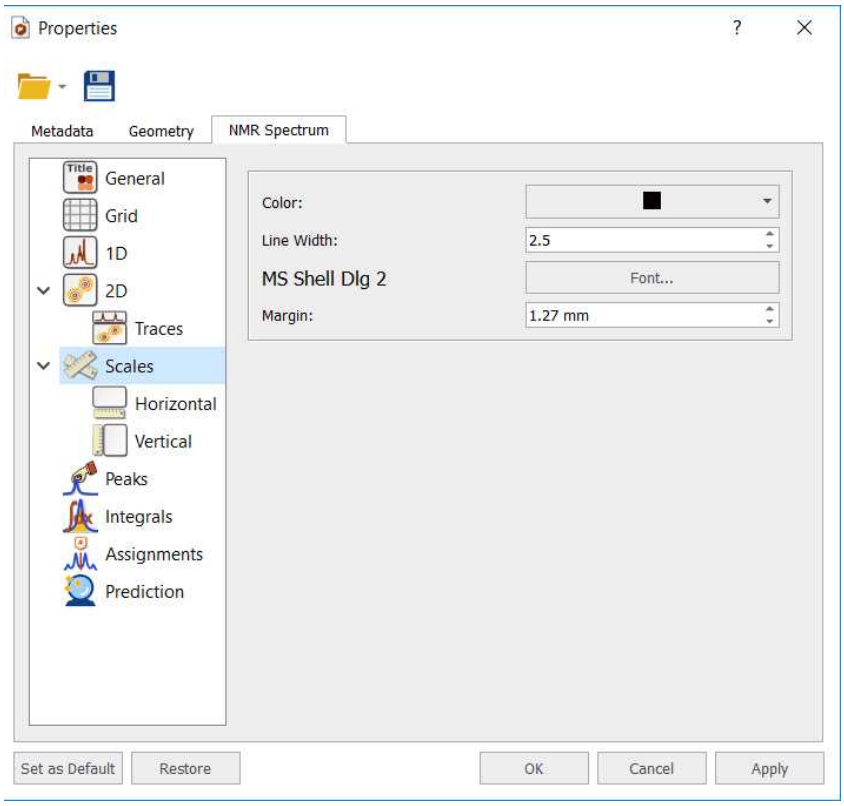
© 2024 MESTRELAB RESEARCH



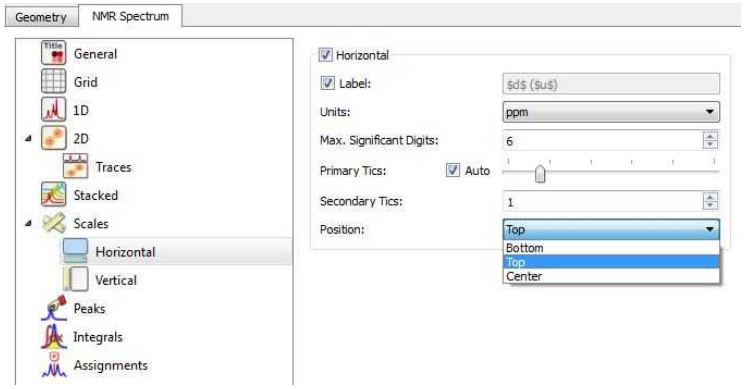
Of course, you can also make your 2D spectrum with a single color (no ramp). Once you are in the 'Palette Editor' dialog box, double click on the coloured rectangle of the level number 1 and select the desired colour, do the same with the level number 10 (bear in mind that you select the same colour) and uncheck any other key mark that could be in the other levels:



You can change the 'Scale Properties':



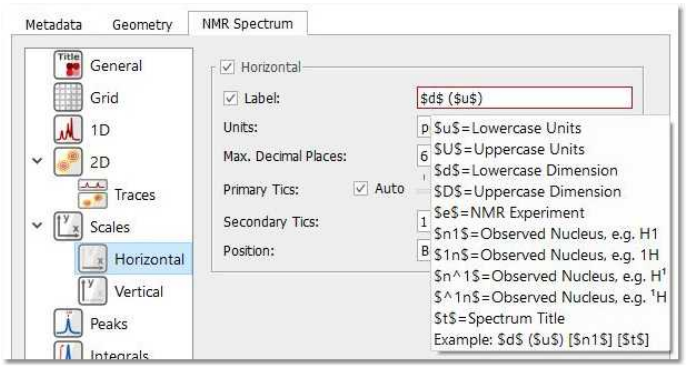
From here the user can change the properties of the horizontal and vertical scales:



The user can include the spectrum title (the title set previously on the spectrometer at acquire time) on any axis label by typing 't' in its applicable edit box. Bear in mind that, Mnova will only show the first line of the original title. This title appears by default on each page of the page navigator.

Hovering the cursor over the label edit box will reveal other commands for including information on the axis label, such as: u = Lowercase Units; U = Uppercase Units; d = Lowercase Dimension; D: Uppercase Dimension; e = NMR Experiment, or $1n$ = Observed Nucleus (e.g. 1H, 13C...).

© 2024 MESTRELAB RESEARCH

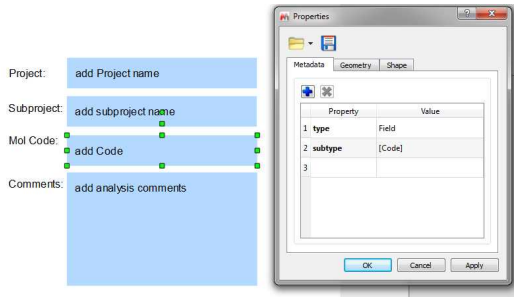


You can select the Maximum significant digits and the number of Primary and Secondary tics of the scales and to select the position of the scales.

The user will be able to save his/her own properties just by clicking on the **Save Properties**' icon. The Saved Properties will be opened by clicking on the '**Load Properties**' icon. You can set the current properties as default by clicking on the 'Set as Default' button. Clicking on the 'Restore' button, will restore the predefined properties.

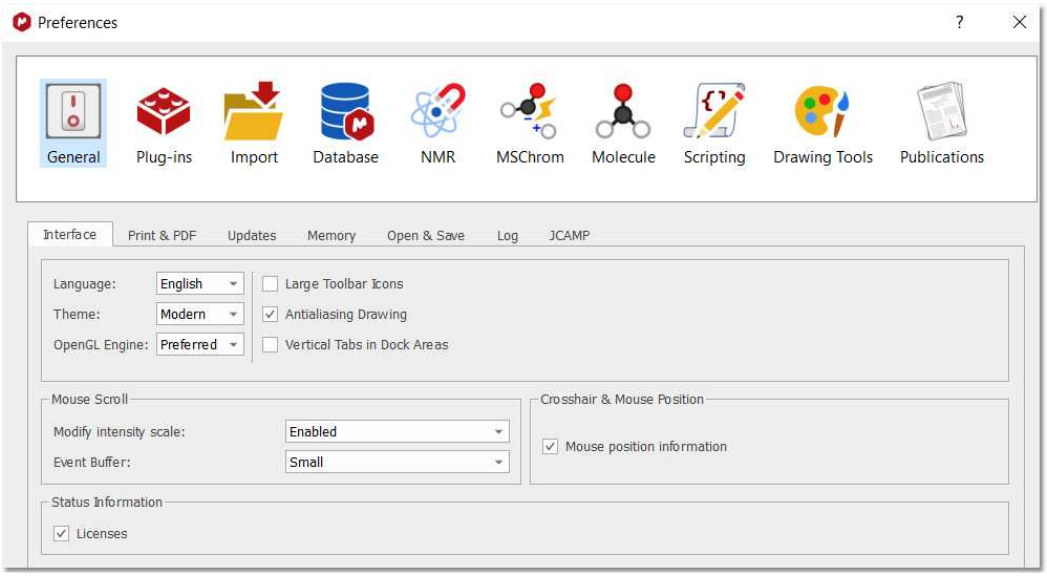
The user can also modify many other features such as the parameters and properties of the peaks or integrals. We will not go into further detail here since all the property pages should be self-explanatory and a description of each one can be found in its applicable section of this manual.

The text boxes will also have its own 'Properties', even we can add text properties to the page items and edit them by selecting the 'Metadata' tab:



6.5 Preferences

Following the menu 'Edit(or File)/Preferences' ('MestReNova/Preferences' in Mac), you will be able to change the options of the Mnova interface, plugins, NMR. mass, molecule, scripting and drawing tools.



To keep the preferences in your registry, just click on the OK button. Once you have them in your registry, you can export them by using the Save button, which includes a scroll down menu to export all settings as an .ini file.

From the General Preferences, you will be able to choose between large or small toolbar icons.

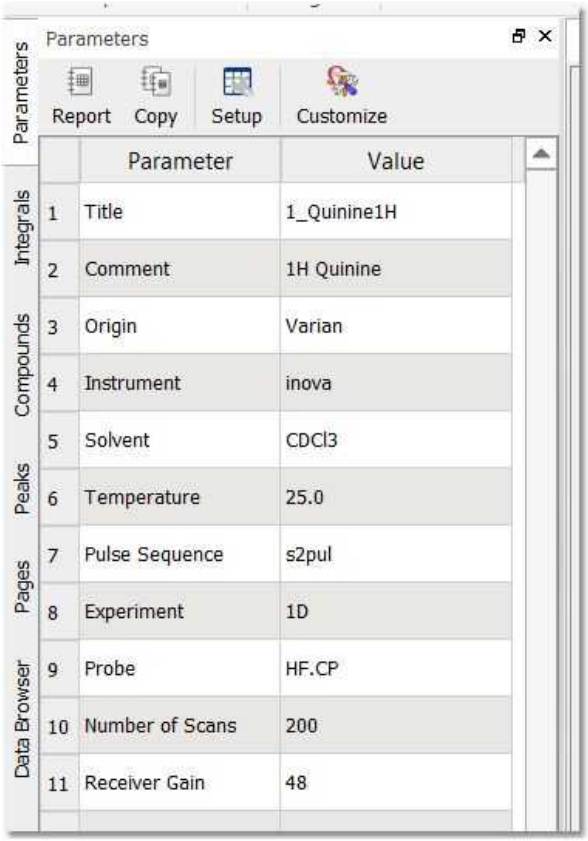
'Antialiasing': This is the technique used to minimize the distortion artifacts known as *aliasing* when representing a high-resolution signal at a lower resolution.

Aliasing is an effect that causes different continuous signals to become indistinguishable when sampled. *Aliasing* also refers to the distortion or artifact that is caused by a signal being sampled at high resolution (on the spectrometer) and then rendered on the PC video-display at lower resolution.

The **'Antialiasing'** is checked by default in Mnova.

Vertical Tabs in Dock Areas: it will display the tabs vertically in the dock widgets (useful when having a lot of dock widgets opened in the lateral dock and most of the tabs displayed horizontally are not visible):

© 2024 MESTRELAB RESEARCH



Language: English, Japanese and Spanish versions of Mnova are currently available.

Theme: it will allow you to select between the ribbon interface (modern) or the classic one.

OpenGL Engine: These libraries will be used by the 3D molecule panel. The options are listed below:

- Preferred: It's the default, leaves Qt to choose one in behalf of you.
- Native: It uses the native OpenGL Windows drivers (It usually means the drivers provided by the graphics card vendor and hardware accelerated)
- ANGLE: It uses the ANGLE - Almost Native Graphics Layer Engine which is a engine which translates OpenGL ES API to DirectX (usually better supported).
- Software: It uses a Qt's software implementation of OpenGL.

Mouse Scroll: The 'Modify Intensity Scale' enables/disables changing the spectra intensity/threshold via the mouse wheel. It also allows to enable it but requiring that the Alt key is pressed.
The 'Event Buffer' will only affect to high sensitive mouses/trackpads (Like the magic mouse and magic trackpad from Mac) in any other device the change of this setting won't affect. It sets the event buffer size (or even disable it).

Check the license box to display the licenses status information in the right down corner of Mnova GUI.

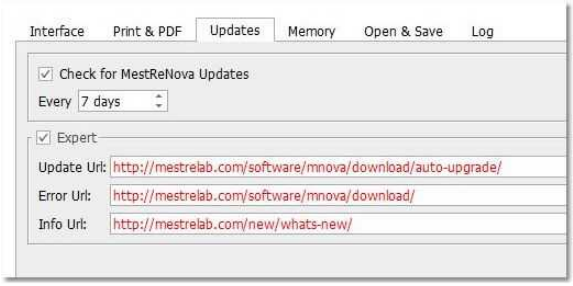
Print&PDF Exporting: From here you can select the PDF export resolution or the use of polylines when printing.

© 2024 MESTRELAB RESEARCH



Metafile exporting/copying: By default the option is checked and the text will be drawn by the GDI+ engine natively as text. If the option is unchecked then the GDI+ engine will draw the text as paths.
The differences between both options are:
With the native text drawing option, the resulting metafile could be slightly different from what you see in Mnova due to differences on how GDI+ renders text.
With the other option, the text should be shown exactly as Mnova shows, but the texts objects in the metafile aren't no more text objects, they are vector graphics objects, so user couldn't edit the text by typing/deleting characters

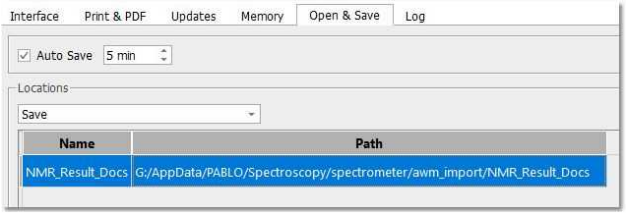
Updates: Use this dialog to select the interval to check for the new Mnova updates.



Memory:
If you work with huge datasets, in order to avoid **memory** issues, you can uncheck the memory options to be able to copy your spectra to clipboard or to disable the Undo feature.



Open&Save: to set one or more locations as default open/close directories. A document can be saved as pdf in one of these default directories (via 'Save As' and choosing the pdf filter). The default open and save directories can be accessed via File/Open from Location and Save to Location.



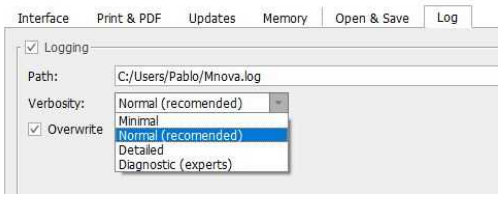
© 2024 MESTRELAB RESEARCH

From here you could also select the 'Auto Save' interval. By default Mnova will create save documents every 5 minutes and keep them at the %APPDATA%\Mestrelab Research S.L\MestReNova\AutoSave folder.

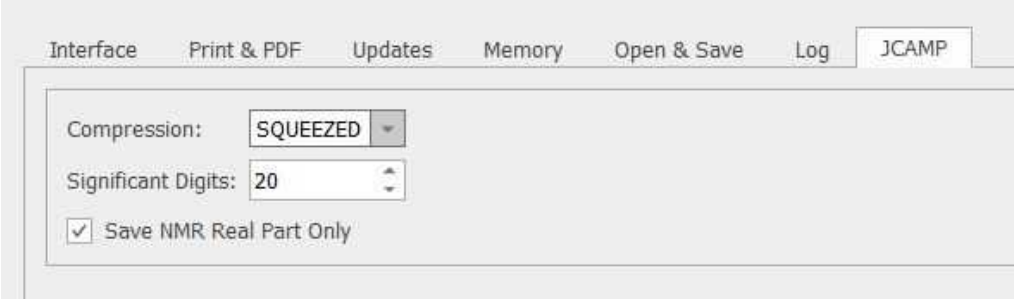
Log: to specify the log file and log's level:

- Minimal -lv 1 - messages(-1), errors(0)
- Normal (recommended) -lv 2 messages(-1), errors(0), warnings(1)
- Detailed -lv 3 messages(-1), errors(0), warnings(1), info(2)
- Diagnostic (experts) -lv 4 messages(-1), errors(0), warnings(1), info(2), debug(3).

This option is disabled by default:



JCAMP: options to save the datasets as JCAMP format:



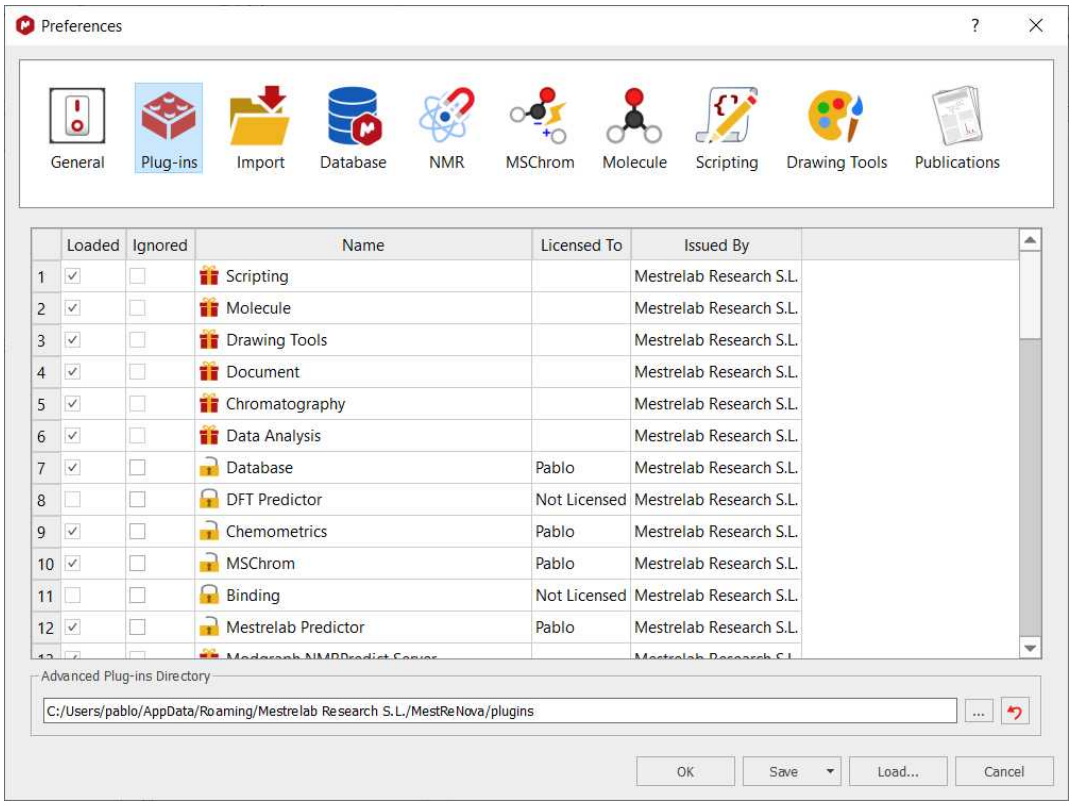
The format "PACKED" to save the data points in JCAMP was added as defined in JCAMP 4.24 section 5.2. This format will not compress the data, it will save the data points as text so the resulting file will be bigger than using the "SQUEEZED" format but, keeping the original dynamic range between saves.

You can decide the real point precision (by default 20) when saving the file with the capability to save or not the NMR Spectra as real only (by default true).

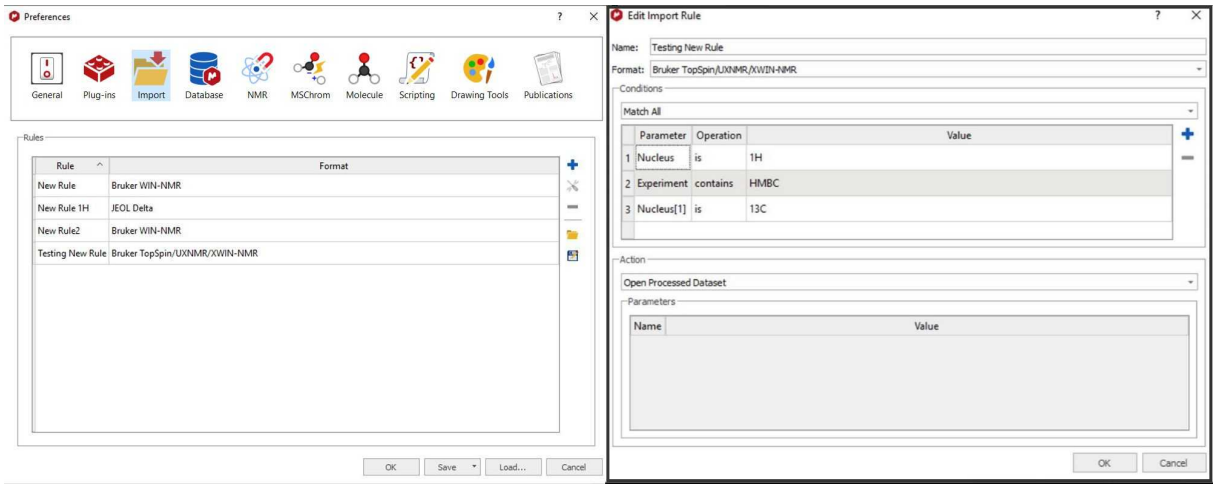
From the '**Plugins Preferences**' dialog box, you will be able to ignore any plugin, which will be very useful in the case of concurrent (floating) licenses, in order to 'liberate' the license of those plugins which will not in use.

In any case, please bear in mind that Mnova allow users to only take the plugins they are ***using*** as opposed to the plugins which are ***installed***.

© 2024 MESTRELAB RESEARCH



Click on the 'Import' button to select the desired options to load your processed NMR spectra (1r, 2rr, etc) instead of raw datasets (fid, ser, etc) or even the **time domain raw data** (original FID):

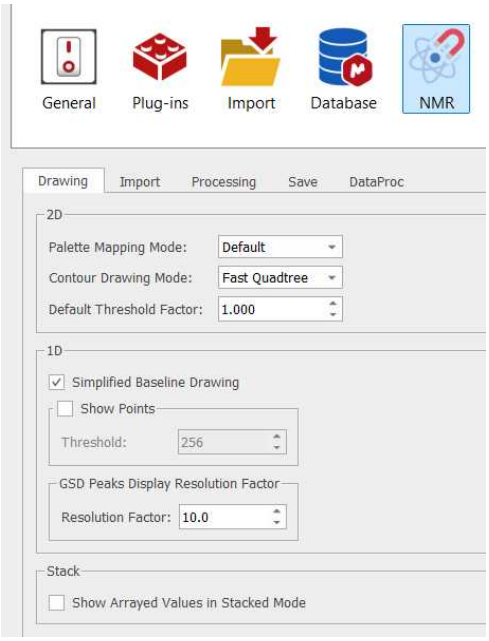


When adding a rule, you can set condition(s) in order to make an action. This condition(s) can be “match all”, “match any” or “match none”. By default “match all” is selected.

You will have the option to apply a processing template after having loaded the processed NMR spectrum.

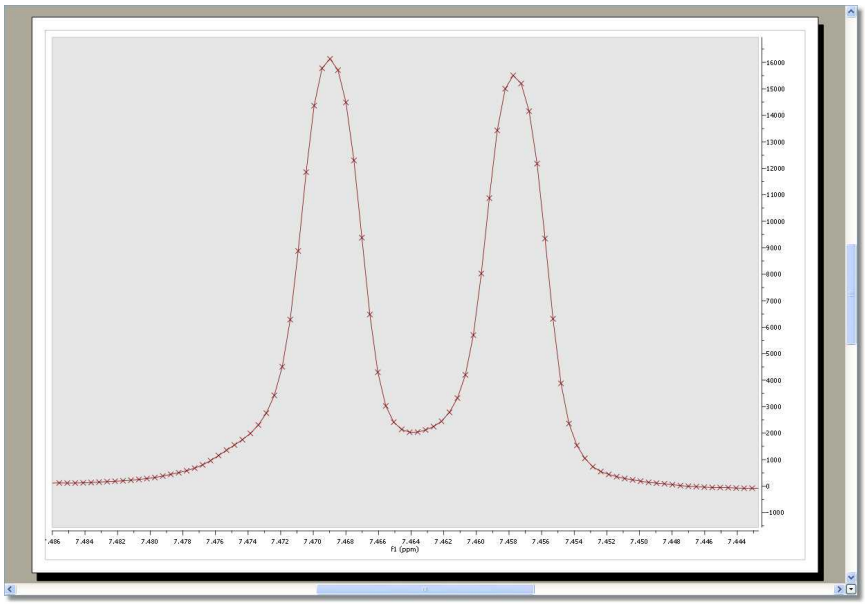
From the '**NMR Preferences**' dialog box, you will be able to select the mode of the '2D palette mapping' (default, absolute and dynamic), the contour drawing mode (Fast, Fast Quadtree and High resolution) and the default threshold factor (useful for 2D plots).

© 2024 MESTRELAB RESEARCH



The Simplified Baseline Drawing box will draw the baseline of spectra with less noise ("simplified" noise) so they will be plotted faster. It will be useful for stacked plots (of 1D and 2D spectra).

From this dialog box, you will also be able to select threshold of the limit of the number of points showed in the spectral window. For example if your spectral window has less than 128 points (and you have this option checked), you will see the points (as crosses) drawn on it; as you can see in the picture below. If the spectral window has more than 128 points, you will see the spectrum as usual.

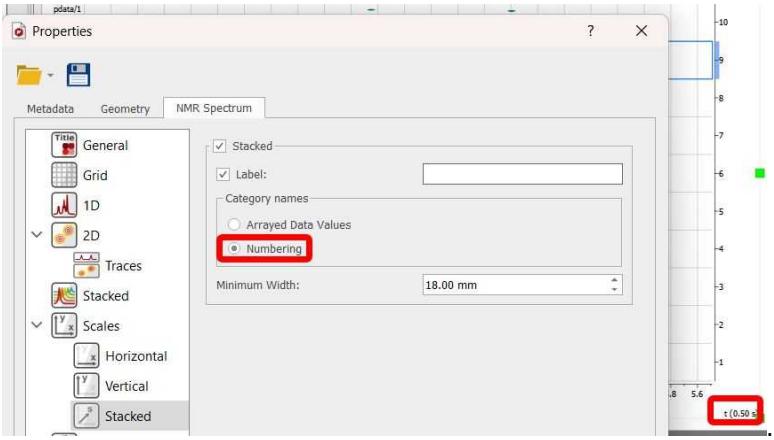


From this Preferences menu you can also select the GSD display resolution factor.

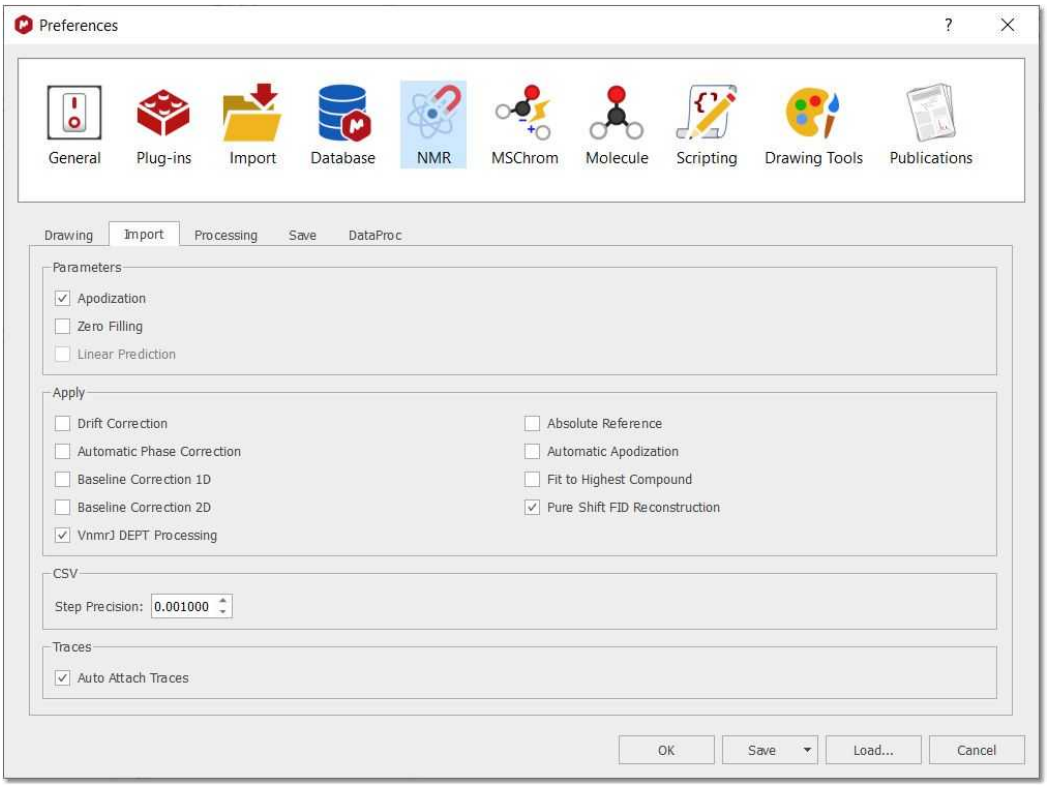
The GSD peaks extend over all the spectra but when drawing we are cutting the display at some point to speed up the drawing. By default Mnova uses the peak width by a factor to "cut" the peak display. This

behavior is the default one but is optional. The user will be able to change it in the preferences (NMR tab) just by checking "GSD Peaks Display Resolution Factor". And if it's checked you can adjust the factor (10 by default) to be multiplied by the width.

Checking the 'Show Arrayed Data Values in Stacked Mode' will display the arrayed values when importing a pseudo spectrum. This option is unchecked by default because it can also be selected/unselected in Properties/NMR Spectrum/Scales/Stacked/Category names:



From this menu, but selecting the Import tab, the user can select the option to import the apodization, zero filling, linear prediction options from the spectrometer and also the default phase correction method (Imported or Automatic). From here you can also select to apply automatic baseline corrections, apodization, absolute reference, fit to highest compound, VnmrJ DEPT processing or pureShift FID reconstruction :



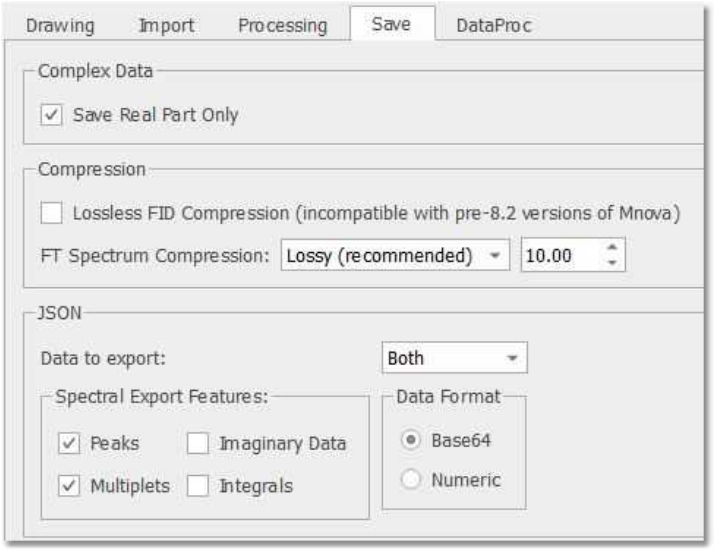
© 2024 MESTRELAB RESEARCH

Note about Apodization:
1. Import Apodization ON, Apply Automatic Apodization OFF: Mnova tries to import apodization parameters. If no apodization is imported, no other apodization is applied.
2. Import Apodization OFF, Apply Automatic Apodization ON: Mnova applies an automatic apodization to each type of spectra.
3. Import Apodization ON, Apply Automatic Apodization ON: Mnova tries to import the apodization parameters, but if no apodization is imported, automatic apodization is applied.
4. Import Apodization OFF, Apply Automatic Apodization OFF: no apodization is applied in any case.

If the the 'Auto Attach Traces' box is checked; the 1D spectra of the document will be used as projections of a 2D.

Under the 'Processing' tab, you will have a preference to use multiple CPU cores to speed up the 'processing&analysis' of stacked spectra.

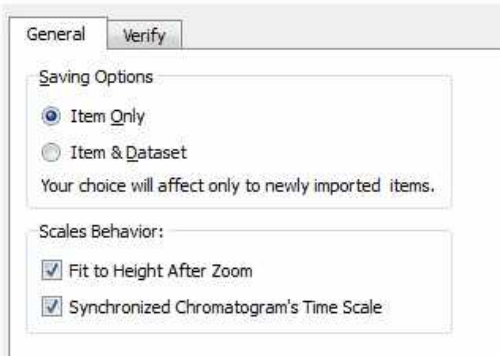
Selecting the 'Save' tab will allow you to compress the data on storing. From this tab you will be also able to select if you want to keep the real part only or both imaginary and real parts when saving an NMR spectrum into an Mnova document.



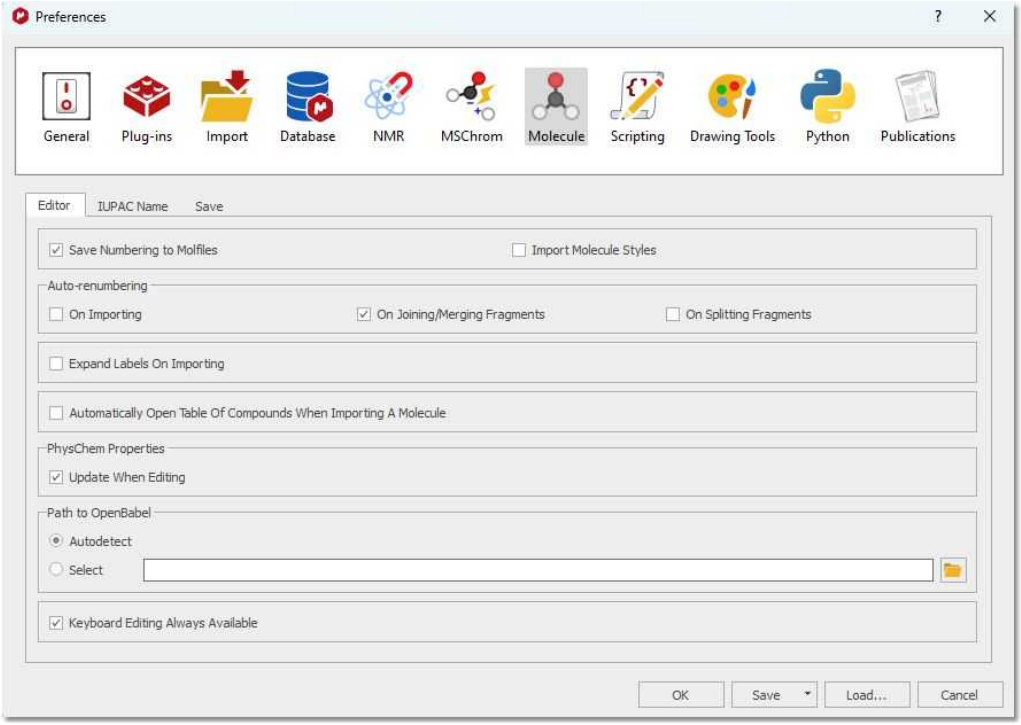
From the same dialog, you can select the options to export your spectra as .json files (from the 'File/Save As' dialog). You will have the capability to export the FID or the processed spectrum (or both). You can export the peaks, multiplets, integrals and imaginary data in 'Base64' (faster but not human readable) or 'Numeric' (slower but human readable) format.

From the '**Mass Preferences**', the user will be allowed to select the saving options (item only or item+dataset). When the option 'Synchronized chromatogram's Time Scale' is selected, you will be able to zoom, pan and cut to all showing chromatograms. You will find also an option to fit to height after zoom (selected by default). When this option is checked, a fit to height is applied after making a horizontal zoom in a mass spectrum or a chromatogram. In case of the other zoom modes (vertical or in both dimensions), a fit to height is never applied.

© 2024 MESTRELAB RESEARCH



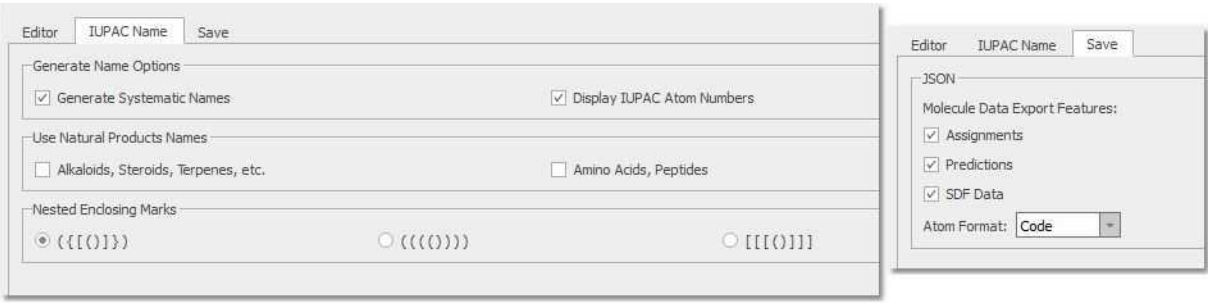
From the **'Molecule Preferences**', you will be able to save the numbering when you export the molecule to a .mol file (via File/Save as'), 'import molecule styles' (when you paste special) and auto-renumbering on importing or joining fragments.



From here you can also select if you want to expand the labels on importing, to automatically open then table of compounds when importing a molecular structure, update the physchem properties when editing, to select a path to OpenBabel or to have the keyboard available for editing the structures.

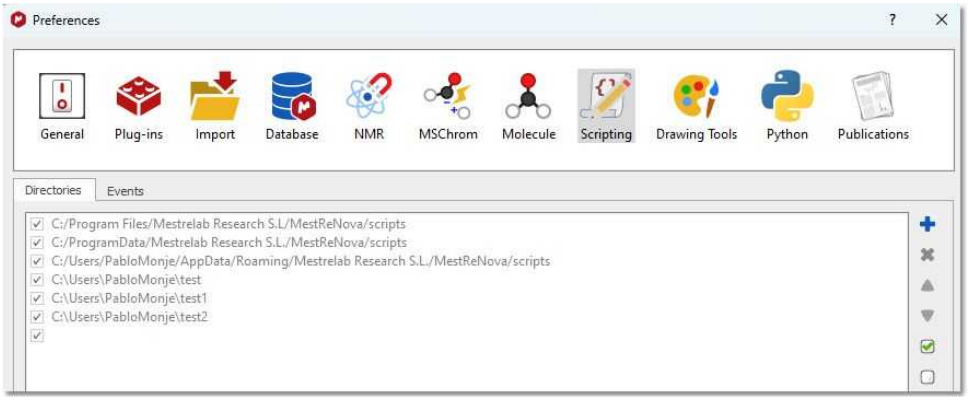
From the IUPAC Name tab, you can select several options to generate the names and from the 'Save' tab, you will be able to choose what information you want o include in the molecules when generating a JSON file:

© 2024 MESTRELAB RESEARCH

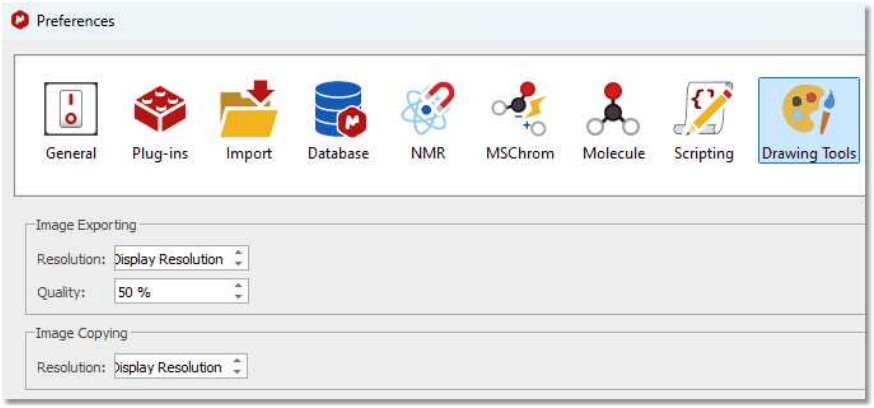


Options related with the "Atom Format" affect only how the assignments and predictions are stored in the JSON file (atom index or atom code).

From the **'Scripting Preferences**', you will be able to select the scripting folders and also to create events to run script events by default:

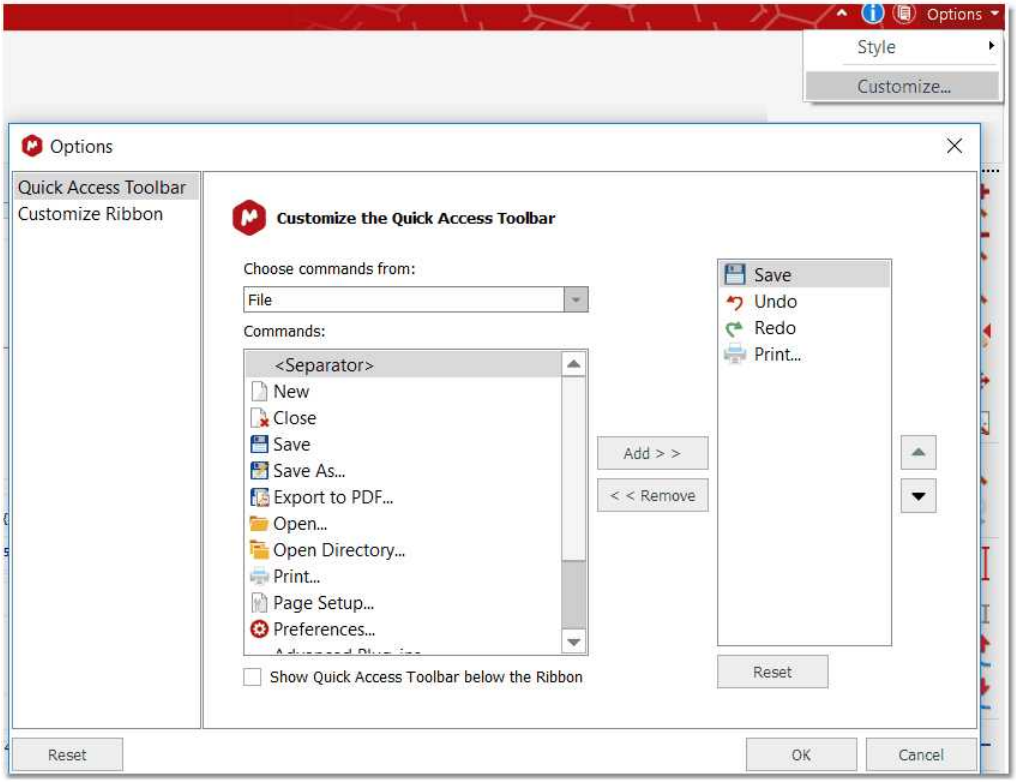


From the '**Drawing Tools Preferences**', you will be able to select the exporting 'Resolution' and 'Quality' of the graphics and also the resolution of the image copying:



6.6 Ribbon Customization

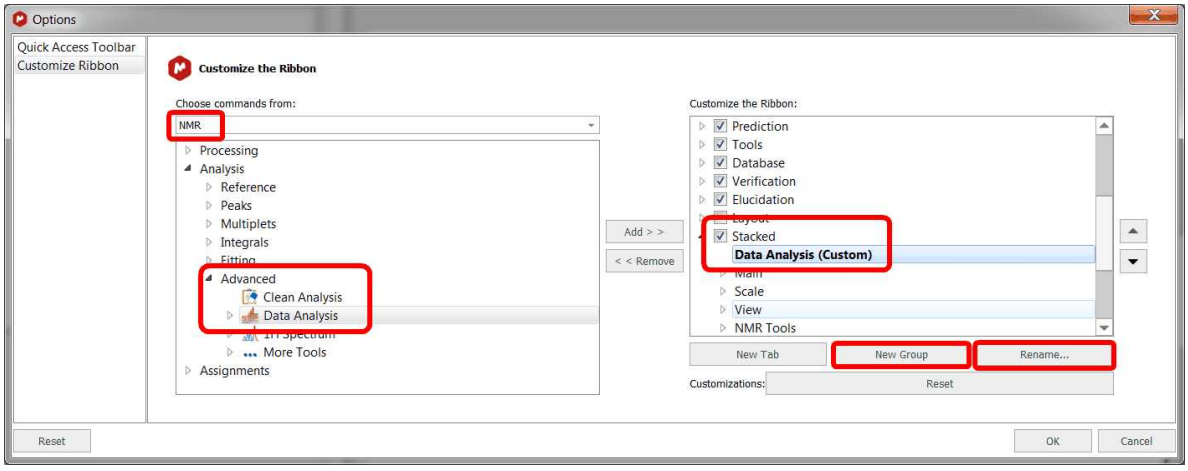
Mnova allows you to customize the ribbon by following the menu: 'Options/Customize' (on the top right corner) or by right clicking on the toolbar and selecting 'Customize':



From here, you will be able to customize the 'Quick Access Toolbar' or the selected Ribbon by using the add/remove buttons.

Let´s see how to add the 'Clean Analysis' feature to the 'Stacked' ribbon.

- Select the 'NMR' command to locate the 'Analysis/Clean Analysis' tool:

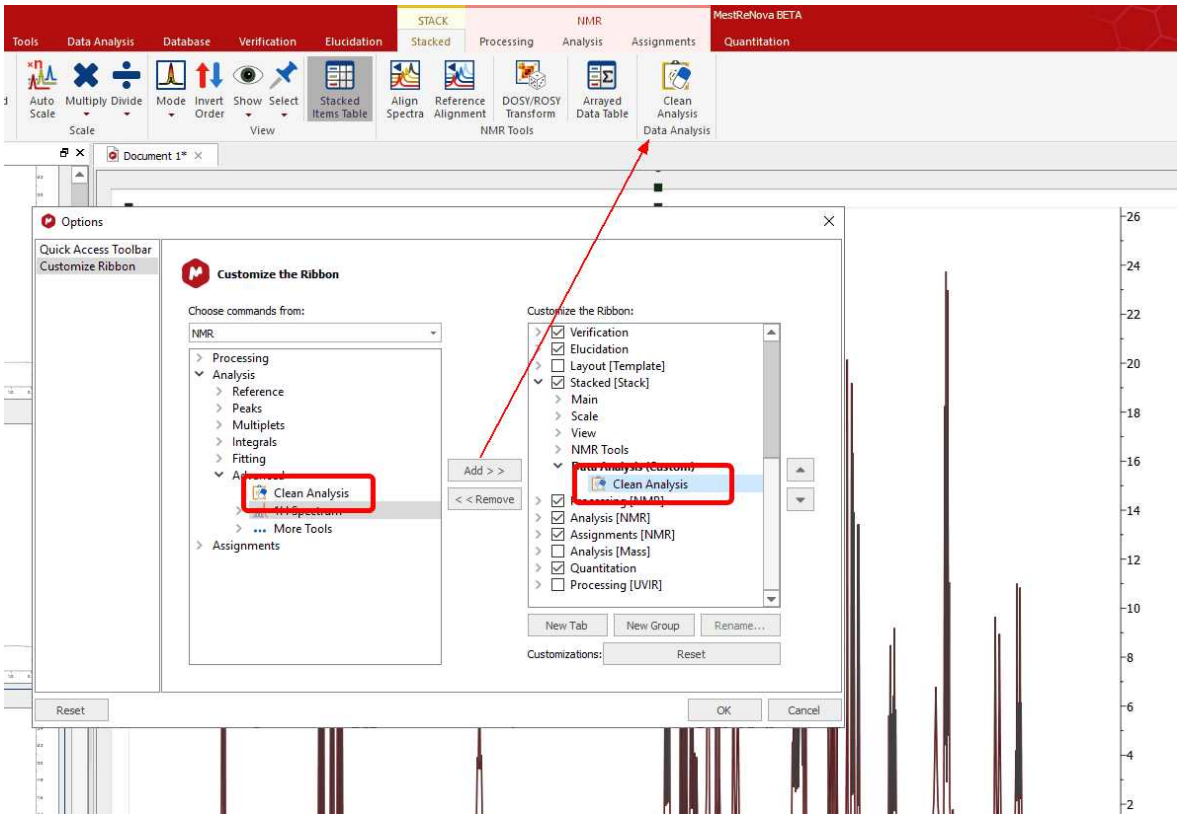


- Next, find the 'Stacked Ribbon' on the right panel and click on the 'New Group' button.

- Click on the 'Rename' button to type the desired name.

- Finally, click on the 'Add' button to include the Clean Analysis feature to the Stacked ribbon:

© 2024 MESTRELAB RESEARCH



You can use the up&down arrow buttons to move the button along the ribbon.

6.7 View Ribbon

This ribbon contains zoom in modes, crosshair, 3D NMR viewer, tables and layout templates features.



6.7.1 Zooming

A number of ways of zooming are available in **Mnova**.

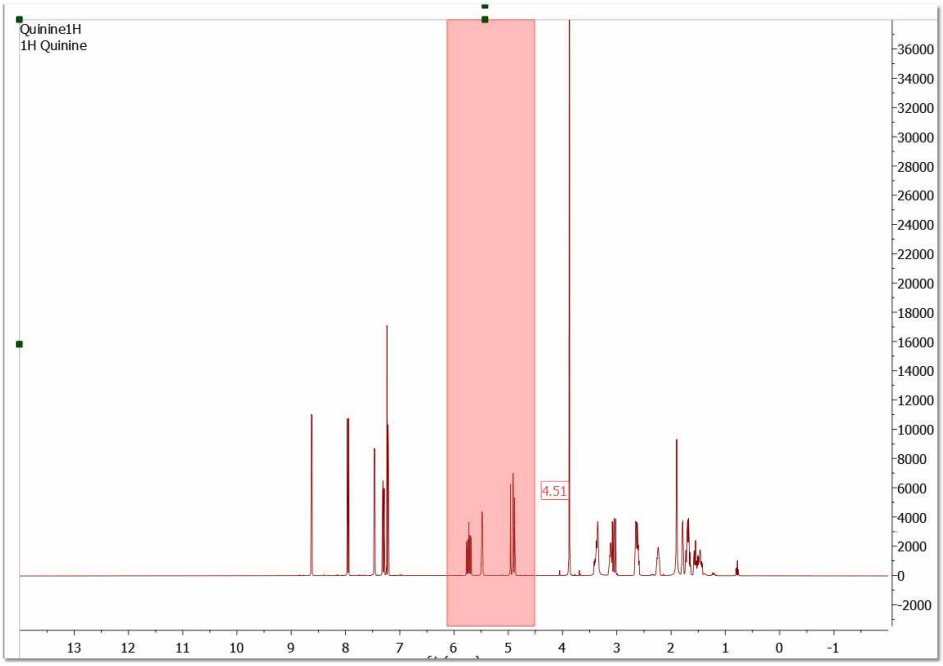


The most common type of zooming in spectroscopy applications is the **'Zoom In'** mode. This mode can be entered by following the menu 'View/Zoom/Zoom In' or by using the **'Zoom In'** icon on the toolbar.

In this area, Mnova presents a significant innovation, allowing the user to apply 'horizontal', 'vertical' and 'box' zooms, as illustrated by the figures below.
Horizontal Zoom. Click the **'Zoom In'** icon on the toolbar (or press **<Z>** key), hold down the left mouse button on the spectrum display and drag the *magnifying glass cursor* (with a red horizontal segment) across the area you want to zoom in to; a highlighted rectangle indicating the new spectral region to be

displayed once the mouse is released, will follow the cursor. Use 'Undo' to revert to the previous display. A zoom lens of a 20% will be obtained by clicking on the left mouse button.

The zoom cursor shows information about the position (same units as in spectrum scales). Once the starting position is selected, only the values for the ending position (f1, f2 or both dimensions, depending on the type of the zoom applied) are displayed.

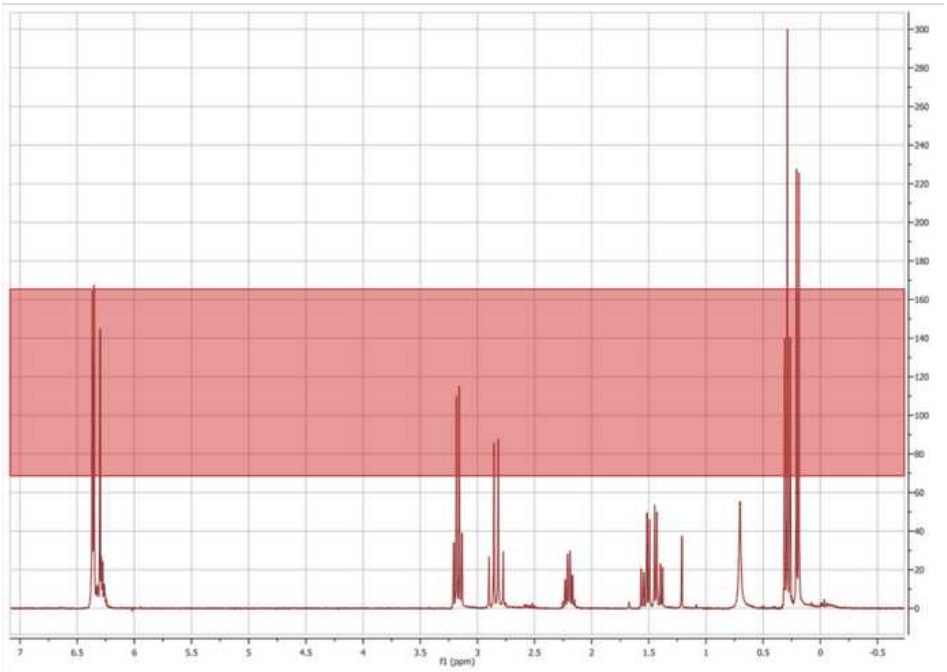


Please note that holding down 'Ctrl+Space bar' (in Mac: 'Space+Cmd') will activate the zoom in temporarily.

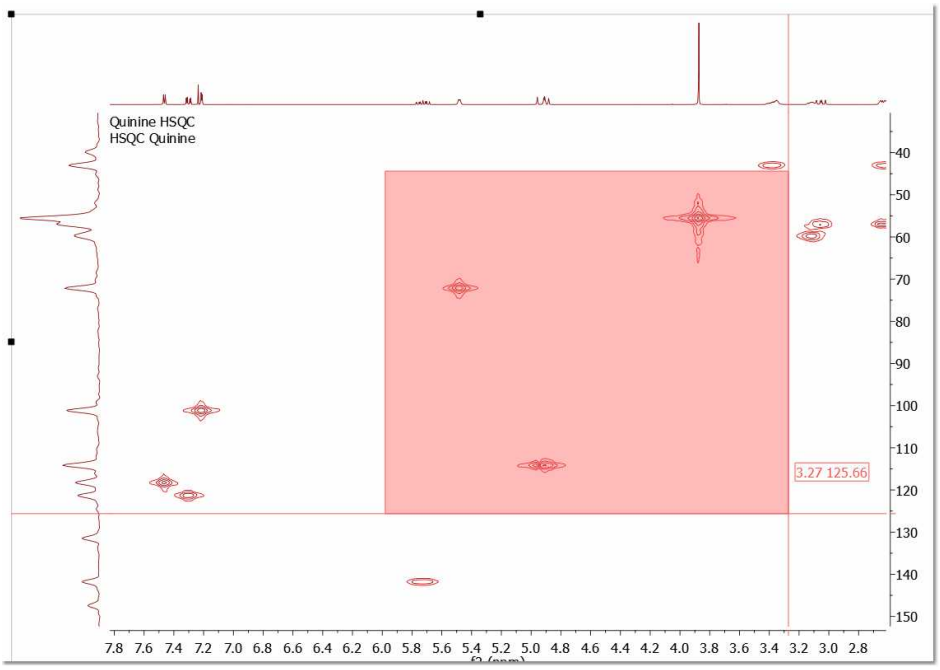
A fixed zoom area can be moved around the spectra using Alt+Arrow keys

Vertical Zoom. Click the **'Zoom In'** icon on the toolbar and then press the **<Z>** key (or just press twice the 'Z' key). You will see a red vertical line segment beside the mouse pointer) while the cursor is located on the spectrum display and then click and drag the cursor vertically over the area you want to zoom in on; once again you will see highlighted red rectangle.

© 2024 MESTRELAB RESEARCH



Box Zoom. Click the **'Zoom In'** icon on the toolbar and then press **<Z>** key twice with the mouse pointer on the spectrum display (or just press three times the 'Z' key), then click and drag the cursor diagonally over the area you want to zoom in (highlighted rectangle in red). This zoom is very useful for 2D-NMR.



In order to switch between these three types of zoom, just press the **<Z>** key whilst in the **'Zoom In'** mode.

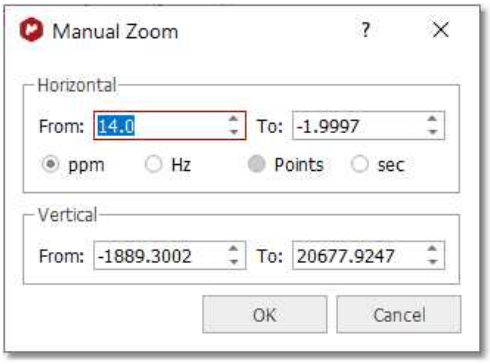
Zoom Out: Click the **'Zoom Out'** icon on the toolbar and click on the left mouse button to obtain a zoom lens out of a 20%.

© 2024 MESTRELAB RESEARCH

Back/Forward Zoom: You can back/forward to previous/next zoom(s) level(s) by using the shortcuts: Shift+LeftArrow(previous)/Shift+RightArrow(next) or the buttons "Previous/Next Zoom".

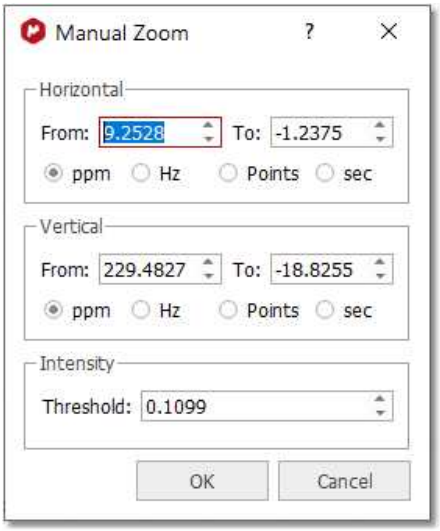
Manual Zoom: Click 'Manual Zoom' on the toolbar or by following the menu 'View/Zoom/Manual Zoom'. The manual zoom is slightly different in 1D to that in 2D NMR spectra where you may select spectral spectral limits for both dimensions.

1. If you are working on a 1D-NMR spectrum, a dialog box like this will open:



You can select the limits of the spectral region of interest in ppm, Hz, points or sec and also for the intensity.

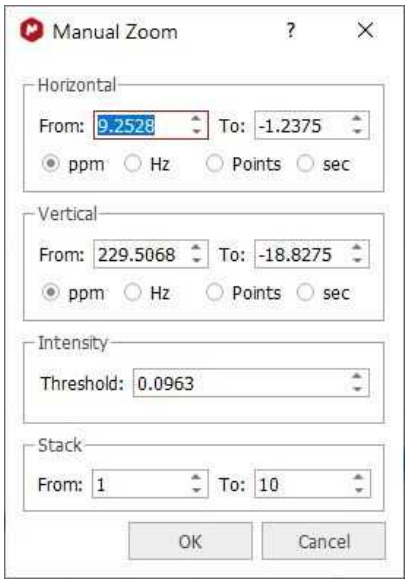
2. If you are working on a 2D-NMR spectrum, the dialog box will look like this:



Select the horizontal, vertical limits and intensity of the region of the spectrum that you want to zoom in on, and click 'OK'.

In stacked plots, you will be also able to select the traces to be used:

© 2024 MESTRELAB RESEARCH



OTHER ZOOMING MODES

1. FULL SPECTRUM: This tool may be used to revert to a full spectrum from a zoomed view. This may be done from the menu 'View/Zoom/Full Spectrum' or by using the **'Full Spectrum'** icon on the toolbar.

2. FIT TO HIGH: The Fit to High function adjusts the display of spectral intensity in both 1D and 2D spectra so that the strongest signal is optimally displayed.

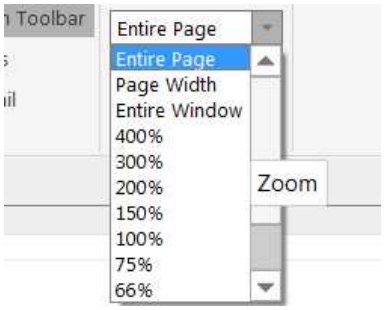
3. FIT TO HIGHEST COMPOUND: The Fit to Highest function adjusts the display of spectral intensity in both 1D and 2D spectra so that the strongest compound signal is optimally displayed (discarding solvent or impurity peaks). You can select this mode by default from the 'File/Preferences/NMR/Import' menu.

4. PANNING: While the spectrum is in a zoomed state, click on the **'PAN'** icon on the toolbar (or hold down the space bar, or hover the mouse over an axis), hold down the left mouse button and drag the *hand cursor* in any direction to navigate throughout the spectrum. In order to select vertical or horizontal panning only, press the <P> key whilst in the **'Panning'** mode.

You will be able to toggle the Full View window by using the F2 key. This will allow you to get a 'Super Fast' panning by using the Full View window.

You could also use the 'Alt' key plus arrow keys (up&down) to apply a fixed panning in 2D-NMR.

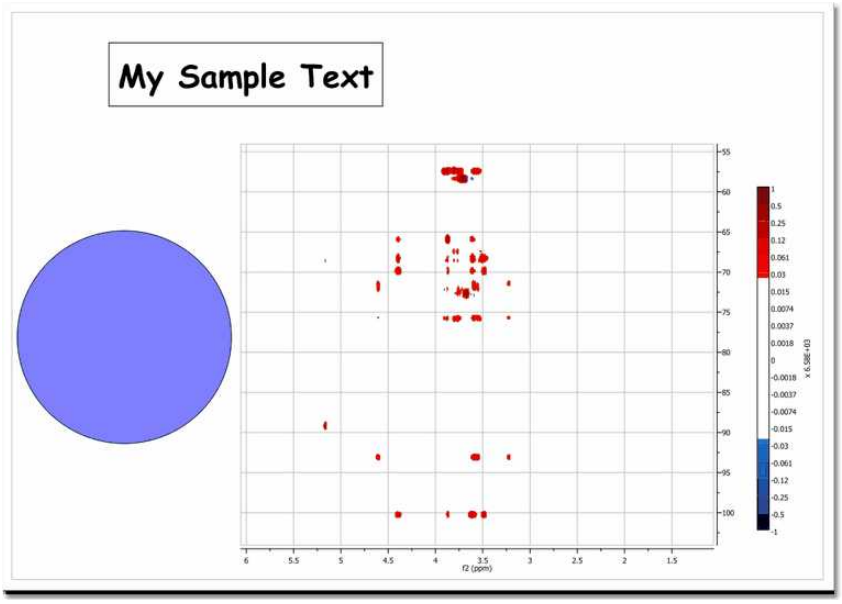
4. ZOOMING COMBO BOX: In addition to the traditional **'Zoom In'** mode, **Mnova** also allows the user to apply different zooms, including Zoom Out (from a zoomed state), by using the zooming combo box.



These zooming modes are:

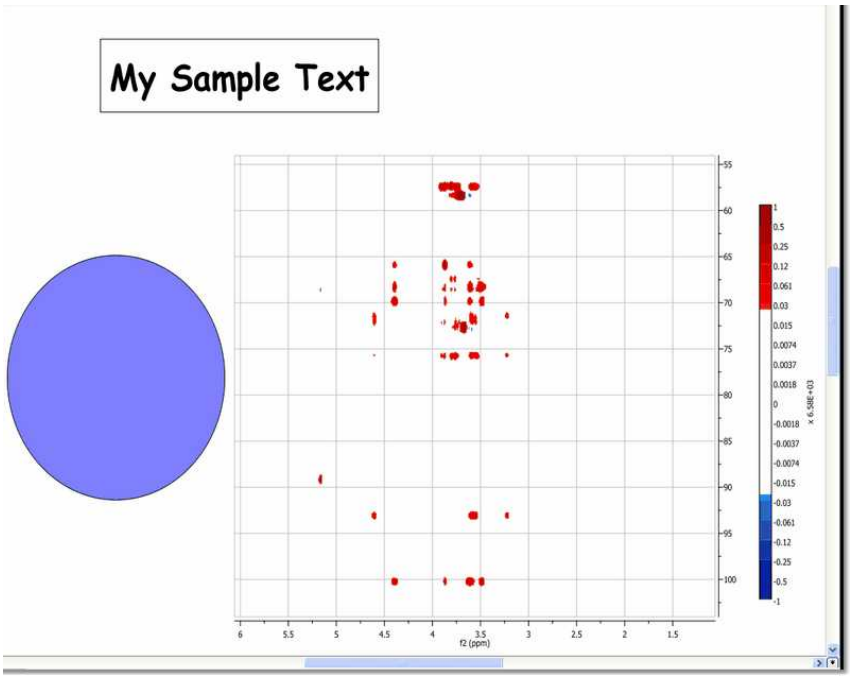
- ✓ **Percentage zooms**: These allow different size views of the object, by selecting the different percentages available (from 400 to 25%).
- ✓ **Entire Page**: This is the standard view, which can be seen in all the figures above.
- ✓ **Page Width**: In this view, the program will fit the size of the paper to the window width, whilst preserving the original paper proportions.
- ✓ **Entire Window**: This is a special view within Mnova. It will fit the size of the paper to the size of the available window, to optimize the available screen area. This view represents an exception to the WYSIWYG concept, since in this case the proportions of the screen paper may differ from the proportions of the printer paper and therefore of what you will get when printing the page.

The **Entire Window** concept deserves further comments. For example, consider the following scenario in which the page zooming mode is set to **Entire Page:**



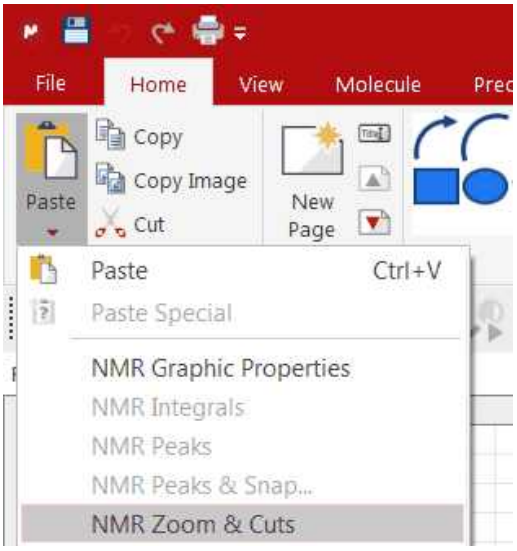
As seen in the figure above, Mnova is a fully **WYSIWYG** application. This can be appreciated in the blue circle which was created with the **ellipse** tool and then its dimensions set to form an actual circle. However, in this case there is a significant space of the window which is not covered by the page (see gray regions surrounding the page) which can be considered as wasted space. In order to make the most of the available space, we can use the so-called **Entire Window** zooming mode. In this mode, the page stretches to fit all the available space. Of course, this means that paper proportions (i.e., width/height ratio) have to be changed and thus the WYSIWYG principle is broken. As always, a picture is worth a thousand words:

© 2024 MESTRELAB RESEARCH



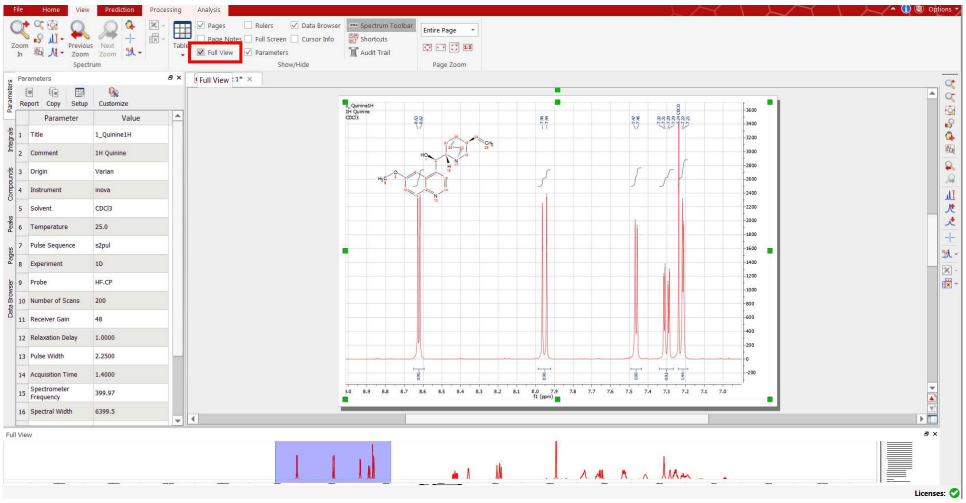
Note that all the objects are now distorted. However, this mode is very convenient to work with spectra on the screen as the graphical spectral proportions are rarely an issue. Nevertheless, if you print a document in this non WYSIWYG mode, you will get exactly the same results as when working on real WYSIWYG modes.

Please bear in mind that you can copy the zoom areas from one spectrum to another just by doing Ctrl/Cmd+C in the first one and 'Home/Paste/NMR Zoom&Cuts':



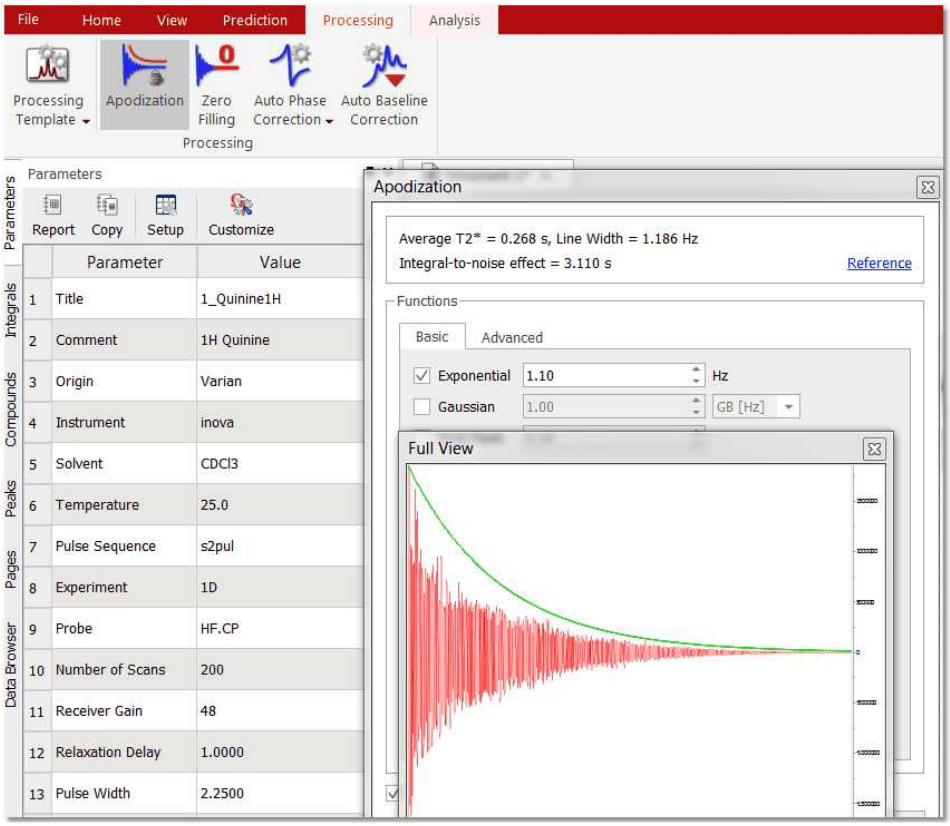
6.7.1.1 Full View

'Full View': (Not to be confused with 'Full Spectrum) will bring up a miniature view of the entire working document with the active area of the spectrum highlighted in blue, as you can see in the picture below:



You will be able to toggle the Full View window by using the F2 key. This will allow you to get a 'Super Fast' panning.

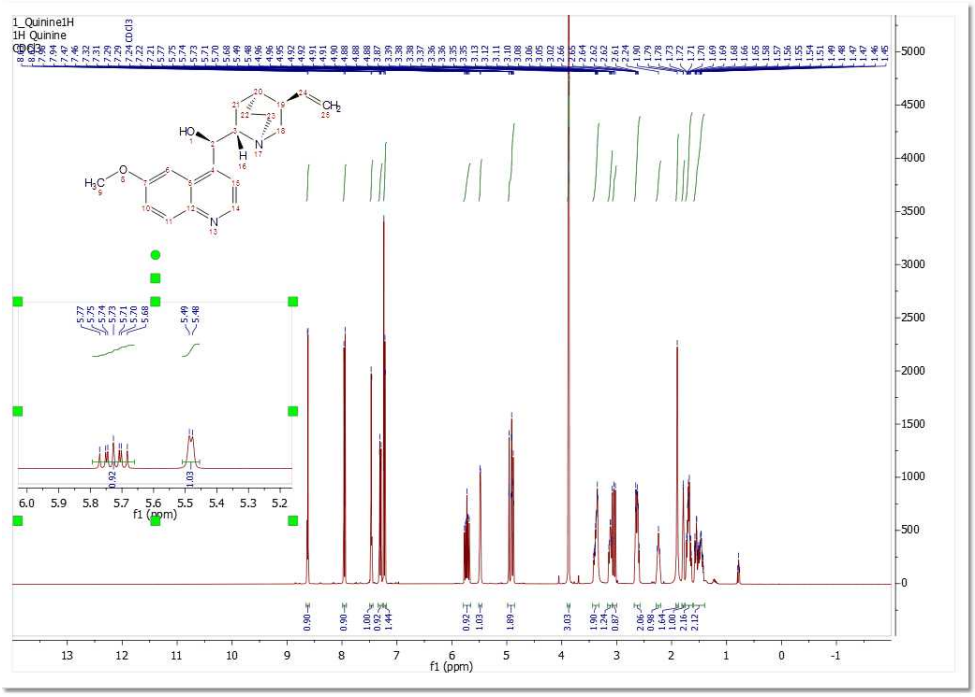
If **'Apodization'** is selected from the Processing menu (or from the toolbar icon ) then the **'Full View'** switches to show the FID and the effective window function. This allows you to manipulate the FID (using apodization for example) and observe the effect on both the FID and the spectrum.



© 2024 MESTRELAB RESEARCH

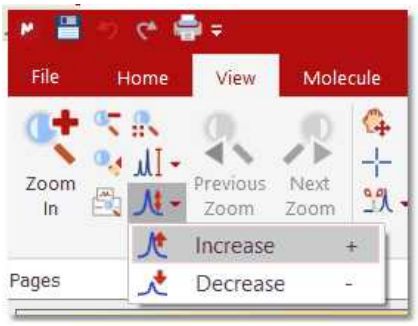
6.7.1.2 Create Expansions

You can create expansions with Mnova by clicking on the corresponding icon of the toolbar  (or by pressing the **<E>** key) and then holding down the left mouse button and dragging the *magnifying glass cursor* (with a red horizontal segment) over the area of interest. A highlighted rectangle indicating the new spectral region to be expanded once the mouse is released, will follow the cursor. You can see in the picture below an example of this feature:



6.7.2 Increasing and Decreasing Intensity

The two buttons under the 'View Ribbon' allow the user to interactively scale the data of the NMR spectrum. This operation does not modify the acquisition or the MestreNova data file; it only magnifies or diminishes the representation of the data which you see on your screen. You can also use the '+ key' to increase or the '- key' to decrease the intensity and the same effects may be obtained by rotating the scroll wheel of the mouse.



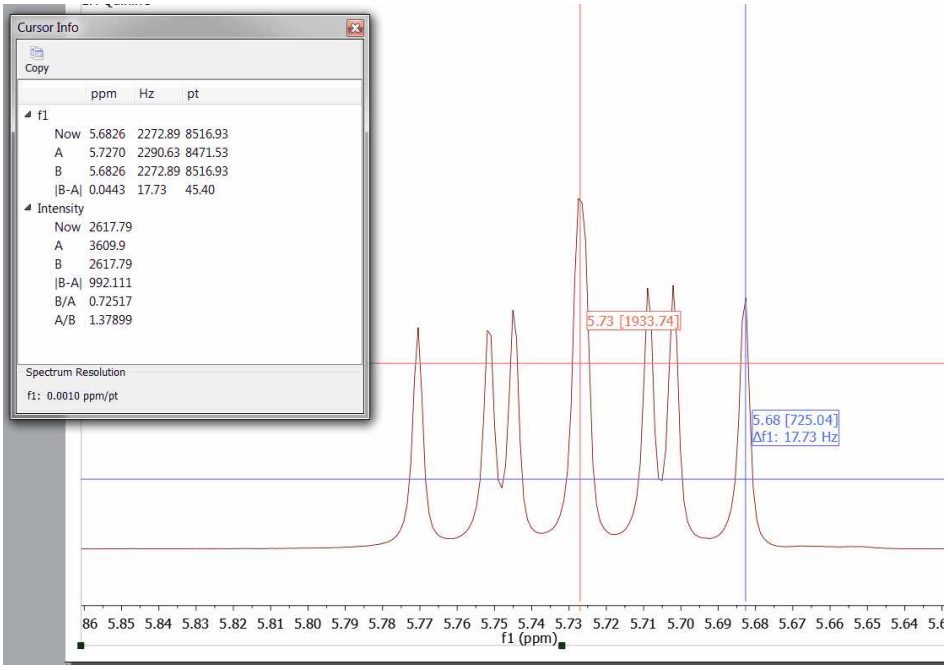
The intensity increases/decreases 10 times slower if the user press the Ctrl/Cmd key while moving the mouse wheel.

6.7.3 How to measure coupling constants? The Crosshair Tool

The Crosshair:  These two orthogonal lines forming a crosshair are available on the 'View' menu (or by pressing the <c> key) for reporting positional information, and for help in visualizing peak alignments. This tool can also be found on the 'Zoom' toolbar .

How to measure coupling constants?

The crosshair includes information about the chemical shift and the intensity of the peak (as you can see in the picture below) and also allows the user to measure distance between peaks (or **coupling constants**) by clicking on the left mouse button and dragging to the desired peak. Activating the crosshair tool will open a 'Cursor Info' dialog box, where the user will be able to see the units distance expressed in ppm, Hz or points. This dialog box will also display the distance between the desired peaks, as well as the intensity ratio. The info View window also contains information about the Spectrum Resolution.

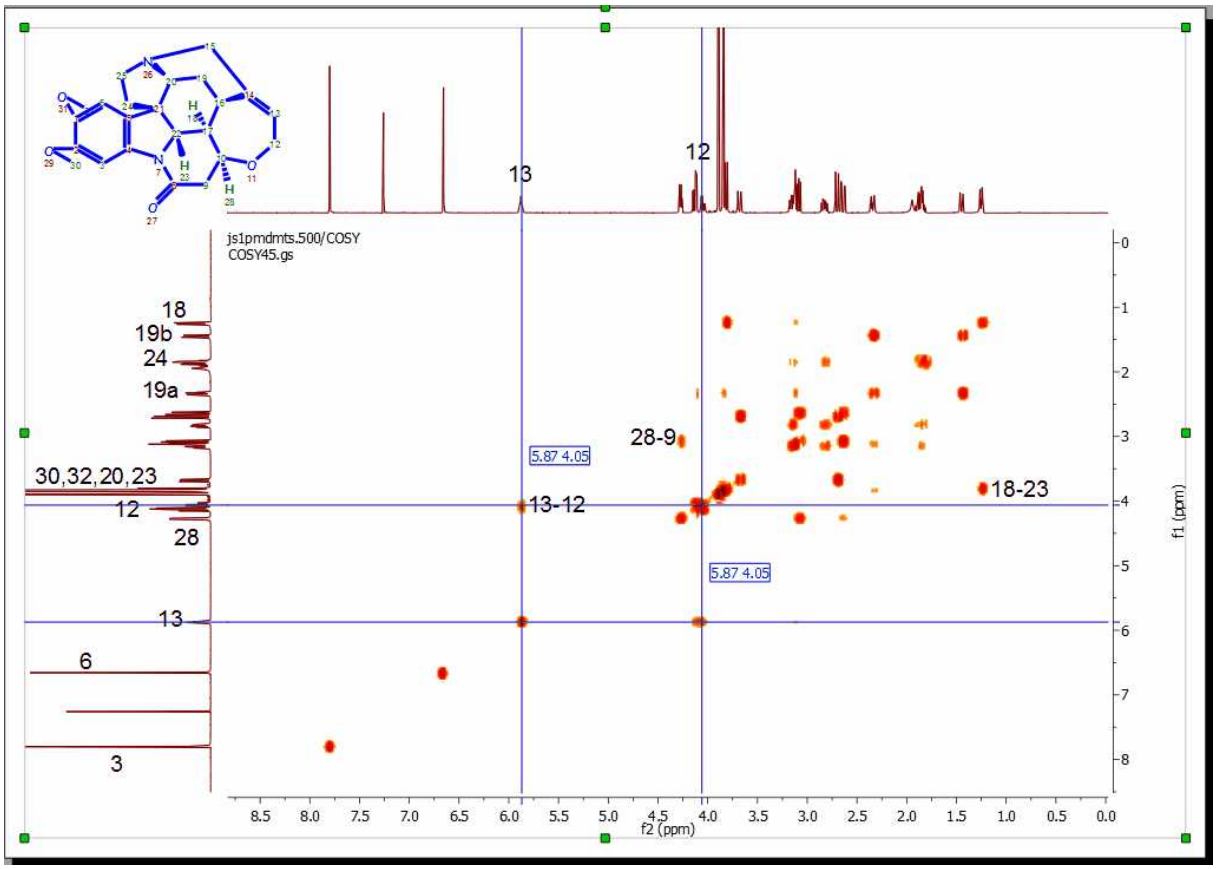


Click on the 'Copy' button to copy to clipboard the information displayed in the 'Cursor Info'.
The cursor will "ride" along the 1D-NMR spectrum simplifying the location of peak maxima and minima by pressing the 'Shift' key.

6.7.3.1 Multiple Crosshair for 2D analysis

Double Crosshair: If you are working with COSY-like spectra (homonuclear spectra) or an INADEQUATE, you will obtain the **double crosshair** tool just by pressing the 'C' key twice, as you can see in the picture below (the crosshair gets deactivated after clicking key C a third time):

© 2024 MESTRELAB RESEARCH

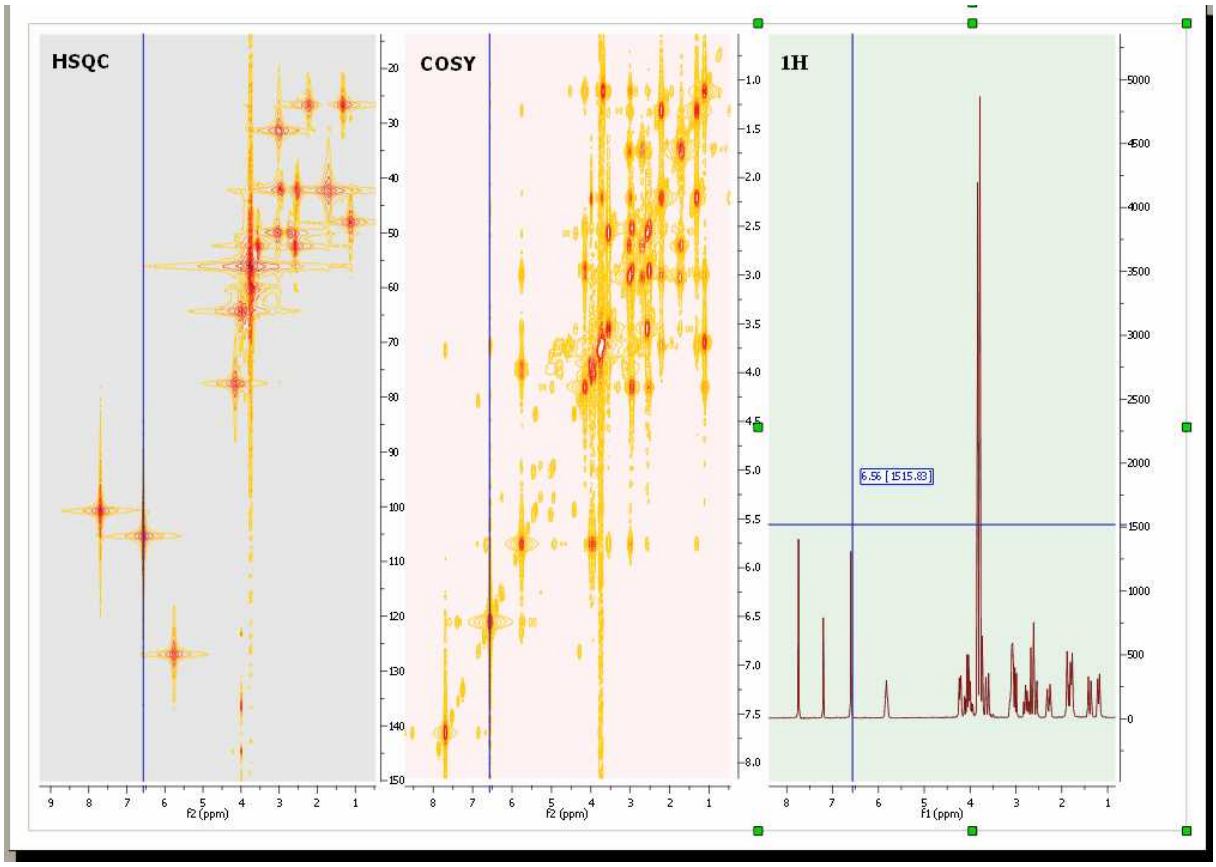


NOTE:

Normal Peak by Peak mode is activated by clicking Ctrl+K only once. Double 'Peak by Peak mode' is activated by clicking Ctrl+K a second time. The tool gets deactivated after clicking Ctrl+K a third time. In double mode, the peak by peak tool adds a peak at the 2 positions, either when the cursor is pointing at lowfield or highfield peak.

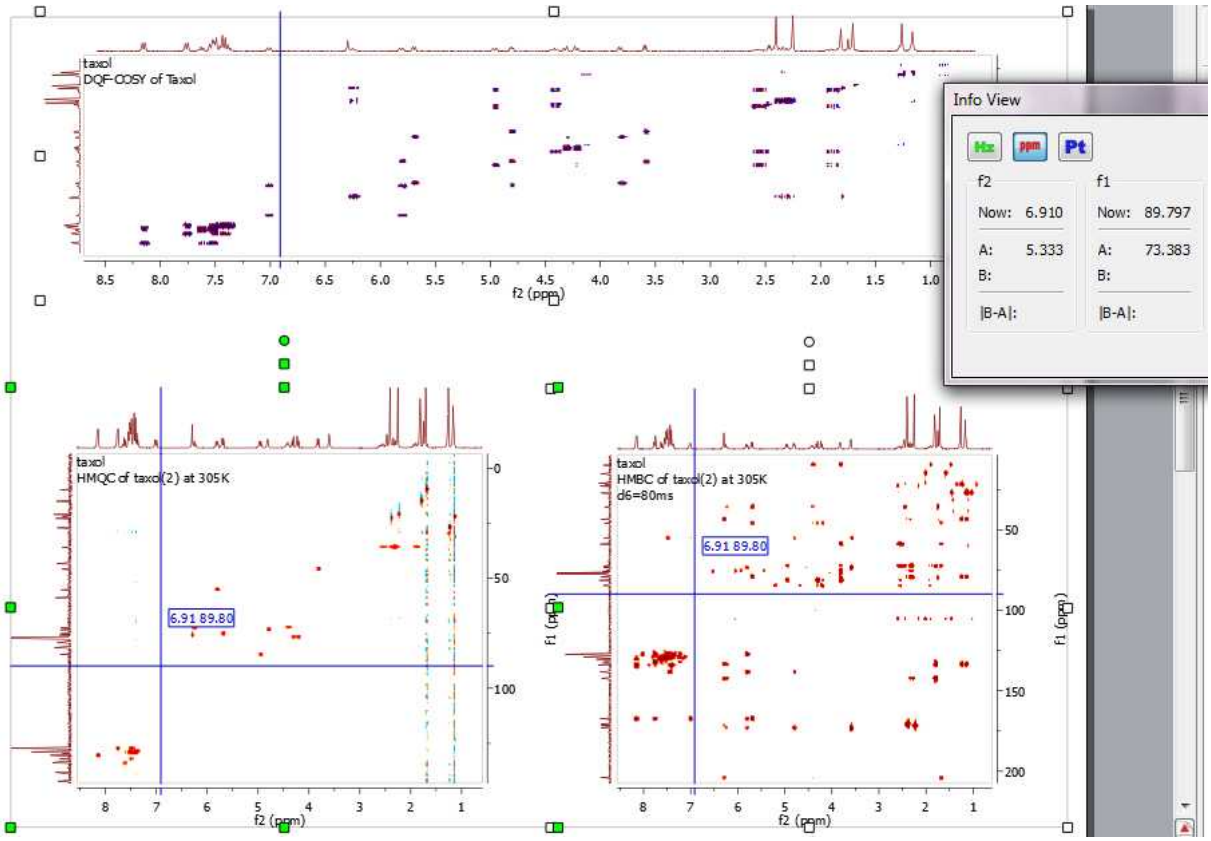
Multiple Crosshair: If you have several spectra displayed on the same page, you can tie the crosshair cursors together over all the experiments by selecting the crosshair feature in one of them. In the example below you can see how the multiple crosshair works in a HSQC, COSY and a 1H spectrum.

© 2024 MESTRELAB RESEARCH

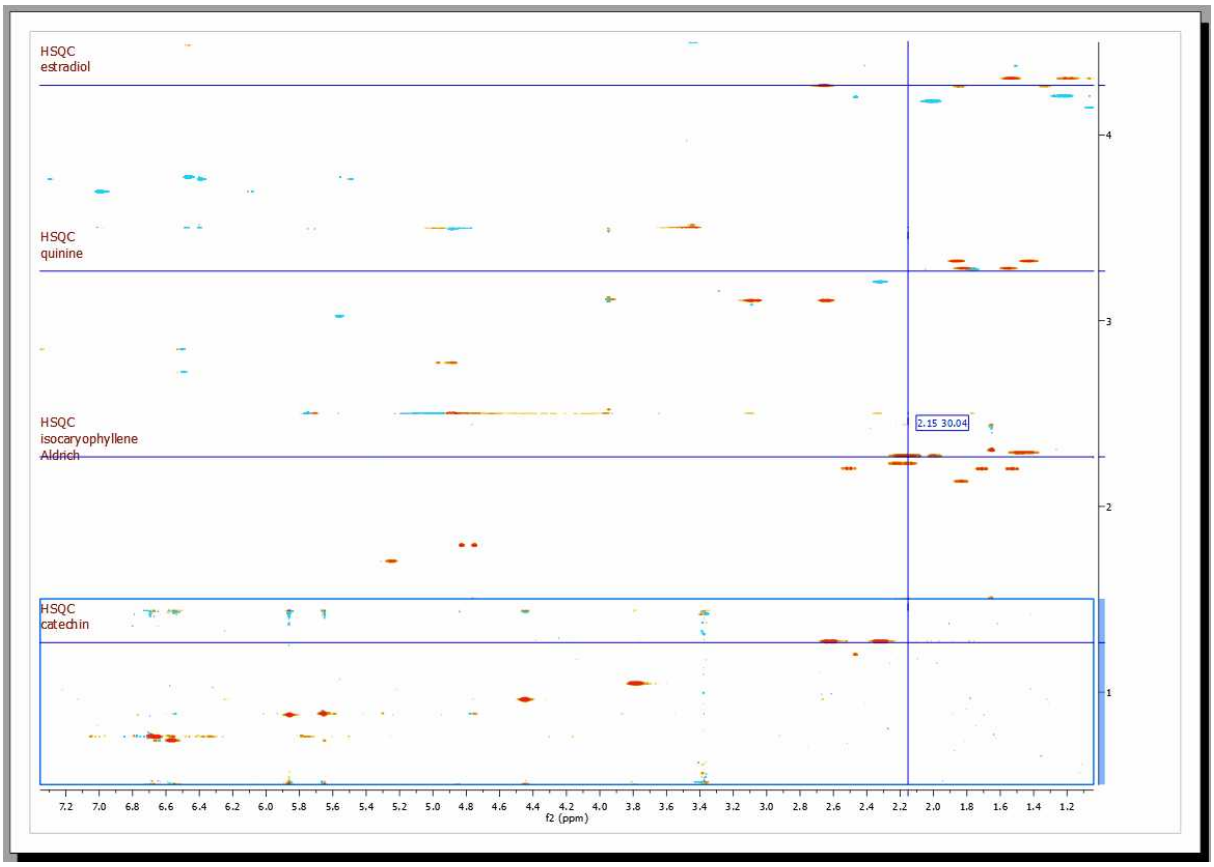


Here you can see a COSY, HMQC and HMBC (with the multiple crosshair):

© 2024 MESTRELAB RESEARCH



You can get correlated cursors in stacked mode. To disable/enable the correlated cursor, just press the 'C' key:



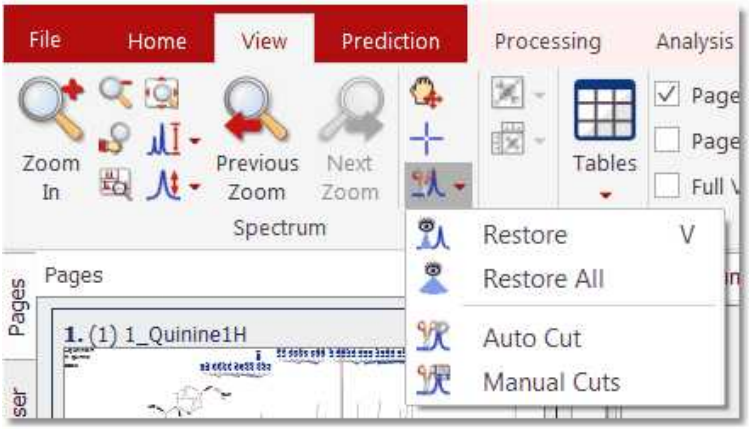
6.7.4 Cutting Spectra

THE SPECTRUM CUTTING TOOL 

This is a tool that most **Mnova** users will not have seen before (with the notable exception of **iNMR** users). It is important to note that this tool is in no way related to the "copy", "cut" and "Paste" object manipulating tools found in this and most Windows programs as Icons and in the Edit drop-down window. The **'Cutting'** tool allows the user to create **dark** regions, i.e., to hide areas of the spectrum the user is not interested in seeing (these could be noise regions or other regions which are of no interest at a specific time). By hiding these regions, areas of interest become larger on the screen without a need to create numerous expansion windows. The Cutting Tool is basically a way of split zooming, as opposed to the more limited traditional zooming in a single block. The hidden areas are shown on the scale and on the grid. The software also implements an **Auto Cut tool**, which will hide all noise-only regions of the spectrum at the click of one button.

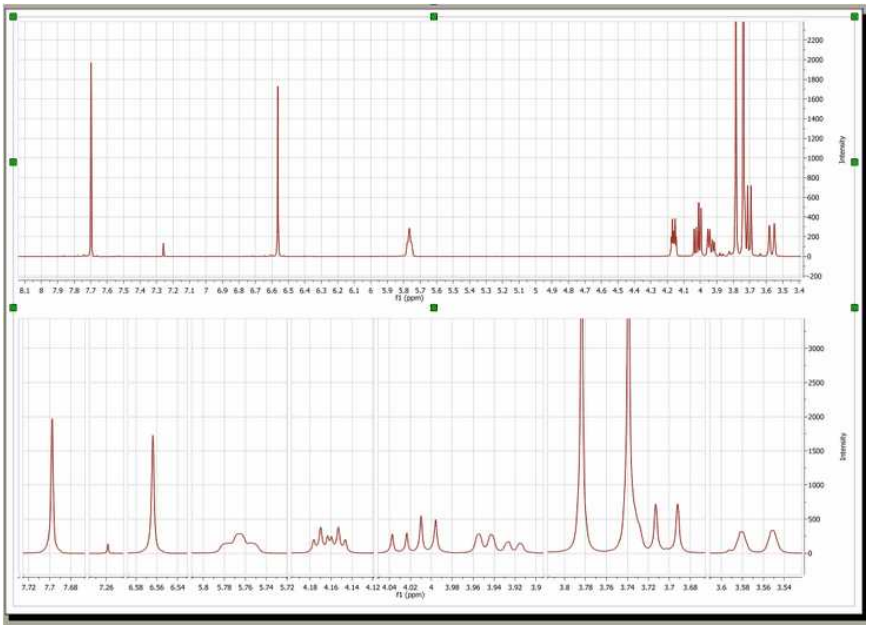
It is important to stress that this tool is not eliminating the information, but simply not displaying it. The 'Cut' and 'Auto Cut' options are available from following the menu item 'View/Cuts/Cut' or 'View/Cuts/Auto Cut'. Manual cutting can also be accessed by clicking on the **'Cut'** icon (or simply, by pressing the <X> key) as can be seen in the figure below. Auto Cut can be accessed via the drop-down menu which can be displayed by clicking on the side-bar of the Cutting Icon.

© 2024 MESTRELAB RESEARCH



You can **restore** one specific region ('Restore', or <V> key), a limited region ('Manual Restore') or all regions ('Restore All'). These options can be selected from the menu 'View/Cuts/Restore', 'View/Cuts/Manual Restore' and 'View/Cuts/Restore All' or by selecting 'Restore', 'Manual Restore' or 'Restore All' in the scroll menu shown in the figure below.

By the way, you should also note that the 'Cut' mode is available in 2D, along direct, indirect or both dimensions. Once again, switching between modes is achieved by simply pressing the <X> key.



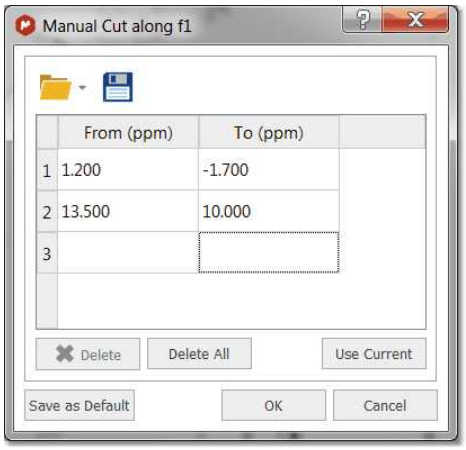
The cutting tool is aware of **dimension** and **nucleus** of the selected items. For example, if you have selected several pages in the same document (i.e 1H, 13C, COSY and HSQC):

- If you cut the 1H spectrum: the cut will be applied to the HSQC in the f2 dimension, and to the COSY in both dimensions.
- If you cut the 13C spectrum: the cut will be applied to the HSQC in the f1 dimension.
- If you cut the COSY (in both dimensions): the cuts in f1 and f2 will be applied to the HSQC in f2 dimension and to the 1H spectrum. The 13C spectrum will be unaltered.
- If you cut the HSQC (in both dimensions): the cut in f1 will be applied to the 13C spectrum, the cut in f2 will be applied to the 1H spectrum, and to both dimensions in the COSY.

© 2024 MESTRELAB RESEARCH

Manual Cutting

The user will be also able to use the 'Manual Cut' to select the limits of the dark regions.



The user will be able to predefine the cuts ranges to automate the cutting analysis. **Clicking on OK, will cut the current spectrum with the desired ranges and keep the regions to apply further cuts to additional spectra.**

The user will be able to save these regions for later uses, by clicking on the **'Save Cuts'** icon  which will be loaded by clicking on the **'Load Cuts'** icon 

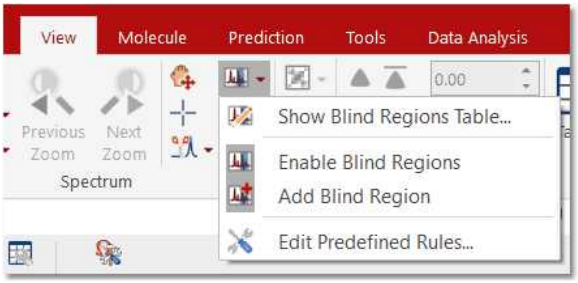
6.7.5 Blind Regions

This feature will be used to select regions from your spectra which will be skipped by the calculation of the automatic phase correction and analysis algorithms such as integration, peak picking or multiplet analysis. Please note that the final Phase correction will be applied to the entire spectrum, but it will not take into account the blind regions for the calculation.

Conversely, blind regions will not be saved or searched in the Database. This will allow users to ignore areas of the spectrum which present particular challenges, whilst still being able to use the rest of the information in the spectrum (for example, ignore non-phasable signals in solvent suppressed spectra when doing quantitation).

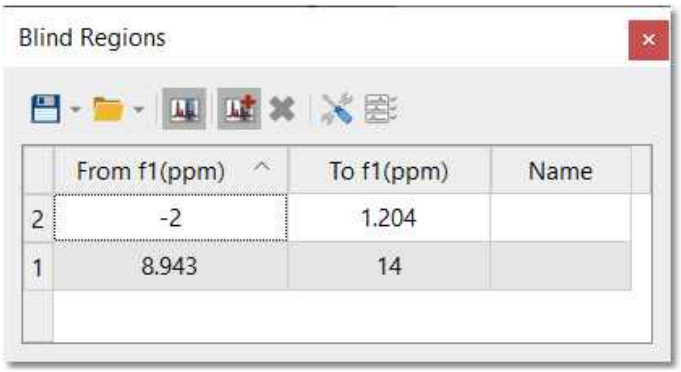
You will be able to select the Blind Regions by following the menu 'View/Blind Regions/Add Blind Region' and clicking&dragging on the regions of interest.

In the example below, you can see how we have added a blind region to the dispersive signal of the water in order to apply an automatic phase correction to the spectrum without taking into account that signal. We can get similar result by using the cutting tool (to hide the solvent signal):



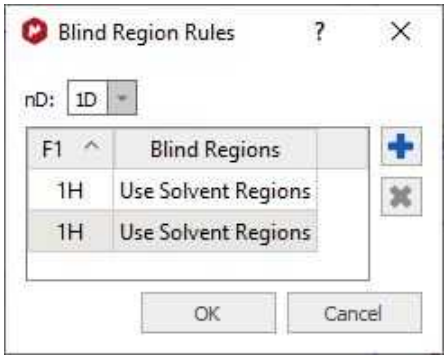
© 2024 MESTRELAB RESEARCH

Selecting the 'Show Blind Regions Table' will display a dialog box which will allow you to save the regions, to load another 'blind regions' file, to enable/disable the highlight of the blind regions or to add additional blind regions:

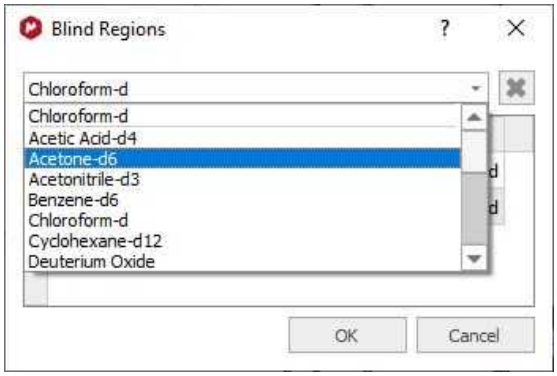


Right clicking on any cell of the table will allow you to delete or disable the applicable blind region. You can also delete them by clicking on the red cross button 

If you have previously saved any blind regions, you can use it as 'Predefined Rule' :



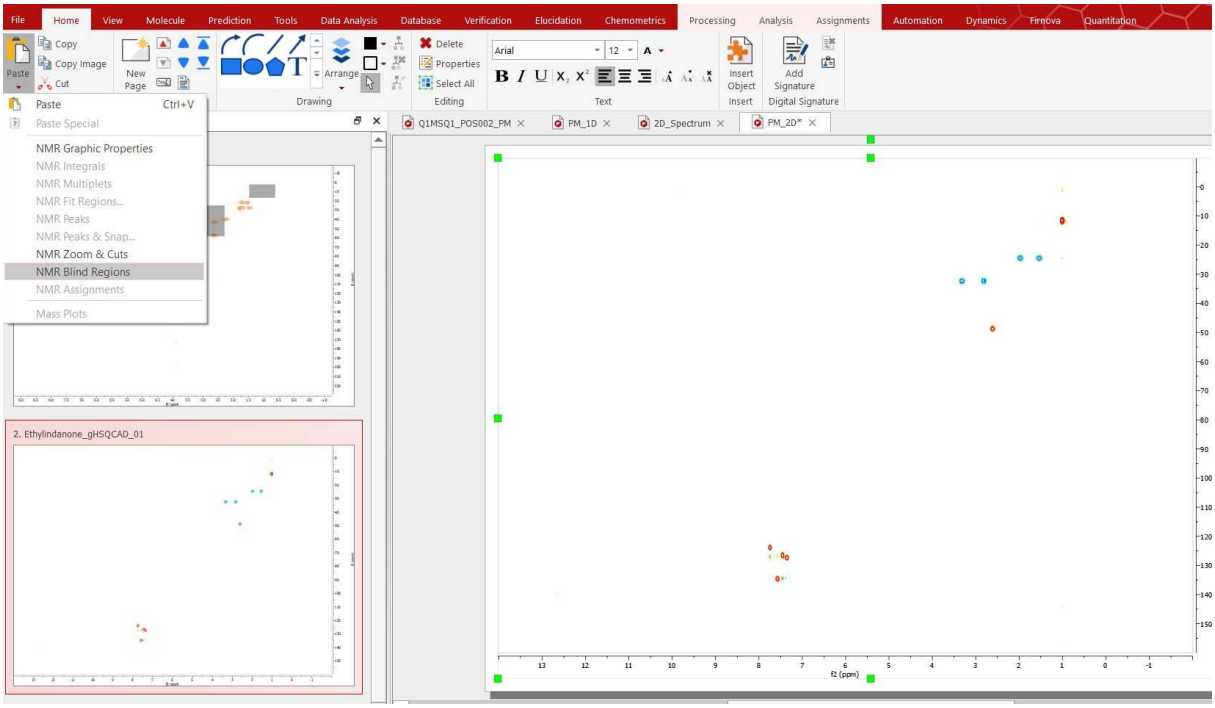
Clicking on the 'Open scroll down menu, you will be able to open any saved regions (including some templates for the common solvents by default).



Selecting the 'Import' option in the 'Open' scroll down menu, will allow you to load XML files containing the blind region limits.

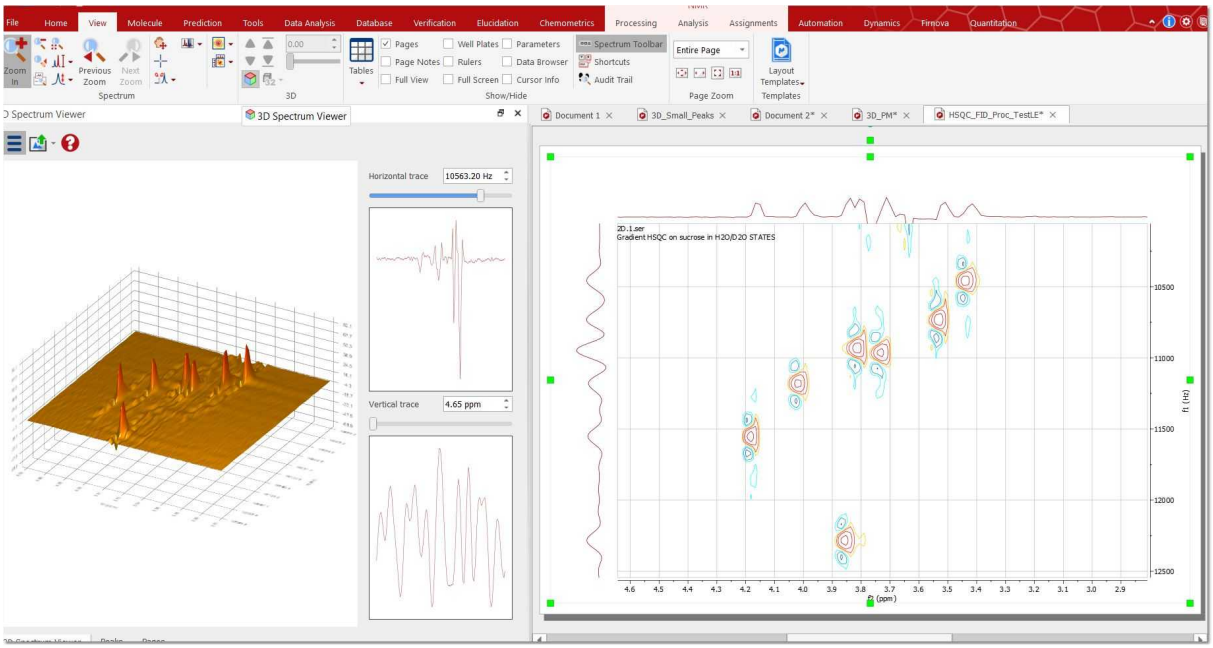
You can use the Intelligent NMR Clipboard feature to copy the blind regions from one dataset (by Ctrl+C) and paste them into the selected pages by following the menu 'Home/Paste/NMR Blind Regions':

© 2024 MESTRELAB RESEARCH



6.7.6 3D Spectrum viewer

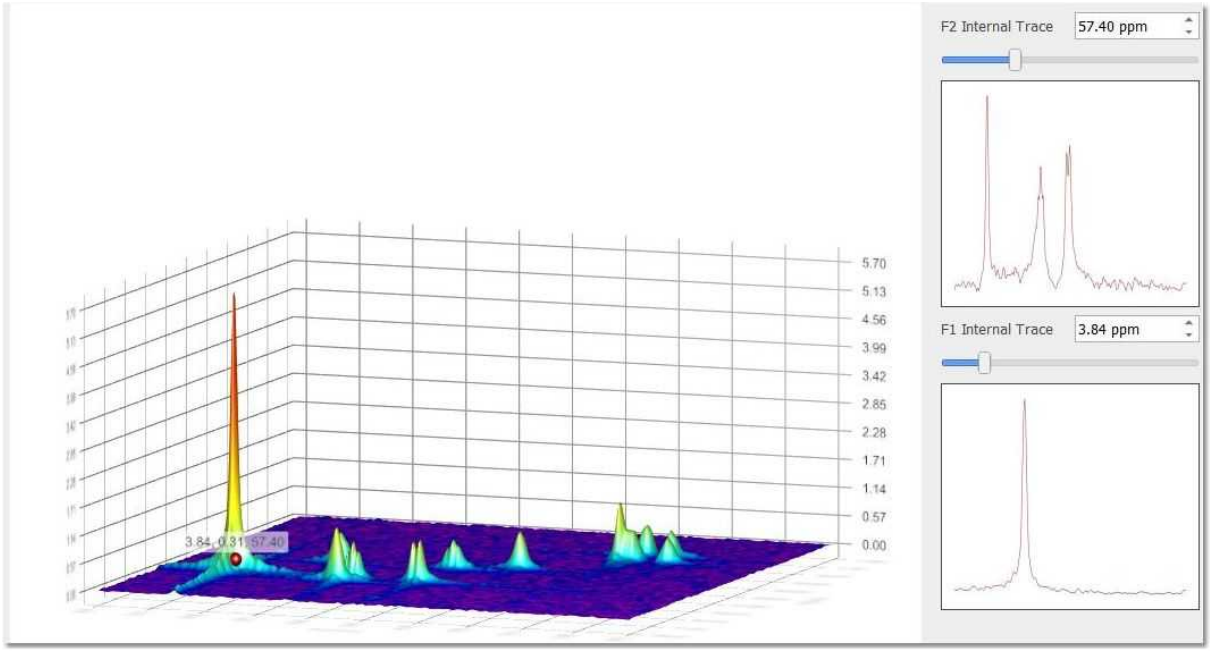
You can display a **2D NMR** in 3D just by clicking on the 3D Spectrum Viewer button:



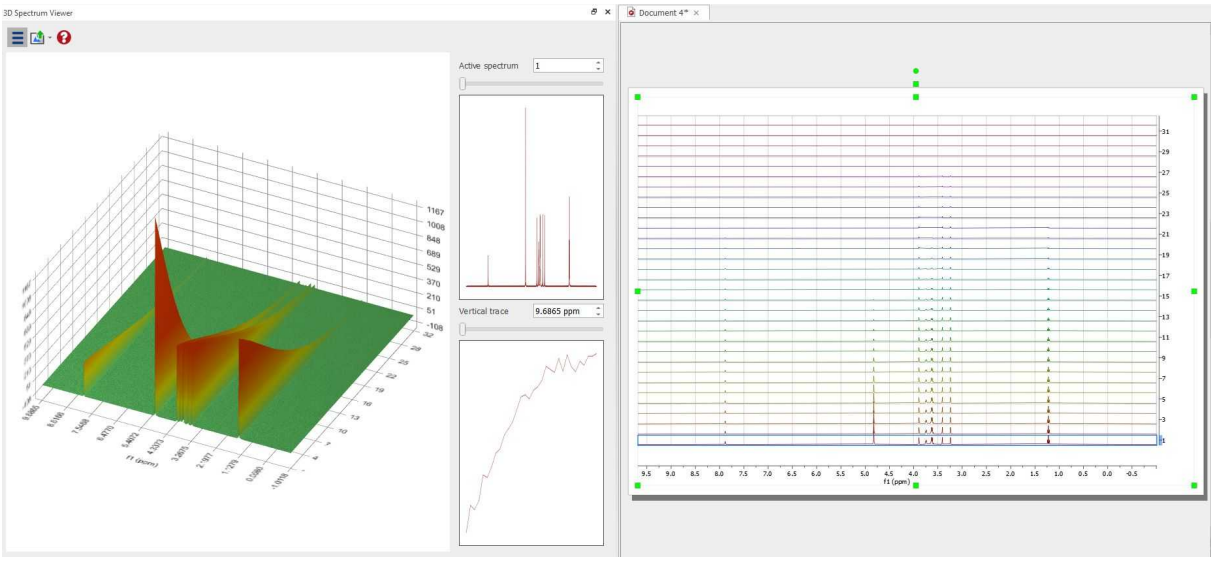
In this dialog, the 3D spectrum is displayed on the left and a panel with two plots of the corresponding internal current traces (f2 and f1) is displayed on the right. The current internal traces can be changed by using the sliders or by editing the corresponding values in ppm (the default value is the highest one on the spectrum scale).

© 2024 MESTRELAB RESEARCH

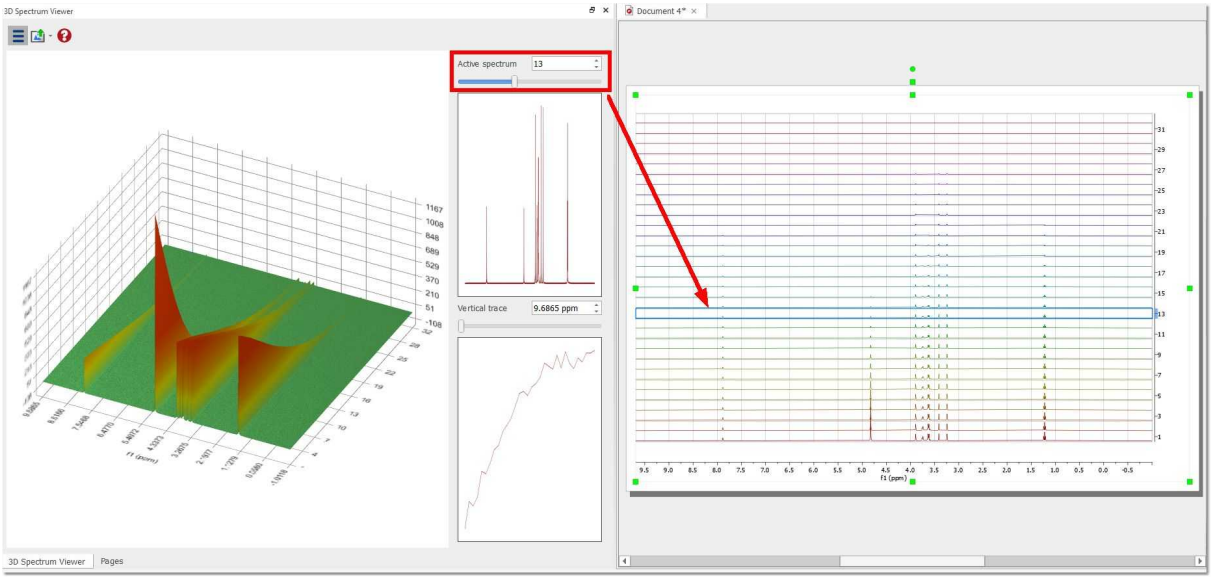
Zoom in and out can be done by dragging the mouse wheel forward or backward. 3D spectrum can be rotated by left-clicking and dragging the mouse. The traces can be selected graphically by right-clicking on a peak on the surface. The peak is highlighted with a red ball (same color as the traces on the canvas) and can be deselected by right-clicking anywhere on the surface:



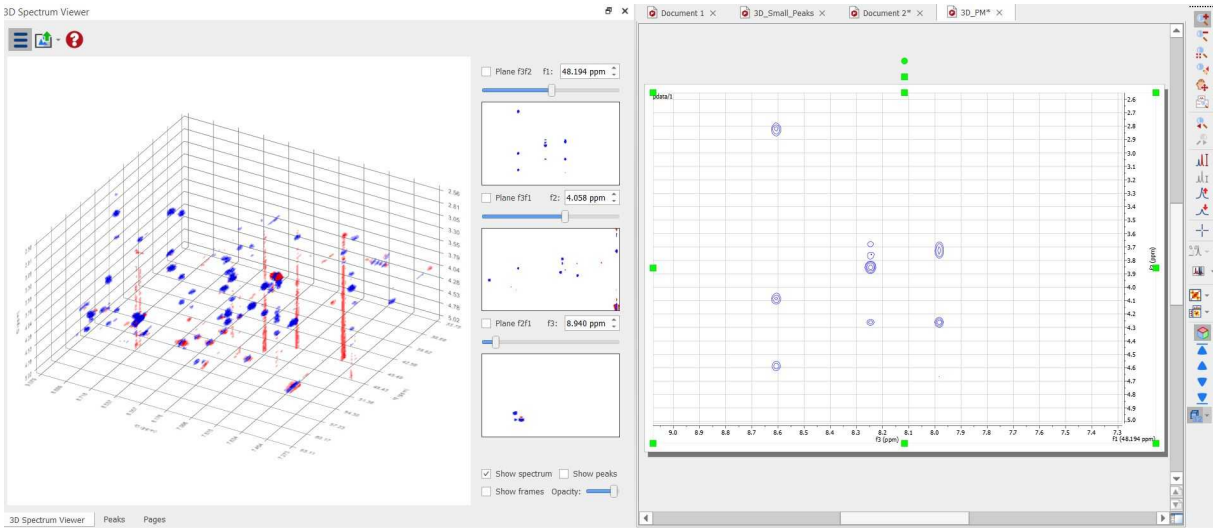
Loading a **stacked plot** of 1D NMR spectra will also display the result in the 3D viewer:



The active spectrum can be found in the left panel. You can use the spinbox or the slider to select the active spectrum (which will be updated in canvas):

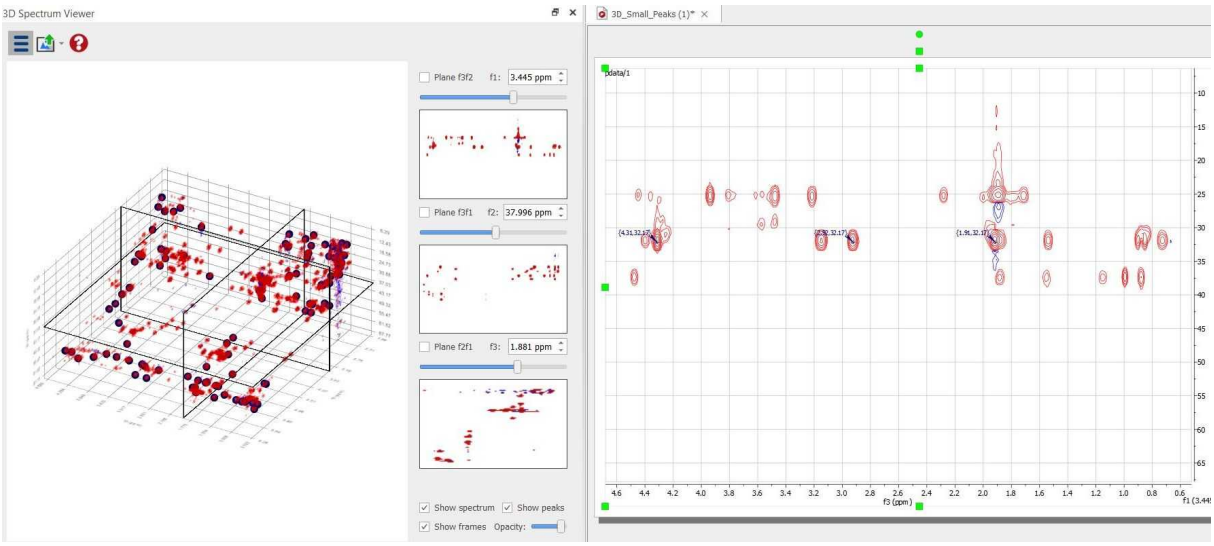


Pure **3D-NMR** spectra can also be loaded:



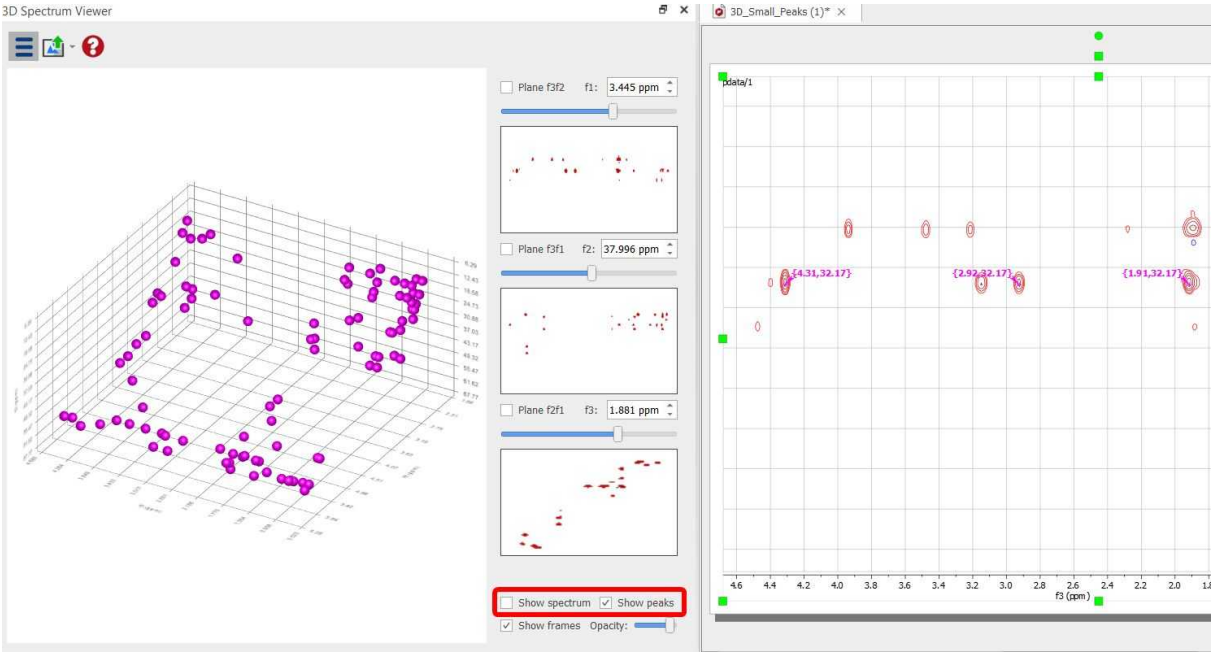
If you have peaks displayed in the 2D plot, those peaks labels will appear as well in the 3D viewer (with the same colour as the 2D peak labels):

© 2024 MESTRELAB RESEARCH



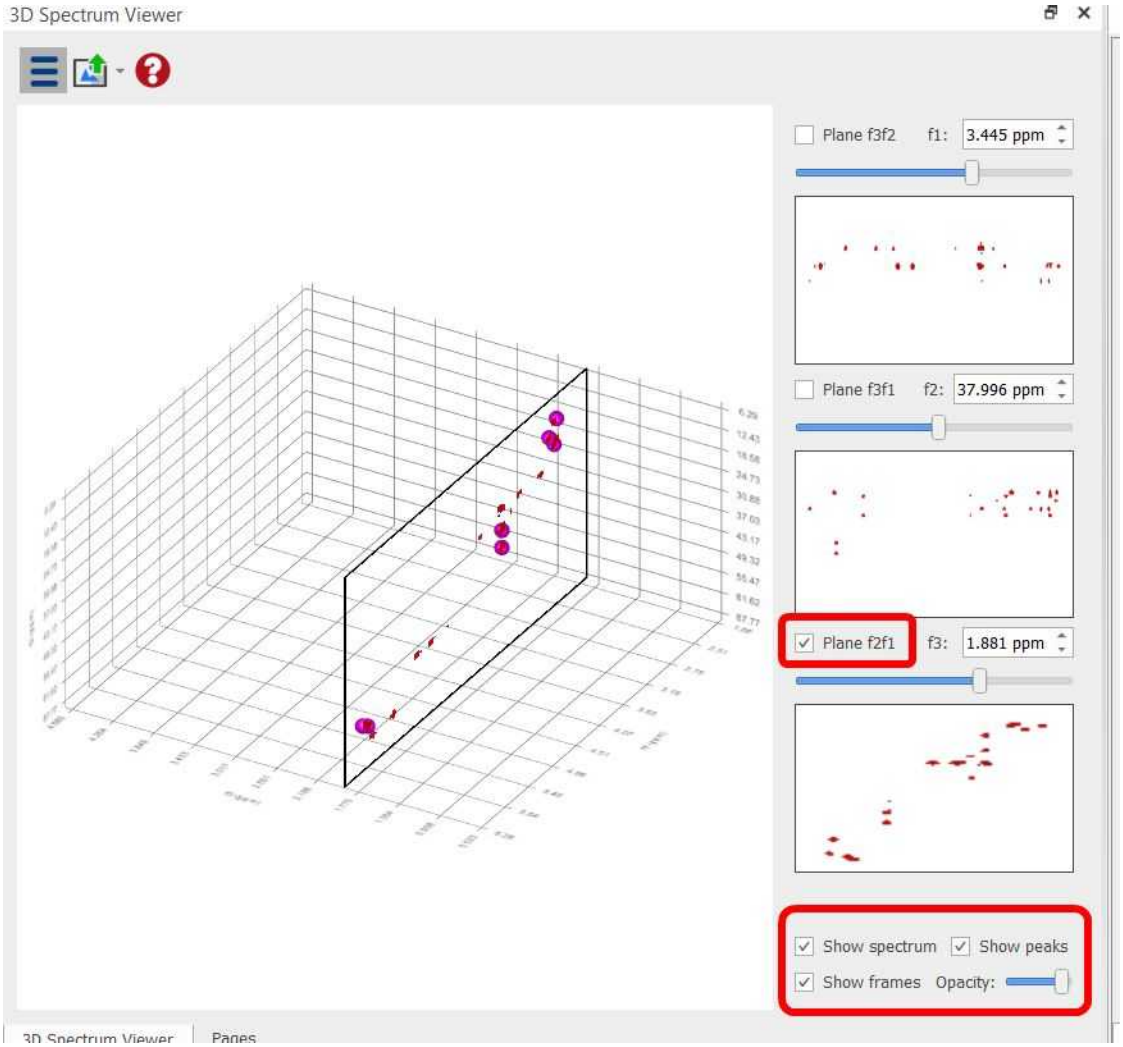
If you do not want to display the peak labels in the 3D viewer, just uncheck the 'Show Peaks' box.

When the "Show Spectrum" option is unchecked and "Show Peaks" is checked, only the peaks will be displayed in the viewer:



When "Show frames" is checked and a plane is chosen, only the peaks that belong to that plane are shown, so that when the plane is changed, you can see the change of the peaks shown in the frame.

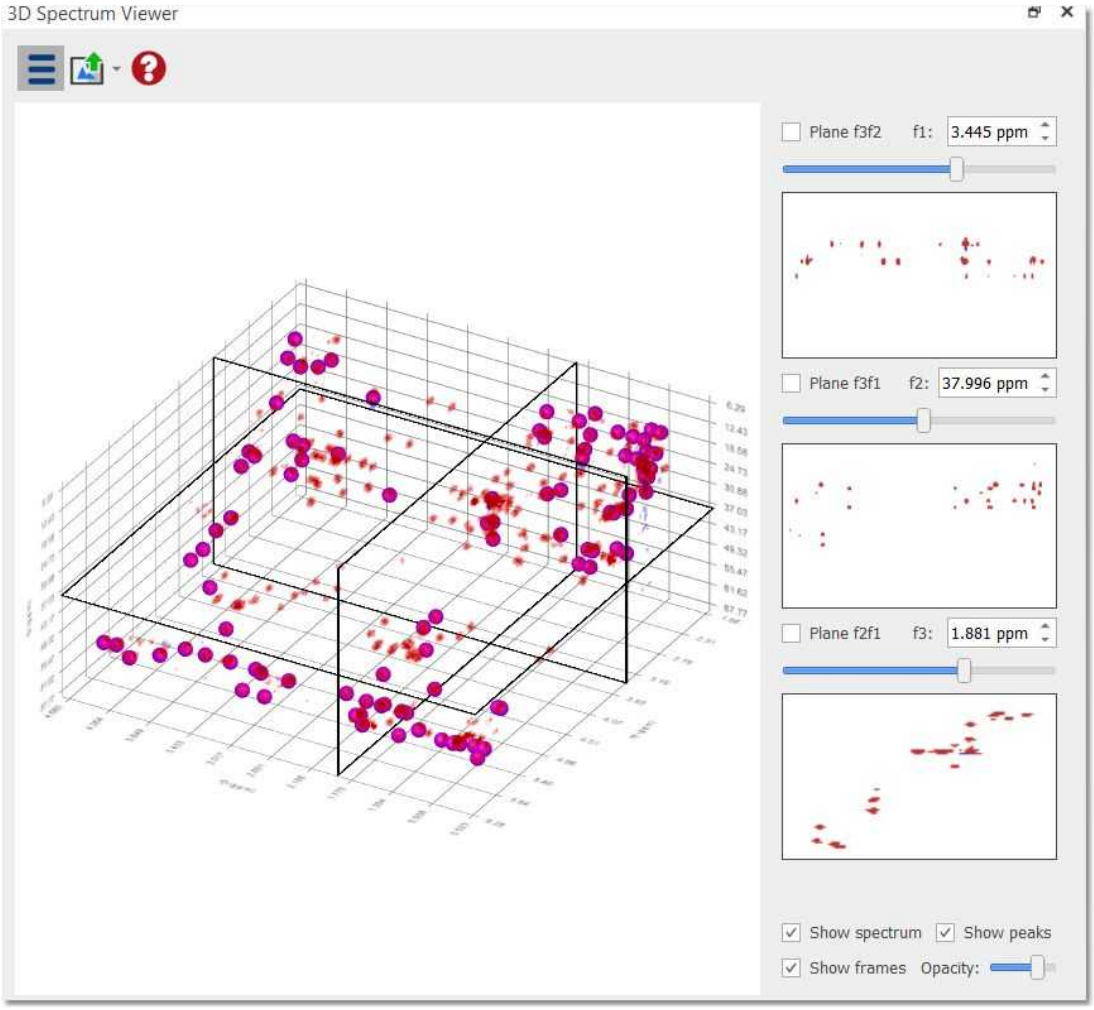
© 2024 MESTRELAB RESEARCH



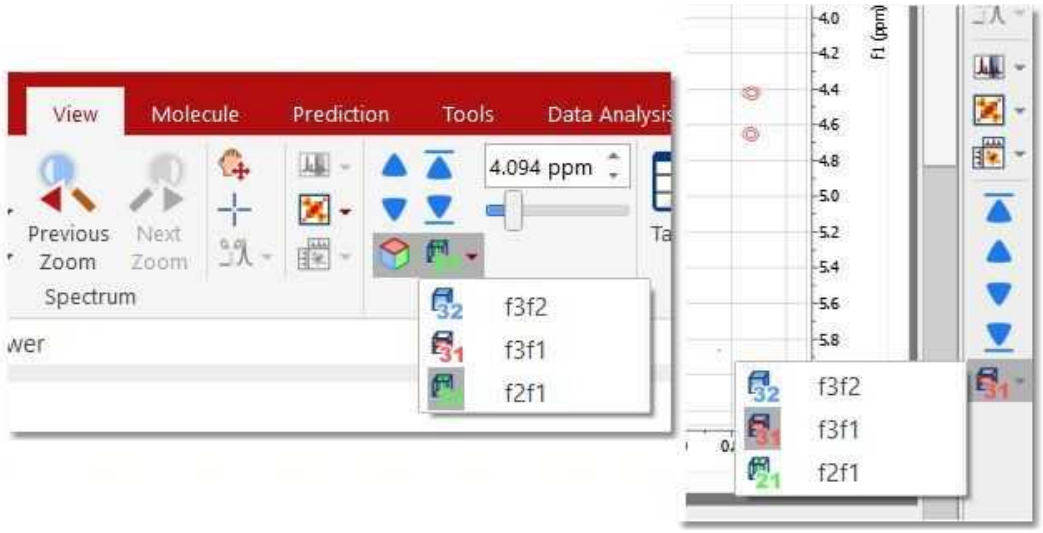
As you can see i the picture above, the 2D slices have a check box to be selected. When no 2D slice is selected, the full 3D spectrum is displayed, but when any 2D slice is selected, the corresponding 2D plane is displayed.

When no plane is selected, all peaks and frames are displayed.

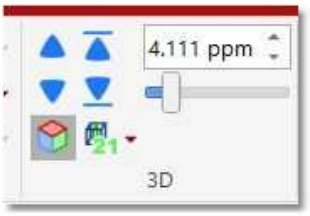
© 2024 MESTRELAB RESEARCH



You can select which 2D plane is shown in canvas by using the '3D panes' scroll down menu from the View ribbon or from the 'Spectrum' toolbar:



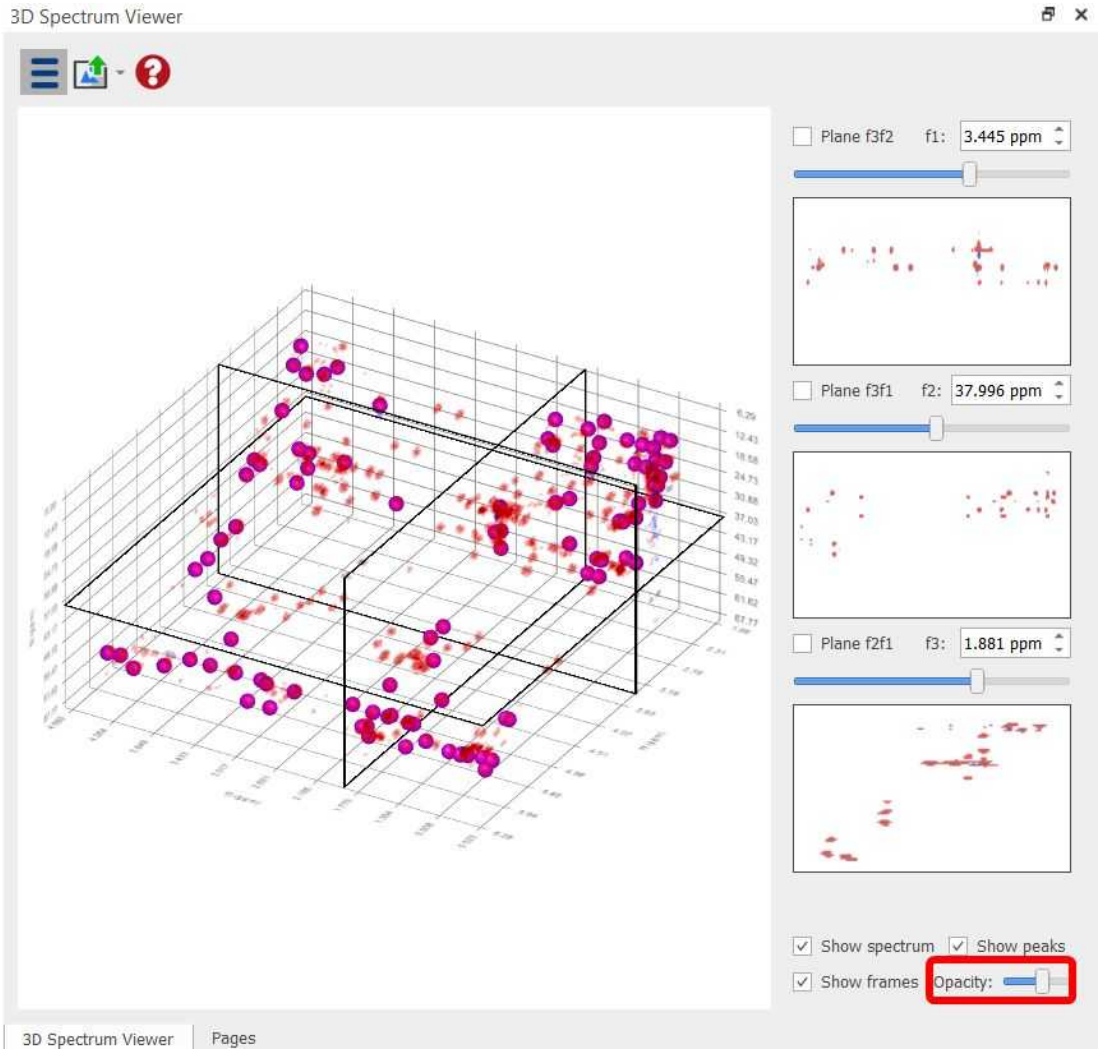
Use the blue arrow buttons, the spin box or the slider to navigate though the 2D panes in the canvas.



Shortcuts are available for these arrow buttons:

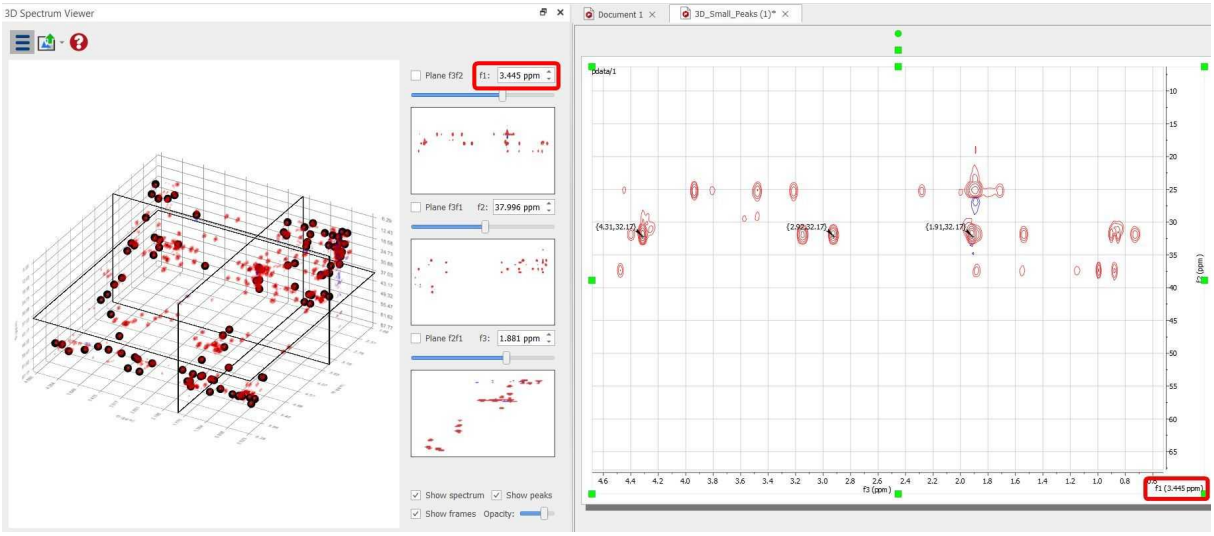
- Shift+Down to Previous 2D plane
- Shift+Up to Next 2D plane
- Alt+Down to First 2D plane (in the corresponding slice)
- Alt+Up to Last 2D plane (in the corresponding slice)

An 'Opacity' slider will allow you to modify the transparency of the colors, to help you to detect the signals with the highest intensity in the spectrum.

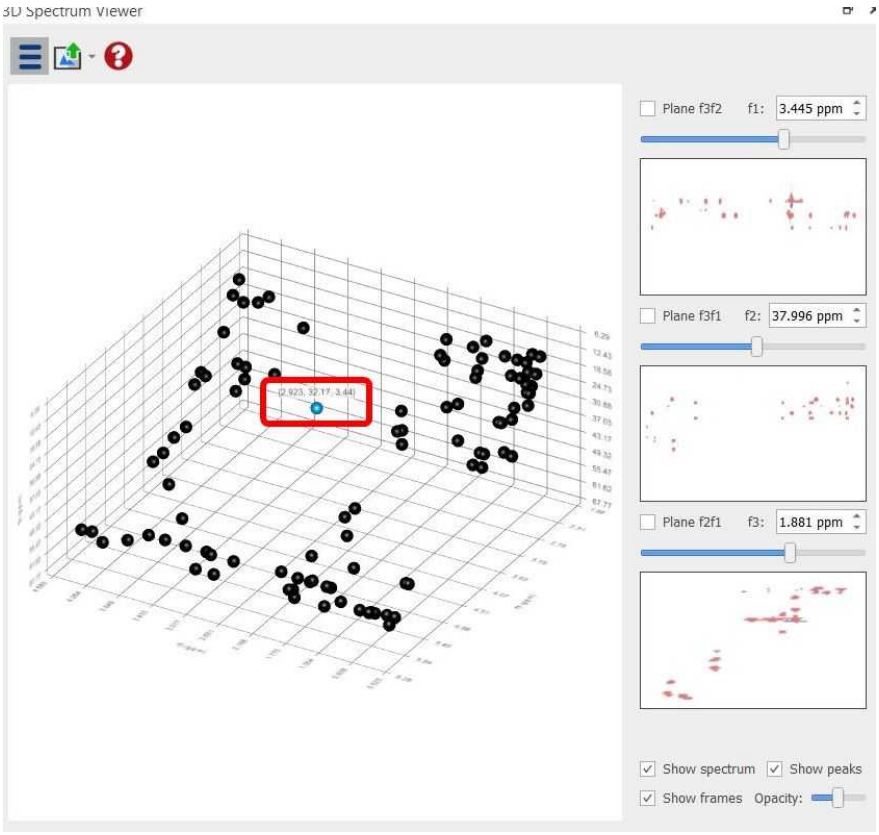


© 2024 MESTRELAB RESEARCH

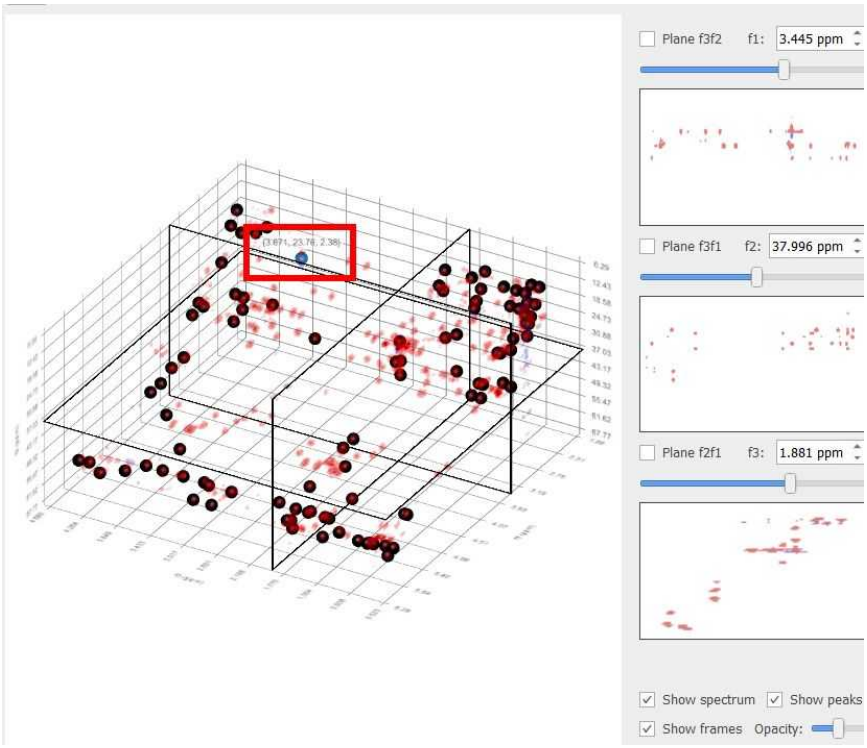
In the canvas, the X and Y axes correspond to the dimensions of the specific 3D view, and the chemical shift of the third dimension is displayed at the bottom right.



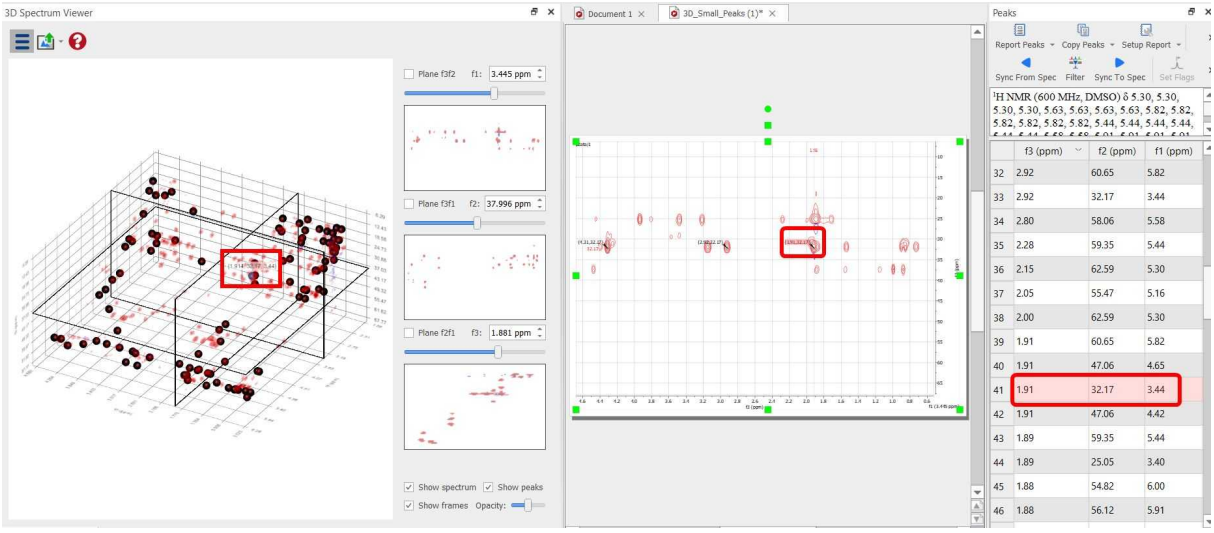
When you right click (and hold down) on the peak, the spectra cross peaks and frames disappear from the panel and the peak will be highlighted (as a light blue ball), with the corresponding label (with 3D coordinates).



Releasing the right click button will recover the spectra crosspeaks (and frames) but keeping the highlighted peak:

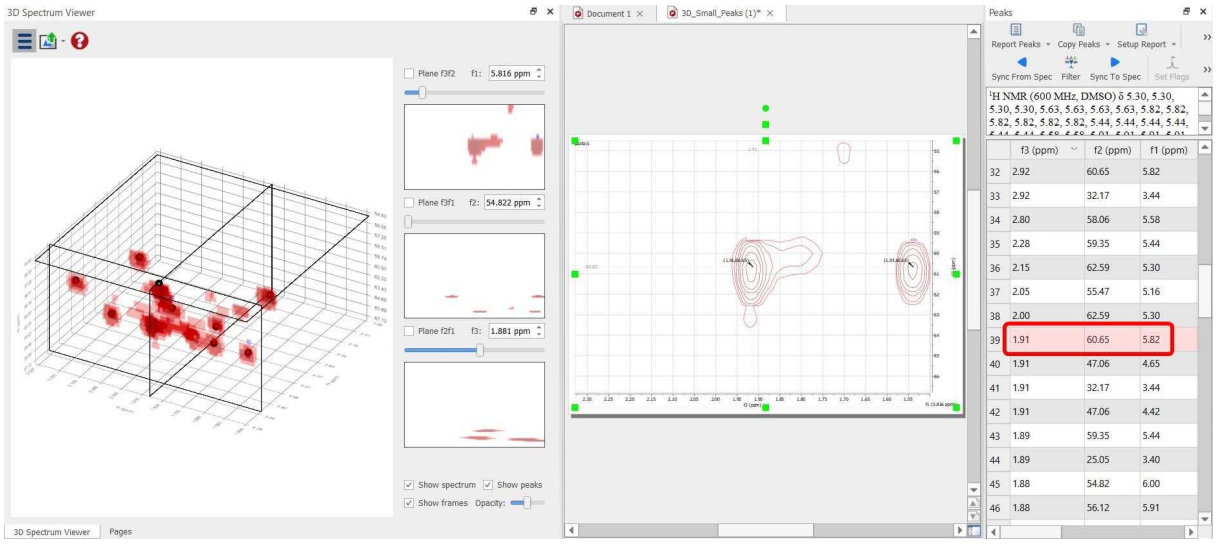


When a peak is selected in the canvas, the same peak will be highlighted in the "Peaks" table and in the "3D Spectrum Viewer":



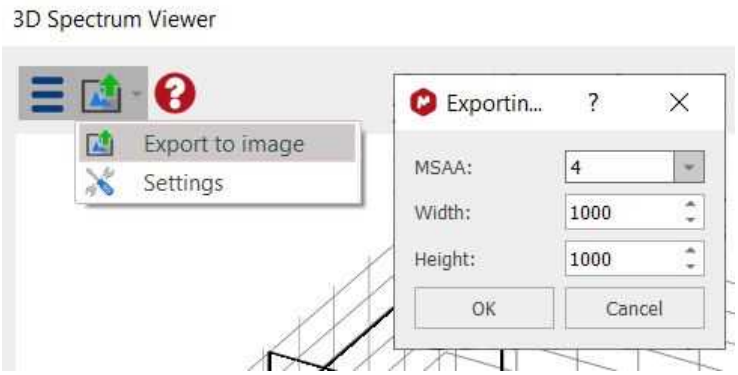
When a row of the peaks table is selected (by double clicking on it), two cross lines with the corresponding values will be displayed on the canvas (no peaks will be highlighted in the 3D viewer or in the canvas and the zoom in the 3D viewer will be the same as in the canvas and the the panel windows).

© 2024 MESTRELAB RESEARCH

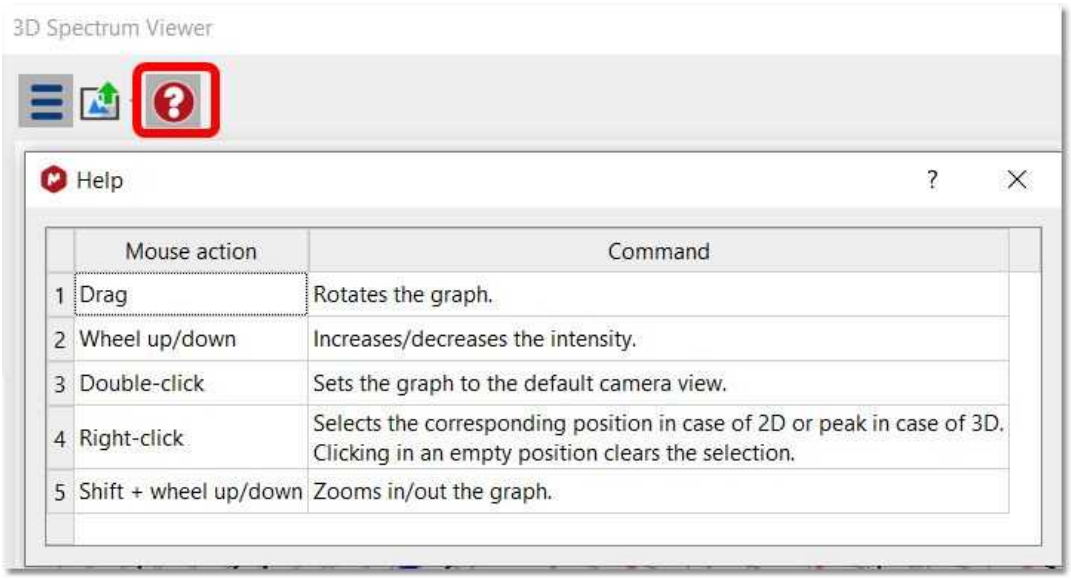


Please note that when zooming/panning in the canvas, the 3D NMR viewer will be updated.

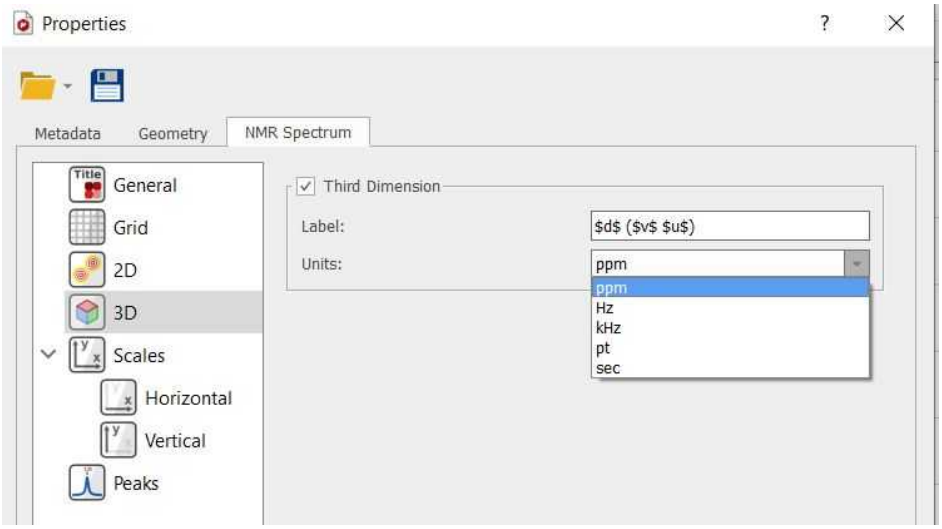
Use the toggle button  to show/hide the right panel. You can export the 3D spectrum as an image file by using this button:



Click on the Help button to display the shortcuts for the 3D viewer:

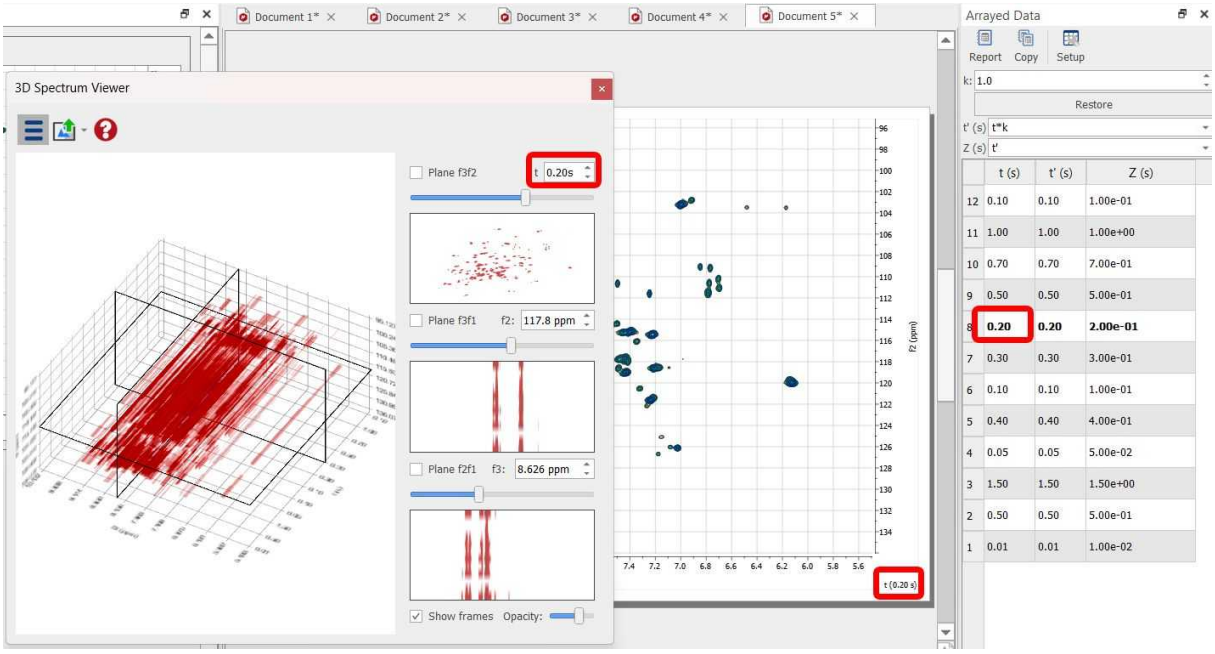


From the Properties dialog box, you can change the label and the units for the third dimension:



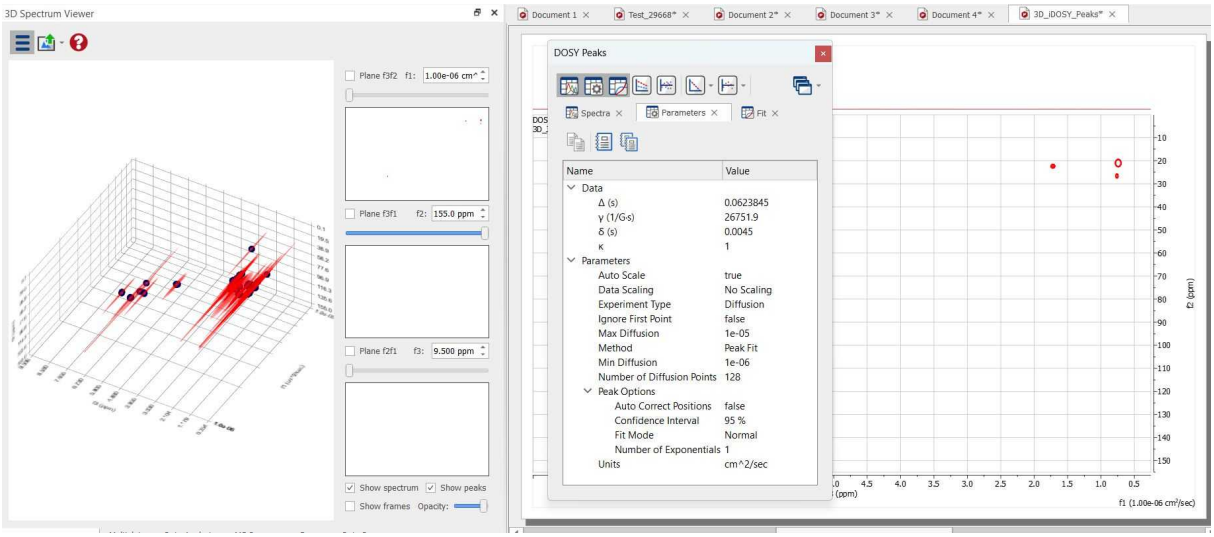
Pseudo 3D spectra (composed by a set of 2D spectra) can also be loaded. In this case, the third dimension is not a frequency (f1 in normal 3D spectrum), but a temporal serie. The values of this serie will be taken from the Arrayed Data Table. These values will be displayed in the third dimension label of the canvas, in the current plane spinbox of the ribbon and in the corresponding scale and spinbox in the 3D viewer:

© 2024 MESTRELAB RESEARCH



.

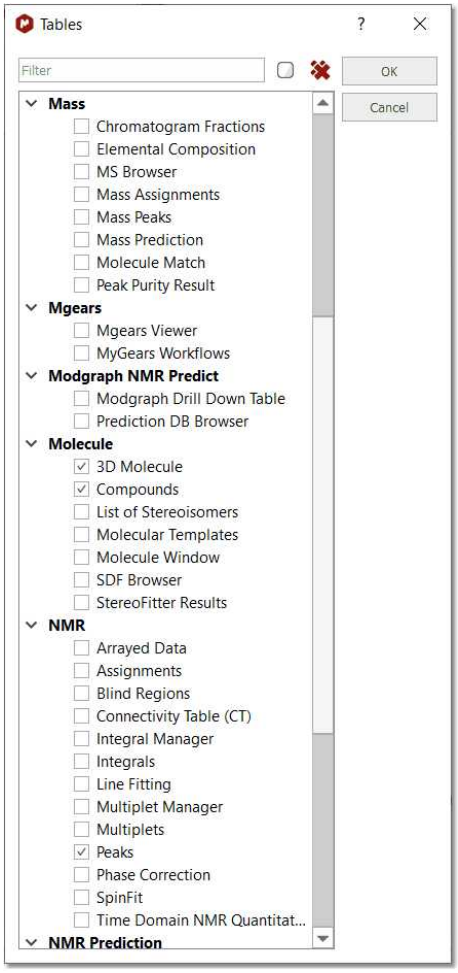
You can run DOSY transforms with Pseudo 3D i-DOSY experiments:



See also this video: https://youtu.be/Y85-wg_qbVc

6.7.7 Tables

Mnova groups all spectra relevant information into the so-called **Tables window**, available from the ***View*** menu or by right clicking on any toolbar.



From this set of tabbed pages, the user can find information, about:

- Cursor Info
- Audit Trail
- Data Browser
- Parameters
- Stacked Items
- Mass Prediction (Mass plugin)
- MS Browser (Mass plugin)
- Elemental Composition (Mass plugin)
- Molecule Match (Mass Plugin)
- Mass Peaks (Mass plugin)
- Peak Purity Result (Mass plugin)
- 3D Molecule
- Compounds
- Molecule Window
- SDF Browser
- StereoFitter Project Viewer
- Arrayed Data (DOSY, T1/T2, etc... processing)
- Assignments
- Integrals

© 2024 MESTRELAB RESEARCH

- Multiplets
- Peaks
- Blind Regions
- Line Fitting
- Data Analysis
- Integral Manager
- Multiplet Manager
- PCA Results
- Phase Correction
- Time Domain NMR Quantitation
- ^{1}H Prediction (Prediction plugin)
- ^{13}C Prediction (Prediction plugin)
- X-Nuclei Prediction (Prediction plugin)
- Prediction DB Browser (Prediction plugin)
- Spin Simulation (Prediction plugin)
- Structure Elucidation
- Verification

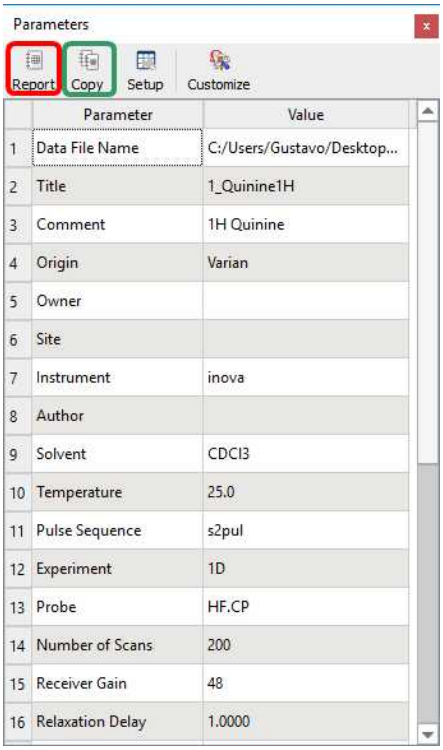
Spectral Parameters: This table shows information about:

- Experiment Title
- Data File Name
- Origin of the Experiment (type of spectrometer used)
- The Owner
- Solvent Used ($CDCl_3$ in the example below)
- The Frequency of the Spectrometer Used to Carry Out the Experiment
- Number of Acquired Data Points
- Spectral Width
- Spectrum Size After FT
- Lowest Frequency (in Hz) Used for Referencing Purposes, etc.

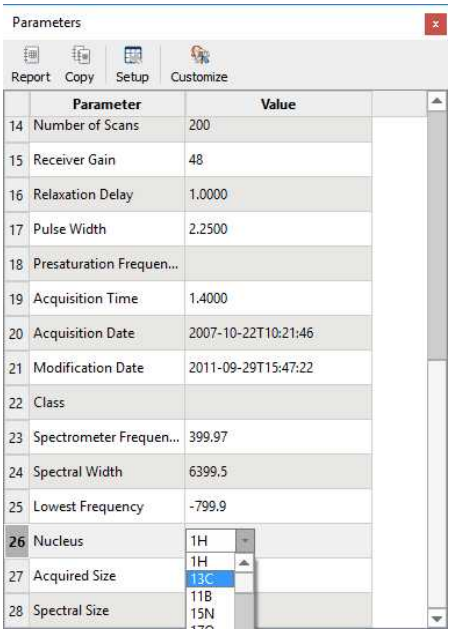
Please note that Mnova includes the ability to add more parameters into this list and customize the way it is displayed on the screen. **See also:** Parameters Table

The user can directly paste the spectral parameters table on the spectrum by clicking on the **'Report'** icon (red square in the capture below). You can also export the table to another document by clicking on the **'Copy to Clipboard'** (green square in the screenshot below).

© 2024 MESTRELAB RESEARCH

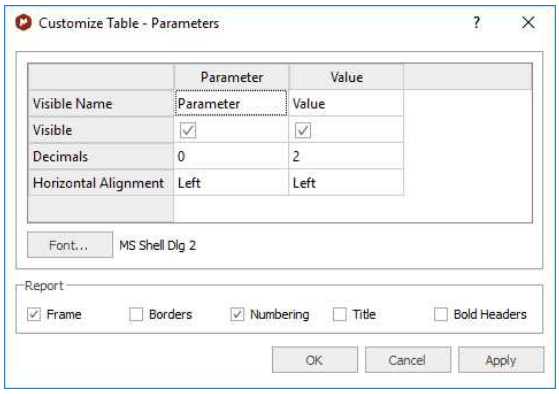


The user will be able to modify some of the parameters (Spectrometer frequency, Spectral Width, Lowest Frequency, Nucleus...) by just double clicking on the value as is shown in the picture below:

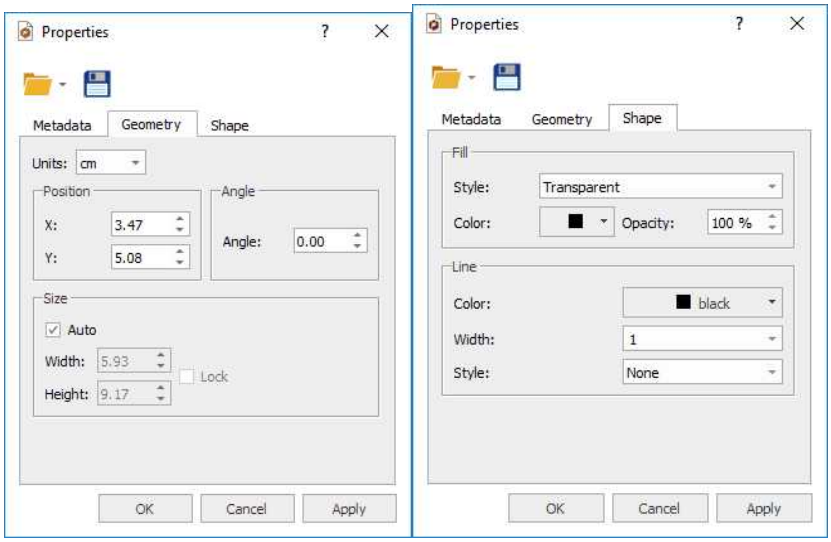


You can customize the table, by clicking on the **'Column or Row Setup'**. The 'Customize Table' dialog box will be displayed and will allow you to change the name of the columns, hide them, change the number of decimals as well as the horizontal alignment.

© 2024 MESTRELAB RESEARCH



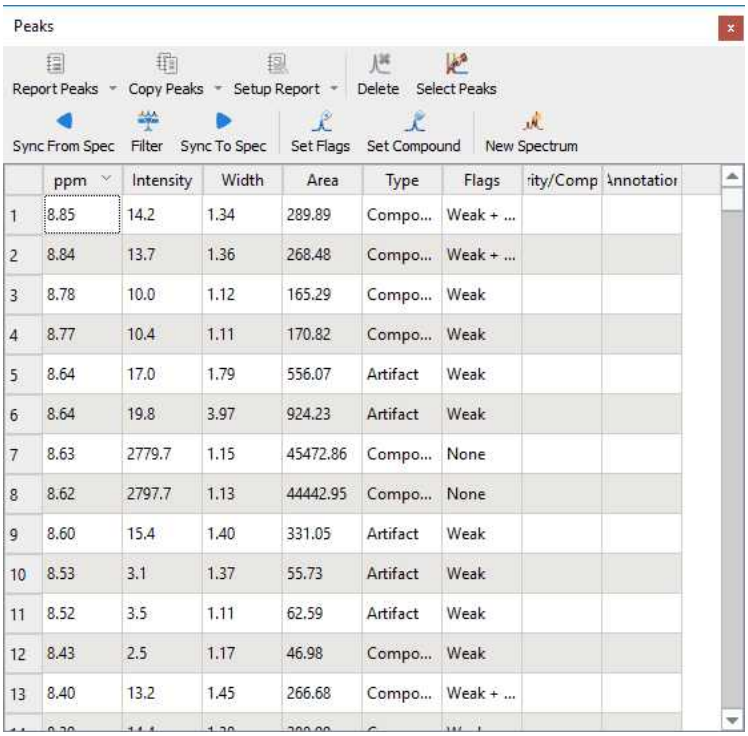
Once any table has been reported, you can change its properties (position, size, fill and border) just by double clicking on it:



See also:
Parameters Table

Peak Table: By default, peak frequencies are listed directly on the spectrum but they can also be accessed via the *Peak Picking table* by following the menu 'View/Tables/Peaks'.

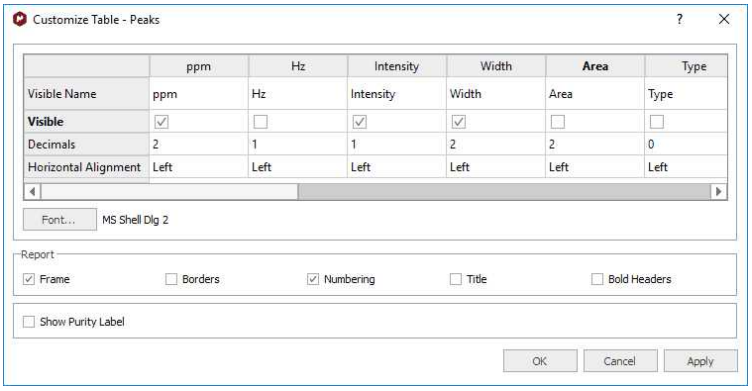
© 2024 MESTRELAB RESEARCH



	ppm	Intensity	Width	Area	Type	Flags	rity/Comp	Annotatior
1	8.85	14.2	1.34	289.89	Compo...	Weak + ...		
2	8.84	13.7	1.36	268.48	Compo...	Weak + ...		
3	8.78	10.0	1.12	165.29	Compo...	Weak		
4	8.77	10.4	1.11	170.82	Compo...	Weak		
5	8.64	17.0	1.79	556.07	Artifact	Weak		
6	8.64	19.8	3.97	924.23	Artifact	Weak		
7	8.63	2779.7	1.15	45472.86	Compo...	None		
8	8.62	2797.7	1.13	44442.95	Compo...	None		
9	8.60	15.4	1.40	331.05	Artifact	Weak		
10	8.53	3.1	1.37	55.73	Artifact	Weak		
11	8.52	3.5	1.11	62.59	Artifact	Weak		
12	8.43	2.5	1.17	46.98	Compo...	Weak		
13	8.40	13.2	1.45	266.68	Compo...	Weak + ...		

This table displays the list of peaks, including the following information: chemical shift in ppm and Hz, intensity (height), width (width, in Hz, at half maximum of the peak), type (the user can select between 'Compound', Artifact', Impurity' or 'Solvent') and 'Annotations'.

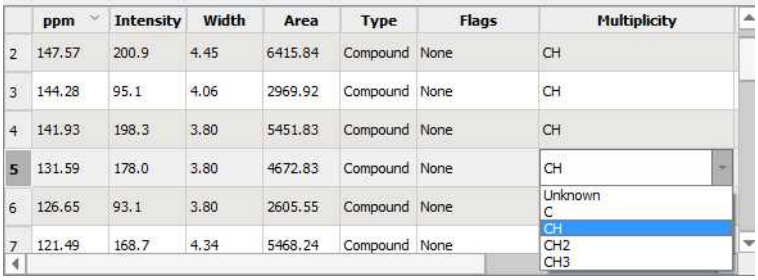
The user can report the Peak table directly on the spectrum by clicking on the **'Report Table'** icon (under the Report Peaks scroll down menu) You can customize the appearance of this report (an also of the table), by clicking on the 'Setup Table' button Setup (under the Setup Text scroll down menu) to display the 'Customize Table-Peaks' dialog box:



This dialog box will allow the user to change the name of the columns, hide them, change the number of decimals as well as the horizontal alignment. You can change the Font Family and also to customize the appearance of the table report (Frame, Borders, Numbering, Title and Bold Headers).

The user will also be able to select the multiplicity of the carbon signals, just by double clicking in the applicable cell, as you can see in the picture below:

© 2024 MESTRELAB RESEARCH

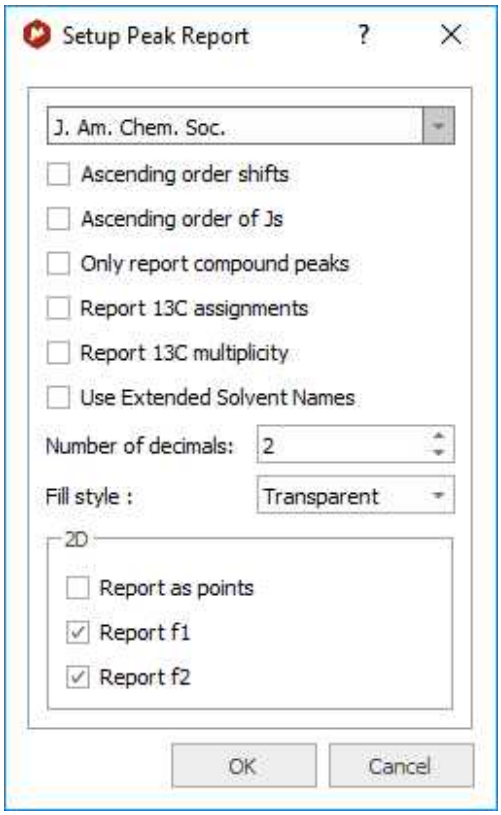


	ppm	Intensity	Width	Area	Type	Flags	Multiplicity
2	147.57	200.9	4.45	6415.84	Compound	None	CH
3	144.28	95.1	4.06	2969.92	Compound	None	CH
4	141.93	198.3	3.80	5451.83	Compound	None	CH
5	131.59	178.0	3.80	4672.83	Compound	None	CH
6	126.65	93.1	3.80	2605.55	Compound	None	
7	121.49	168.7	4.34	5468.24	Compound	None	

To delete an undesired peak, click on the **'Delete'** icon  of the Peak List, or select 'Delete Manually' on the Peak Picking scroll bar icon

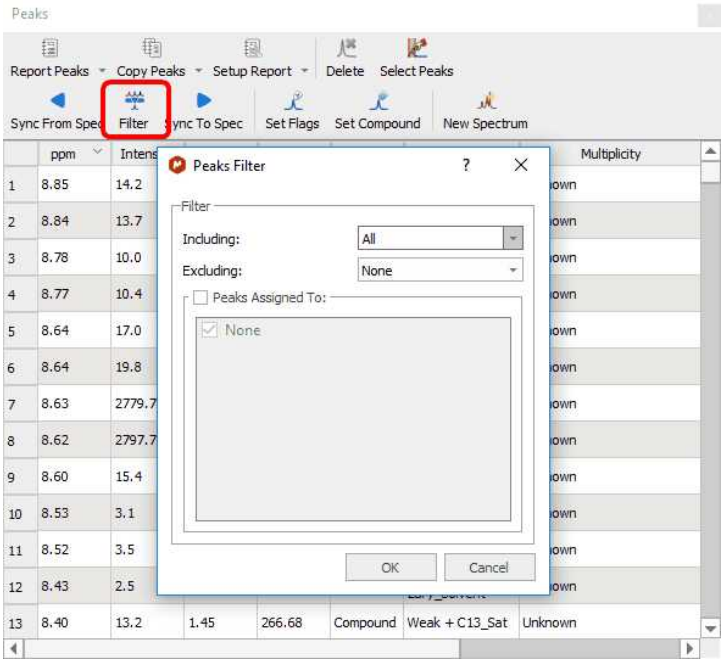


By default, peak frequencies are listed directly on the spectrum but they can also be accessed via the *Peak Picking table* and also as a **'Peak List'** by clicking on the **'Report Peaks'** icon . The user will be able to select the 'Peak Report Template' between several journal formats by selecting 'Setup Text' in the 'Report Special' scroll down menu.



From here you can select the order of the report or only report the compounds peaks.

From the Peaks table, you can apply some filter (by clicking on the applicable button) to show for example only Compounds Peaks:

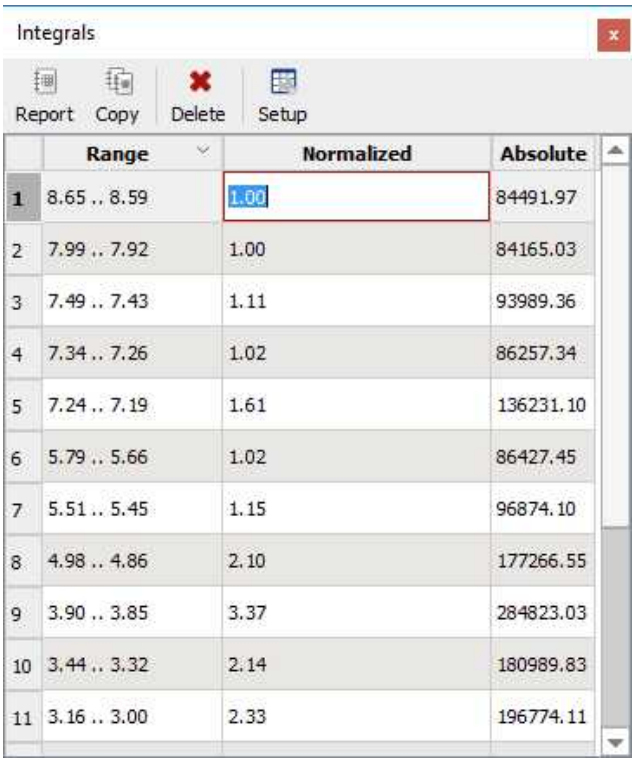


You can synchronize the type of the peaks in the table (i.e: only Compounds peaks) with the peaks showed in the spectrum (by using the button 'Sync from Item') or viceversa, synchronize the spectrum peaks with the peaks showed in the table ('Sync to Item' button).

Integral List: The Integrals Table includes information about the integral range (in ppm) and the normalized and absolute values of each integral. In this table the user can normalize the integrals by double clicking on the normalized value of the integral which the user would like to set (in the red square on the capture below). Once it is highlighted, the user has to overwrite the desired value, press return, and all integrals will update with reference to the chosen one. You will obtain the same effect by double clicking directly on the integral label on the spectrum.

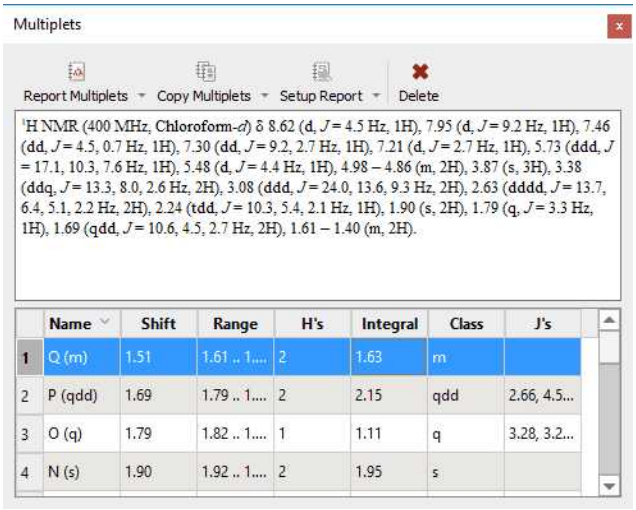
The user can also delete an integral from this table by clicking on the **'Delete'** icon  when the undesired integral is highlighted. To paste the integral list on the spectrum, just click on the **'Report'** icon .

© 2024 MESTRELAB RESEARCH



	Range	Normalized	Absolute
1	8.65 .. 8.59	1.00	84491.97
2	7.99 .. 7.92	1.00	84165.03
3	7.49 .. 7.43	1.11	93989.36
4	7.34 .. 7.26	1.02	86257.34
5	7.24 .. 7.19	1.61	136231.10
6	5.79 .. 5.66	1.02	86427.45
7	5.51 .. 5.45	1.15	96874.10
8	4.98 .. 4.86	2.10	177266.55
9	3.90 .. 3.85	3.37	284823.03
10	3.44 .. 3.32	2.14	180989.83
11	3.16 .. 3.00	2.33	196774.11

Multiplets: The multiplet table includes information about the name (by default Mnova named the multiplets following the alphabetical order, but the user is able to change it in the multiplet manager), the chemical shift (ppm), the range (ppm), number of hydrogens, integral value, type of multiplet and coupling constant values (Hz).



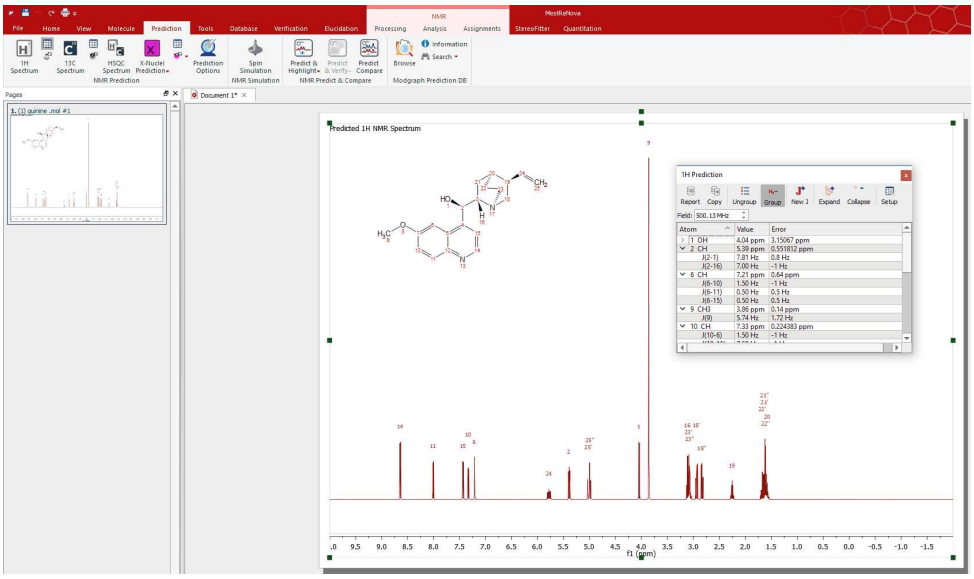
	Name	Shift	Range	H's	Integral	Class	J's
1	Q (m)	1.51	1.61 .. 1....	2	1.63	m	
2	P (qdd)	1.69	1.79 .. 1....	2	2.15	qdd	2.66, 4.5...
3	O (q)	1.79	1.82 .. 1....	1	1.11	q	3.28, 3.2...
4	N (s)	1.90	1.92 .. 1....	2	1.95	s	

See also 'Multiplets Table.

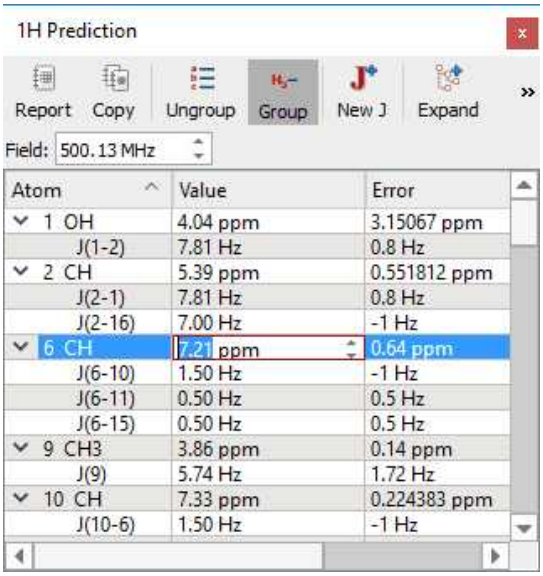
1H Prediction: In this table, the user can visualize the ^{1}H assignments of the predicted shifts (in NMR Predict mode). As you can see in the picture below, the carbon atoms are labeled to make easy the proton assignment. The user can also select the frequency of the desired predicted spectrum (on the frequency edit box) as well as do (or not) groups magnetically equivalents (by clicking on the

suitable icons: 

) and also expand or collapse the predicted values by clicking on the applicable buttons 

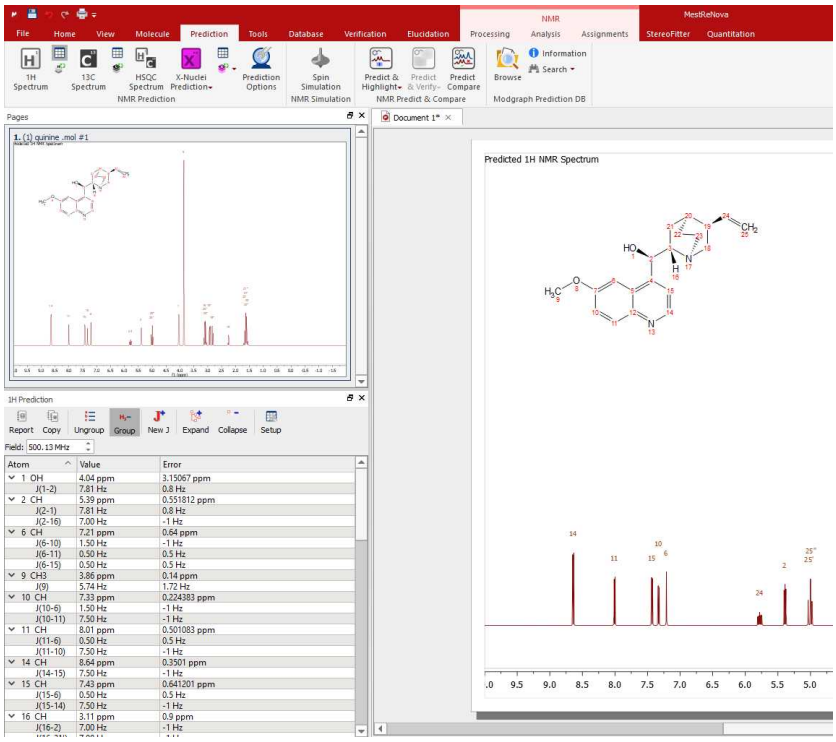


You can also modify the chemical shift and coupling constant values directly from this tables, just by double clicking on the applicable box and entering the desired value.

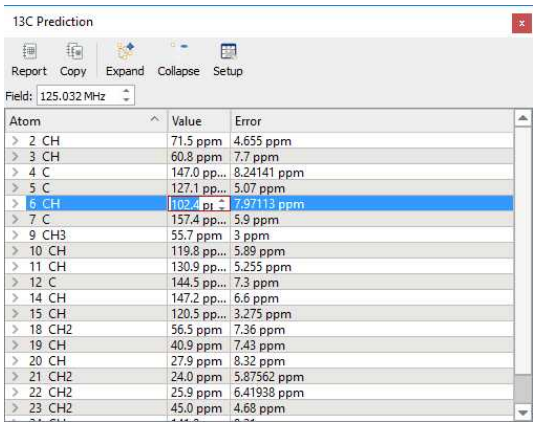


Please bear in mind that you can also put the table on the 'Page Navigator' just by clicking on the table and dragging it to the 'Page Navigator', as you can see in the picture below:

© 2024 MESTRELAB RESEARCH

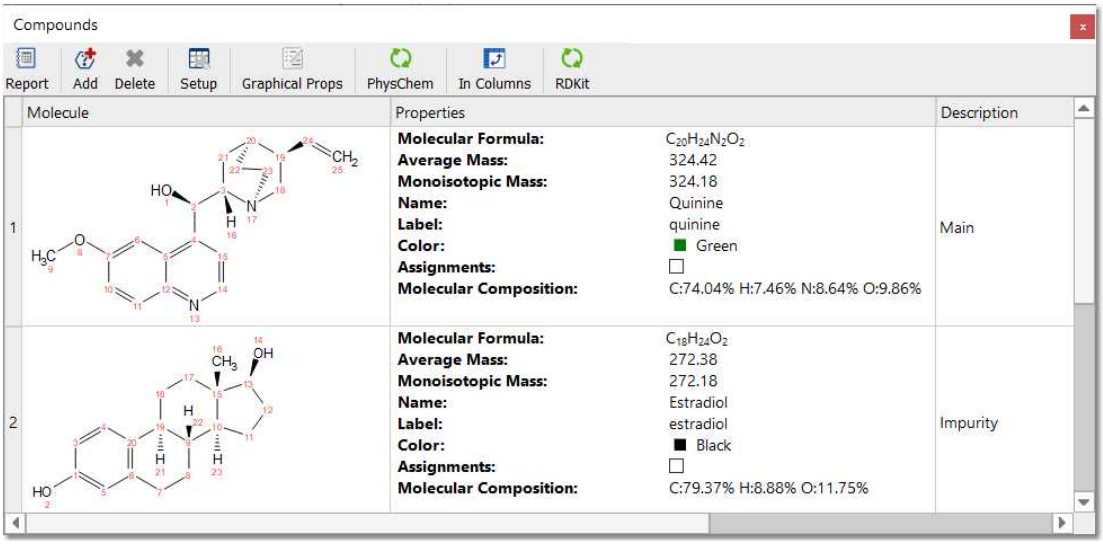


^{13}C Prediction: In this table, the user can visualize the ^{13}C assignments of the predicted shifts (in NMR Predict mode). You can also select the frequency of the desired predicted spectrum (on the frequency edit box). The user can paste the ^{13}C Prediction table directly on the spectrum by clicking on the **'Report'** icon  or copy it to the clipboard by clicking on the applicable icon . You can also customize this table by clicking on .



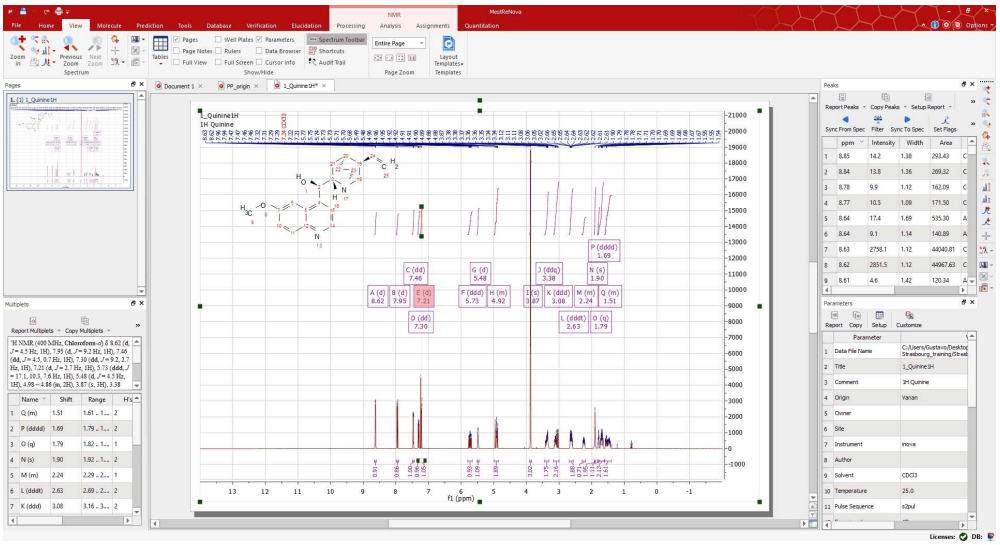
Compounds: This table will show all the molecules present in the document, with information about the chemical formula and molecular weight, name.

© 2024 MESTRELAB RESEARCH



You can copy any molecule from this table to another page just by selecting it and clicking on the 'Report' button. Please bear in mind that the information about predictions and assignments is linked to the molecule and will be kept after having pasted it to another slide.

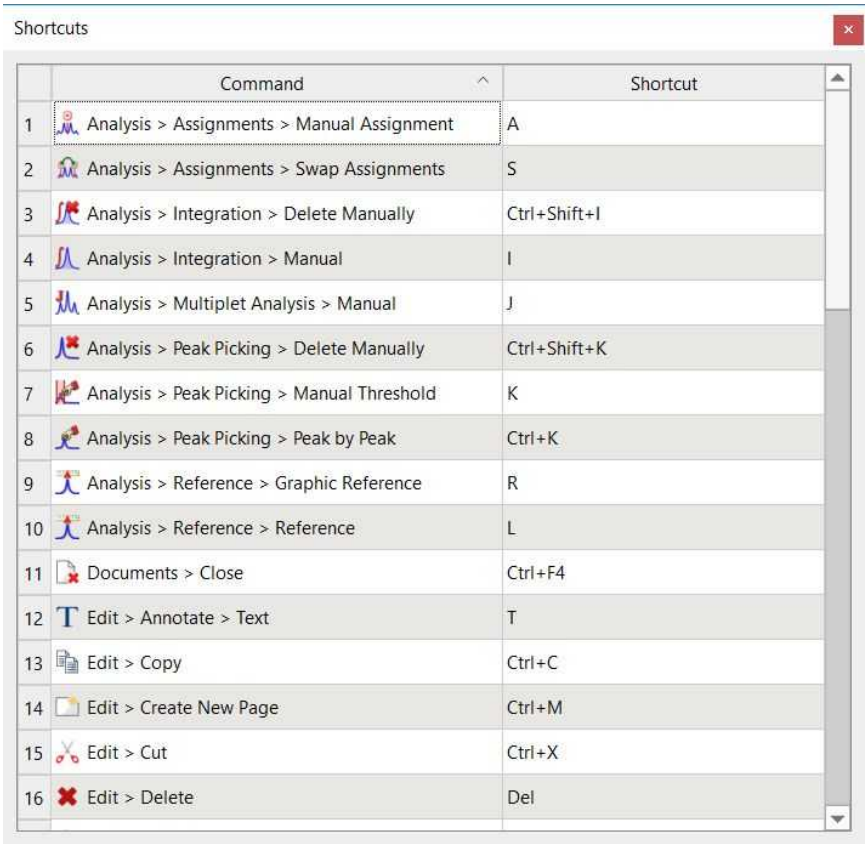
You will be able to dock any table to the right or left side of the spectrum just by clicking on it and dragging to the desired place:



6.7.8 Shortcuts

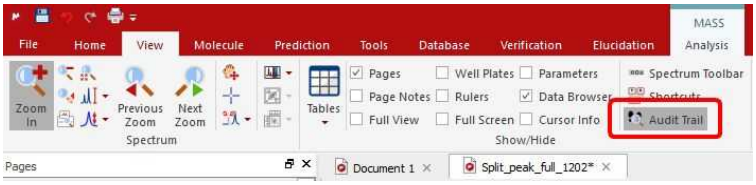
Mnova provides shortcuts for finding commonly sought tools. You can see a list of these commands by following the menu 'View (or Help)/Shortcuts':

© 2024 MESTRELAB RESEARCH



6.7.9 Audit Trail

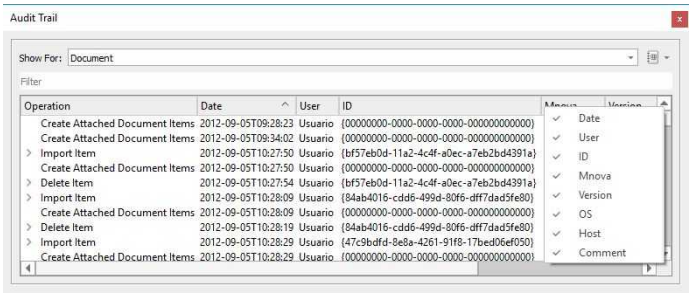
Mnova provides an audit trail tool that records into a log file every action carried out in Mnova. You can use Audit Trail to find any processing operation performed on your open Mnova document. You can find this tool by following the menu 'View/Audit_Trail':



After that, you will get all the operations performed on your document. You can:

- filter the operations by text and also by selection.
- customize the fields displayed just by right clicking on the headers.
- report the displayed content to the Mnova document or to any other application, as you can see in the image below. The table contains information about Operation, Date, User and Version of Mnova.
-

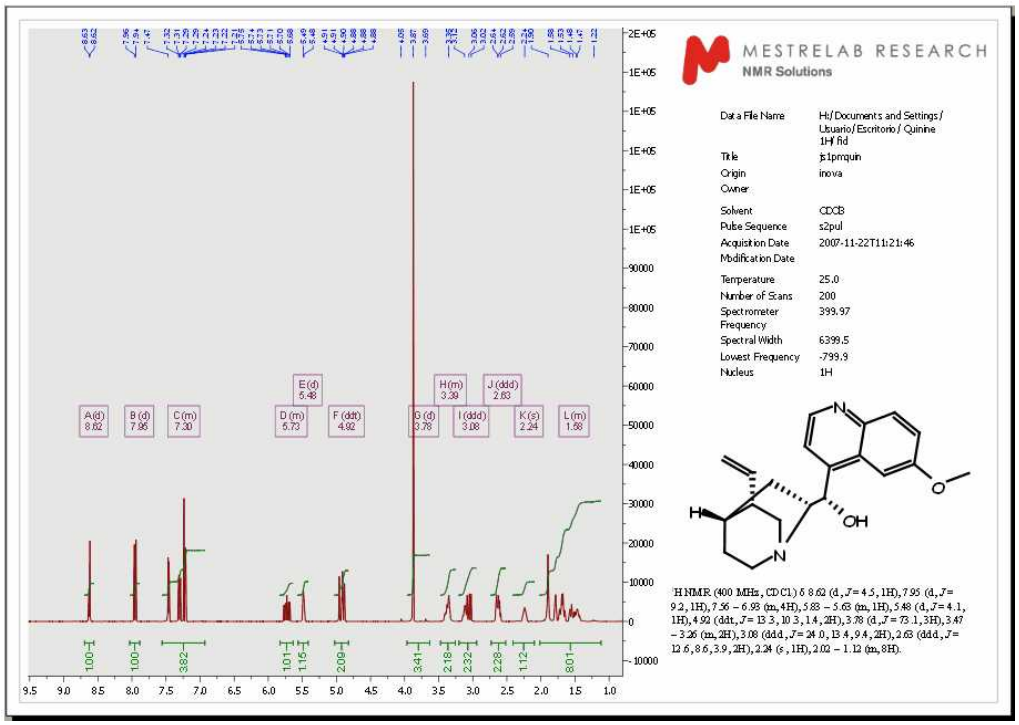
© 2024 MESTRELAB RESEARCH



6.7.10 Layout Templates

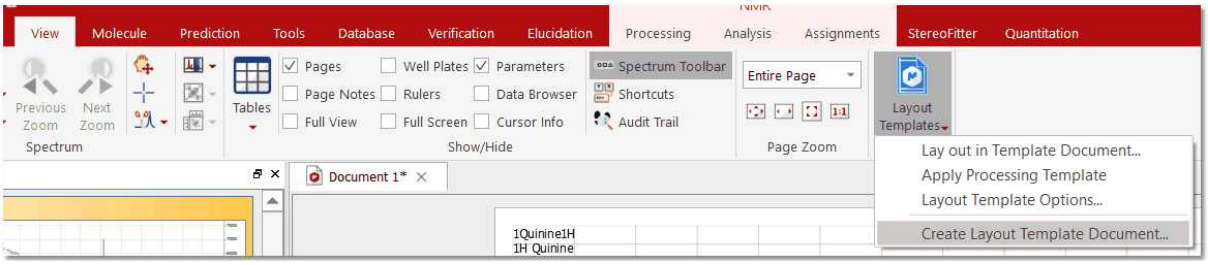
You will find a sample Layout Template in the installation folder of Mnova (under the 'File/Help/Download Example datasets' menu)

This feature will allow you to create templates for your NMR, MS and Optics spectra (including processing) with the location of the spectrum, spectral width, molecular structure, images, tables and multiplet reports (including journal format). To create a template with Mnova, open a document and select the desired location and properties for the different objects present in it. The picture below shows a 1H-NMR spectrum, with the applicable molecular structure, multiplet report, images and the Parameters table:



To save the created layout as a template, just follow the menu **'View/Layout Templates/Create Template Document'** and select the location of the new template.

© 2024 MESTRELAB RESEARCH



Once saved, a template can be easily opened by double clicking on the .mnova file. Please note that drag and drop also works in this instance.

ATTENTION

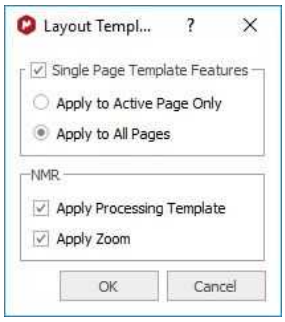
The layout template will keep all the properties of the objects (draws, annotations, spectra and molecules), the processing (optional) and the analysis features used in the original spectrum ('peak picking', 'integrals', 'multiplets' or 'cuttings'). Double click on each item to check the embedded properties.

The picture below shows the layout created previously:



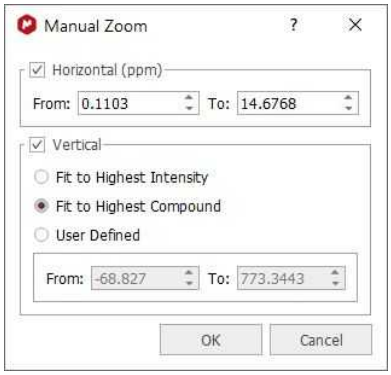
You can select if the layout template will be applied to the entire Mnova document (Apply to all pages) or only to the active page (Apply to Active Page Only) by using the Layout template options (View/Layout template/Layout template Options).

© 2024 MESTRELAB RESEARCH



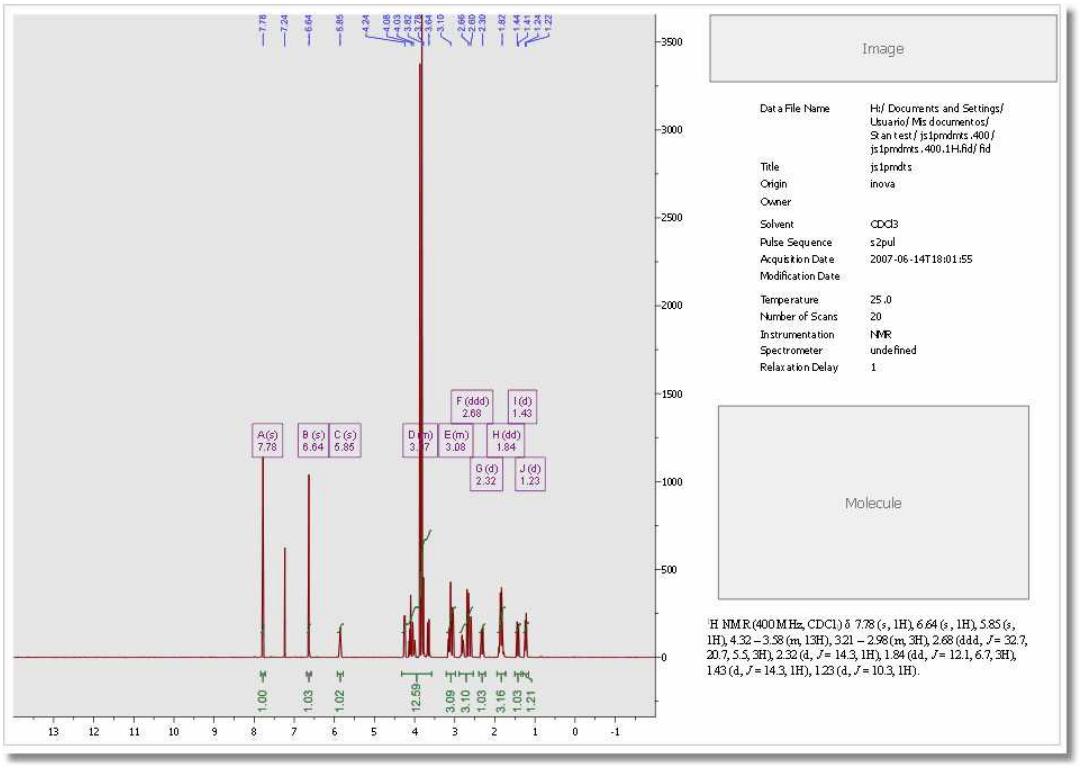
If you check the **'Apply Processing Template'** option, the layout template will apply also the processing used in the original spectrum. If this option is not selected, the layout template will only keep the graphical properties

Check the 'Apply Zoom' box to keep the horizontal and vertical zooms used to generate the template. You can apply a 'Fit to Highest Intensity', or 'Fit to Highest Compound' or even a vertical custom zoom by loading your layout template, selecting 'manual zoom' (shortcut: M) and saving the changes in the resulting layout template document.

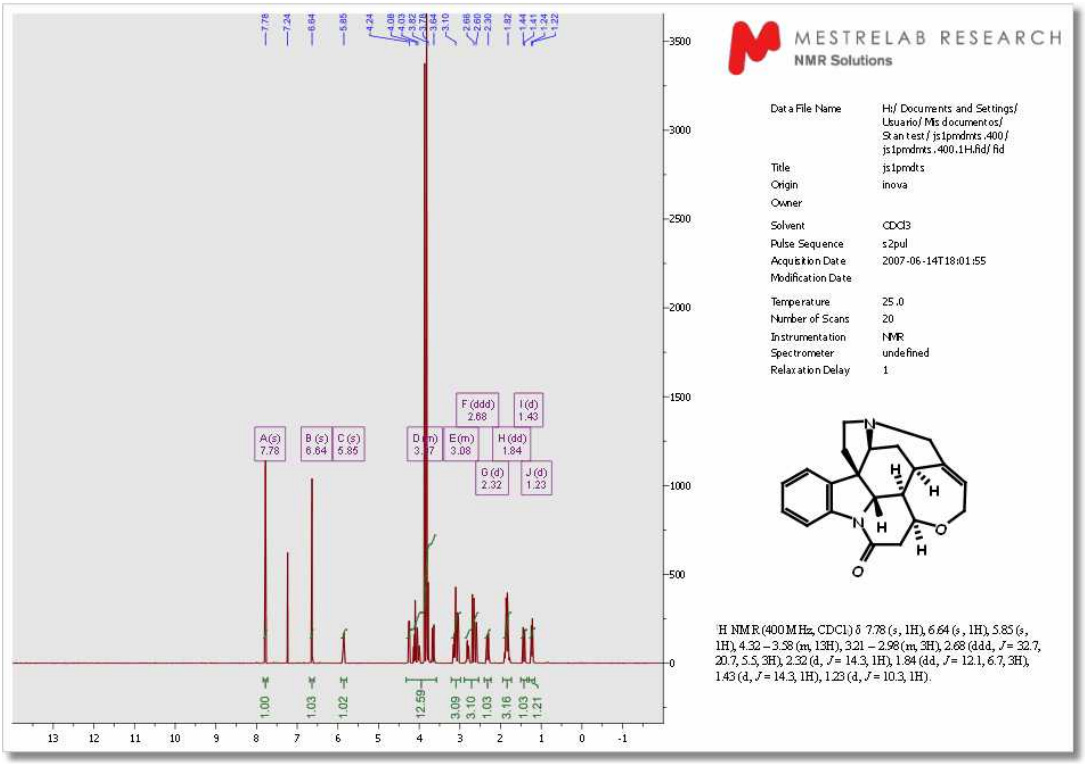


If the user opens a different 1D-NMR spectrum over this layout, it will be obtained in the applicable location, with the corresponding processing and analysis features (multiplet analysis in this case) and also with the applicable object properties, including all the relevant tables and reports:

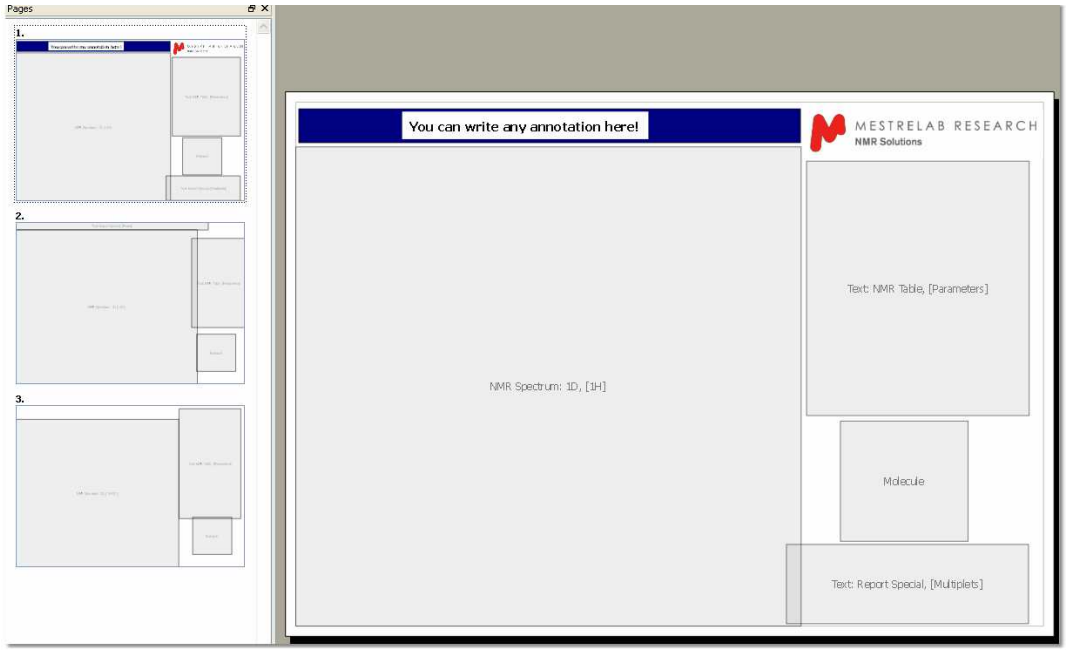
© 2024 MESTRELAB RESEARCH



In the same way, when the user opens or pastes a molecule and an image, they will also be automatically displayed in the corresponding location (with the original properties).



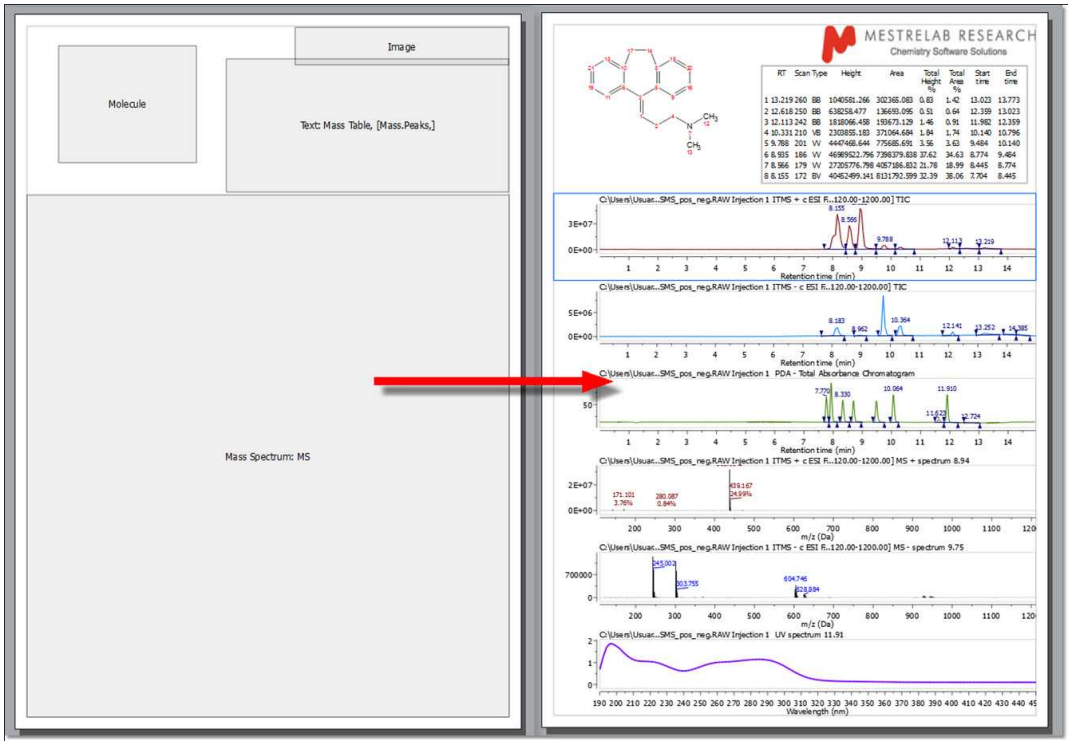
Also note that our concept is designed to be extended to reports with more than one page, to allow for the placing of several spectra on the report (in this case 1H, 13C and HSQC):



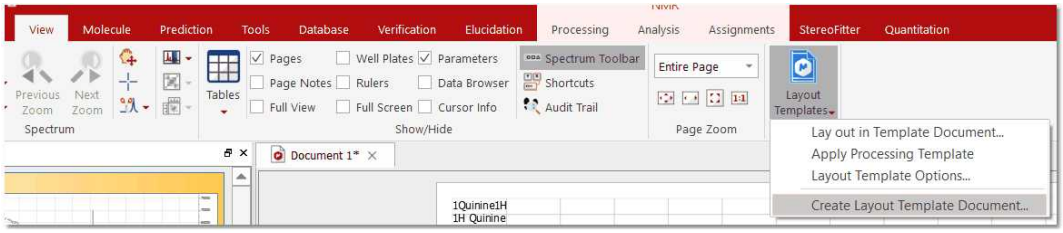
You can modify any layout template. For example in the template above we have added an annotation and a logo and then clicked in the 'Save' button. If you open again the template you will have by default the annotation and the logo on your screen. You can also double click on any item to change any property and then save the changes.

Of course you can use layout templates with your MS documents (or combined with NMR spectra). Here you can see an example of a layout template for MS spectra:

© 2024 MESTRELAB RESEARCH



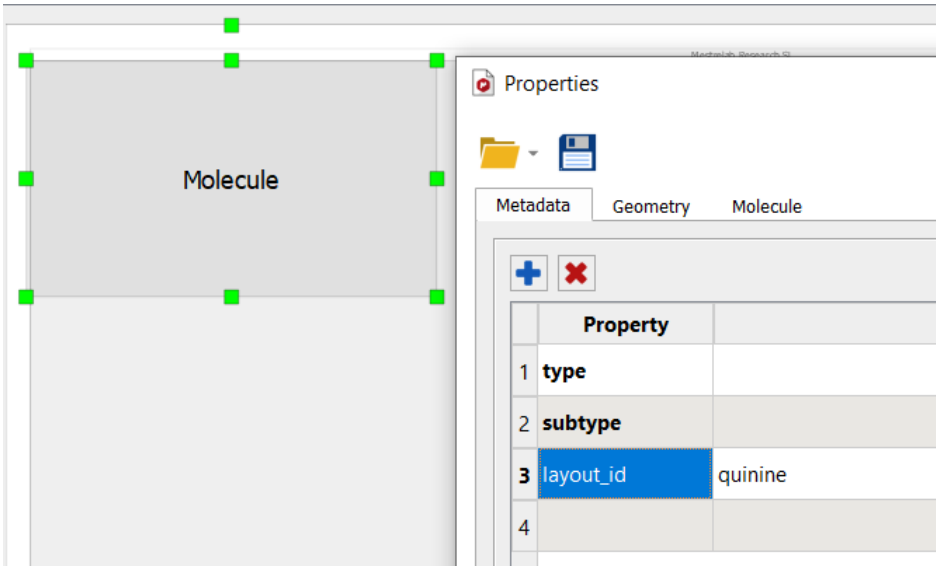
You can also apply a layout template to any opened spectrum, just by following the menu 'View/Layout Templates/Lay Out in Template Document':



If you are interested in a layout template containing the same molecular structure in several pages, you would need to right click on the 'molecule box' (of the layout) select Properties and make sure that you add a field named 'layout_id' wich will be common for all the molecules of the layout.

Alternatively, you could edit the metadata field for one of the molecule boxes and make copies of that box in all the desired pages

© 2024 MESTRELAB RESEARCH

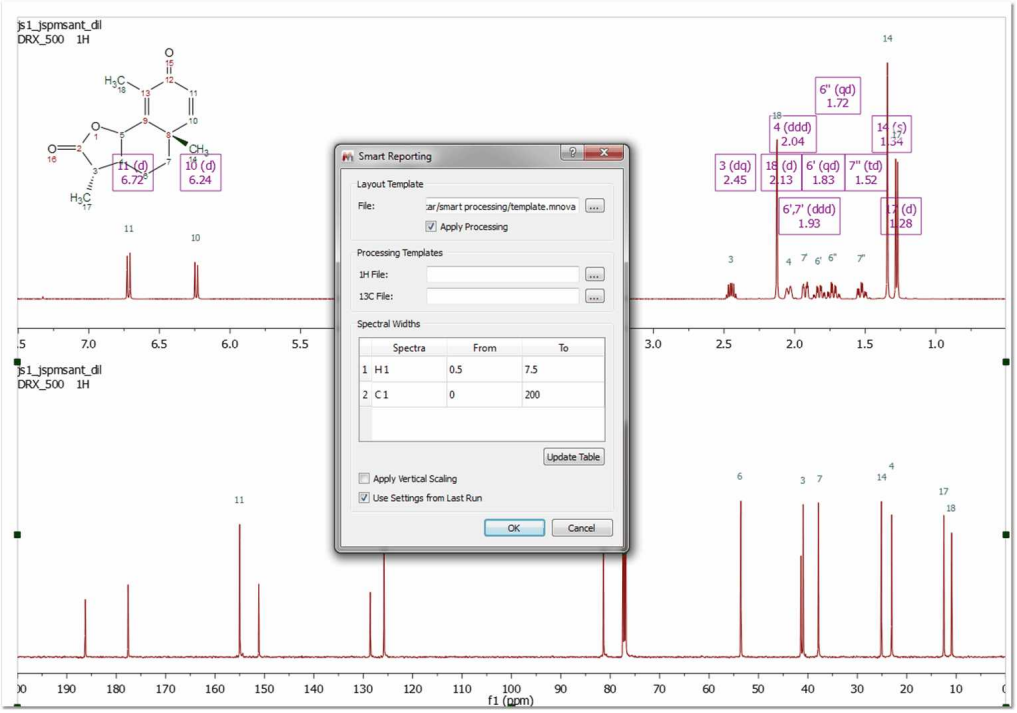


There is a script to load a layout template (see the script called 'applyLayoutTemplateAndPrint.qs' under the examples/scripts installation folder). You will find another example under the 'Tools/Report' menu, to apply a **'Smart Reporting'** which will load a layout template, with the option to include applicable processing template embedded in the layout or to load other processing templates. The script will allow you also to select 'Spectral Widths' for each nuclei:

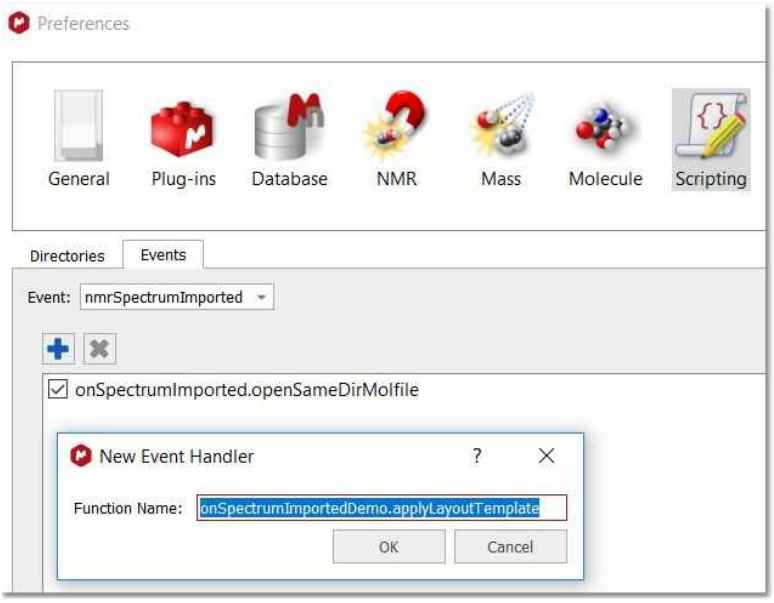


Here you can see an example:

© 2024 MESTRELAB RESEARCH



To use a layout template by default for all of your experiments follow the menu 'Edit(or File)/Preferences/Scripting/Events/nmrSpectrumImported' and add the function onSpectrumImportedDemo.applyLayoutTemplate.



Next, select the Directories tab in the 'File/Preferences/Scripting' dialog and copy the script "onSpectrumImportedDemo.qs" to a script directory of Mnova. Finally, save a layout template document (called 'test.mnova') into "C:\temp\' and restart Mnova. Load your NMR spectra and the layout template will be applied automatically.

© 2024 MESTRELAB RESEARCH

6.8 Undo & Redo



Undo is a very useful command; it erases the last change made to the document, causing the document to revert to an earlier state. The **Redo** command reverses the undo.
Mnova has very powerful **Undo** and **Redo** capabilities. These can be applied to virtually any operations carried out by Mnova, from creation of pages and opening of documents through zooming, panning, and annotating, to any processing or analysis operations. Undo & Redo in Mnova are virtually unlimited (RAM memory limitations may apply). These options can be found as the toolbar icons shown above or by using the shortcuts <Ctrl+Z> (or Cmd+Z for Mac) for Undo and <Ctrl+Y> (or Cmd+Y for Mac) for Redo.

© 2024 MESTRELAB RESEARCH

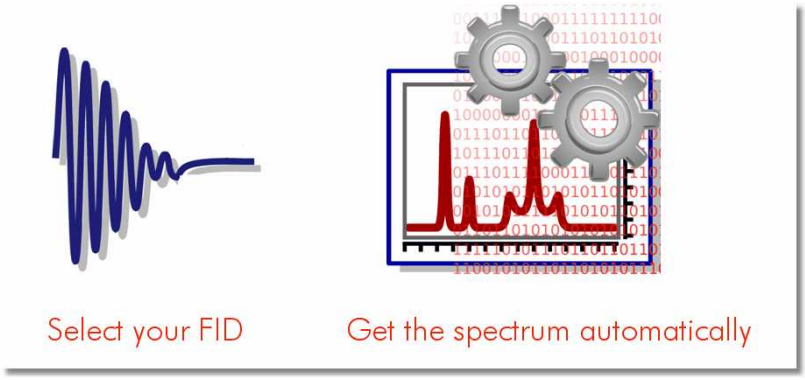
Part



7 Processing Basics

Our aim in developing Mnova has been to make the opening, processing, handling, analysis, saving and printing of NMR data simpler than it has ever been before, so that even the NMR novice can enjoy the software and obtain excellent results from the very start.

Mnova introduces a brand new NMR data processing paradigm; when a raw, unprocessed spectrum (FID) is opened, you will get the fully processed spectrum instantaneously.



This new concept consists of two fundamental points:

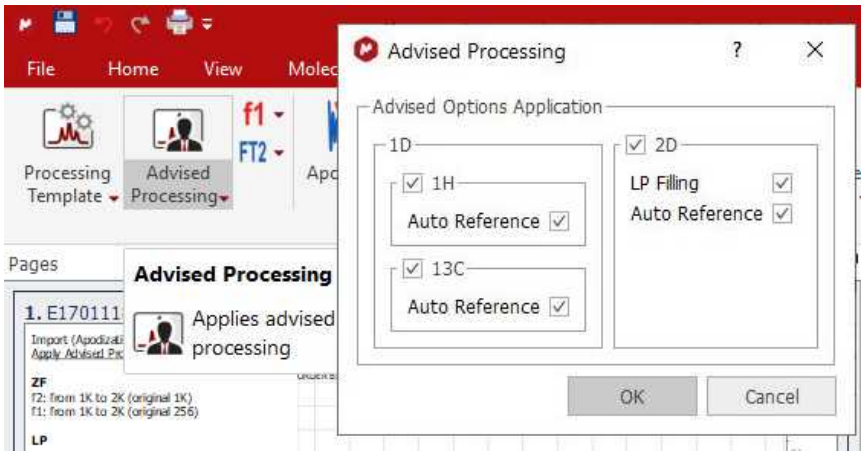
1. **Automatic File Format Recognition:** Once you select the FID, Mnova will automatically identify its origin (e.g. Varian, Bruker, Jeol, etc).
2. **Fully Automatic Processing:** Once the FID is opened Mnova will automatically and optimally process it using the information it discovered in Part 1, so you do not need to bother with processing details.

The two steps outlined above will successfully process most NMR data. However, if you are not completely satisfied with the result you can, at anytime, reprocess using your own choice of processing parameters, changing or adjusting the window function, the FT, the phasing and baseline correction to meet your requirements.

With this scheme, processing of 1D and 2D spectra is essentially identical as you will see in the following two tutorials:
1D NMR Processing
2D NMR Processing

There is also an 'Advised processing' feature which will apply a suggested processing with the option to automatic reference the spectra and to apply a Linear Prediction:

© 2024 MESTRELAB RESEARCH



For 1H it will apply a Stanning apodization of 8.0, a three fold Zero Filling, 'Regions Analysis' phase correction a Bernstein polynomial (order 3) baseline correction. For 13C datasets, it will include an exponential apodization of 2.0 Hz (on top of the Stanning), 'Regions Analysis' phase correction and a Bernstein polynomial (order 3) baseline correction.

For 2D, it will apply a Zero Filling along F2 with a maximum factor of 2 and an exponential apodization of 2.0 Hz and Stanning 4.0 and a 'Fit to Highest Intensity'
For F1 it will apply a Sine Square 90º (first point: 0.5) apodization and a ZF (up tp 3 times).
Magnitude phase correction (only in f2) will be also applied in case of HMBC spectra an in other 2D spectra where FT is None.
In other cases, Regions phase correction will be applied in both F1 and F2.

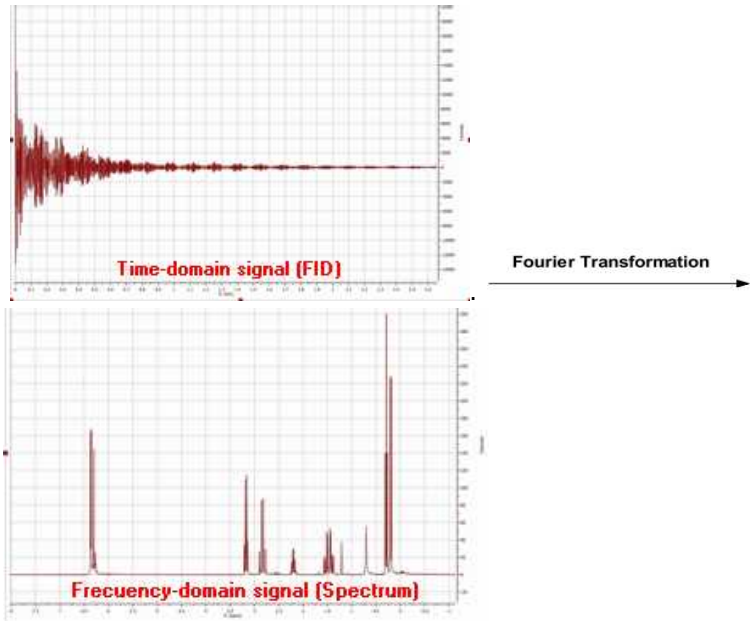
For homonuclear 2D NMR experiments, the advised processing will set the size of the F1 dimension equal to the size along the direct dimension (the goal is to get square matrices so that if symmetrization is needed, this would make the process more efficient so that no interpolation will be needed).

7.1 1D NMR Processing Tour

Opening the data set

In pulsed NMR spectroscopy we obtain what is called a *time-domain signal* since the measured signal is a function of time; all the nuclear resonances decay from the same starting time to form the Free Induction Decay or FID. This kind of signal is not readily interpretable by the human eye, and so it is necessary to transform it (into the frequency domain) using a Fourier transformation (FT) to display the separate nuclear resonances as a frequency spectrum.

© 2024 MESTRELAB RESEARCH



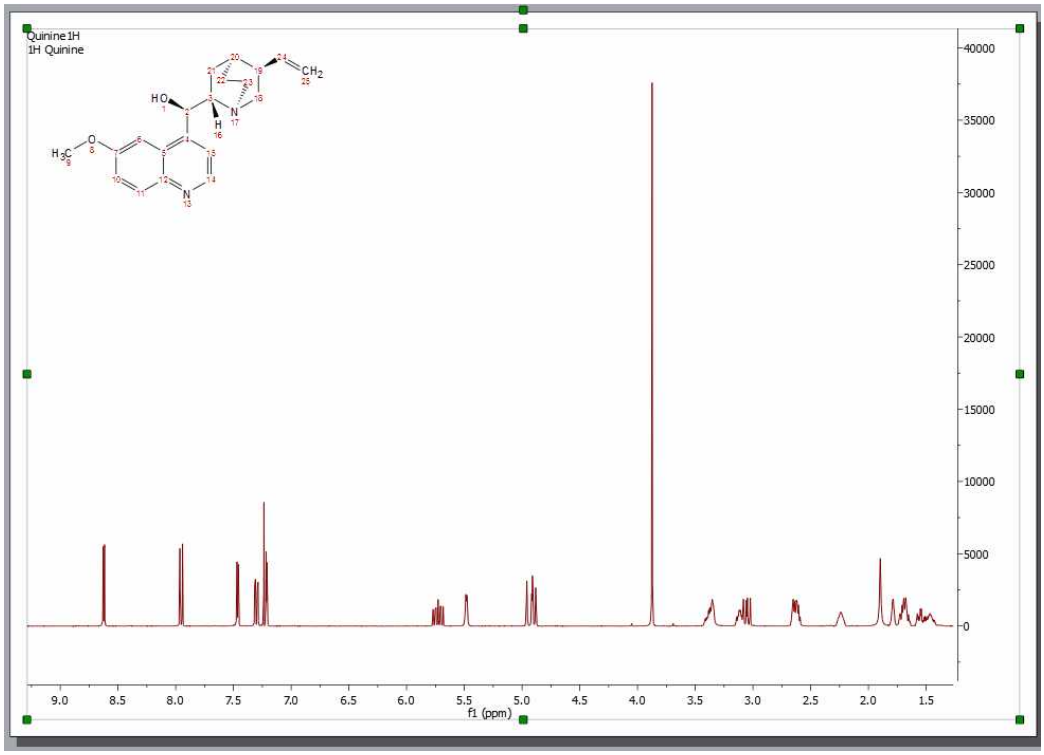
There is one single command for opening all types of files in **Mnova**. It is not necessary for the user to have any knowledge about the way in which the experiment has been acquired. **Mnova** will automatically recognize most common spectrometer file formats and acquisition modes and will apply the necessary processing operations.

To open your data (including mol files and graphics files), just go to ***'File/Open'*** menu, use the keyboard shortcut **<Ctrl+O>** (or Cmd+O for Mac) or click on the **'Open'** icon on the toolbar (note that this is exactly the same for 1D and 2D data). You can also drag&drop into Mnova, the folder containing the FID (the FID must be in the first level of folders)

Once this command has been issued, navigate through your disk to find the NMR experiment of interest and select the desired FID file which is normally named **fid**; however in Bruker 2D experiments the time domain data is named ser (serial fid). After selecting this file, the first thing you will notice is that the spectrum will open **already processed**, despite the fact that you are opening raw data (FID). When opening files, Mnova will apply the necessary processing operations, selecting the correct processing parameters from the spectrometer files. If these parameters are not available (e.g. phase parameters are not available), Mnova will use automatic algorithms (e.g. automatic phase correction) and will apply them to the data set. Thus, even a completely inexperienced chemist, working with NMR for the first time, should be able to get good results when processing both 1D and 2D spectra.

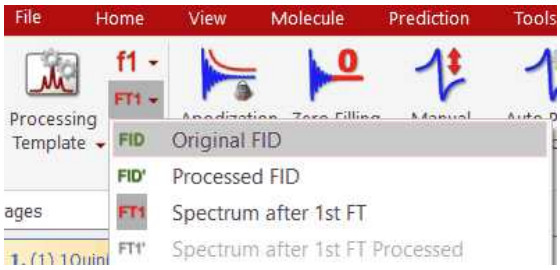
In the figure below you will see what happens when the FID is opened:

© 2024 MESTRELAB RESEARCH



As explained above, the FID is automatically processed. In this case, the native files contained values for phase correction and thus Mnova utilized them for phasing. However, as you can appreciate, the result is not good enough (most likely because the spectrum was processed in the spectrometer computer using an inefficient automatic phase correction algorithm). Later we will show how to improve the phase of this spectrum by applying first the automatic phase correction algorithm implemented in Mnova and finally using manual phase correction. Remember that if the native files do not contain values for phase correction, Mnova will apply an automatic phase correction.

Even though you have opened a FID and you see only the processed spectrum in Mnova, you can see the **original FID and the processed FID** (Processed FID or **FID'**). When we process the raw data on-the-fly, we do not discard these data, we just do not display them. The user can view the FID by just clicking on the '**FID'** icon on the toolbar:



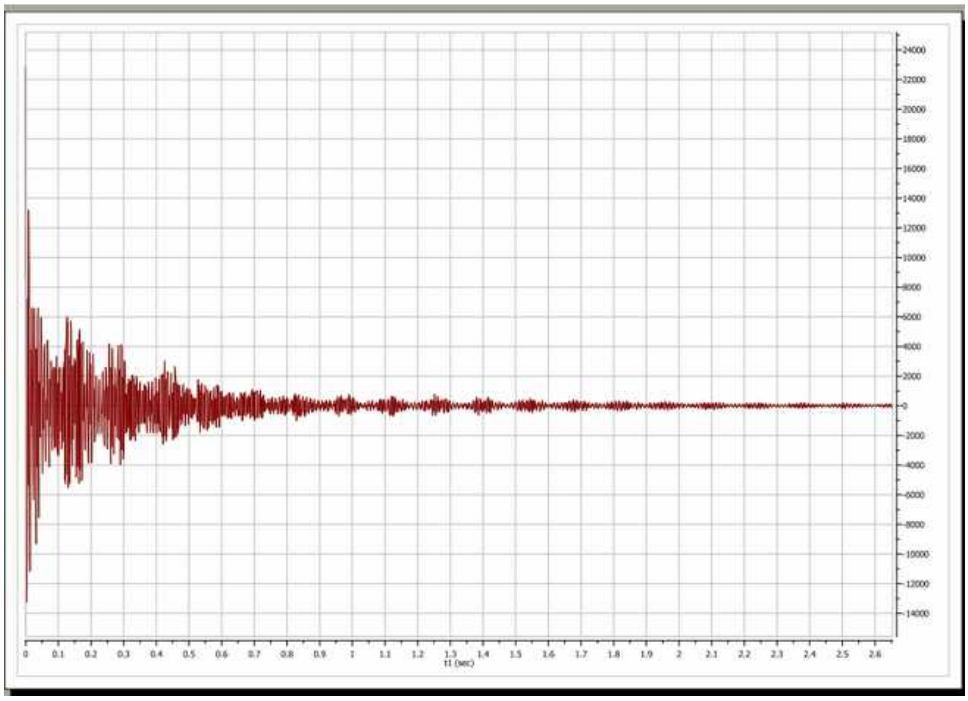
There are several options in the scroll down menu:

- **Original FID**: Clicking on this option will show the original *time-domain signal* (FID) as it was when ported from the spectrometer.
- **Processed FID**: Clicking on this option will show the FID after time-domain processing functions have been applied (i.e., immediately prior to FT)

© 2024 MESTRELAB RESEARCH

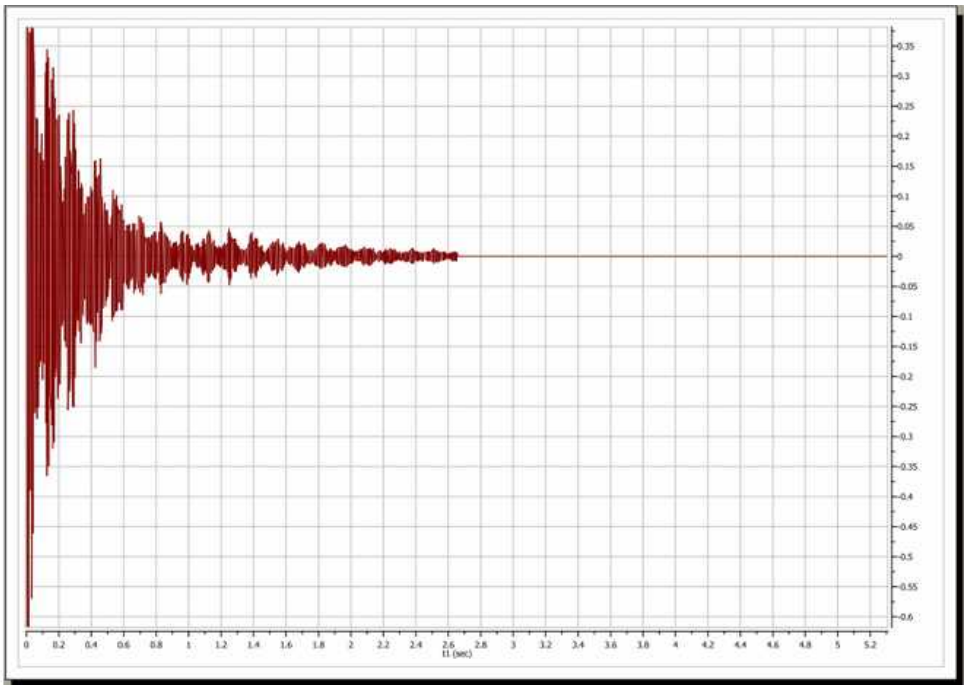
- **FT1**: Clicking on this option will show the data after FT along the direct dimension (spectrum for 1D data, interferogram in 2D data)
- **FT1':** it will display the processed fid before the second FT (in t1):
- **FT2**: Clicking on this option will show the data after FT along two dimensions (2D spectrum)

For example, this is the original FID of our test spectrum:



And this is the so-called **FID'**. In this case, prior to FT, Mnova applies a zero filling operation as can be appreciated in the FID' below. A zero fill increases the digital resolution by adding null data to the FID, increasing the size of the data array.

© 2024 MESTRELAB RESEARCH



You can switch at any time between any of the displays (FID, FID' and processed spectrum).

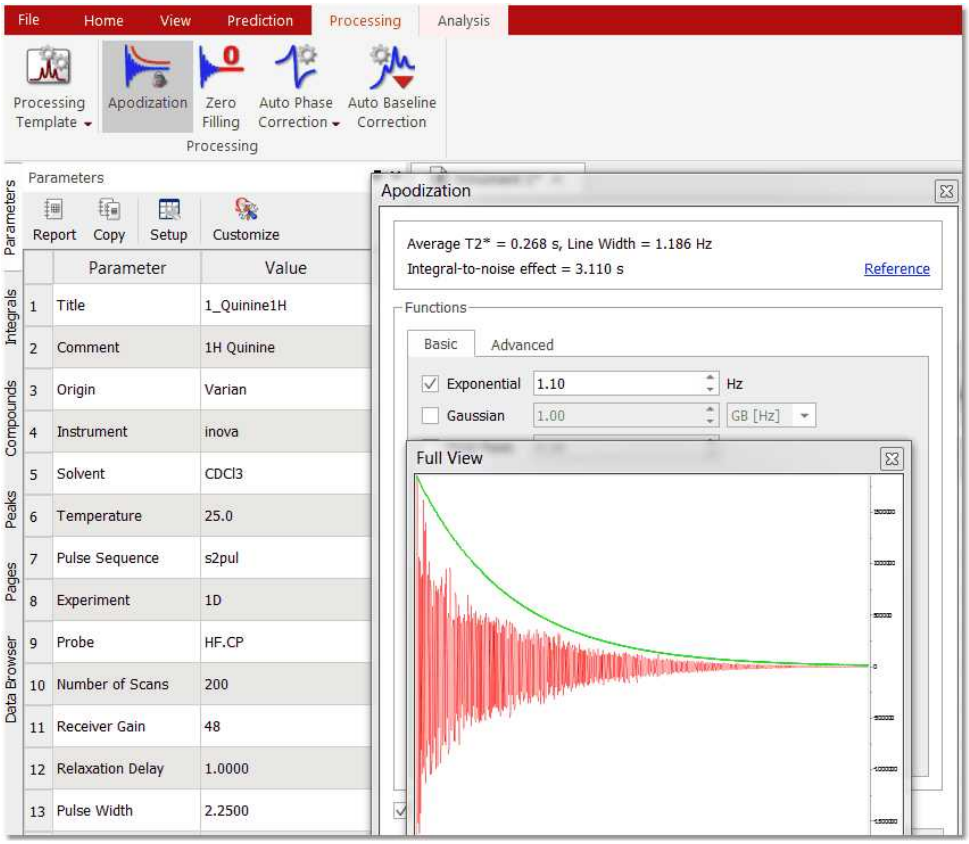
Interactive Processing: Real Time Frequency Domain Processing

As we saw in the previous section, Mnova will keep all pre, during and post processing information together with the spectrum we can see on the screen. This fact, combined with the extremely fast and optimized processing algorithms implemented in Mnova, allows us to introduce to NMR processing the concept of **'Real Time Frequency Domain Processing'**, designed to greatly simplify processing for the user and to ensure faster and better results even when complicated operations are applied to the data.

The way this concept works is very simple: Reprocessing of the spectrum is possible at any time, independently of the time domain and frequency domain operations which may have been applied to the data set (for example, to change the window function after having applied a FT, phase and baseline correction). This is possible because, in the background, Mnova is going back to the FID and reprocessing the spectrum by applying the new functions or parameters as well as all the other functions applied ad posteriori on the previous processing. The very important advantage for the user is that he can see, in **real time**, the effect of any processing operation (including time domain operations) on the resulting frequency domain spectrum (for example, you can apply a more drastic exponential apodization function and see in real time, on screen, what effect this is having on the resolution and sensitivity of the frequency domain spectrum).

In the figure below, you can see the frequency domain 1D spectrum, the FID with the window function which is being applied interactively (which is shown graphically in the 'Full View' window (go to the 'View/Full View' menu to activate), and also the Apodization dialog box - note that the 'Interactive' box is ticked). The user can follow the exact effect of the function s/he is applying on the frequency domain spectrum. This can avoid repeated transformation and reloading of the data while the optimum processing parameters are sought and, of course, act as an excellent teaching and self-teaching tool for those who are not conversant with the effects of different processing parameters.

© 2024 MESTRELAB RESEARCH



This same principle can be applied to all other processing operations, such as Zero Filling, Linear Prediction, Signal Suppression, etc. For example, if we want to increase the digital resolution of the spectrum, we can increase the number of points used in the FT operation by selecting **Zero Filling.**

Of course, standard frequency domain operations such as phase correction or baseline correction can be applied in the traditional way.

See also:
Phase Correction
Baseline Correction

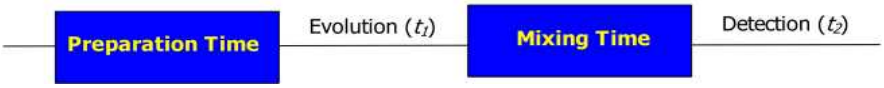
7.2 2D NMR Processing Tour

(Not available in Mnova Lite)

1D experiments contain a wealth of information but this information is often obscured due to signal overlap. Homonuclear couplings are often more readily and rapidly determined by extension into a second dimension. Heteronuclear couplings (1H-13C for example) are much easier to determine using two frequency dimensions. 2D experiments which use one frequency dimension and some other variable on the other axis (the DOSY processed STE experiment for example with diffusion coefficient on one axis) exist but this tour will concern two frequency (chemical shift) dimensions.

These 2D spectra are collected as time domain data dependant upon two time variables (t_1 and t_2) and after two orthogonal Fourier transformations a frequency spectrum which is a function of two frequency variables results. The general scheme for these **two** dimensional experiments is shown in the pulse sequence below:

© 2024 MESTRELAB RESEARCH



In the *Preparation Time*, the sample is excited by one or more pulses and the spin system is placed in a non-equilibrium state which will evolve later. During the *evolution* period, the resulting magnetization evolves freely. Each signal will evolve with its characteristic frequency. Then follows the *Mixing Time* containing a further pulse or pulses; it is in this period that, for example, magnetization, may be transferred from proton to 13C during indirect detection. Finally the signal is recorded (detected) as a function of the second time variable, t_2; the signal has, however, been frequency-labelled as a function of t_1.

After acquiring a bidimensional experiment, you will have a matrix of data; the rows contain data collected at fixed values of t_1 with t_2 varying; they are, in fact, normal 1D data FIDs. The columns contain data collected at fixed values of t_2 with t_1 varying and are "synthetic" 1D FIDs. This matrix will be fourier transformed twice, once for the rows and again for the columns. In this way, the time dimensions (t_1 and t_2) are converted into frequency dimensions (f_1 and f_2).

In a 2D-NMR experiment we can find any nucleus represented on either of the dimensions of the spectrum, so the spectrum can show correlations between the same nuclei ($^1H/^1H$) or different nuclei (such as $^1H/^{13}C$, $^1H/^{15}N$, $^1H/^{31}P$, etc...). In a 2D-NMR spectrum, the acquisition dimension (t_2) is the dimension in which we can obtain the greater number of data points without a considerable increase of the acquisition time. On the other hand, each point acquired in dimension t_1, multiplies the necessary number of scans, considerably increasing the total acquisition time. For this reason, in many cases the number of data points acquired along the t_1 dimension is much smaller than along t_2, although this number of points must always be must always be sufficient to provide adequate f_1 resolution. The "synthetic" f_1 FIDs are generally truncated and may benefit from forward linear prediction.

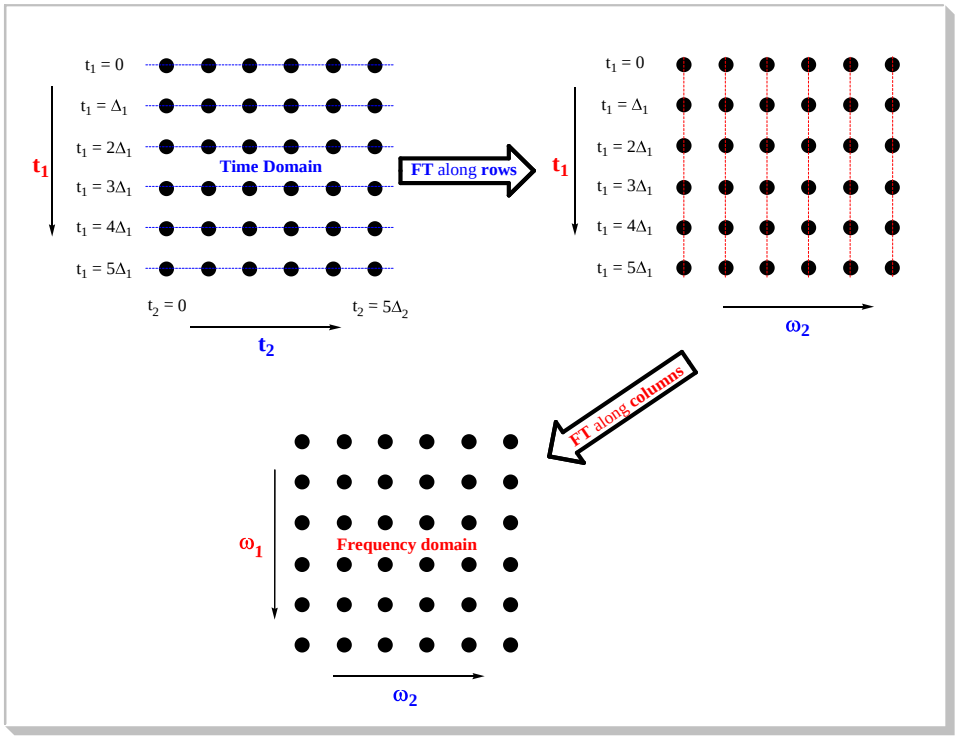
Processing of 2D NMR data sets with Mnova is almost as easy as processing 1D NMR data. You simply select the FID file (or SER file in Bruker) and the 2D spectrum appears on your screen without the need to touch a single button.

In this tutorial we will show how to process a 2D experiment. This tour will guide you through all the necessary steps to process, display, and print out two dimensional spectra with Mnova. Before you attempt to process a 2D spectrum, please read the 1D NMR Processing Tour since you need to be able to process 1D spectra also.

Multidimensional (or bidimensional) data processing requires only a few new concepts when compared with 1D NMR data processing. A 1D-NMR spectrum can be considered as a single vector of data points and its transformation into the frequency domain only requires only one FT. In the case of 2D spectra we have a two dimensional matrix of points and it is necessary to Fourier transform both rows and columns.

In 2D homo- and heteronuclear NMR the matrix of points results from an array of 1D FIDs which are functions of t_2 due to their detection (sampling of the FID at intervals of delta-t_2) and t_1 since each successive FID the value of t_1 which determined the evolution time, is incremented. The first row has t_1=0, the second row has $t_1=\Delta_1$, the third row has $t_1=2\Delta_1$ and so on. The same happens with the data columns, although in this case the sample interval is different (Δ_2).

© 2024 MESTRELAB RESEARCH

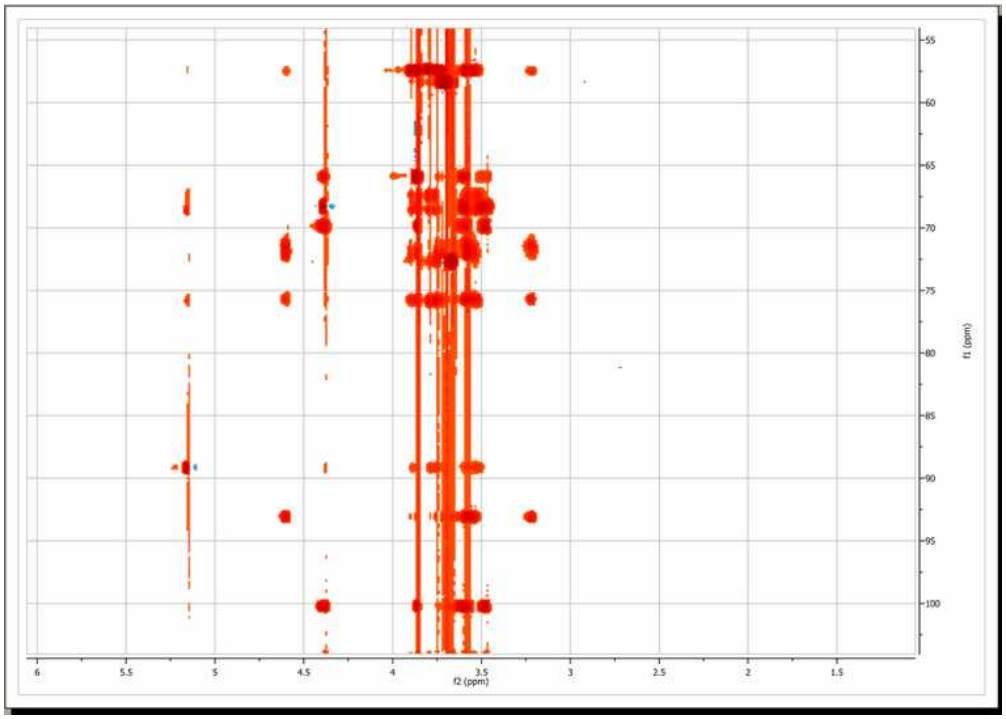


As you can see in the picture above, the first step is the application of the FT along the rows of the matrix; to obtain a new matrix (*interferogram*) where each row corresponds to an increasing value of t_1 and each column to a running frequency variable ω_2. Next the columns are subjected to a FT to obtain the final matrix (2D spectrum) where the frequency axes (ω_1) corresponds to the evolution in t_1, while ω_2 corresponds to the evolution in t_2.

This 2D data can be processed using apodization functions and once you obtain the 2D spectrum, you can apply a phase or a baseline correction as with 1D processing. One important point is that processing operations need to be performed along each dimension. In Mnova, the processing is always started along the acquisition dimension, and finished along the indirect dimension.

In this example we will use a Bruker spectrum so you will need to select the **ser** file. Once you do so, you will immediately obtain the processed spectrum:

© 2024 MESTRELAB RESEARCH

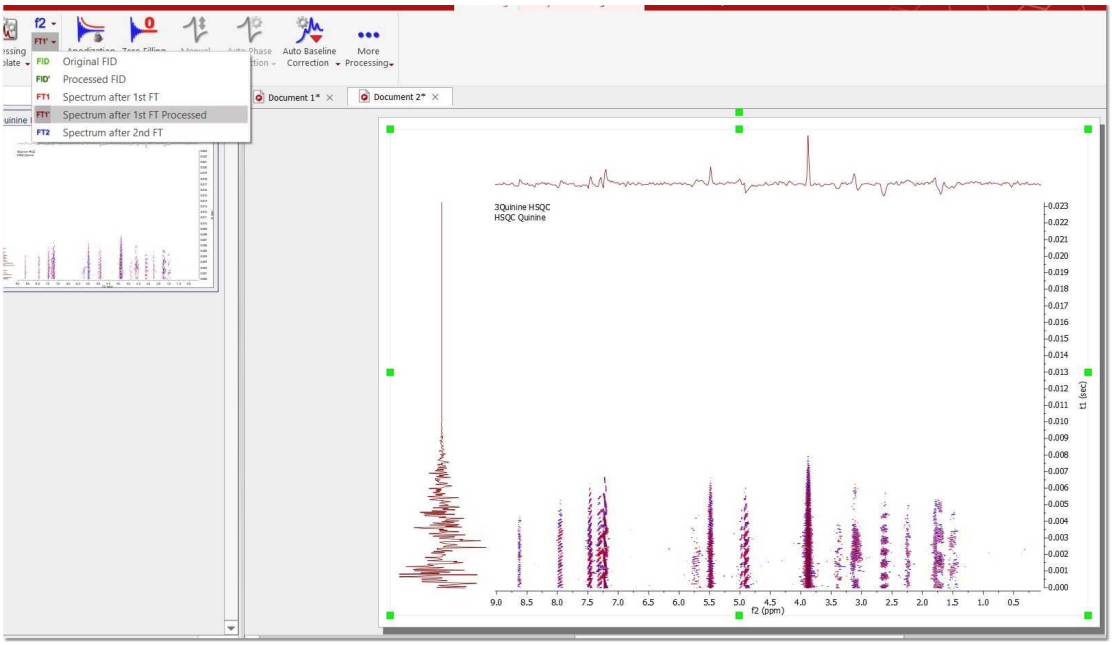


The program has performed the following set of operations on the fly:

1. Automatic file format recognition: The program identifies this file as a Bruker file and uses the corresponding filter to decipher the data
2. For each dimension:
   - Weighting
   - Zero filling
   - Fourier Transform
   - Phase Correction

The user can break the processing after the first FT along the direct dimension and also before the second FT (in t1)

© 2024 MESTRELAB RESEARCH



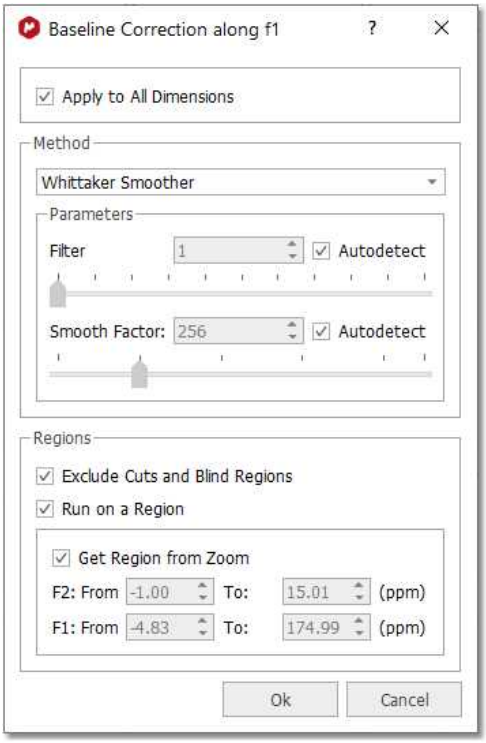
Baseline Correction

From the figure above, baseline artifacts along the indirect (f1) dimension are apparent. They can be easily removed by the baseline correction procedure. Select this option by clicking on the **'Baseline Correction'** icon 

to obtain an automatic **'Baseline Correction'.**

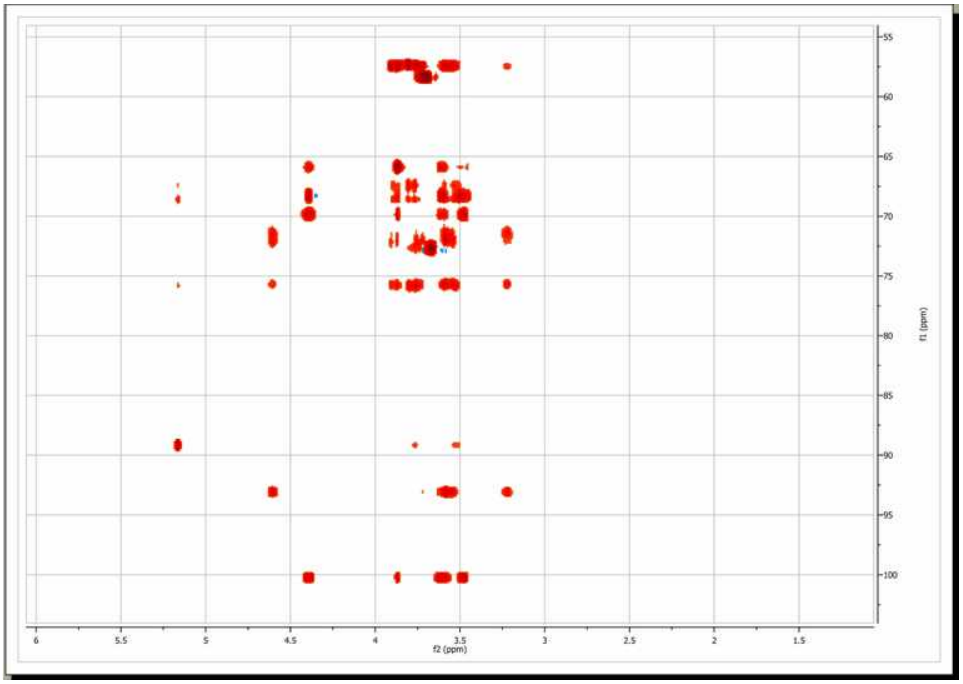
Baseline correction will be applied to the currently **active dimension** unless the 'Apply to All Dimensions' option is checked.

© 2024 MESTRELAB RESEARCH



You can exclude cuts and blind regions or select a region of interest to apply the baseline correction.

In our present situation, as the current processing dimension is **f2**, baseline correction will take place only along this dimension. This is not appropriate for our case as it is evident that baseline distortions are present mainly along the indirect **(f1)** dimension. We can simply set the processing dimension to **f1**, but it is even simpler to check the 'apply to all dimensions' option in the **baseline correction dialog box**. After applying the correction using default values, this is the resulting spectrum:



© 2024 MESTRELAB RESEARCH

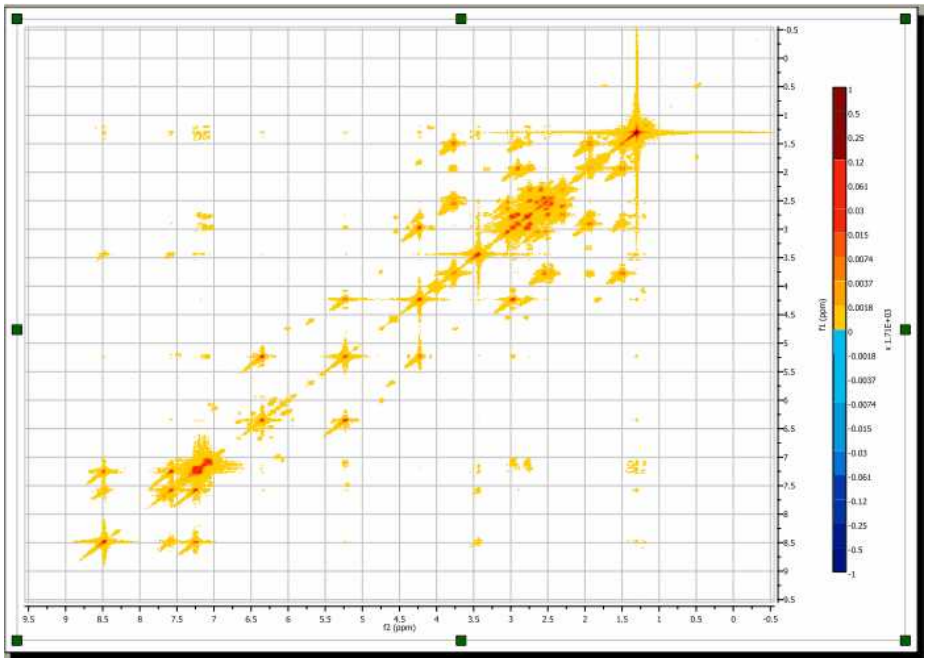
The user can show the traces of the 2D-NMR spectrum, just by clicking on **'Show Traces'** icon . In the same icon, you will find a scroll bar menu to setup the vertical and horizontal traces of a bidimensional spectrum. You can show the traces by default, by selecting the applicable option on the 'Properties dialog box and clicking on the 'Set as default' buton.

See also 'Show traces in 2D-NMR'

More on real time frequency domain processing

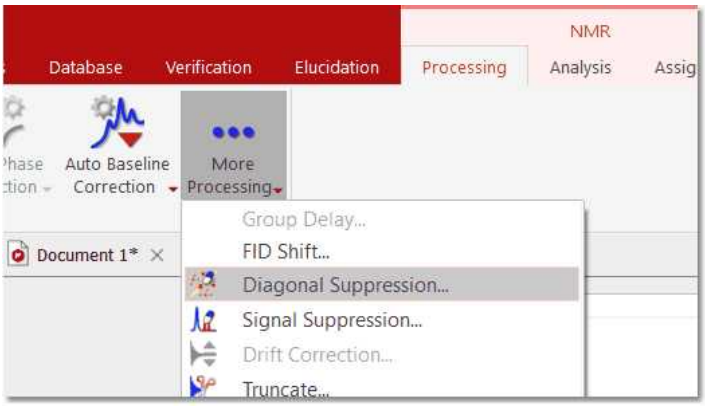
In the 1D NMR Processing section, we described the real-time frequency domain processing concept. **Diagonal Suppression** of homonuclear 2D experiments represents another example of this concept. This technique is typically carried out in the mixed time-frequency domain (interferogram). However, this is something you do not need to know in Mnova. You can suppress the diagonal while the transformed spectrum is on the screen.

For example, the figure below shows a 2D magnitude spectrum:

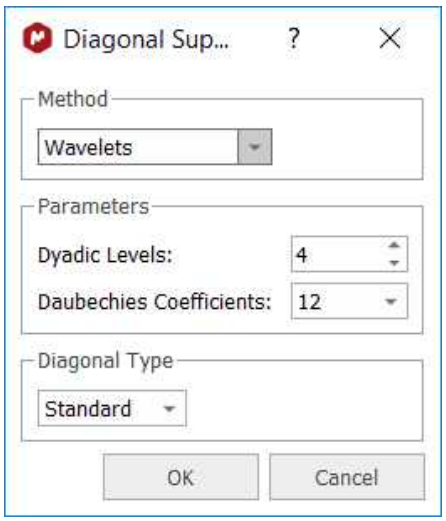


To suppress the diagonal, just click on the small arrow at the right-hand side of the FT toolbar button (see below) and select the **Diagonal Suppression** command:

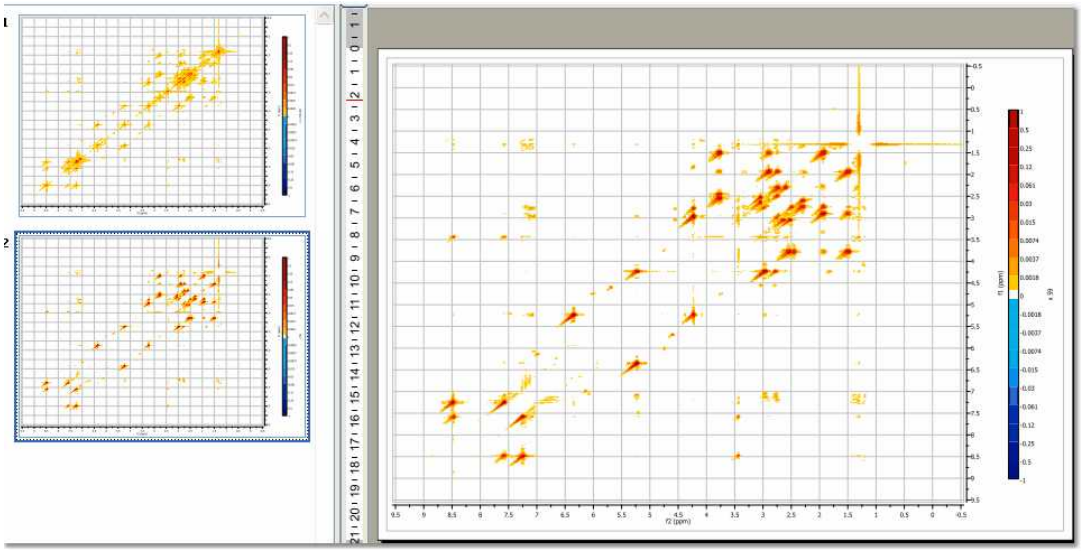
© 2024 MESTRELAB RESEARCH



Mnova provides different algorithms for this task. In this example we will select the Wavelet method



And this is the resulting diagonal-free spectrum.



Note that in the figure above there are two pages, the first one containing the 2D spectrum with its original diagonal, and the second one after the diagonal has been removed.

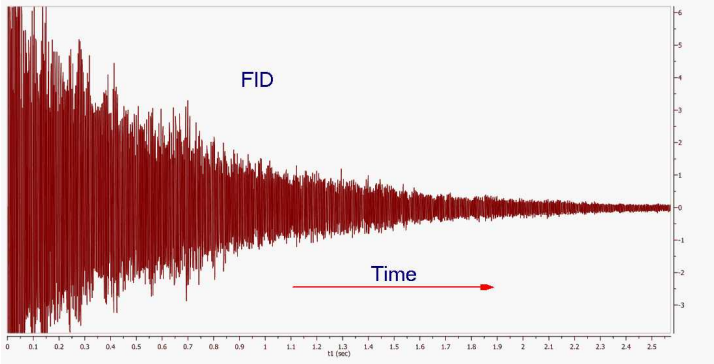
See also
Phase Correction
Baseline Correction

7.3 Fourier Transform

Basic concepts on FT

Modern **NMR** involves pulse RF energy to excite all frequencies at once. Immediately after the pulse, the signal is detected as a time domain interferogram which contains the sum of all the damped-sinusoid signals emitted by the sample at the various nuclear resonance frequencies.

The signal will decay with time as various relaxation mechanisms either dephase the magnetization in the X-Y plane or return the magnetization to the Z axis. The resulting interferogram is called the *free induction decay* (FID, see figure below)

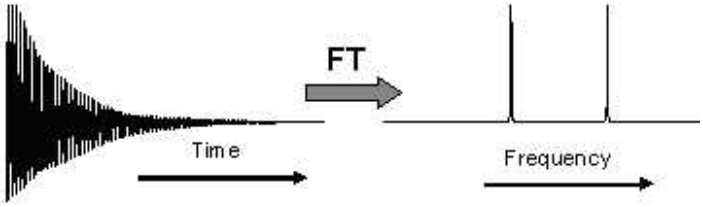


If you pay close attention to the FID shown in the figure above you can appreciate that it is composed of several frequencies. Moreover, if you were able to count the number of peaks and valleys in a given period of time (e.g. 1 second), you might be able to measure the periods and calculate the frequencies of some of the signals composing the FID.

In 'real' life, you don't need to do this conversion by hand. Fortunately, **Jean-Baptiste Joseph Fourier** suggested, while working a study of heat flow, that any function of a variable, can be represented by a sum of multiples of sinusoidal harmonics of that variable. This work was later developed into the Fourier Series.

Fourier's proposals led, in part, to the development of a linear mapping operator which maps a given function to other functions and in our particular case, functions of time to functions of frequency. The operator was named the Fourier Transform in Fourier's honour. In short (math details are beyond the scope of these documents), the Fourier Transform or FT is the mathematical process that converts the time domain function (the **FID**) into a frequency domain function (the spectrum) as illustrated below:

© 2024 MESTRELAB RESEARCH



Thus, FT is a mathematical procedure which generates the spectrum from the FID. It searches through the FID for frequency information and allows a plot of signal intensity versus frequency to be generated. When the frequency information is well defined, the peaks will be sharp, but if the information is not precise, the peaks will be broader.

The Continuous Fourier Transform nitty-gritty involves the equation:

$$A(w) = \int_{-\infty}^{+\infty} A(t)[\cos(wt) - i\sin(wt)]dt$$

where $A(w)$ is the intensity of the signal as a function of frequency, $A(t)$ is signal intensity of the FID as a function of time and i is $(-1)^{1/2}$.

Separating the real from the imaginary part, we have:

$$A(w) = \int_{-\infty}^{+\infty} A(t)\cos(wt)dt - i\int_{-\infty}^{+\infty} A(t)\sin(wt)dt$$

The real part can be taken as the signal component which starts off in phase with the receiver reference oscillator, while the imaginary part is that which starts off 90 degrees out of phase with the receiver reference.

The original Fourier Transform is defined for functions of a continuous variable: To meet the needs of pulse NMR where the FID is sampled at short but discreet intervals, the so called **Discrete Fourier Transform** (**DFT**) is used. When the signal strength has been measured with discrete time-interval sampling, the Fourier integral is replaced by:

$$x(n) = \frac{1}{N}\sum_{n=0}^{N-1}\left[A(n)\frac{\cos(2\pi kn)}{N} - iA(n)\frac{\sin(2\pi kn)}{N}\right]$$

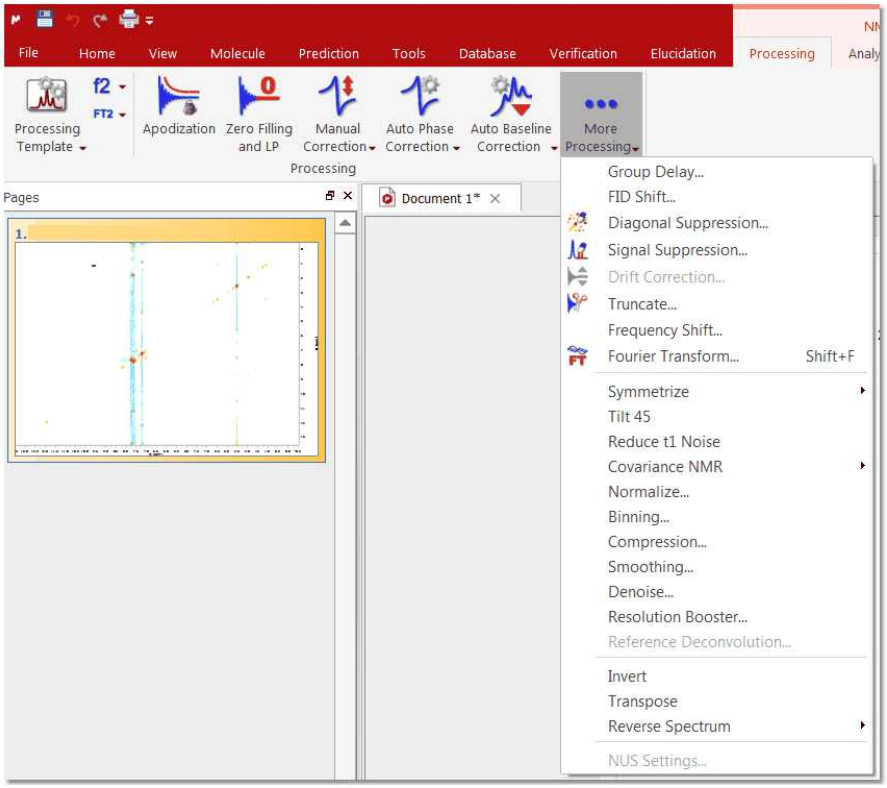
where N is the number of samples, n is the n^{th} sample and $2\pi k$ is a multiplier which converts point n, into the value corresponding to wt (in rad/s). If we have a collection (named A) of N signal points as a function of time, the calculation of the applicable arrays of real and imaginary frequency points is quite straightforward and can be easily carried out with a computer.

The calculations with many points are obviously slow (though with current computers, they are extremely fast), but the more points, the smoother the plots will be. In order to reduce the computation time we can use the **Cooley-Tukey fast-Fourier algorithm** (**FFT**), reducing the number of calculations from $N*N$ for the **DFT** to $Nlog_2N$ for the **FFT**. If N is 1024 samples the reduction is from 1048576 to 7098, a 93% time reduction. This algorithm has the special restriction that N must be a power of 2. (In cases where the number of points is not a power of 2, Mnova will extend the size of the FID by adding zeros to the next higher power of 2).

© 2024 MESTRELAB RESEARCH

The Cooley-Tukey algorithm for the **FFT** achieves this stunning reduction in computation time in part by a process called "bit reversal" which avoids repetitions of internal vector component calculations. The algorithm also saves time by recognizing the orthogonality of certain calculations, allowing the one vector to be calculated from another just by changing an algebraic sign, or reversing sine and cosine terms. This process is an example of the general technique of *divide and conquer* algorithms apparent in many traditional implementations, in which the FT is divided in other more and more small FTs.

Although, as explained in the items dedicated to 1D and 2D NMR Processing, all processing parameters are applied by default by Mnova, prior to Fourier Transform and for Fourier Transform, the software also incorporates a significant number of algorithms which can be applied by the user to try to optimize the results obtained. Thus, on the scroll menu applicable to the '**More Processing'** icon the user can find the following options to apply:



The shortcut for the FT will be the 'Shift+F'.

FT and Mnova

At first sight it might seem that a plain FFT would suffice to obtain the desired spectrum. However, this is not so in many cases. Although Mnova will adjust the number of samples by zero-filling to obtain a power of 2, the user may elect to increase the zero filling further to double the acquisition power of 2 value to obtain optimum 'frequency' resolution, or even further to improve the 'digital' resolution.

Other operations closely related to the FFT algorithm include:

- Quadrature Detection
- Drift Correction
- Digital Filtering
- Phase-sensitive Protocols

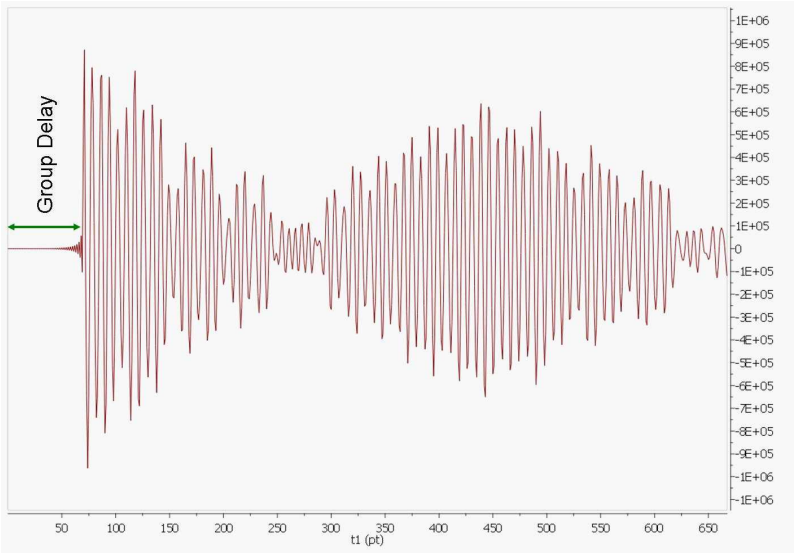
© 2024 MESTRELAB RESEARCH

7.3.1 Group Delay

Most of NMR has finally gone totally digital and is now routinely resorting to high-frequency ADC sampling at rates of several tens of MHz, with a tendency to ever higher sampling frequencies. Such a drastic oversampling has clear advantages in simplifying the front-end electronics. It has also many side benefits due to the fact that the subsequent down-conversion to low frequency range is done digitally and therefore theoretically artefact free. This includes perfect, calibration-free quadrature detection and drastic reduction of quantization noise. By the way, such techniques are routine in other areas of electronics (military, astronomy, audio, …); NMR is just a latecomer to this world.

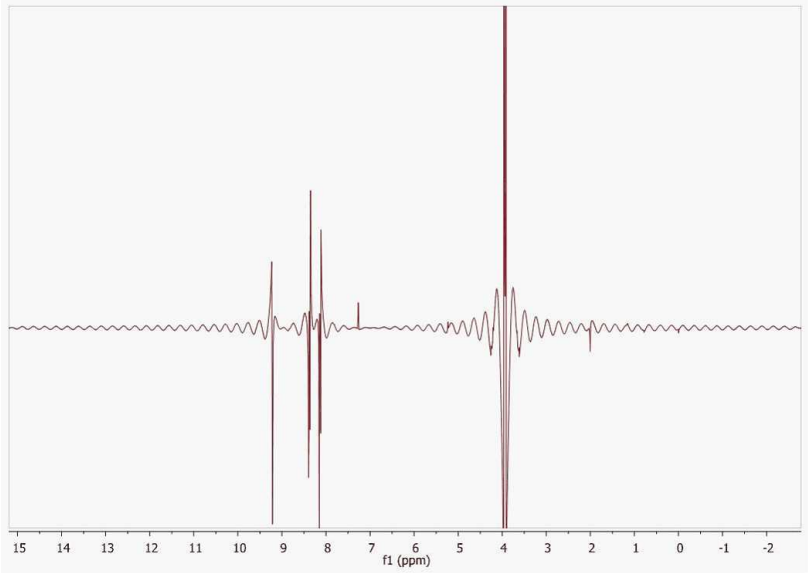
High-frequency (HF) oversampling, however, has also some problems of its own. The digital decimation from the HF range to the audio range and the contextual digital filtering (a combination of CIC and FIR filters) need to be properly implemented in the hardware in order to be completely transparent to the User.

However, in the case of Bruker and Jeol spectra a death time or group delay can be observed in the FID: it starts with very small values and then, after some points (usually between 60-80 points) the normal FID starts.



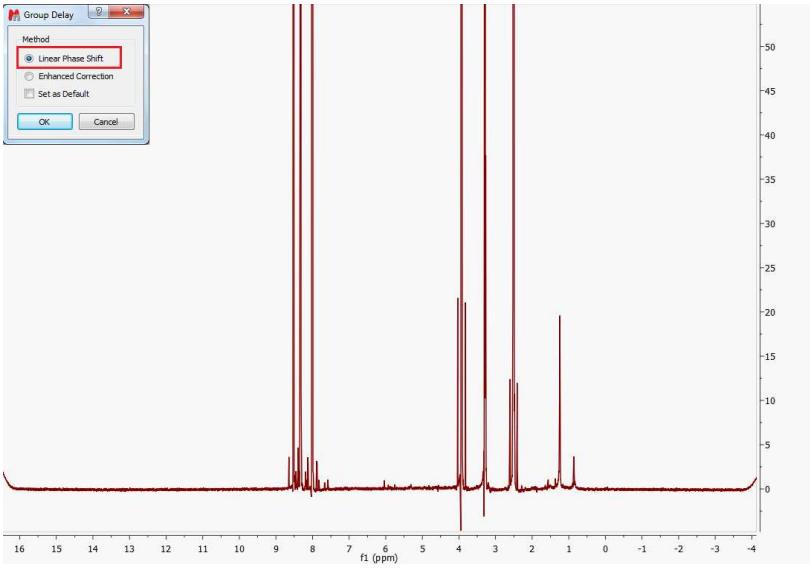
If a plain FT is applied to this FID, we will get a spectrum with a lot of wiggles in the baseline analogous to the convolution with a sinc function centred in the middle of the spectral window. This can be explained by recalling the time shift theorem of the Fourier Transform which says that if the time domain signal is shifted by n points, the frequency domain spectrum corresponds to the standard spectrum (when the FID has not been shifted) multiplied by exp(-i2*pi*w*n). In other words, we have introduced a very large first order phase correction in the spectrum. For example, if the FID is right shifted by 60 points (death time = 60 points), f-spectrum will exhibit a first order phase distortion of 60 * 360 = 21600 degrees.

© 2024 MESTRELAB RESEARCH



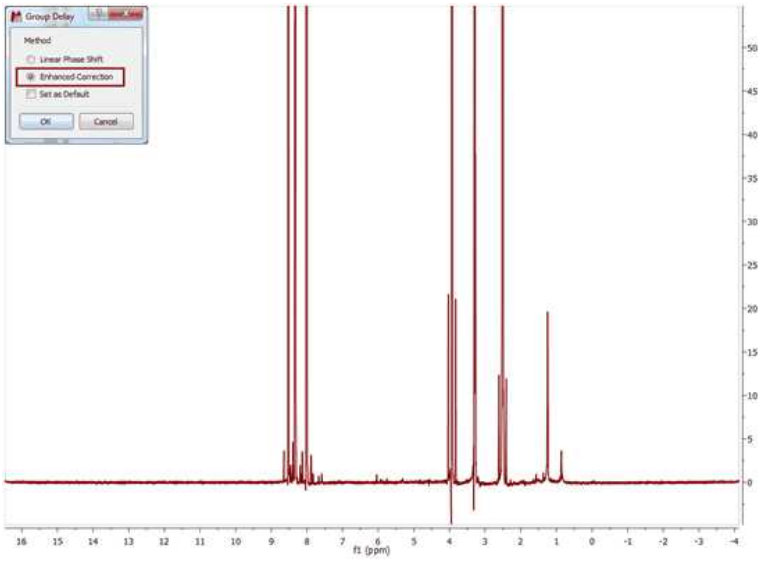
In order to work around this problem, Mnova reads the length of the group delay to be able to calculate the actual beginning of the FID to apply the required correction.

Former versions of Mnova applied a linear phase shift after FT calculated as the product of the group delay length by 360. The problem with this approach is that the resulting spectrum usually shows the so-called smiles at both edges of the spectral width (see figure below)



From version 6.1.0 onwards, Mnova includes a new correction algorithm (Enhanced Correction) which produces a normal and physically correct FID in such a way that the smiles/brownies will not be seen in the f-domain spectrum.

© 2024 MESTRELAB RESEARCH



See also: http://nmr-analysis.blogspot.com/2010/05/bruker-smiles.html

7.3.2 FID Shift

Use this command located under the 'Processing/More Processing' menu of Mnova to right or left shift the FID.

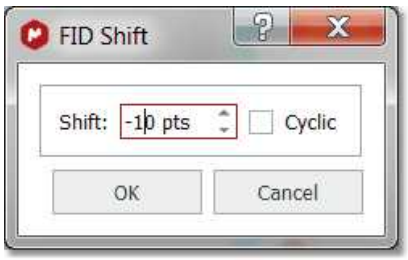
Shifting the FID to the left or to the right is equivalent to shift the phase of the frequency domain spectrum by $-wt_0$.
From a practical point of view this means that if the FID is shifted by one point to the right, the resulting f-domain spectrum will have a linear phase correction of -360º. The same effect, but with the opposite sign occurs if the FID is shifted to the right.

This phenomenon is very easy to understand by considering the well-known property of the **Fourier Transform of a Time Shifted Signal which can be stated as**:
The Fourier transform of a signal which has a simple time shift is:

$$F\{x(t-t_0)\} = e^{-j\omega t_0} X(j\omega)$$

i.e. the original Fourier transform but shifted in phase by $-wt_0$

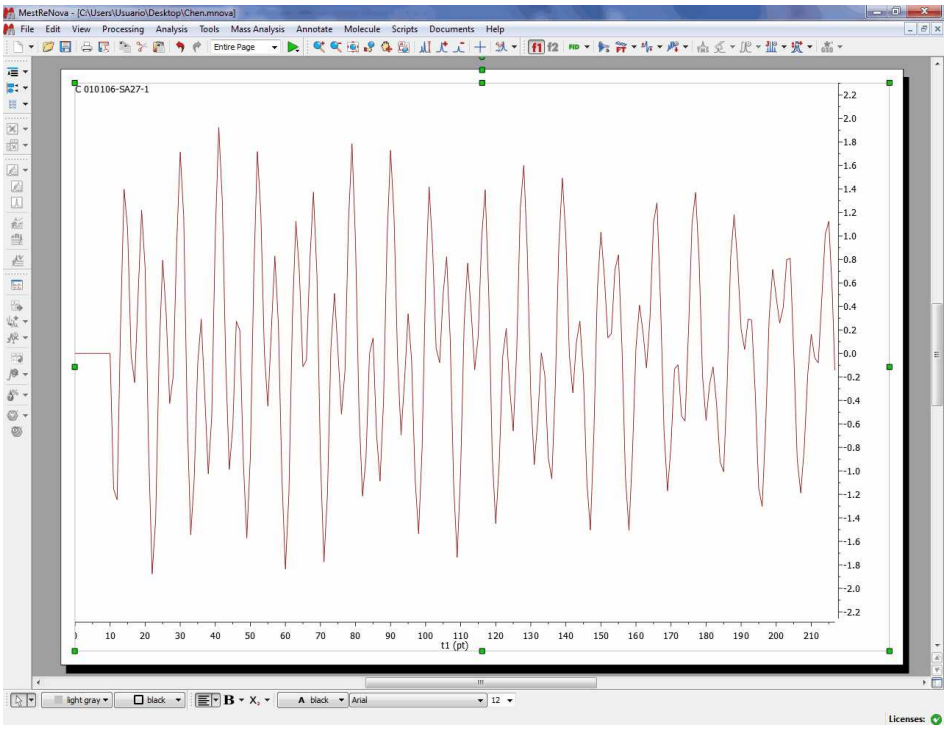
For example: if you type -10 in the edit box, this will be equivalent to apply a linear phase correction of +10*360º.



Basically, in this example, Mnova will not take into account the first 10 complex points of the original FID; shifting the origin of the new FID 10 points to the left and adding 10 zeroes to the end (i.e., left shift 10

points). Clicking on the Cyclic check box will add the original first points to the end of the FID (instead of zeroes).

Here you can see an example of a FID which needs to be shifted +11 points:



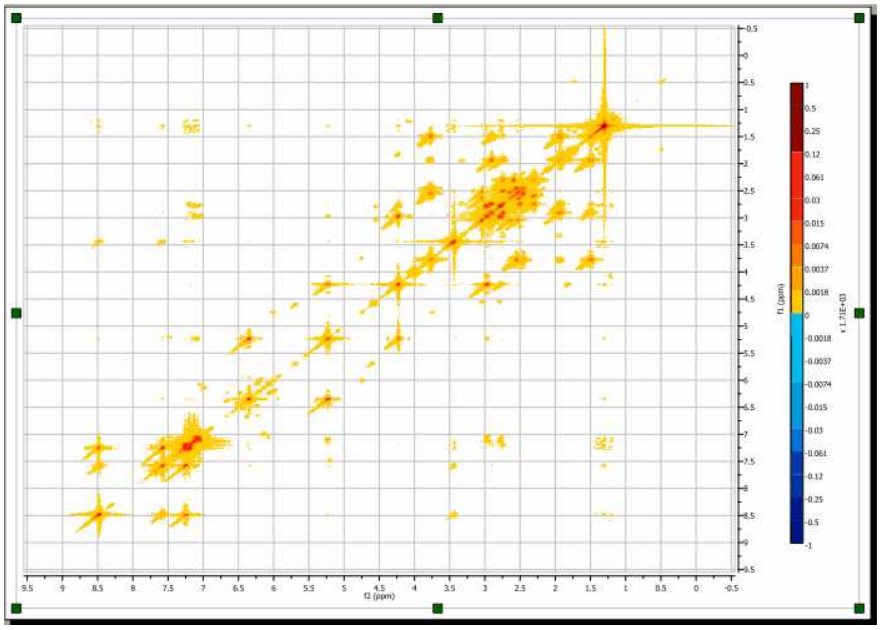
7.3.3 Diagonal Suppresion

Diagonal Suppression (Not available in Mnova Lite)**:**

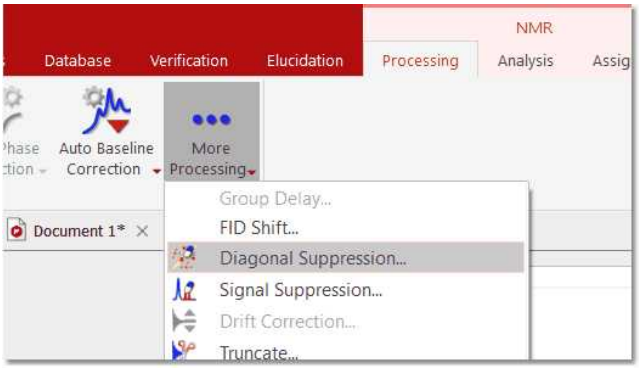
In the 1D NMR Processing section, we described the real-time frequency domain processing concept. **Diagonal Suppression** of homonuclear 2D experiments represents another example of this concept. This technique is typically carried out in the mixed time-frequency domain (interferogram). However, this is something you do not need to know in Mnova. You can suppress the diagonal while the transformed spectrum is on the screen.

For example, the figure below shows a 2D magnitude spectrum:

© 2024 MESTRELAB RESEARCH

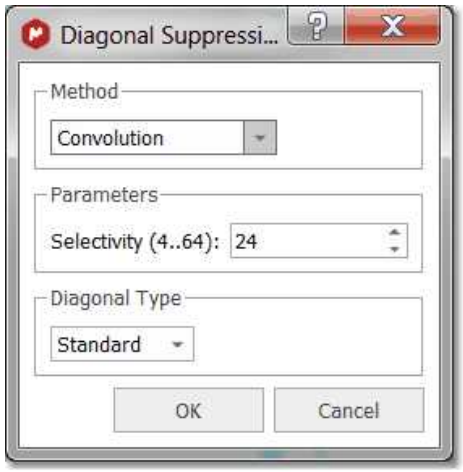


To suppress the diagonal, just select **Diagonal Suppression** command under the 'More Processing' scroll down menu:



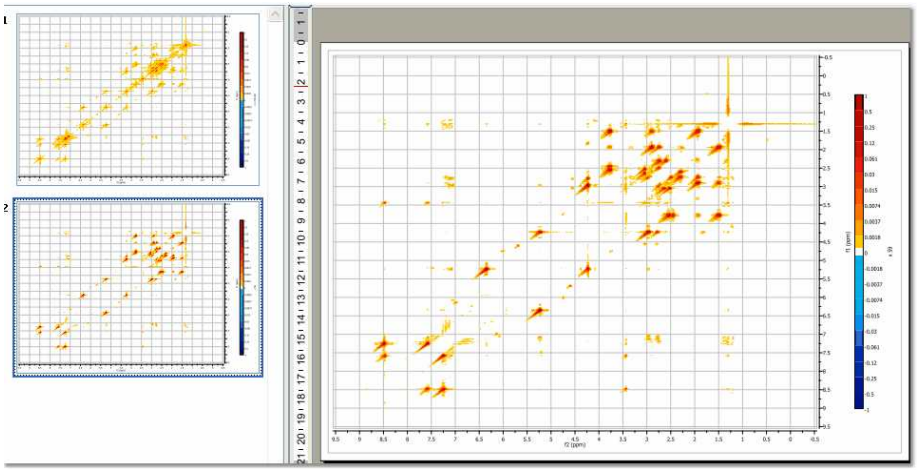
This option offers a couple of well known traditional algorithms (Convolution and Shifted Convolution) as well as a new, in-house developed algorithm based on Wavelets to remove the diagonal from homonuclear spectra. Once you select the 'diagonal suppression' on the FT scroll menu, a dialog box like this will open:

© 2024 MESTRELAB RESEARCH



As you can see, you can select the desired algorithm and also the selectivity of the diagonal supression. In the case where you choose the in-house algorithm based on Wavelets, you will be able to set the 'Dyadic Levels' and the 'Daubechies Coefficients'.

In this example we will select the Wavelet method; and this is the resulting diagonal-free spectrum.



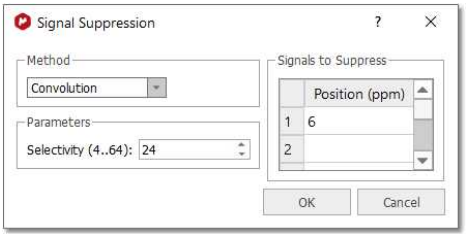
Note that in the figure above there are two pages, the first one containing the 2D spectrum with its original diagonal, and the second one after the diagonal has been removed.

7.3.4 Signal Suppresion

Signal Suppression (Not available in Mnova Lite)**:** Strong solvent resonances with solutes of low concentration can result in a lot of problems relating to spectrum quality. One of the primary challenges in obtaining High resolution NMR spectra of biological samples is the suppression of the water resonance to prevent it from overwhelming the signals of interest.

This option is intended to suppress the solvent signal but can also be used to remove any other signal from the spectrum. To suppress the signal, you can select the method (algorithm) of the suppression and the selectivity (or width of the filter; a higher value of selectivity will suppress a narrower band). You can choose the signal, or signals, by double clicking on the applicable table titled 'Signals to Suppress'.

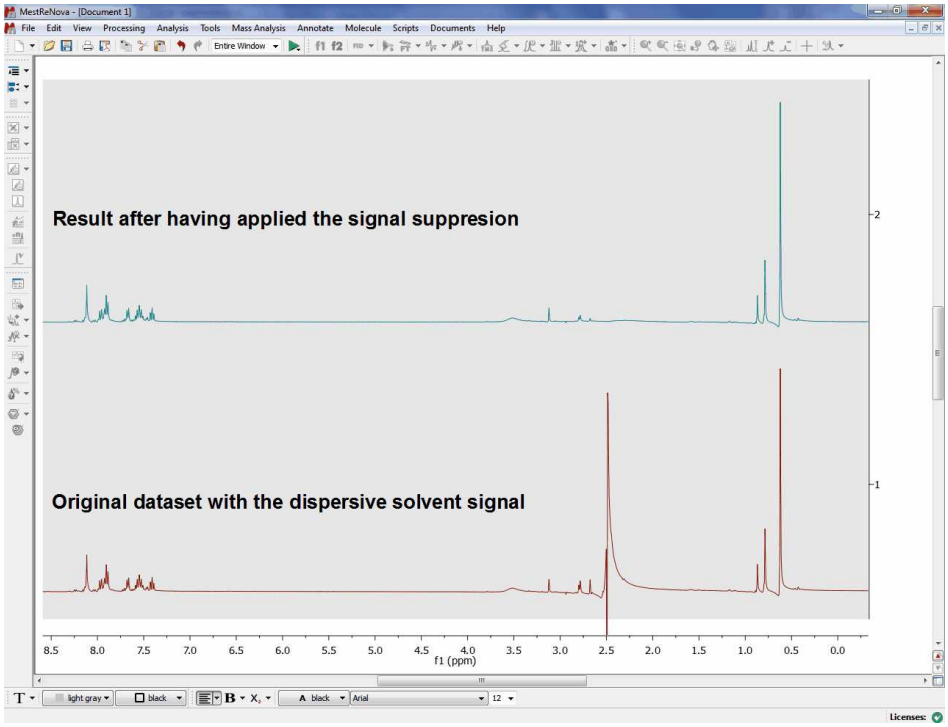
© 2024 MESTRELAB RESEARCH



Utilities:

The 'Signal Suppression' tool is extremely useful when working with proteins, in which spectra are usually measured at very low concentrations in aqueous solutions.

Here you can see an example:



More information about the signal suppression methods can be found here:

Convolution method: *Marion, D.; Ikura, M.; Bax, A. J. Magn. Reson. 1989, 84, 425.*
Wavelets: http://www.ebyte.it/library/docs/nmr08/NMR_Wavelet2DFiltering.html
In particular, the following reference was used to develop the signal suppression algorithm using Wavelets:

Günther U.L., Ludwig C., Rüterjans H.,
WAVEWAT - Improved Solvent Suppression in NMR Spectra Employing Wavelet Transforms
J. Magn. Reson. 156, 19-25 (2002). doi: 10.1006/jmre.2002.2534.

Whittaker Smoother: https://onlinelibrary.wiley.com/doi/abs/10.1002/mrc.4747

© 2024 MESTRELAB RESEARCH

7.3.5 Apodization

Apodization (or Weighting or Windowing): literally, apodization means *'cutting off the feet'* in the original Greek. In this case, the feet are the *leakage or wiggles* which appear when the signal decays rapidly to zero producing an abrupt truncation of the FID, as can happen for example when zero filling. Apodization is a very useful approach to enhance the S/N ratio (sensitivity) or the resolution, or even to remove truncation artefacts, after data has been collected.

The most commonly used apodization function is a decaying single-exponential curve. The magnitude of the decay constant determines how quickly the curve decays. In a regular FID, the resonances due to the sample are found in the earlier parts of the signal. When you multiply the FID by a decaying-exponential function, you favour the early parts of the time domain and attenuate the data at the end of the acquisition period, which is essentially all noise. A single exponential apodization function does not significantly affect the signal at short times, but it greatly reduces the noise at later times; at the expense of an increase in linewidth, that is to say, a decrease in the resolution. On the other hand, if you use an *increasing exponential curve* as apodization, you will obtain finer lines (increased resolution) but amplifying the noise (decreased sensitivity).



Utilities:

Windowing is particularly useful in ^{13}C-NMR spectra and extremely important in multidimensional-NMR to increase the signal/noise ratio or to improve the resolution.

The Window function is applied by multiplying the data vector d, element by element, by a *vector* a. Thus element k of vector d is multiplied by element k of vector a to yield element k of vector d'.

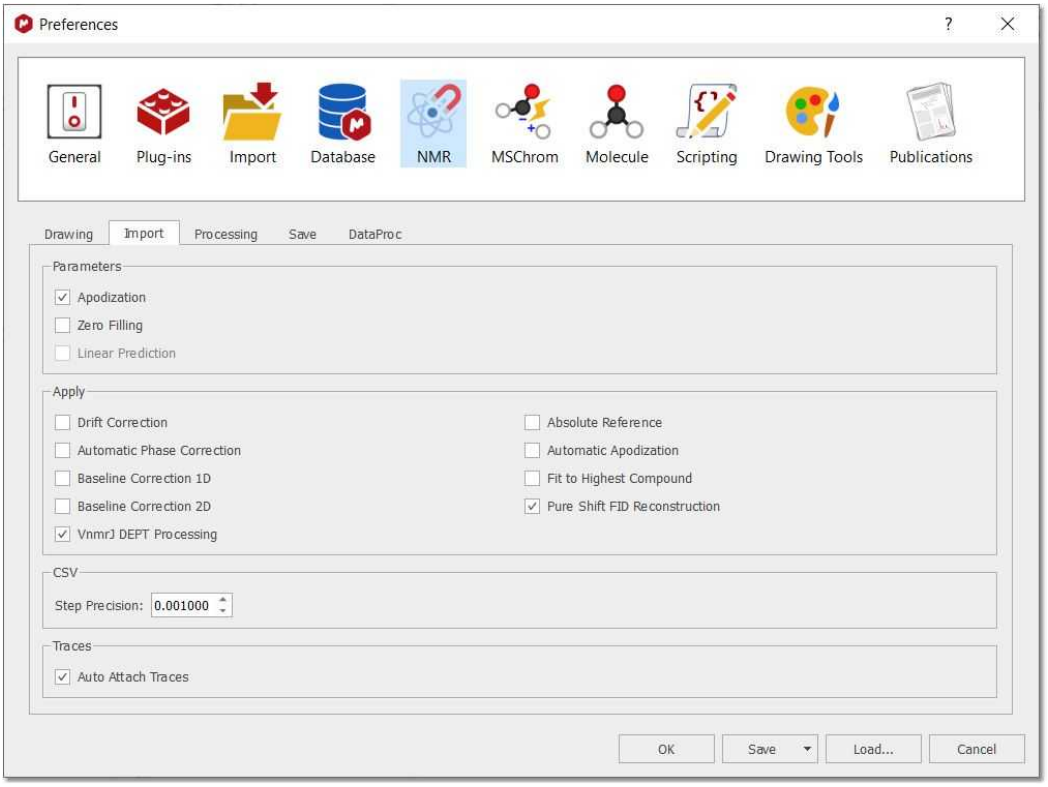
$$d'_k = a_k d_k$$

The challenge lies in finding a vector a, such that the noise in the tail of the FID is attenuated without excessive line broadening or, in the case of apodization, to smooth the truncated data exponentially towards zero, again without excessive line broadening. Theory shows that this is attained when the decay of vector a matches the decay of vector d; in this case we have what is known as a *matched filter*. Mnova makes this particularly easy by allowing the final spectrum to be observed while the decay parameter of the window function is adjusted.

Mnova incorporates many window functions (Exponential, Gaussian, Sine Bell, Sine Bell Square, TRAF, Trapezoidal, Parabolic, Hanning, Convolution Difference and Linear Ramp), which can be combined and can be used interactively (while viewing the FID as well as the resulting spectrum) to improve the sensitivity or the resolution. Unfortunately, it is very difficult to find a function which improves sensitivity and resolution simultaneously (an exception could be the Traficante function, as we will see later).

You can import the apodization used in the spectrometer by following the menu 'File/Preferences/NMR/Import':

© 2024 MESTRELAB RESEARCH

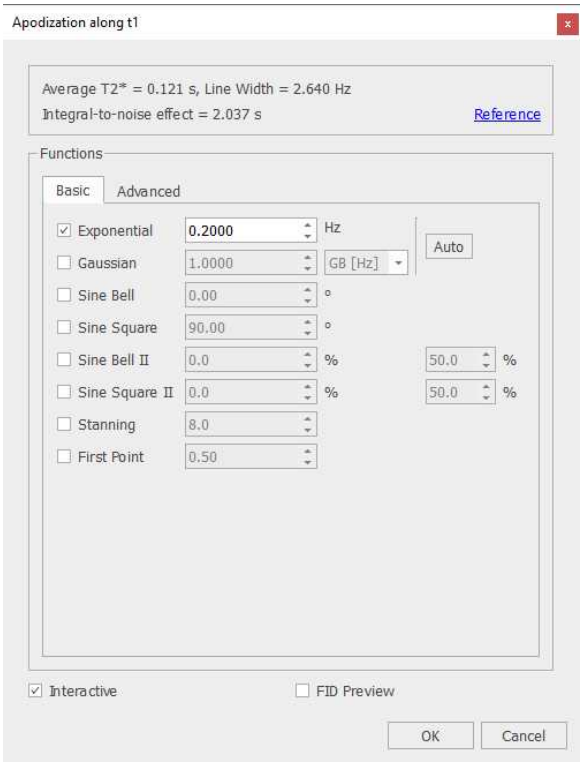


You can also let Mnova to apply an **Advised Processing** by clicking on the applicable button of the ribbon. In that case, Mnova will apply Stanning apodization of 4.0 in 1D (and for 13C it will add an exponential apodization of 2.0 Hz). For 2D, it will apply an exponential apodization of 2.0 Hz and Stanning 4.0 if F2 and Sine Square 90º (first point: 0.5) in F1.

The apodization feature can be found in the Processing ribbon (shortcut: W):



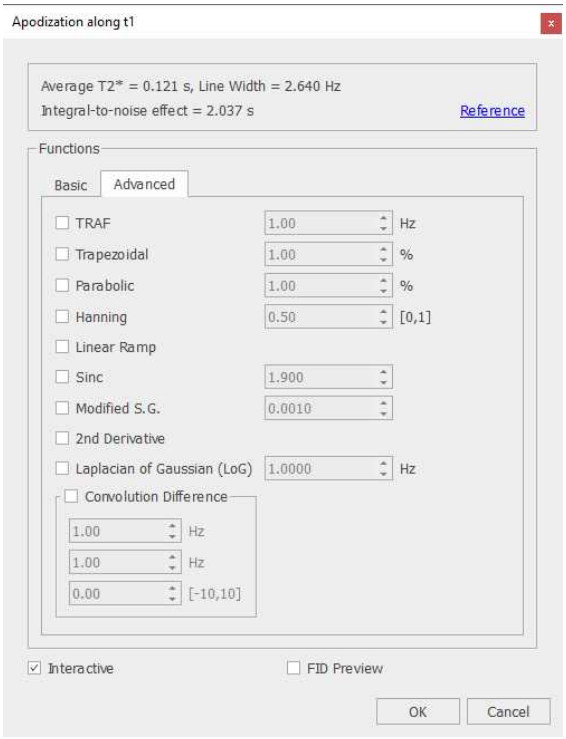
Once you have selected **'Apodization'**, a modeless dialog box will be displayed:



As it is a modeless dialog, you can change the intensity of the spectrum while the dialog is open. In 2D-NMR spectra, you will be allowed to switch from F1 to F2 to select the desired apodization function.

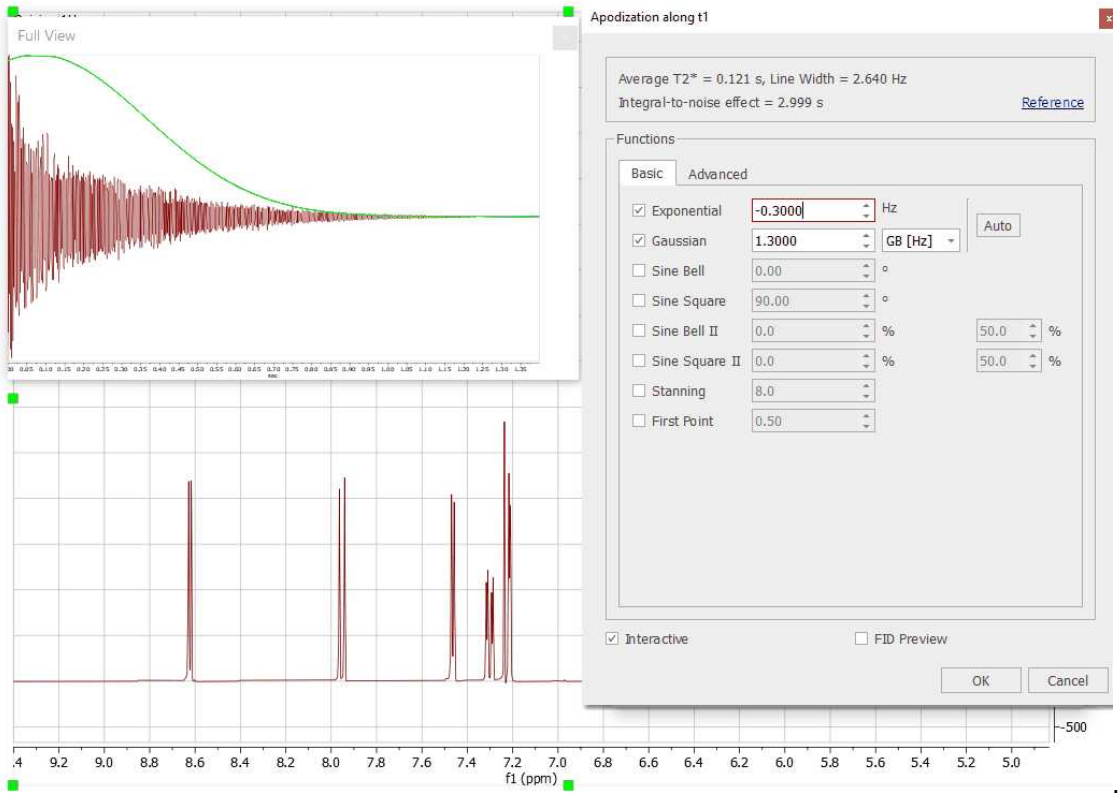
In this dialog box you will find the type of window function which Mnova has applied prior to FT, as well as other Standard Functions (Gaussian, Sine Bell, Sine Square and First Point). If you select the 'Advanced' tab, you can set other apodization functions, as shown below:

© 2024 MESTRELAB RESEARCH



In order to assist in the process of finding the most suitable window function and its parameters, Mnova allows the user to interactively adjust the parameters of window functions, and to view the effect directly on the frequency spectrum. For this purpose, make sure the 'Interactive' box is ticked, you can then follow the exact effect of the function you are applying on the frequency domain spectrum (or on the FID, if you are on the FID' view) by using the buttons to change the desired parameter. If you have checked the 'FID preview' box or activated the 'Full View' window (by holding down the F2 key or by checking the applicable box under the View ribbon) you will be able to see the window function displayed along with the FID.

© 2024 MESTRELAB RESEARCH



You can do the same with a 2D-NMR spectrum (both for f1 and f2):

$$f(t) = 1 - \frac{t-k}{T-k} \cdots\cdots \text{when } t > k$$

We will try to explain in a brief and easy way the applications and the effects of these functions:

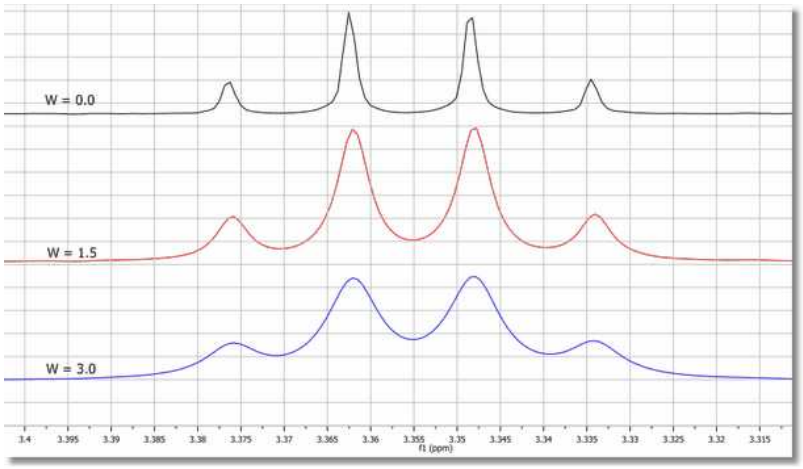
Exponential: A exponential apodization function is defined as:

$$a_k = e^{-\pi W k \Delta t}$$

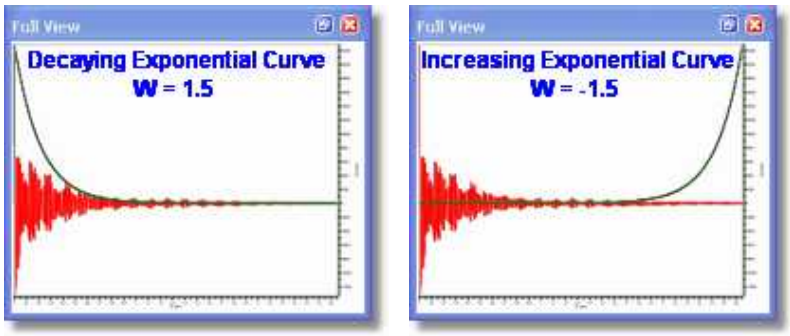
where *W* (the parameter to adjust) represents the line broadening factor expressed in Hz. So, if the original width of the signal is '*L*', the final width of the signal after applying this window function would be '*L+W*'. If *W* is positive we will have a *decaying exponential curve* (and we are giving more weight to the points at the beginning of the FID and suppressing the data at the end of the time series, which is essentially all noise). As we explained previously, this will increase the **'sensitivity'** (Signal to Noise Ratio) of the spectrum at the expense of an increase in linewidth (decreased resolution).

The figure below shows the effect of *W* on the linewidth. The black trace has no window function applied. The red trace shows a taller signal due to application of an **Exponential Function** and also an increase of the Signal to Noise Ratio (matched filter), while the blue trace shows that if the **Exponential Function** is too severe the broadening of the lines reduces the signal size again.

© 2024 MESTRELAB RESEARCH



If *W* is negative, we will have an *increasing exponential curve*, thus obtaining the opposite effect (increased **resolution**, with finer lines, and a decrease in sensitivity, i.e., a lower S/N ratio). You can see below the graphical difference between an *increasing and a decaying exponential curve* over the FID (shown in the 'Full View' window). Note that in this case an *increasing exponential curve* will not be a good choice (it is normally used in combination with other functions, such as Gaussian).



In most of the literature this parameter is symbolized by LB (*Line Broadening*).

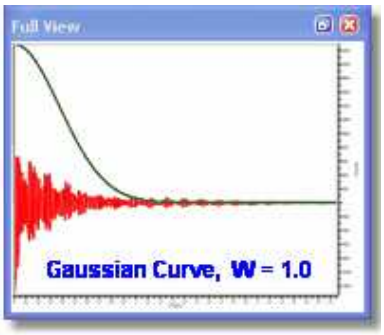
Gaussian: In Gaussian window function resembles the Gaussian Probability Distribution function used in statistical analysis. The gaussian function rises quasi exponentially to its single maximum and then falls back to zero in the same manner and so may be regarded as almost two back-to-back exponentials.

In Gaussian apodization, each data point of the FID is multiplied by the vector:

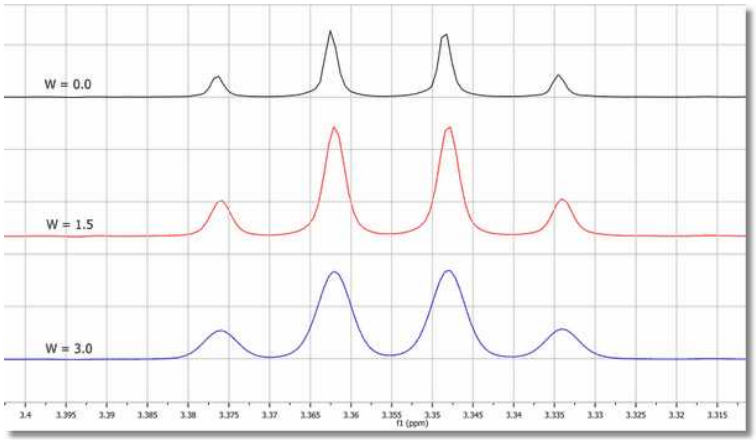
$$a_k = e^{-W(k\Delta t)^2}$$

where *W* is the same widening factor used in exponential apodization. This function is similar to the exponential but decays comparatively slowly at the beginning of the FID and then quite rapidly at the end. For this reason, it causes less line broadening than the exponential multiplication.

© 2024 MESTRELAB RESEARCH



In the next picture, you can see the influence of *W* over the linewidth:



Gaussian plus Exponential: Lorentzian-Gaussian apodization is one of the most recognized apodization functions for its ability to enhance the resolution of spectra without causing a significant loss in SNR. This is achieved by combining an increasing exponential apodization function, EM, with a Gaussian apodization function (GM).

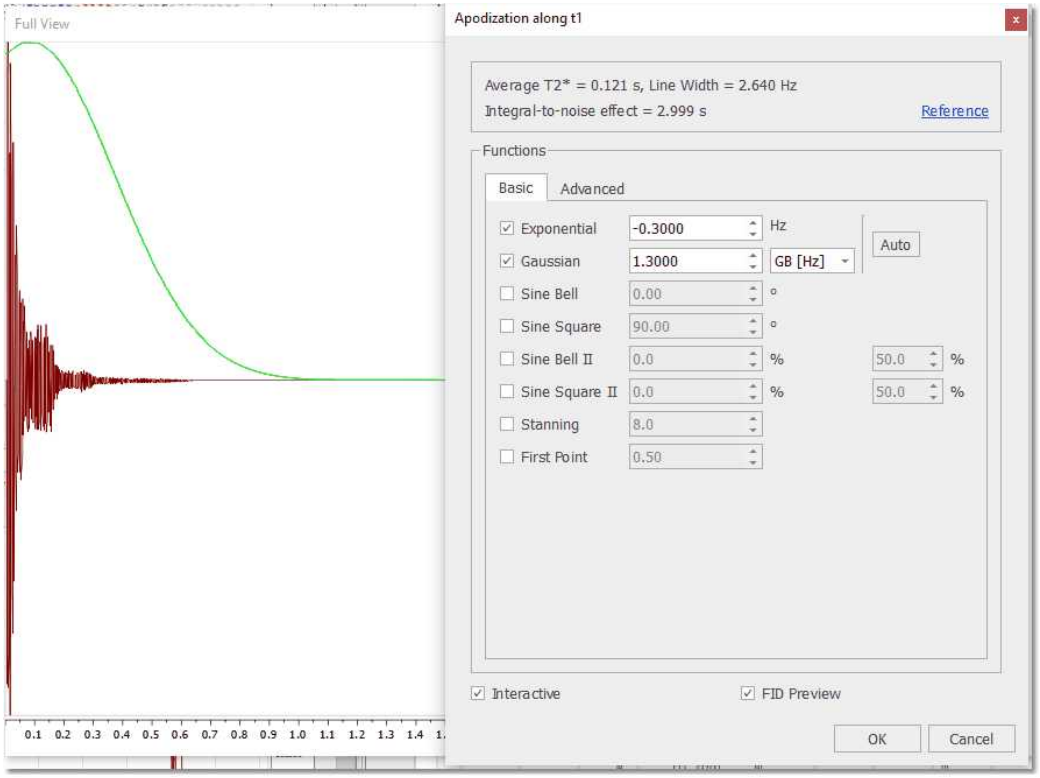
The synergistic effect of both functions accomplishes this. On the one hand, the increasing exponential function improves the signal resolution by giving more weight to the latter part of the FID compared to the beginning, but as a drawback, the noise increases unacceptably. To control this loss of SNR, the Gaussian function is applied simultaneously, which has the effect of damping the SNR loss caused by EM.

Thus, this weighting function combines a rising exponential function with a decreasing Gaussian function; then, the resulting vector will be:

$$a_k = e^{\pi W k \Delta t} e^{W(k\Delta t)}$$

Usually, a process of trial and error is adopted with the parameters being adjusted until the best result is obtained. You can follow (by ticking on the 'Interactive' box) the exact effect of the function you are applying on the frequency domain spectrum or on the FID window (shown graphically in the 'Full View' window), as you can see in the screenshot below.

© 2024 MESTRELAB RESEARCH



GF (Gaussian Factor) is a number between 0 and 1 and defines where the maximum of the function will be, as a fraction of the acquisition time.

Therefore,you have to handle two parameters, EM and GM, and this is something that can be challenging for many users who are not experts in signal processing. If this is the case, you can use the 'Auto' button to automatically set Lorentzian-Gaussian apodization parameters, which will perform the following two actions:

1) Set the value of EM to the average line width of the FID (in Hz). This value must be negative since by definition, the exponential apodization function is already negative, and as we want it to be positive (increasing), we have to use a negative value. For example, if the average width is 1.5 Hz, we would make EM = -1.5.

2) Set the value of GM to 0.8 times the average line width.
That is, if the average width is 'reflw' (here referring to line width), we would have:
EM = -reflw;
GM = 0.8*|EM|

Sine Bell (Not available in Mnova Lite)**:** This function is very useful in those NMR experiments where the signals of interest are not maximal at the beginning of the FID and very convenient for magnitude spectra because it reduces the dispersive part of the line shape.
In COSY experiments the cross peaks are sine modulated while the diagonal peaks are cosine modulated. Sine bell windowing thus emphasizes the cross peaks and attenuates the diagonal signals. Unfortunately due to differing relaxation times for some signals possibly not all cross-peaks will be maximal at the same time; in this case the sine-bell may be shifted to yield the best compromise spectrum. The unshifted sine-bell also removes dispersive signals from the absolute value COSY, providing the sought for absorption line shape.

© 2024 MESTRELAB RESEARCH

Utilities:

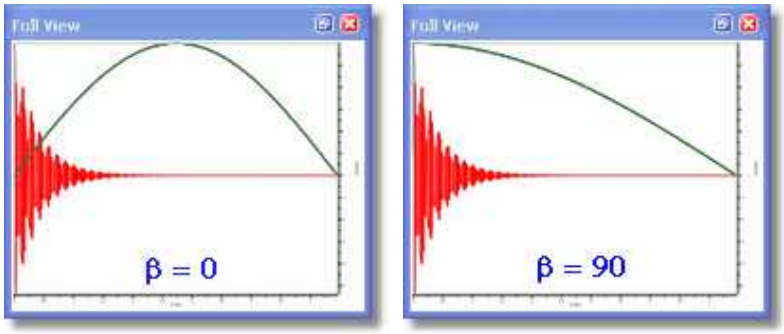
The **Sine Bell** window function is very useful for **COSY** experiments because it emphasizes the sine-modulated cross peaks compared with cosine-modulated diagonal peaks.

Avoid using this function with 1D spectra since it decrease the spectral intensity. If used with phase sensitive experiments the sine bell can result in signals with negative side lobes and near zero integrals.

This function consists in the multiplication of each data point of the FID by the next vector:

$$d_k = sin\left(\beta + \frac{(\pi - \beta)t}{t_{acq}}\right)$$

Mnova requires you to enter the β parameter, whose input is expressed in degrees. When β = 0; the equivalent function is a sine-bell, while when β = 90 the equivalent function is a cosine-bell. In the pictures below you can see the representations of the sine bell functions resulting from different values for β:



The cosine-bell apodization (β = 90) is very convenient in 2D-HSQC experiments:

Utilities:

The **cosine-bell** weighting function (β = 90) is very convenient in 2D-HSQC experiments.

You can use the "VNMR-like version of sinebell apodization" which uses % of acquisition time (instead of seconds) in order to make the parameters applicable to other spectra with the saved processing template.

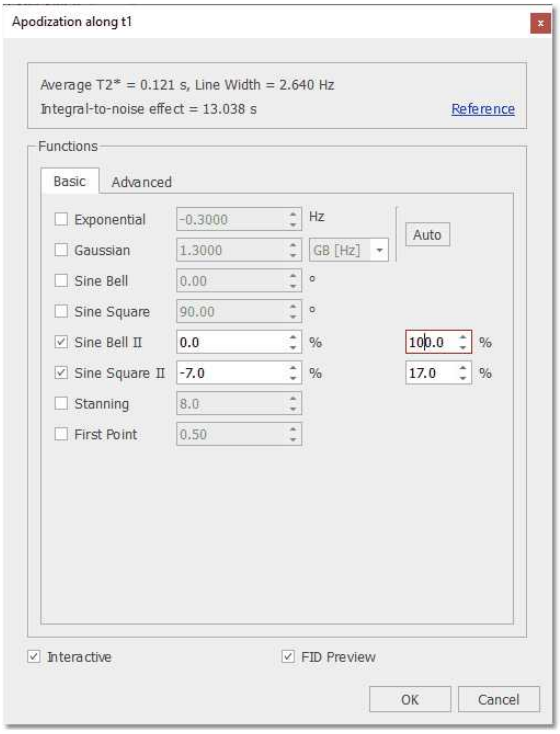
The full formula for the shifted Sine II is sin(((t - sbs)*pi)/(2*sb)).

Where sbs = sbs Mnova /100*T AQ; sb = sb Mnova/100*T AQ

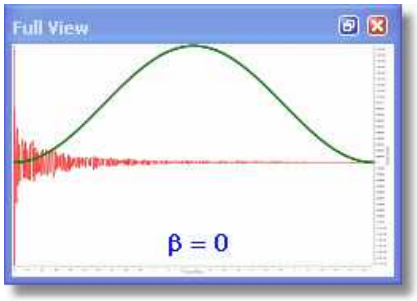
For function values less than zero, the function is truncated (i.e., only the first set of positive values are used).

As you can see you can also adjust the time-period of the SineBell (like the "sb" parameter in VNMR)

© 2024 MESTRELAB RESEARCH



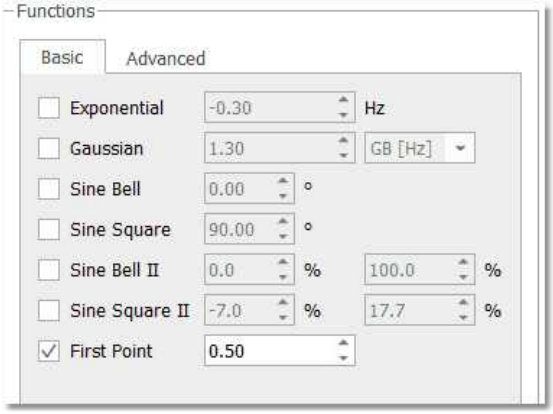
Sine Square (Not available in Mnova Lite)**:** The shape of the Sine Bell weighting function is altered by squaring it, resulting on the **Sine Bell Squared** function, which is more concentrated over the maximum. It is very useful to use a 90° shifted sine-bell squared to apodize 2D phase-sensitive experiments.



First Point Correction: There is an important difference between the *discreet* FT and the *continuous* FT. The *discreet* FT generates a constant vertical displacement of the baseline, which can be due to a distortion of the first point of the FID. It treats the FID as a function which is repeated periodically, thus the ordinate at t=0 would represent the algebraic average of the first and the last point. In most FIDs, the value of the last point is very close to zero, and thus the first real point would have to be divided by two, before introducing it in the FT algorithm. It is often necessary to multiply the first point of the FID by 0.5 before FT in order to ensure that the value of the integral over the spectrum is equal to the value of the first point of the FID.

You can alter this multiplication parameter by entering the desired value (it should be 0.5 by default, as you can see in the FT dialog box below).

© 2024 MESTRELAB RESEARCH



The functions which follow are accessed via the [More >>] button in the Apodization dialog box.

Traficante (Not available in Mnova Lite)**:** The Exponential Multiplication Gaussian and the combination of both can improve spectral resolution at the expense of some loss in SNR. The Traficante window function endeavours to improve resolution without noticeable loss in SNR. Traficante enhances the middle part of the FID and then uses a matched filter to attenuate noise in the latter part of the time-series. The process involves the division of the FID by the sum of the squares of the FID and its reverse. If there are N data points, the k^{th} point in the original FID is point k while the applicable point in the reversed FID is point (N-k). As the resulting FID is symmetrical (with respect to the midpoint), we can multiply it by a function to obtain a new desired FID which does not decrease.

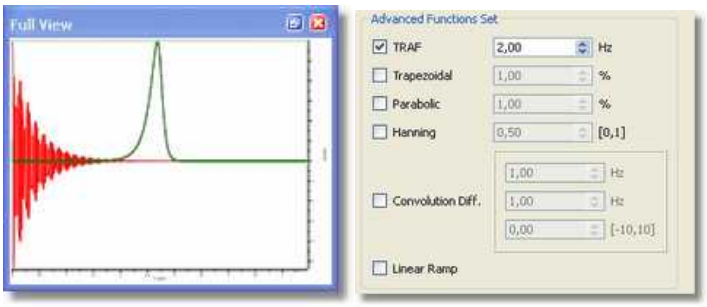
So, if we named the original FID *E*, and the reversed FID ε, we would get:

$$E \cdot g(t) + \varepsilon \cdot h(t) = 1$$

And Traficante apodization will consist of the multiplication of the FID by the following vector:

$$a_k = \frac{e^{-\pi W k \Delta t}}{(e^{-\pi W k \Delta t})^2 + (e^{-\pi W (N-k) \Delta t})^2}$$

In the picture below, we can see a Traficante function represented (with $W = 2$) applied to the FID



© 2024 MESTRELAB RESEARCH

IMPORTANT:

Traficante apodization will be only useful if the FID decays exponentially and, moreover, the value of W must be chosen with care.

Trapezoidal (Not available in Mnova Lite)**:** This function multiplies the FID by a trapezoidal shaped function;

$$f(t) = 1 \cdots\cdots \text{when } t \le k$$

$$f(t) = 1 - \frac{t-k}{T-k} \cdots\cdots \text{when } \; t > \text{k}$$

where T is the acquisition time and *k* is the percentage of the total length of data

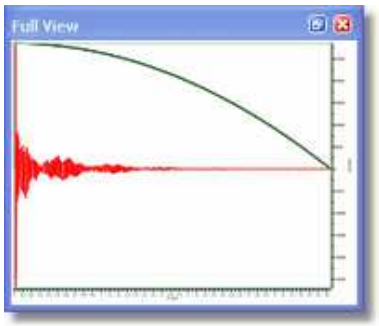
This option is useful to avoid the "sinc" artifacts resulting from truncation of the FID. You can select the percentage of the total length of data between 0% and 100% (*k*), in the advanced functions of the FT menu.

Parabolic (Not available in Mnova Lite)**:** This function multiplies the FID by a parabola shaped function, where

$$f(t) = 1 \cdots\cdots \text{when } \; t < b$$

$$f(t) = 1 - \frac{t-b}{[t(\max) - b]^2} \cdots\cdots \text{when } \; t > b$$

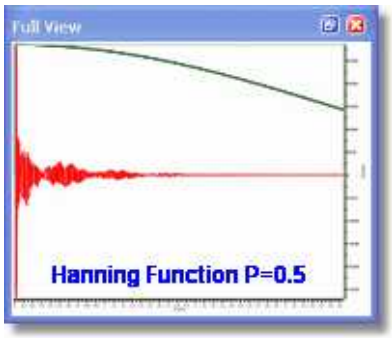
You can also set the length of the parabola by selecting (on the FT scroll menu) Apodization, click "More", select parabolic and then choose the desired value for the parameter (*b*, expressed as the total length of data). Again, you can see the exact effect of the function you are applying on the frequency domain spectrum or the effect on the the FID by (pre-)selecting the Full View" window:



Hanning (Not available in Mnova Lite)**:** Mnova adopts the following generalized equation:

© 2024 MESTRELAB RESEARCH

where *T* is the acquisition time, and P can be a value from 0 to 1. You can set this parameter on the advanced Functions set of the FT dialog box. If ***P = 0.5*** we will have a Hanning function and if ***P = 0.54*** we will apply a Hanning function, producing 10 and 20% line broadening respectively. The Hanning window, more correctly called the Hann window and the Hamming window are part of a family of functions known as "Raised Cosines" and also includes the Gaussian Window. These functions, like a cosine bell, have a maximum value; Mnova generally implements these functions with their maxima at zero time. The Hanning Function is sometimes used to reduce aliasing. Aliasing arises when signals are insufficiently sampled (violating the Nyquist Criterion), resulting in spectral folding. It results in peaks appearing at incorrect positions and generally not phasing along with the authentic signals. This arises due to incorrect experimental set up (signals beyond the high frequency end of the spectral width), and the ability to correct for it after acquisition is useful indeed.



Convolution Difference (Not available in Mnova Lite)**:** This weighting function multiplies the FID by :

$$W(t) = 1 - e^{\left(-0.5t\pi\frac{LB}{SW}\right)}$$

where *LB* is the line broadening and *SW* is the spectral width in Hz. You can also access this function from the scroll menu of the FT icon where you will be able to set the value of LB.

Linear Ramp (Not available in Mnova Lite)**:** This command is used to multiply the data points of the FID by an increasing linear ramp, resulting in the first derivative of the Fourier transformed spectrum { d(intensity)/d(frequency) }. This emphasizes the faster rising parts of the spectrum at the expense of SNR. If the initial spectrum had an excellent SNR this function can dramatically improve resolution. Mnova will soon offer derivative performed using wavelet processing which produces even better spectra.

This weighting function gives *more weight* to the end of the FID, amplifying the noise (decreasing the sensitivity), and for that reason we recommend its use only in combination with another function which gives more weight to the beginning of the FID (increasing sensitivity). In practical terms, add the ramp function to a spectrum that has already been windowed by, for example the EM function for 1D or the sine-bell function for a 2D in order to improve the selectivity.

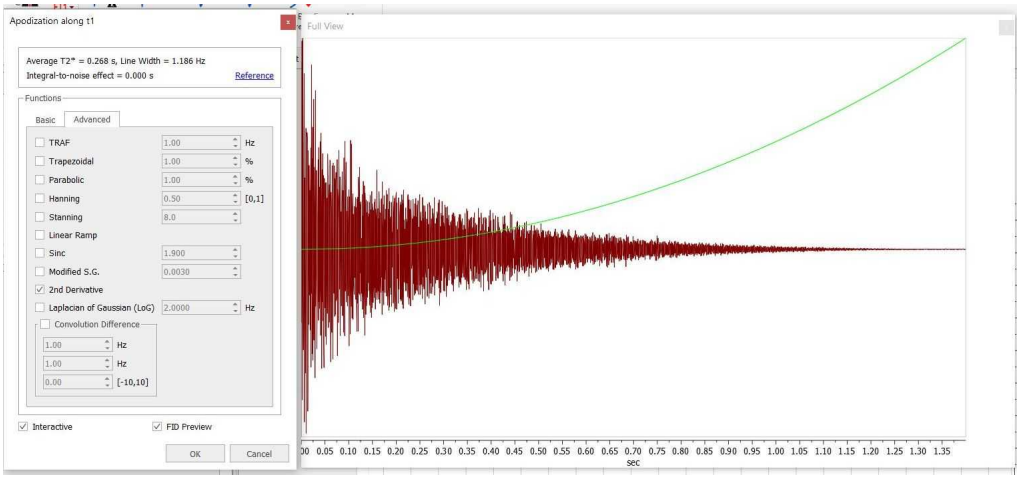
Modified Savitzky-Golay (in the time domain) Recognising that the Savitzky-Golay window is zero at the start of the signal, we propose to add a decreasing ramp to it. That is the modified Savitzky-Golay window becomes:

$$w_{msg}[k] = 2\cos\left(\frac{2\pi}{L}k\right) - 2 - \alpha(K-k)$$

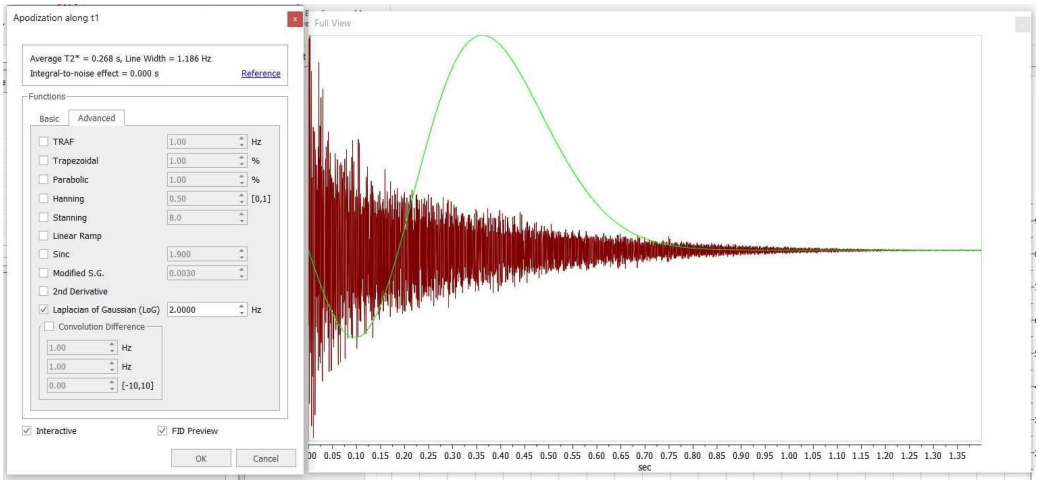
© 2024 MESTRELAB RESEARCH

where α is the weight of the ramp. The larger α is, the more we weight the original signal and the higher the SNR gain will be at the expense of worse resolution enhancement.

Second Derivative: as an extension to the existing first order (that was already implemented years ago and that consists of the application of a linear ramp).

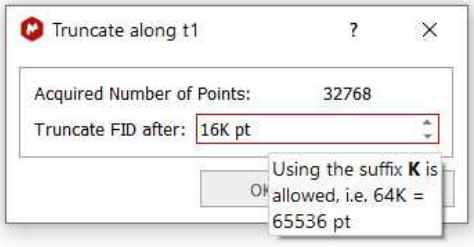


The **Laplacian of Gaussian** The second derivative window is of the form $w_2[n] = k^2$



7.3.6 Truncation

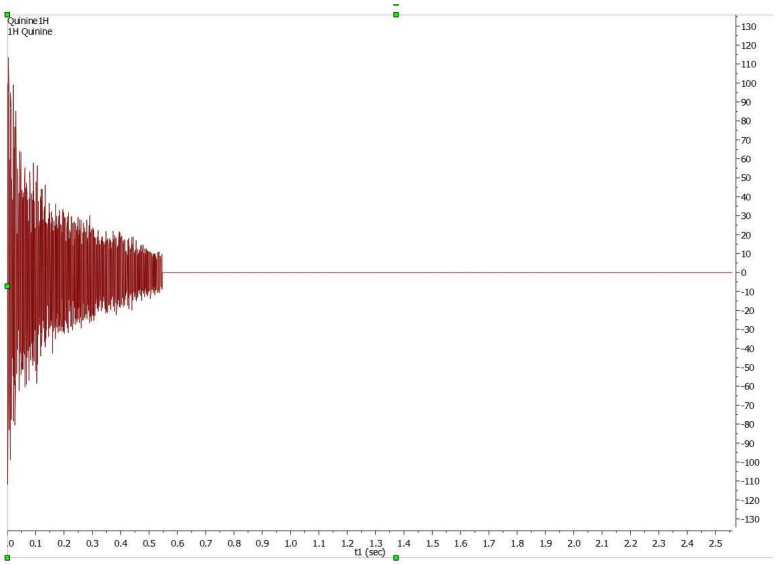
Truncation: This technique causes part of the time series, from a given time onward to be set to zero. For convenience Mnova truncates the time series after a given number of acquired points. The user may access this function either via the menu 'Processing/More Processing' menu:



This technique may be used to shorten the acquisition data time series. If the FID, for example, decays to zero in 0.5 seconds and the acquisition time was 2 seconds, the FID consists of 25% signal and 75% noise. Since both 0 and a 90 degrees phase shifted spectra are collected a time series data length of twice the FID time is required for maximum information content. Truncating the FID at about 1.0 second will reduce the amount of storage required with no data loss. At a later time Zero Filling may be used to cosmetically adjust the spectrum's appearance.

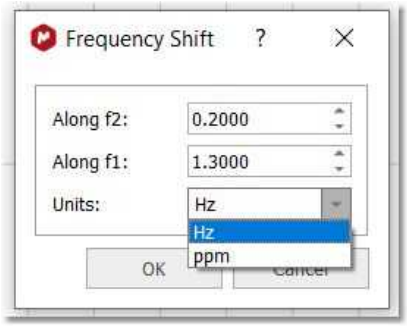
This tool is used when the acquisition time has been set much longer than the FID, resulting in the acquisition of a long period of signal-free noise. Truncation sets the signal free latter region of the time-series to zero. This does NOT amount to zero-filling, and the appending of zero-s to twice the original number of acquisition points will still reveal the intrinsic resolution.

Here you can see an example of truncated FID:



7.3.7 Frequency Shift

Follow the menu ""“Processing/More Processing/Frequency Shift” to shift the frequency in f1 and f2.

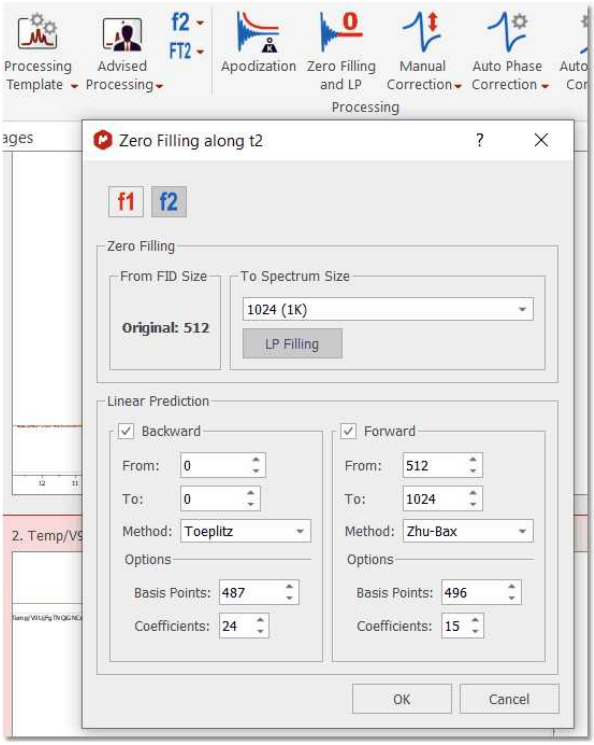


When applying for example 100 Hz in f2 or f1, a shift is applied in f2 or f1 dimension, respectively.

© 2024 MESTRELAB RESEARCH

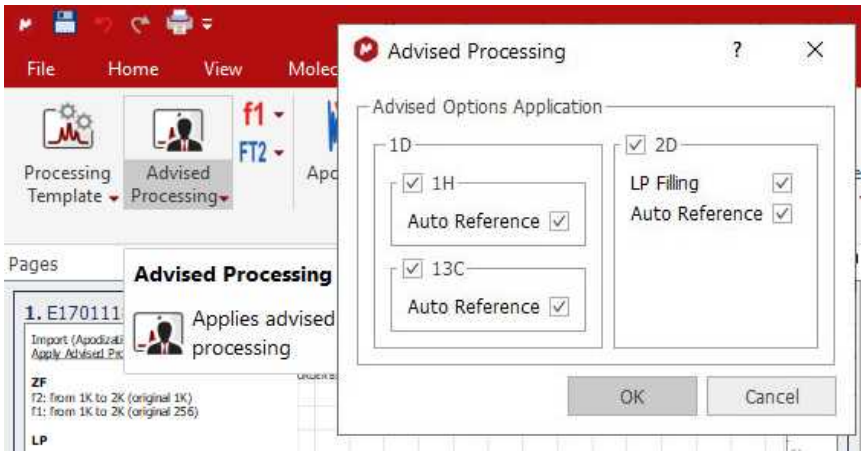
7.3.8 Zero Filling and Linear Prediction

Zero Filling: This option can be used to set the size of the Fourier Transform. It can be used either to increase the digital resolution or to truncate the FID. When the time-domain signal is subject to a FT, the resulting spectrum is represented by a set of data points (by joining the points to plot the spectrum as a smooth line). As a Fourier transformation preserves the number of data points, if we take the original FID and add an equal number of zeroes to the end of it, the applicable spectrum will have double the number of points, increasing therefore the 'apparent' digital resolution of the spectrum. The zero filling process is equivalent to a frequency-domain interpolation. This command is available in the Processing ribbon:



Mnova will be able to import the Zero Filling and Linear Prediction from the spectrometer by following the menu 'File/Preferences/NMR/Import' and checking the applicable box.

There is also an 'Advised processing' feature which will apply a suggested processing with the option to automatic reference the spectra and to apply a Linear Prediction:



For 1H it will apply a Stanning apodization of 4.0 and a three fold Zero Filling. For 13C datasets, it will include an exponential apodization of 2.0 Hz (on top of the Stanning).

For 2D, it will apply a Zero Filling along F2 with a maximum factor of 2 and an exponential apodization of 2.0 Hz and Stanning 4.0 and a 'Fit to Highest Intensity'
For F1 it will apply a Sine Square 90º (first point: 0.5) apodization and a ZF (up tp 3 times).
Magnitude phase correction (only in f2) will be also applied in case of HMBC spectra an in other 2D spectra where FT is None.
In other cases, Regions phase correction will be applied in both F1 and F2.

In some cases, the FID does not fulfill the requirement of the number of data points corresponding to an entire power of 2. In these cases, Zero Filling must be applied to the immediately higher entire power of 2 for FT to be possible. Mnova will automatically carry out this Zero Filling when the number of data points is not an entire power of 2. By default, Mnova proposes a duplication of the original number of FID data points. For example, if the FID has 16384 data points (or a number of data points between 16385 and 32768), the Zero Filling open dialog would show us the value of 32768 (32K) data points, which indicates that Mnova will add up to 16384 data points at the end of the FID.

Linear Prediction (Not available in Mnova Lite)**:** Linear prediction is a mathematical procedure where a missing part of a FID can be constructed in order to increase the digital resolution of the spectrum; it is a tool which examines the free induction decay, extracts a set of coefficients, and extrapolates, either forward or backward, to predict what the data would have been had it been collected. This is a potentially valuable technique for extraction of useful information from marginal data.

Linear prediction is based in this fundamental principle: each value in the time series can be represented as a linear combination of the immediately preceding values. Mathematically, the basic LP equation postulates:

$$d_m = \sum_{p=1}^{p} a_p d_{m-p}, m = p,....., N-1$$

where a_p are the LP Coefficients and the number 'p' corresponds to the order of the prediction. Thus, you can extrapolate the series beyond the point *N*. This equation is known as *Forward Linear Prediction* (extrapolates forward, predicting the new values from the older ones).

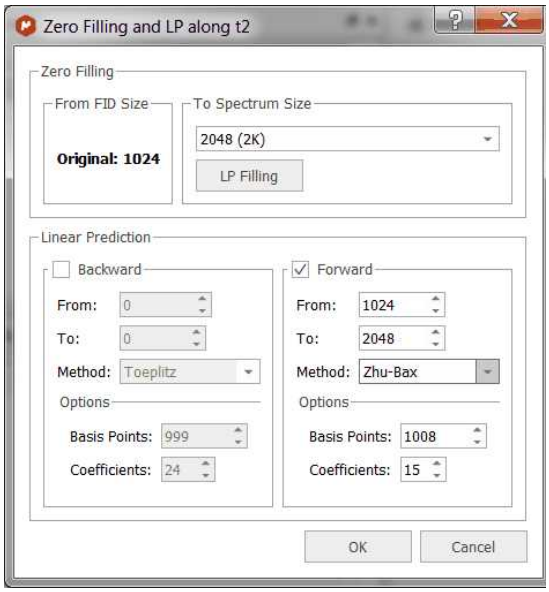
Backward Linear Prediction will be represented in the following equation:

© 2024 MESTRELAB RESEARCH

$$d_m = \sum_{p=1}^{P} b_p d_{m+p}, m = 0,....., N - P - 1$$

This command is available in the same menu as zero filling. If you need to repair the baseline distortions (due generally to the first points of the FID) you have to tick Backward LP and select the limits; this will predict the first points of the FID.

Forward Linear Prediction is a better alternative to correct the last part of the FID than *zero filling;* this will extrapolate the FID in the direction of the advance, predicting the new data points from the older. If you select 'Linear Filling', a Forward LP from the end of the original FID up to the number of points defined in the 'Spectrum size' combo box, will be executed. You can also set the number of Basis Points and Coefficients of the algorithm that you want to use for Linear Prediction.



Method: Linear Prediction is extremely useful in 2D NMR as a way of reducing the acquisition time or increasing the quality of already existing data sets. Also, in 1D NMR it is useful when the FID has not decayed to zero before experiment completion. This often happens when working with slowly relaxing nuclei (such as quaternary carbons, in ^{13}C-NMR) and in experiments with a wide ppm area (spectral width).

One of the most common causes of the corruption of the first data points of the FID is the security time or *pre-scan delay* (time which exists between the pulse and the beginning of the acquisition) which in many cases may not be enough to allow the detector to recover fully from the effect of the pulse.

Our LP implementation is based in a very fast orthogonalization of Toeplitz matrices which is one of the fastest implementation of LP algorithms used in NMR. It gives very good results with phase sensitive data, in particular with HSQC and related spectra. However, it does not work very well with spectra in magnitude (or non phase sensitive) mode. In fact, Toeplitz method does not make it possible to reflect the roots of the LP coefficients within the unit circle because the resulting new LP coefficients get worse.

With such problems in mind, we decided to try the well known Zhu-Bax approach. In short, the algorithm calculates the forward and backward LP because in theory they should be identical. However, in presence of noise they are different. Zhu and Bax proposed to reduce the contribution of the random

© 2024 MESTRELAB RESEARCH

errors induced by noise by simply averaging the two sets of coefficients (fwd & bwd). Finally new roots are computed based on the average coefficients.

Zhu-Bax LP algorithm has been speeded up and it will be the default algorithm for forward LP as it is numerically much more stable and produces more artifacts-free spectra.

The Burg method is probably the first implementation of Linear Prediction developed by John Burg many years ago and it was extensively used in geophysics, but in general, should not be used with NMR data.

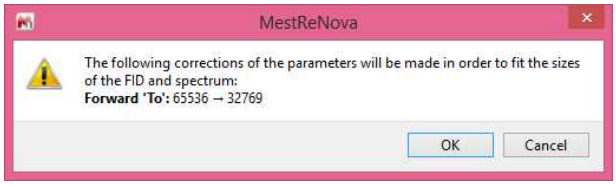
Coefficients: number of theoretical sinusoids. The number of the coefficients should be equal to the number of points in the FID in an ideal case. By default Mnova sets the number of coefficients according to:

Number Of Coefficients = points in the spectrum/16

Basis Points: they are the number of 'good' experimental points to be entered in the calculations. The number of basis points should be at least twice or three times greater than the number of coefficients.

MIST: This option is aimed at extending the FID using a modified Iterative Thresholding algorithm. Its performance has not been studied thoroughly so its application must be used with caution.

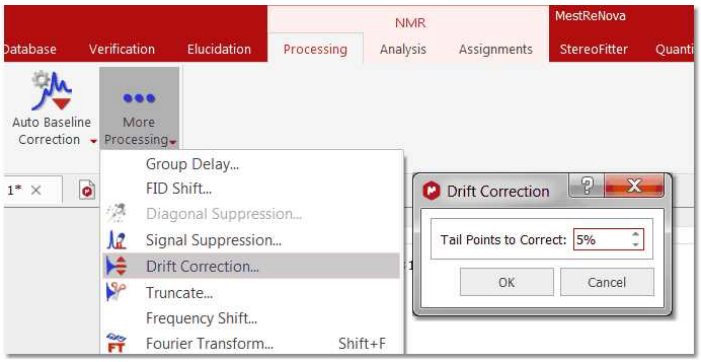
If some LP parameters don't match the sizes of the FID and spectrum then instead of auto-fitting them, a message box with the suggested corrections will be appear:



7.3.9 Drift Correction

Drift Correction: The DC (Drift Current) component is the zero frequency (F_0) of the spectrum, which is proportional to the sum of all data points in the FID; if F_0 is different to zero; a glitch signal (spurious peak) will appear in the centre of the spectral window. Both real and imaginary parts of the FID should decay to zero at the end of acquisition, but sometimes this "zero" is different and the offset may not be the same for both channels. Mnova corrects this spurious peak by default prior to Fourier Transform, although you can find and customize this tool (by selecting the number of tail points of the FID to determine the mean DC level), as you can see below:

© 2024 MESTRELAB RESEARCH



You can automatically apply a drift correction when loading your NMR datasets by following the menu 'File/Preferences/NMR/Import' and checking the applicable box.

7.3.10 The FT Command

Fourier Transform: The FT command transforms the acquired data of the current data set, which may already have been processed using a filter function. Depending on the way a spectrum was recorded (e.g. simultaneous vs sequential sampling, quadrature settings, etc) the FT can be real or complex and some additional pre-processing may be required. All these options can be set in the Fourier Transform dialog box, following the main menu item 'Processing/More Processing/Fourier Transform' (shortcut: SHIFT+F).

If you click on the F1 icon 

(to select the indirect dimension) and then on the FT option, you will obtain the following dialog box:



Protocol: This option corresponds to the data shuffling algorithm required to process phase sensitive 2D spectra

None: If your spectrum is not phase sensitive, then the correct choice is: **none** protocol. (i.e. when only the
magnitude or power spectrum is to be obtained). It corresponds to the simpler 2D experiment in which a scan is collected with a 0º pulse of the mixing pulse, and then another is collected with a phase of 90º and finally combined before to storage in memory.

Bruker: QF mode
Varian: p-type: f1coef='1 0 0 -1 '
n-type: f1coef='1 0 0 1 '

Phase sensitive: this kind of experiments are used to get higher resolution and more information out of a 2D dataset. You can obtain better resolution and better S/N ratio by

generating quadrature phase detection in the second dimension, but at the expense of longer acquisition times and larger datasets.

Echo-antiecho: You should select this option for phase modulated experiments with gradient pulses (used for coherence pathway selection), where pairs of slices are added and subtracted to generate complex "fids" in the indirect dimension. Each slice contains both sine and cosine terms in t_1. This means that the data must be handled differently from hypercomplex data, in which odd numbered slices contain only cosine t_1 terms, and even numbered slices contain only sin t_1 terms.

Bruker: ECHO-ANTIECHO
Varian: f1coef='1 0 -1 0 0 1 0 1'
f1coef='1 0 -1 0 0 -1 0 -1'

Swap Halves (Quadrature): the negative frequencies are distinguished from the positive ones by the detection and summation of the in-phase and 90º out-of-phase magnetizations (two-channel mode).

Mirror Image (Invert): this command performs a complex-conjugate of the data before the protocol and FT is aplied. It is used to generate a mirror image of both the real and the imaginary parts of the spectrum, with respect to a vertical line positioned in the middle of the spectrum; i.e., the spectrum is reversed.
You will need to check/uncheck this option when you get the mirror image of your expected spectrum along any dimension.
Most Varian spectra will require this option to be checked whilst most Bruker spectra will not except those experiments acquired using the TPPI scheme.

Real FT: it allows to execute a real Fourier Transform as opposed to the conventional Discreet Fourier Transform (normally, this is only necessary for Bruker spectra acquired by sequential mode). Modern spectrometers acquire spectra in a way which avoids the use of this option.
Typically, quadrature detection involves the use of two digitizers (ADCs) to sample at the same time the real and imaginary components of the signal. However, quadrature detection can also be done with a single digitizer using the Redfield trick in which the real and imaginary parts are sampled sequentially in time but doubling the sampling rate in order to get the same spectral with. In this case, a Real Fourier Transform is needed. This option is typically used in 1D Bruker spectra acquired in sequential mode (QSEQ) and also it is also necessary along f1 for 2D spectra acquired in TPPI mode.

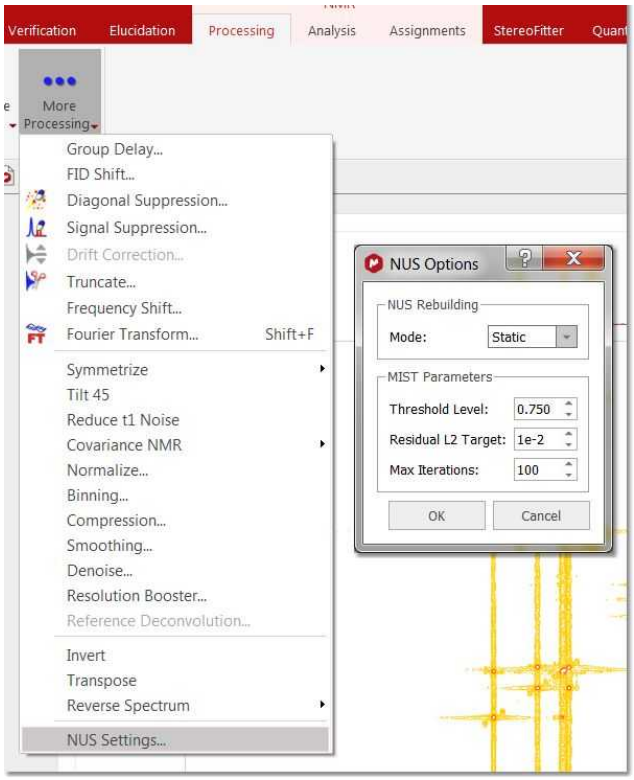
Swap IR/II Before FT: to cover Relay_CH coefficients 1 0 1 0 0 1 0 1

Change Sign II Before FT: to cover Relay_2CH coefficients 1 0 -1 0 0 1 0 -1

7.3.11 NUS Processing

Mnova is able to process 2D data that has been acquired using Non Uniform Sampling (NUS). Under the Processing menu, you will be allowed to select the settings (Max iterations and Threshold level) for the MIST algorithm (Modified Iterative Soft Thresholding):

© 2024 MESTRELAB RESEARCH



Generally speaking, NUS processing is fully automatic and there is no need to touch any parameter. The most critical one is the processing mode, Static vs dynamic. The static mode is the one that should always be used for performance considerations, otherwise everytime any processing in Mnova is issued, it will try to reconstruct the missing data points. The other parameters are related to the way in which IST works.

For further information about NUS and IST, please visit this link, this paper and also this post.

7.4 Phase Correction with Mnova

NMR spectra are usually represented in absorption mode, which offers a greater resolution and proportionality between the integral of peaks and the number of nuclei which cause the signal, in comparison with other representations such as dispersion mode, magnitude or power of the spectrum. Nevertheless, the absorption and dispersion curves are often mixed, giving rise to curves which lose the original symmetry. This problem, combined with other deficiencies, such as delays between the initial pulse and the beginning of data acquisition, cause a phase shift which varies linearly with frequency. This phase errors can be easily rectified in software by applying a lineal combination of the real and imaginary components of the spectrum in order to obtain pure phase lineshapes.

This correction is composed by a *frequency independent* parameter, or zero phase correction (α) and a first order phase correction (β) or *linear dependent* on the frequency parameter:

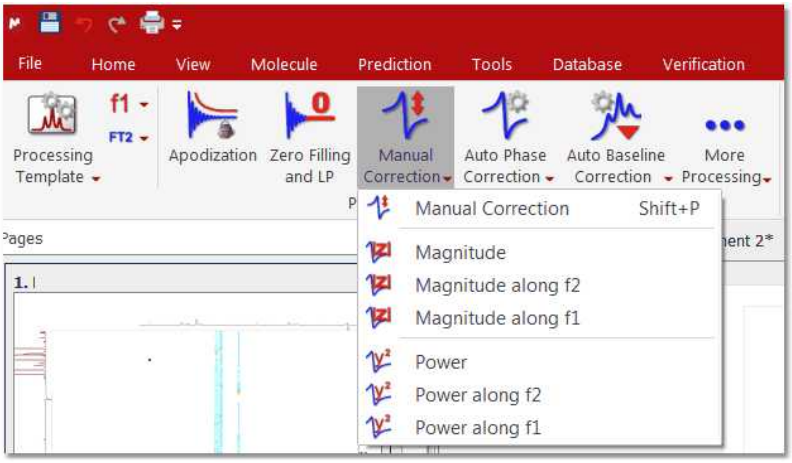
$$\mathrm{Re'}_k = \mathrm{Re}_k \cos(\alpha + \frac{k}{N}\beta) - \mathrm{Im}_k \sin(\alpha + \frac{k}{N}\beta)$$

© 2024 MESTRELAB RESEARCH

where k = 0,.....,N-1; Re_k and Im_k are the real and imaginary components of the data points (k), Re'_k and Im'_k are the new components after correction and N is the total number of points.

Mnova implements both manual and automatic **Phase Correction algorithms.**

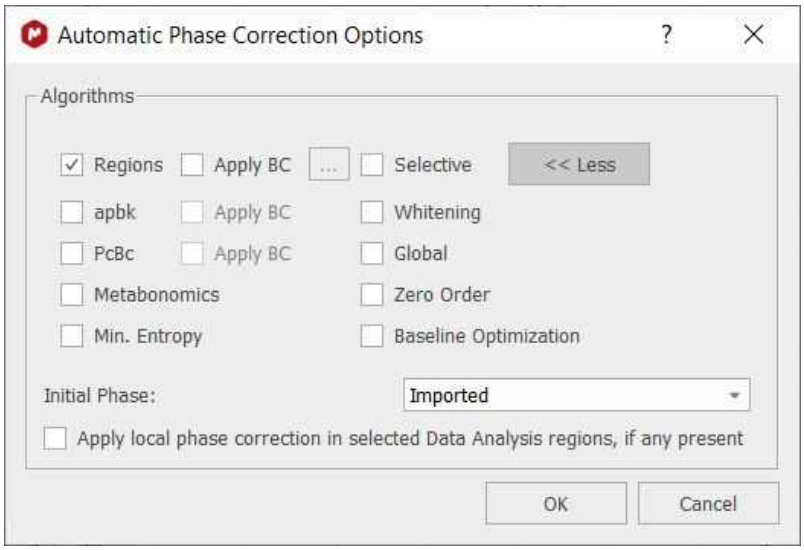
The Phase Correction scroll menu is located on the Processing ribbon:



The software implements three algorithms for automatic phase correction, which can be applied both to 1D or to 2D data sets, and a manual algorithm, which allows for very easy, eye driven, real time zero order and first order phase correction. Once the user selects a Phase Correction method, Mnova will store it for future uses, highlighted with a black square.

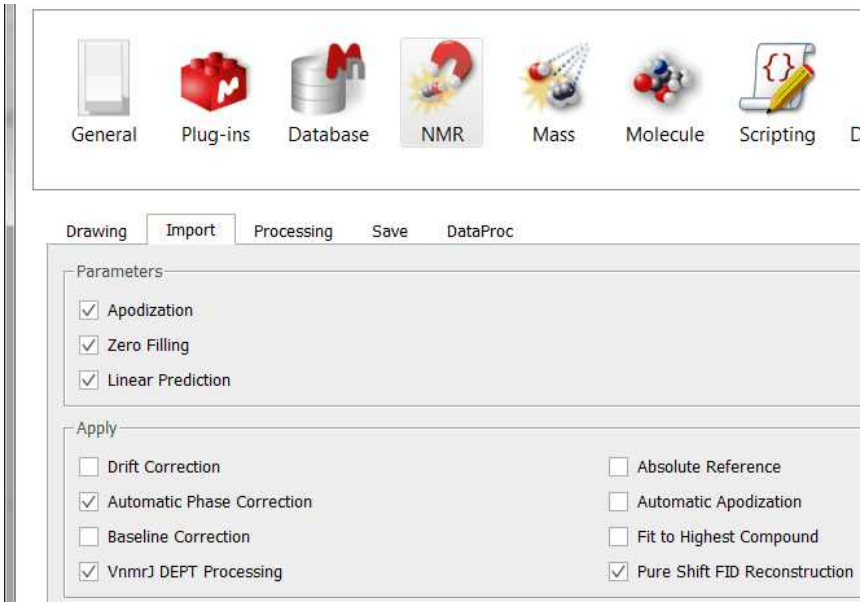
Automatic Phase Correction

Mnova gives the user the choice between several auto-phasing algorithms for higher flexibility. Indeed you can select more than one algorithm at the same time and the algorithms will be applied consecutively:



© 2024 MESTRELAB RESEARCH

The user will be able to select the initial phase correction to run the Automatic Phase Correction algorithm (Zero,Imported, Current). By default is the imported phase from the spectrometer (you can change the default Phase Correction from the 'File/Preferences/NMR/Import' menu):

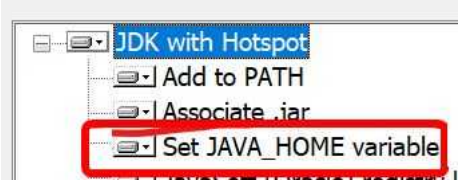


Regions Analysis: An autophasing routine which uses complex fit with phase on the peak regions.

Selective Method (Not available in Mnova Lite)**:** This automatic method is intended for spectra containing negative and positive peaks (e.g. DEPT, APT, etc) or in the presence of baseline distortions. Mnova creates a list of the highest peaks in the spectrum and then uses symmetrization criteria to obtain the optimal phasing parameters (α and β).

Mnova will automatically execute the better Phase Correction in each case prior FT.

apbk: algorithm for 1H Bruker NMR datasets, with the capability to apply a baseline correction. It requires to have a Java virtual machine installed.You can install this OpenJDK, making sure that you enable the environment variable JAVA_HOME during the installation:

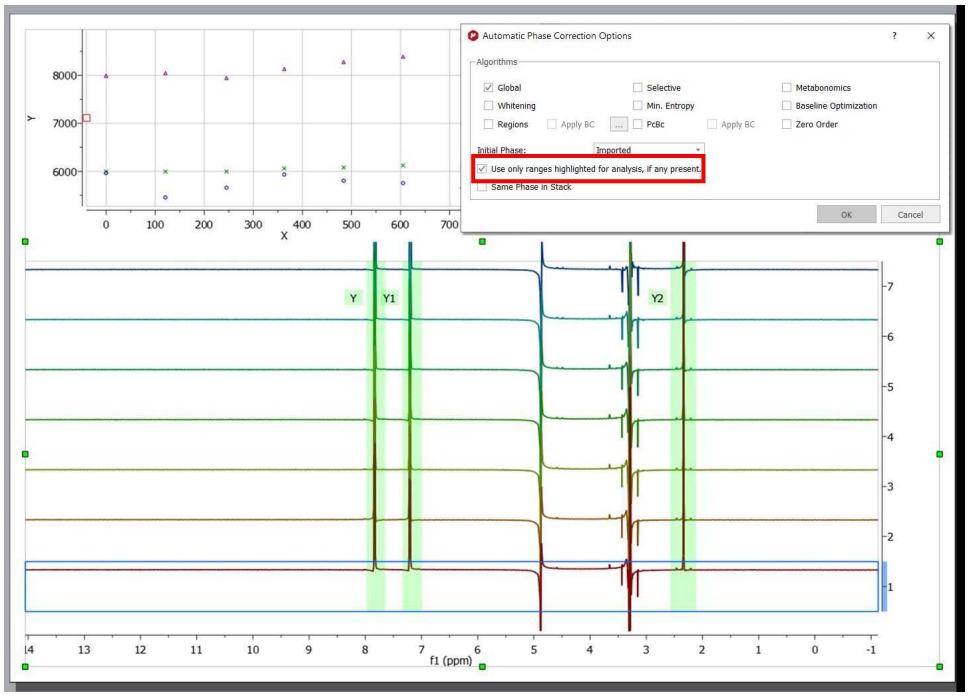


Whitening: A method for fully automated phasing of Fourier transform NMR spectra based on a combination of the whitening and the metabonomics method. First of all, the whitening method is applied in order to get good starting phase correction parameters even in spectra that have both positive and negative peaks. Then the most negative peaks are removed and a simple baseline correction is applied. Finally, the metabonomics method is applied.

If you are working with the Data Analysis feature in stacked plots, you can check the option "use only regions highlighted for analysis" to extract values only from the selected regions to be used by the phase correction algorithms. Please note that the final Phase correction will be applied to the entire spectrum.

© 2024 MESTRELAB RESEARCH

So for example, if the user has selected 3 regions in a spectrum for data analysis, auto phase correction will exclusively use those 3 regions to apply the phase correction to the entire spectrum.

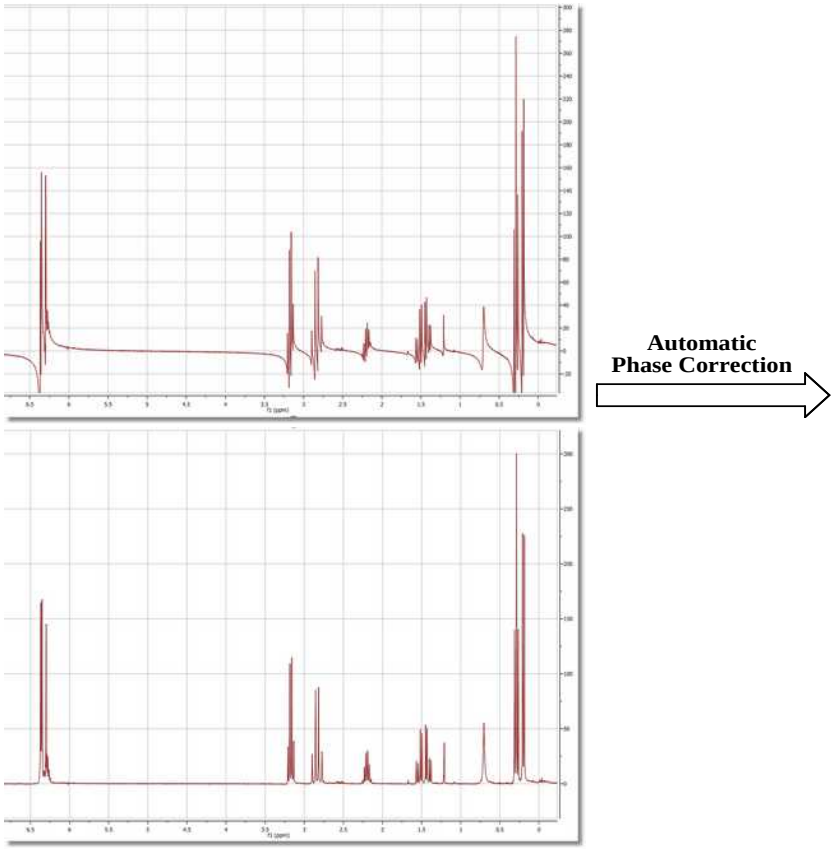


PcBc: only available for 1D, it will allow the user to apply a phase and baseline correction at the same time.

Global method: This method automatically finds phasing parameters (α and β or PH0 and PH1) using an iterative process in which the intensity of the lowest point in the spectrum is optimized. This method is not appropriate for experiments like DEPT or APT, where we can find both positive and negative peaks, but is highly efficient for most ^{1}H spectra. However, it is sensitive to bad baselines and low Signal/Noise ratios. Mnova will automatically execute this Phase Correction.

You can see below the effect of an automatic phase correction on a ^{1}H-NMR spectrum with a very bad phase:

© 2024 MESTRELAB RESEARCH

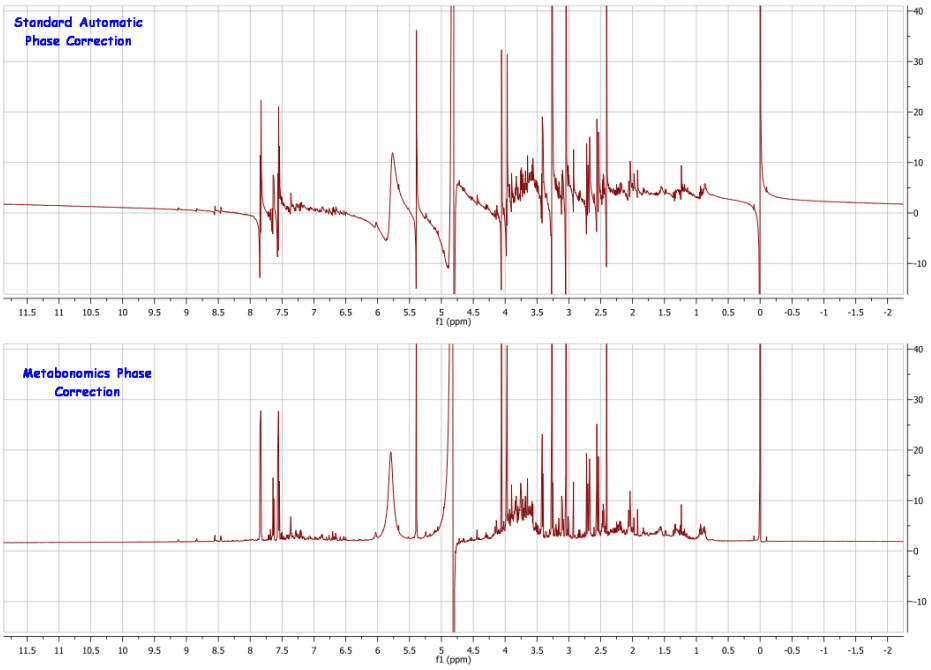


Metabonomics. A new Automatic phase correction algorithm optimized for biofluid-like spectra (Not available in Mnova Lite).

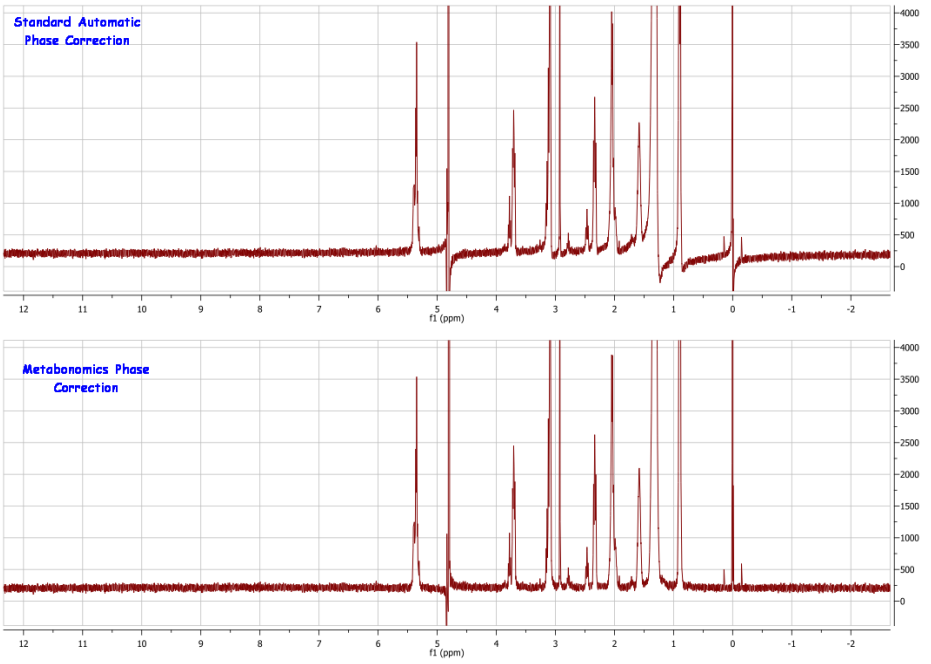
This algorithm is an extension of the standard automatic phase correction algorithm optimized to deal with Metabonomics-like spectra in which most of the signals are concentrated towards the center of the spectral width. In addition, it can also efficiently deal with situations in which a dispersive peak is present in the center of the spectrum, as is usually the case with water in urine samples. It is also tolerant to small first order baseline distortions (including V-shaped baselines).

The figure below shows a spectrum of rat urine with the standard automatic phase correction algorithm applied to the top spectrum and with the new automatic phase correction algorithm applied to the bottom spectrum.

© 2024 MESTRELAB RESEARCH



This algorithm is not restricted to Metabonomics spectra, as it is also good for any other spectra satisfying the two conditions outlined above. For example, the figure below shows the difference in performance between the standard automatic phase correction algorithm (top) and the new one (bottom). In this case, the water signal cannot be perfectly phased and thus a standard automatic phase correction fails. The new method yields a much better result as can be seen in the figure.



Zero Order: allows to make an automatic phase correction in PH1 with a zero value. Please note that the auto-phase correction is performed by running the selected algorithms **one by one** in a predefined order. The *Zero Order* phase correction is the last algorithm, so, even if it doesn't modify the PH1, all the other algorithms do (they adjust both phases, PH0 and PH1, together). Therefore, if you select also

© 2024 MESTRELAB RESEARCH

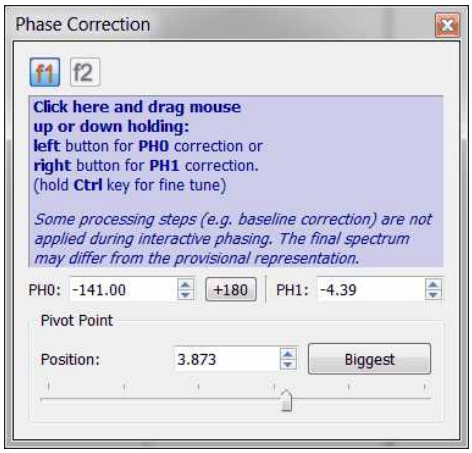
other algorithms different to *Zero Order* when running Phase Correction, these algorithms (*Global*, *Whitening*, etc.) will change PH0 and PH1, and then, the *Zero Order* algorithm will just adjust again the resulting PH0.

Min. entropy: An algorithm for automatic phase correction of NMR spectra based on entropy minimization. See this paper for further info

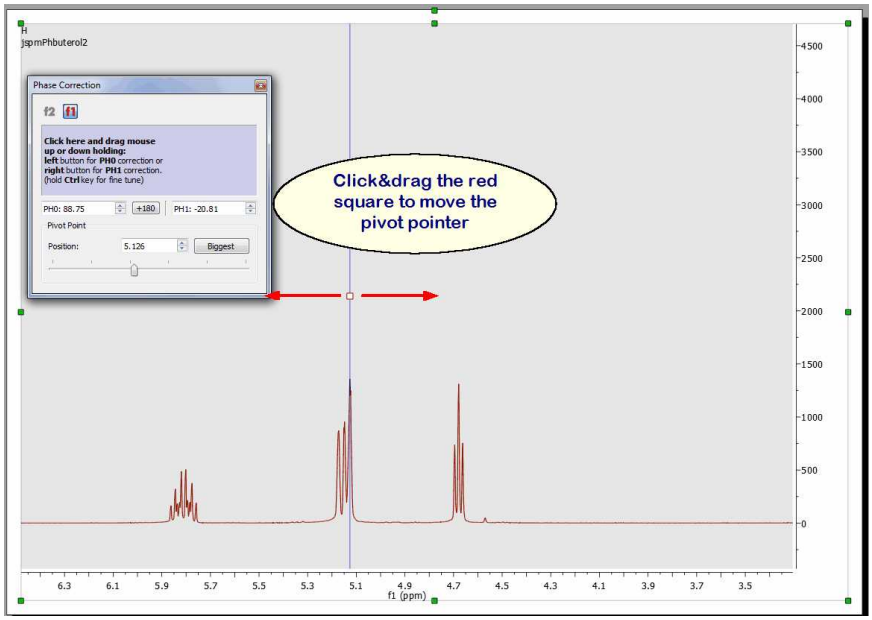
Baseline optimization: A method for fully automated phasing of Fourier transform NMR spectra based on a baseline optimization technique. See this paper for further info.

Manual Phase Correction (shortcut: Shift+P)

The manual phase correction is based in finding the appropriate α and β phase correction parameters. This option is very easy to use. Just select 'Manual Correction'.



Once the dialog box is open, establish a pivot for the adjustment by just using the scrolling bar provided. The pivot will maintain an invariable phase in the peak where it is located, while a first order correction is carried out. Click on the *Biggest* button to put the pivot point on the biggest peak of the currently displayed spectrum region. You can also place it in a position of your choosing by clicking and dragging the pivot point marker or by selecting the desired position in the edit box:



Next, correct the zero order phase (by clicking with the left mouse button anywhere on the *Phase Correction* dialog box and dragging the mouse up or down without releasing to correct the phase of the reference peak) and finally do exactly the same whilst right clicking to correct the phase of the remaining peaks (*First Order Phase Correction*). For a fine tuning, hold the 'Ctrl' (or cmd key in Mac) key while dragging the mouse.

You can use the 'Ctrl' key in a Mac machine to simulate the right mouse button in order to phase PH1.

The Phase Correction dialog box incorporates two edit boxes to introduce the values of α (PH0) and (PH1) β for the correction and a button to apply +180º to PH0

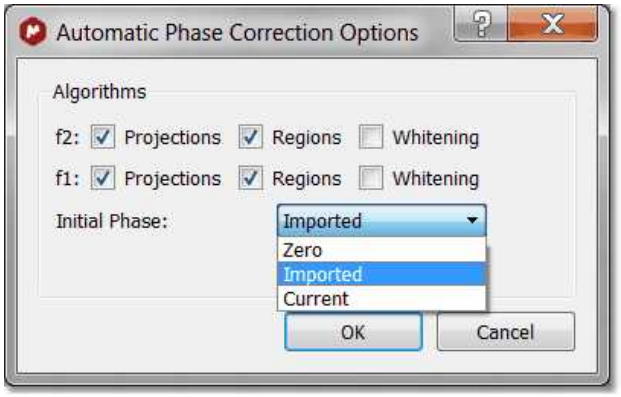


Please note that the Phase Correction dialog is **modeless**, so you will be able to use any other tool (i.e. zooming) whilst you are applying the correction.

The power and speed of the manual phase correction algorithm implemented in Mnova becomes obvious when correcting the phase of 2D experiments. This can be done directly on the 2D matrix, and the phase can be quickly corrected along one and then the other dimension (Note that for all operations in 2D NMR, the active dimension is indicated by the **'f1'** and **'f2'** icons in the upper toolbar).

Phase Correction for 2D-NMR spectra

The phase correction feature for 2D NMR spectra includes powerful algorithms which can be accessed via the 'Auto phase correction scroll down' menu. Clicking on 'Options' will display a dialog box which will allow you to select the desired algorithm:



The **Projections** algorithm will calculate the 1D projections of the 2D-NMR spectrum and will apply a 1D automatic phase correction over both projections. These values will be used as initial values for the 2D adjustment when you have selected also the **Whitening** algorithm. If you only select the Projections algorithm, only 1D phase corrections values (of the projections) will be used for the 2D correction.
This method involves the calculation of the internal projection as the sum of all columns (or rows) in the 2D matrix. In the case of homonuclear correlation spectra, the projection is analogous to the diagonal. The phase correction calculated by an automatic 1D algorithm can be used as a rough estimate of the phase correction of the whole 2D matrix. This method is satisfactory in the case of homonuclear correlation, but inapplicable in the case of an edited HSQC (with both positive and negative peaks and much more noise).

Regions: the phasing in each direction is applied on slices, selected by peak picking. All phases are combined for a single linear regression analysis per each dimension.

© 2024 MESTRELAB RESEARCH

The **Whitening** algorithm is based on the *whitening* concept formulated as the 'maximization of the number of white pixels into a bitmap that corresponds to the spectrum'. This process of maximization can be factorized along the individual axes of the spectrum and this property makes the method robust and fast. It employs a statistic measure based on a large number of spectral data points and, for this reason, is very tolerant to low SNR and local artifacts. The algorithm can efficiently phase either homonuclear or heteronuclear experiments and, unlike other previous methods, it can also process automatically spectra containing positive or negative peaks so that it is not necessary to deal with individual or special cases.

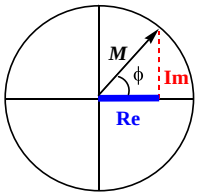
NOTE: After having applied a baseline correction in a 2D dataset, the manual phasing will be grayed out. To undo the baseline correction, follow the menu "Processing/Processing Template' and turn off the Baseline Correction for either dimension, click on OK and you will be able to correct the phase manually.

Alternatives to Phase Correction. Magnitude and Power.

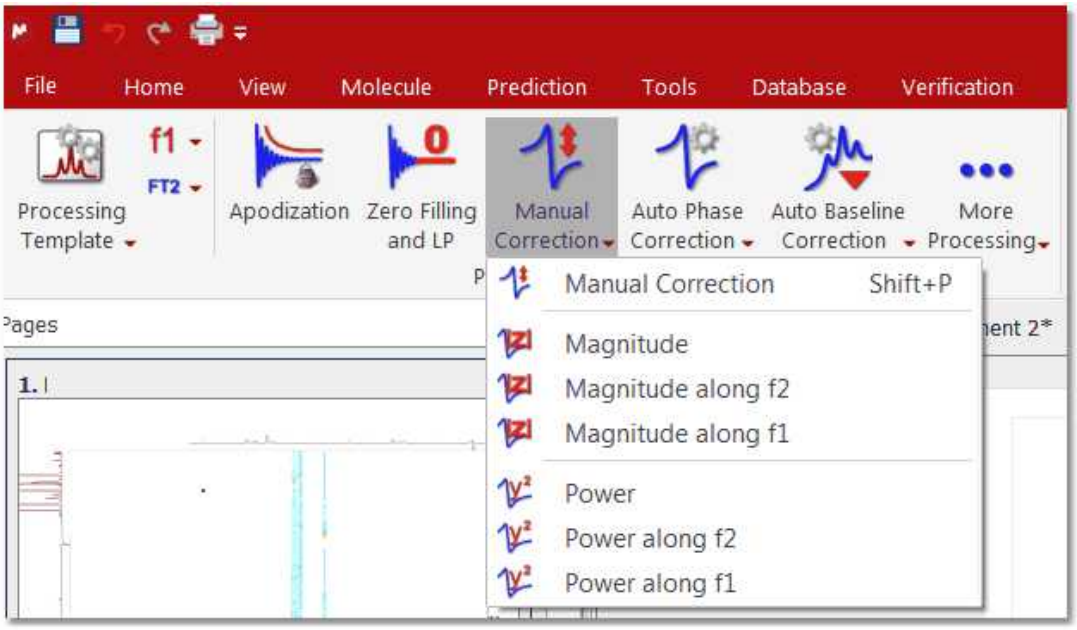
There are other easier alternatives if you only need positive peaks: representations in 'Magnitude' or in 'Power'.

Magnitude: It determines the representation of the final spectrum. If this option is checked you will see the absolute part of the spectrum; if it is unchecked you will only see the real part. As a spectrum is composed of complex vectors, its magnitude or module ***M*** can be calculated by the following expression:

$$M = \sqrt{\mathrm{Re}^2 + \mathrm{Im}^2}$$



To represent the spectrum in *Magnitude* with Mnova, just select Magnitude under the 'Manual Correction' scroll down menu:



Mnova will replace the real part of the spectrum by the magnitude (***M***) of each complex vector and the imaginary one by the phase angle of the vector ϕ, thus allowing reversal of this option. That is to say:

$$\mathrm{Re'}_i = M = \sqrt{\mathrm{Re}_i^2 + \mathrm{Im}_i^2}$$

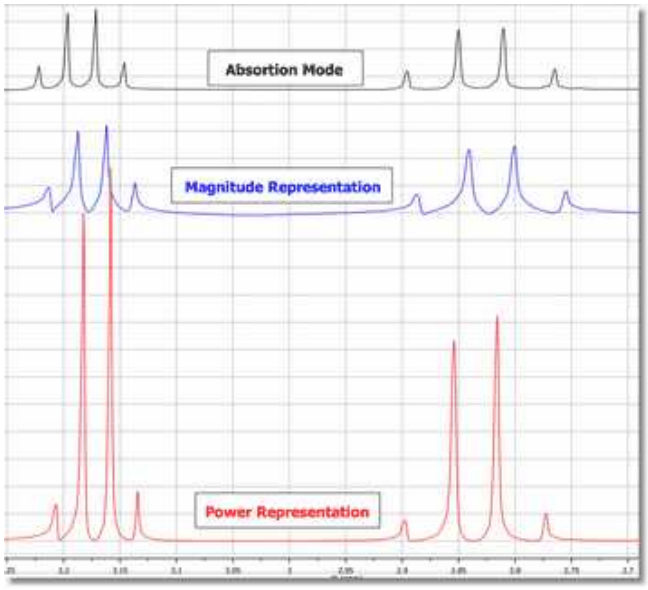
$$\mathrm{Im'}_i = \arctan\left(\frac{\mathrm{Im}}{\mathrm{Re}}\right)$$

Power: the power of the spectrum (***P***) is defined as the square of the magnitude:

$$P = M^2 = \mathrm{Re}_i^2 + \mathrm{Im}_i^2$$

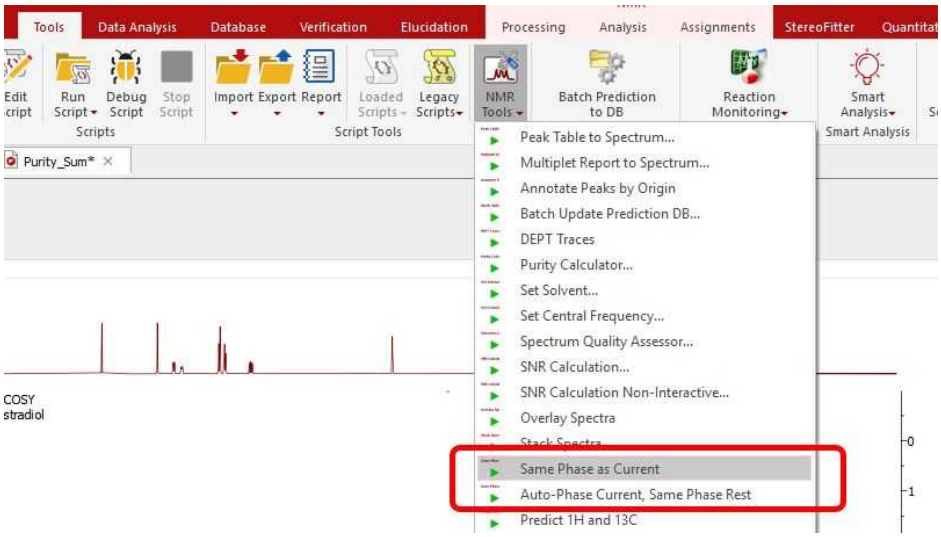
To represent the power of the spectrum, select **'Power'** under the 'Manual Correction' scroll down menu . The **'Power'** as well as the representation of the **'Magnitude'** are reversible options.

The **'Magnitude'** representation produces an increase in linewidth, whilst the **'Power'** representation distorts the intensities of the peaks:



Scripts to autophase importing autophase stacked spectra when importing.
Following the menu 'Tools/NMR Tools', you will find a couple of scripts to apply automatic phase correction to a stacked plot:

© 2024 MESTRELAB RESEARCH



- **Auto-Phase Current, Same Phase Rest**: the active spectrum is automatically phase corrected, and the resulting values of PH0 and PH1 are applied to the other spectra in the stacked.
- **Same Phase as Current**: PH0 and PH1 values of the active spectrum are applied to the other spectra in the stacked.

7.5 Baseline Correction with Mnova

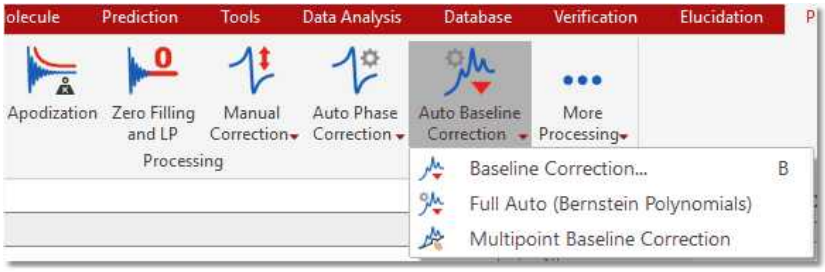
The baseline of a monodimensional spectrum is the theoretical line which connects the spectrum points which are not peaks (nor artefacts). When this baseline is not flat and/or it is offset from zero, many problems arise. Quantitative measuring of high resolution NMR demands a precise signal integration. These integrals are very sensitive to slight deviations of the spectrum baseline. The deviations are often due to a distortion of the first points of the FID (see First Point Correction and Linear Prediction). The origin of this kind of distortion is often the *transmitter breakthrough* effect (the detector needs a period to recover from the pulse effect in spite of being switched off when the pulse is applied). This could distort the first points of the FID producing a vertical displacement of the baseline, or even undulations, and, consequently, errors in the signal integrations. You can use a ' Backward Linear Prediction' to solve this problem on the time domain, or a 'Baseline Correction' as a better alternative.

Modern spectrometer hardware uses oversampling and digital signal processing to improve the baseline, but some undesirable broad signals arise from real sources. Thus, a more general solution would employ an efficient post processing baseline correction in the frequency domain. In fact, this is the most common approach found in NMR literature.

2D spectra suffer from baseline problems which may cause difficulties in the visualization of the full data set at once, since the signals of interest may be smaller than the baseline distortions; specially for phase-sensitive experiments and when a large residual solvent signal distorts the baseline. Thus, it turns out that baseline correction is a very important processing step to obtain good quality spectra.

Baseline correction is an area in which Mnova is particularly strong. The software implements Manual Baseline Correction and several automatic Baseline Correction algorithms (Polynomial Fit, Bernstein Polynomial Fit and our in-house developed algorithm, the Whittaker Smoother, which gives exceptional results in 1D and 2D data sets, is extremely fast and in the vast majority of cases fully automatic and is tolerant to varying linewidths along the same spectrum and to poor signal to noise ratios).
The Baseline Correction interface is also very simple. Just click on the **'Auto Baseline Correction'** icon and this will execute a 'Full Auto' Baseline Correction. If you click on the scroll menu, you will be allowed to choice between the algorithms available:

© 2024 MESTRELAB RESEARCH

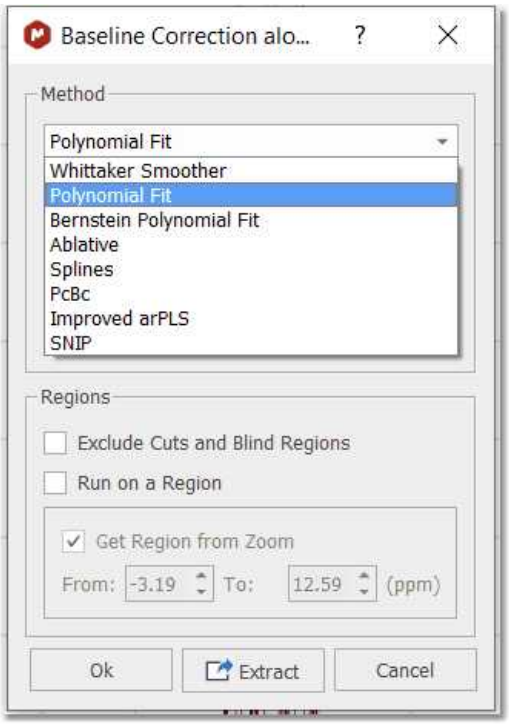


Novelty:

Using **Mnova**, you will able to adjust the phase after baseline correction, because Mnova will not discard the imaginary part of the spectrum after a baseline correction.

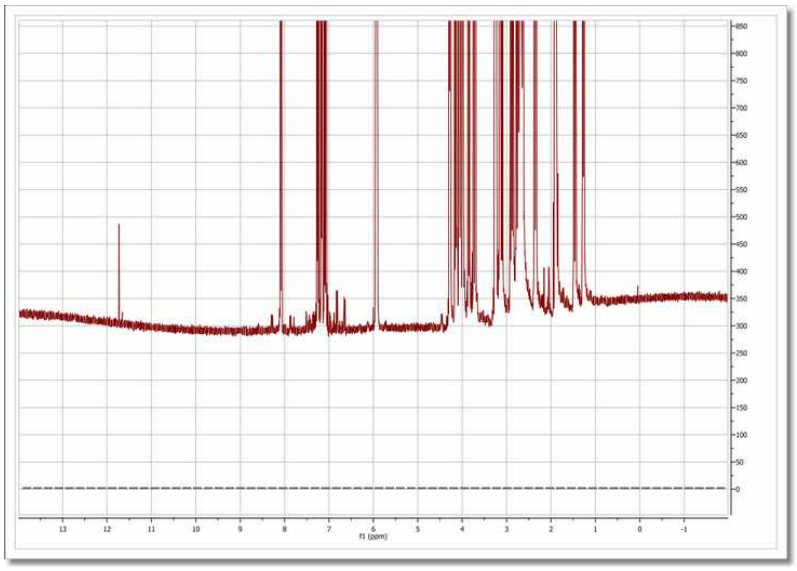
Mnova will be able to import the baseline correction from the spectrometer by following the menu 'File/Preferences/NMR/Import' and checking the applicable box.

THE BASELINE CORRECTION DIALOG BOX

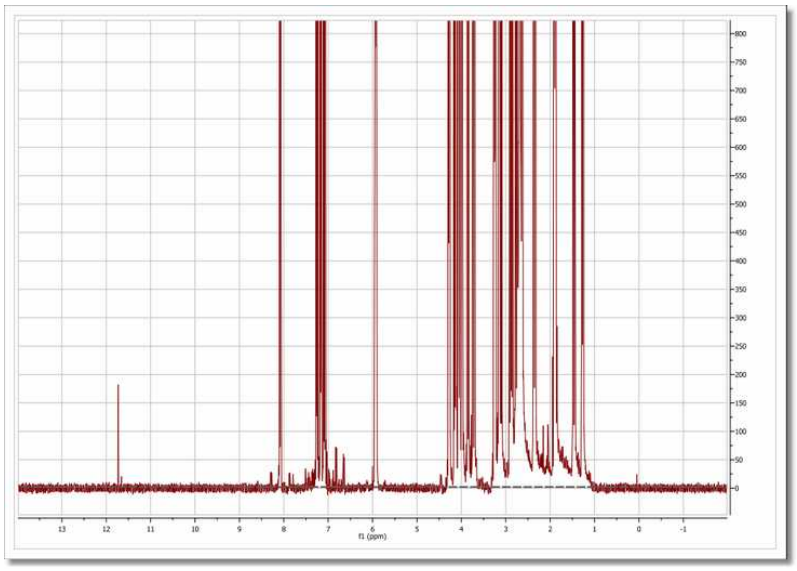


As a simple example of baseline correction, in the figure below we show a 1D spectrum in which the baseline distortions are evident. Note that the baseline not only deviates from the 0 level (represented by the gray dotted line), but it does also contain a significant rolling. You can choose the parameters of the baseline correction by dragging the parameter control (in the red circle in the 'Baseline Correction' dialog box above) or by introducing the values of the parameters directly into the edit boxes. You can also select 'Autodetect' if you prefer Mnova to calculate the best parameters for your spectrum.

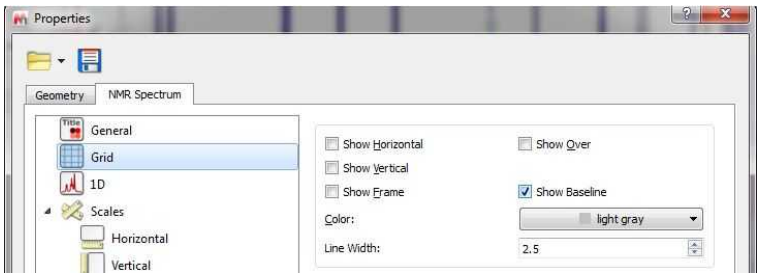
© 2024 MESTRELAB RESEARCH



After baseline correction (fully automatic), using the **Whittaker Smoother** algorithm, the baseline becomes perfectly flat as depicted in the figure below



NOTE: Follow the menu 'Properties/Grid' to draw the baseline by selecting the applicable option (very useful if you have removed the vertical scale):



Whittaker Smoother Algorithm: it is a new procedure for automatic baseline correction of frequency-domain NMR data sets which we show to be highly effective on 1D and 2D spectral datasets, and which preserves the range of component line widths which are present in the sample.

The algorithm consists of two independent processes:

1. Automatic baseline recognition (signal-free regions) based on a Continuous Wavelet Derivative transform (CWT) followed by iterative threshold detection in the Power mode domain.

2. A baseline modelling procedure based on the Whittaker smoother algorithm.

Overall, the algorithm has been designed to afford perfect baselines in NMR data sets spanning a wide range of possible baseline topographies and signal-to-noise ratio (SNR) conditions. In most cases, it can be applied successfully without any operator interaction, but further versatility is assured by two parameters which could be adjusted to guarantee an optimum outcome. The procedure can therefore be ‘‘tuned’’ to achieve more accurate baseline recognition of signal-free regions, or to increase smoothness at the expense of spectral fidelity, or vice versa.

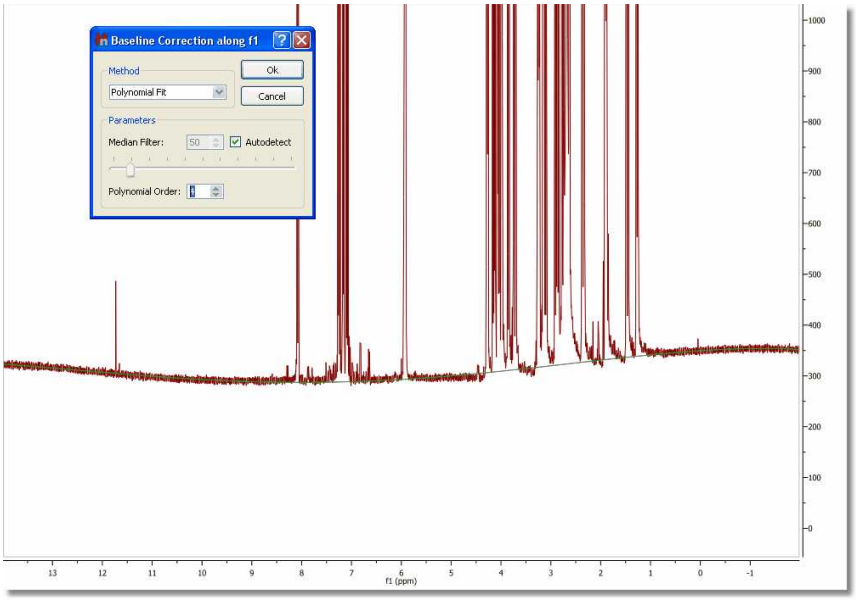
You can find more information about this 'in-house' developed algorithm in this reference: Cobas, J.C. *et al*. *J. Mag. Res*. *183*, **2006,** 145 and this mini review.

Polynomial Fit: In some cases, the employment of polynomial interpolations offers the advantage of smoothing the corners of each section. The user can adjust the polynomial from first to 20th order as well as the so-called 'Median Filter' (the user can see in **real time** the exact effect of the algorithm by a green line drawn on the spectrum display).

The polynomial used in this interpolation is:

$$Y = a_1 + a_2 x + a_3 x^2 + a_4 x^3 + \cdots\cdots + a_N x^{N-1}$$

where a_1-a_N are the coefficients to be fitted to the baseline and x the coordinates along the spectral axis.



© 2024 MESTRELAB RESEARCH

Bernstein Polynomial Fit: This interpolation is defined by the following polynomial:

$$Y = a_1(1-x)^{N-1} + a_2 x(1-x)^{N-2} + a_3 x^2(1-x)^{N-3} + \cdots\cdots + a_N x^{N-1}(1-x)^1$$

The user only needs to select the order of the polynomial (a_1-a_N) and then click 'Ok'.

For a 2D example, see the 2D NMR Processing Tutorial.

Please bear in mind that you can extract the baseline correction when you are working with 1D-NMR spectra by just clicking on the 'Extract' button. Once the baseline correction has been extracted, you can use it to correct the baseline of any other spectrum by using the 'Arithmetic' feature. You can see in the picture below that we have applied the previously extracted baseline correction of the sample 'js1pmquin' to the sample named 'STANDARD 1H OBSERVE' by applying a subtraction of the spectrum minus the baseline (Spectrum-Baseline=Spectrum with Baseline Correction).

Ablative Baseline Correction: powerful algorithm for 1D-NMR spectra without negative peaks.

The ablative baseline correction works in frequency domain on NMR spectra which do not have negative peaks, nor prominent negative artifacts. It works best on spectra with relatively sharp peaks (though typical “broad” labile peaks are generally not affected).

It works, basically, by repeatedly “shaving off” positive peaks. Since it does it by alternating up- and down- sweeps, it is reminiscent of the carpenter’s ablative tool used to produce smooth surfaces (which is where it takes its name).

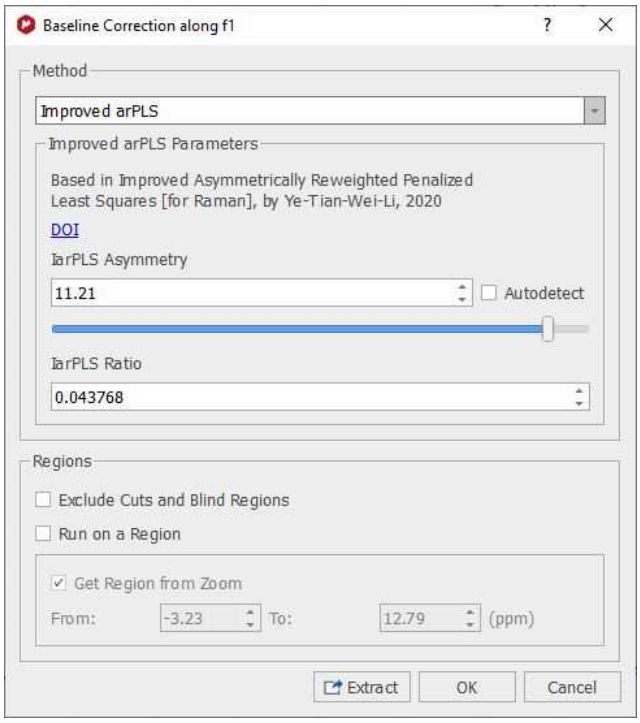
The algorithm gets negatively affected by negative-pointing peaks, including artifacts (like those from saturated water). In both cases the result is an improperly handled area extending over a significant portion of the spectrum. In many cases there is nothing of interest in that area, so the result is still perfectly usable. But sometimes it disturbs, in which case one should first get rid of the undesired features.
The algorithm gets also affected badly by Bruker smileys. Because of that, 2% of the spectrum on both ends are always set apart and considered as their own baseline. The result is that in the final spectrum, they get cut off.

Splines: it uses smooth curves to link the points. This method comprises two stages: First, it uses an algorithm that detects signals-free regions which are then connected, in a second stage, with splines to build the baseline model which is then subtracted from the original spectrum.

PcBC: this is an experimental method that works by simultaneously correcting the phase and baseline of a 1D spectrum. While it can be used for both purposes, it can also be applied.

Improved arPLS: a baseline correction method based on improved asymmetrically reweighted penalized least squares.

© 2024 MESTRELAB RESEARCH



The algorithm is very sensitive to the presence of downward pointing peaks (this may limit its applicability to poorly phased NMR spectra). It has an internal step (z-scores) that attempts to ignore sharp downward spikes when computing the baseline. Note that this may not work for not-too-sharp downward peaks.

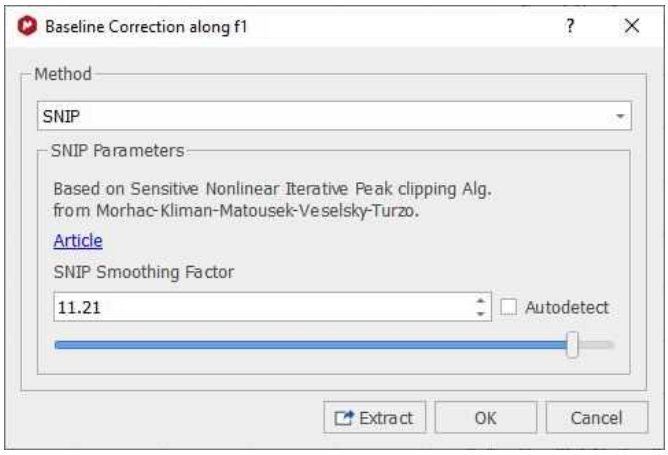
The "ratio" parameter is set to 1e-6. This value should work for most situations, and should not approach 1e-1 except for some 2D cases for which the number of iterations (limited to 50) are to be kept as low as possible for speed reasons. Otherwise relatively large values may lead to nonsense results.

The Log10(lambda) parameter modulates how closely should the baseline follow the spectrum values (not its "stiffness"), and is intended to be tuned by the user for each particular case.

This algorithm is based in this one (implemented for Raman): https://doi.org/10.1364/AO.404863

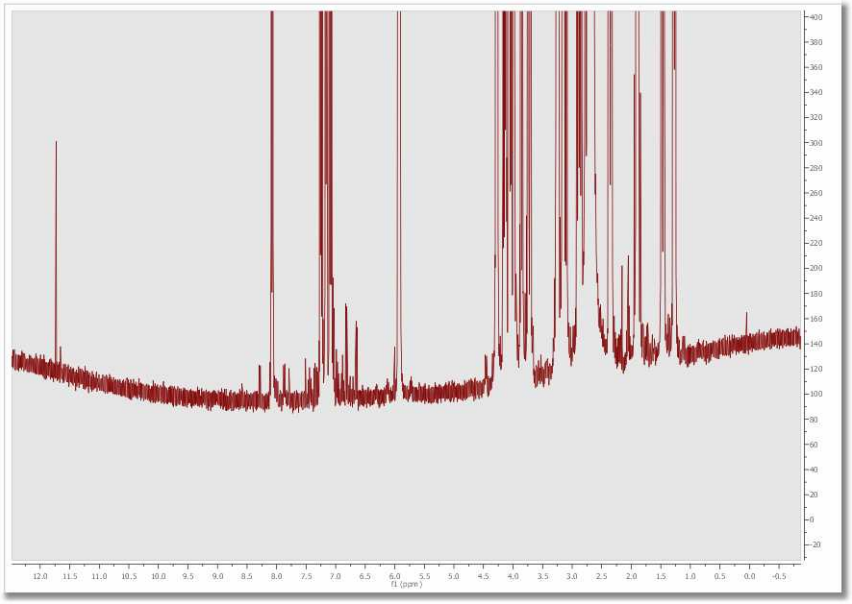
SNIP: Sensitive Nonlinear Iterative Peak baseline correction algorithm has been implemented based on this paper.

© 2024 MESTRELAB RESEARCH



Multipoint Baseline Correction: This method provides a way of modeling the baseline by selecting a well-distributed set of points that fall on the baseline and then interpolating between those points to complete the model. Please keep in mind that this method is not scriptable and can not be included in the Processing templates.

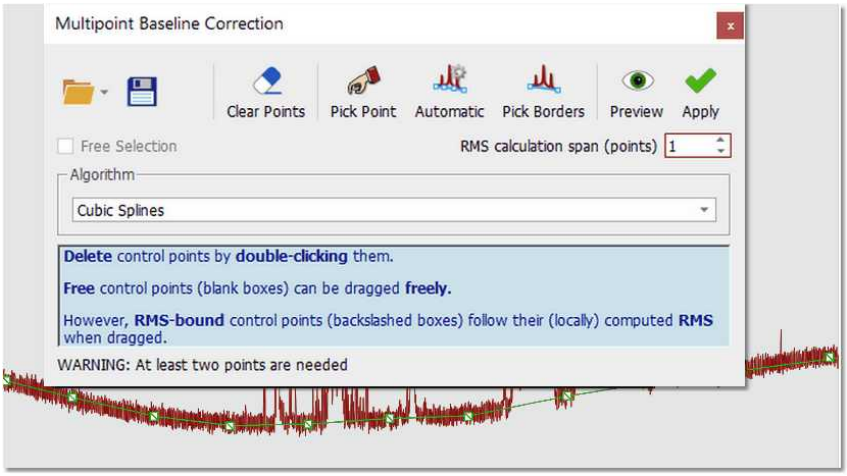
The following spectrum shows significant baseline distortion:



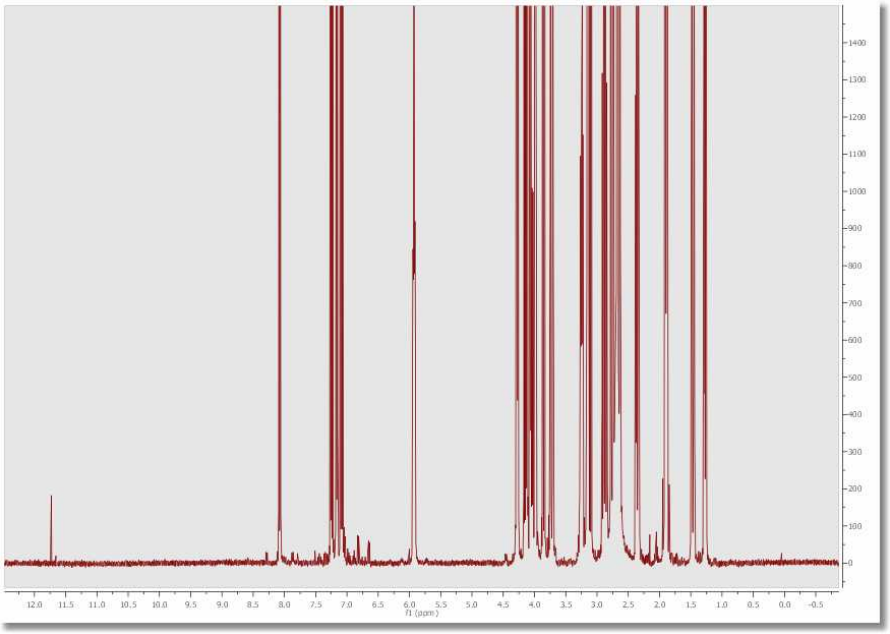
Next, select the 'Multipoint Baseline Correction' under the 'Auto Baseline Correction' scroll down menu.

This will display the 'Multipoint Baseline Correction' dialog box which will allow you to put down points along the baseline (by clicking on the 'Pick Baseline points' button ) to help the program to find the correct coefficients for the baseline correction equation that it will subtract from the spectrum. If you want to remove any undesired pick, just double click on it (click and drag if you want to change the location of the point).

© 2024 MESTRELAB RESEARCH



After subtracting the estimated baseline model, this is the resulting spectrum:



Let´s see the functionality of each button of the "Multipoint Baseline Correction' dialog box:

'**Load and Save':** to import and export multipoint baseline correction settings and points.

'Pick Baseline points': allows the user to pick the points. To remove any selected point (highlighted in green), just double click on it.

'Automatic' automatically add points for the baseline correction

'Pick Borders': allows the user to pick the first and the latest points.

'Clear all baseline': click on this button to delete all the selected points.

'Toggles Preview Showing': to see a preview of the baseline correction.

'Apply Baseline Correction': to apply the correction.

© 2024 MESTRELAB RESEARCH

'Free Selection' : to pick a point anywhere.

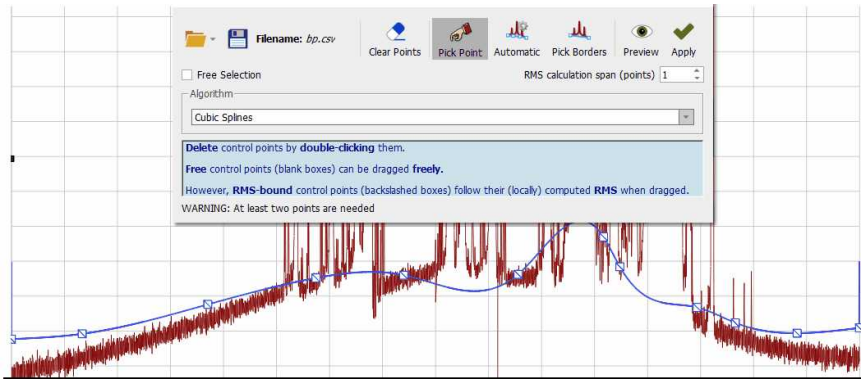
'RMS Calculation span (points): The multipoint baseline algorithm builds a baseline model using the points selected; but if you select this option; instead of use the specific points selected in the spectrum when clicked, it uses an average value calculated from the point and the surrounded points given by the "average window" value. For example, and average window of 0 will use exactly the clicked/selected point, and a average window of 2 will use the average of the clicked/selected point and 2 point from the left and 2 points from the right, ie, in total it will use 2*n+1 points where n is the average window

'Algorithm': from this drop down menu, we can select the algorithm for the baseline correction:

Segments: it simply connects the points with flat lines. This baseline will not be smooth, it just abruptly links the points with segments.



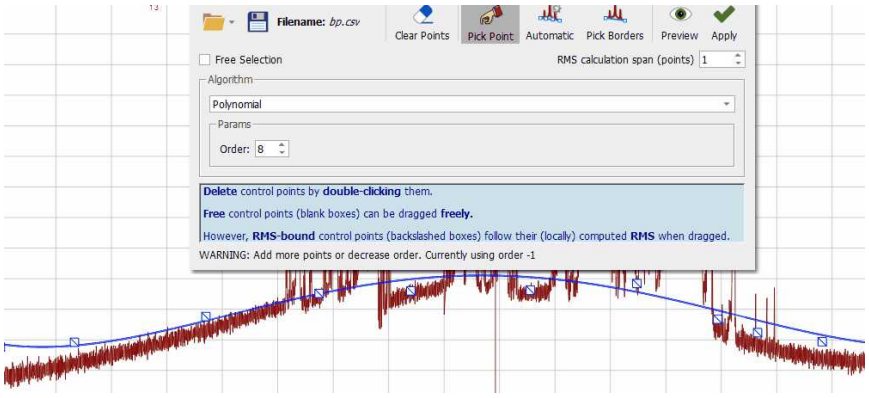
Cubic Splines: it uses smooth curves to link the points



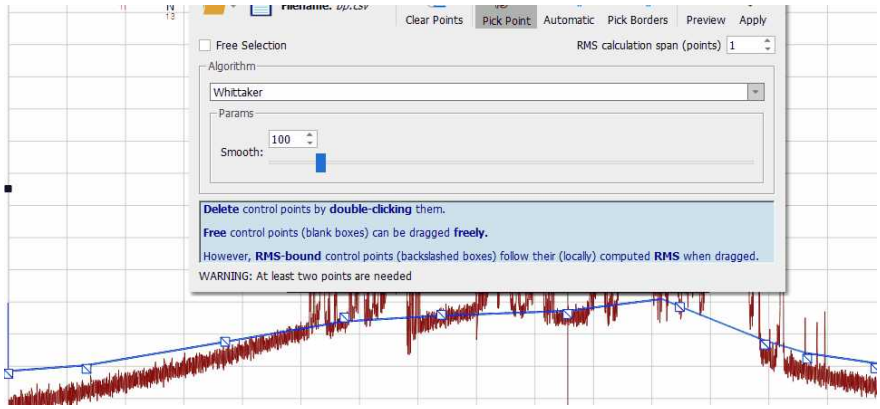
With segments and splines the baseline go thorough all the points defined by the user. This will not happen with the remaining algorithms:

Polynomial: the baseline will be adjusted to a polynomial function

© 2024 MESTRELAB RESEARCH



Witthaker: it will use the Witthaker Smoother algorithm to fit the baseline.



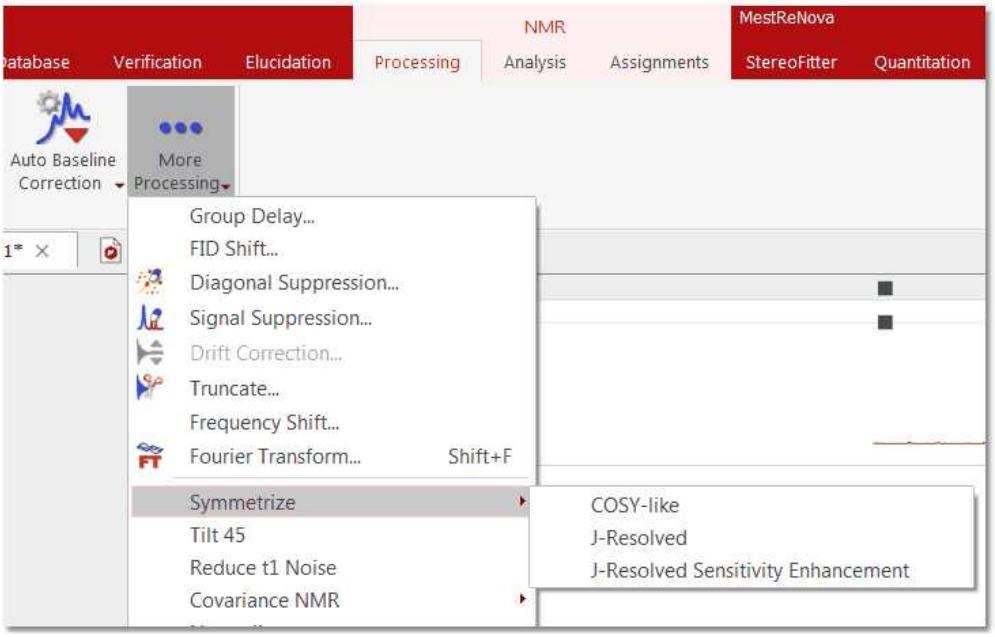
NOTE: After having applied a baseline correction in a 2D dataset, the manual phasing could be greyed out. To undo the baseline correction, follow the menu "Processing/Processing Template' and turn off the Baseline Correction for either dimension, click on OK and you will be able to correct the phase manually.

7.6 Symmetrization

Symmetrize (Not available in Mnova Lite):

This function, used in homonuclear correlation spectroscopy, replaces all signals which are symmetrically positioned about the diagonal by the smaller of the two signals. In this way unsymmetrically positioned noise and artifacts are removed. This technique should be used with caution since any symmetrical noise or artifacts will be preserved and may be mistaken for genuine signals. The data can be cleaned up considerably by using this tool in square data sets (homonuclear experiments).

© 2024 MESTRELAB RESEARCH



There are three modes available:

Cosy-like mode
The most common type of symmetrization is performed on COSY type data where cross-peaks are symmetric on either side of a diagonal. This type of symmetrization is performed by following the menu 'Processing/More Processing/Symmetrize/Cosy-like'. The command replaces the larger of two symmetrical points with a point that has the same magnitude as the smaller one. The user must know that this kind of symmetrization must not be used on phase sensitive data (because of the potential introduction of distortions to the cross peaks).

J-Resolved mode
For J-Resolved type data where the spectrum is symmetric on both sides of a horizontal line running through the middle of the matrix. The data point with the larger magnitude is replaced by one which has a magnitude equal to that of the smaller data point. This command is available by following the menu 'Processing/More Processing/Symmetrize/J-Resolved'.

J-Resolved Sensitivity Enhancement mode
For the new Sensitivity-Enhanced J-resolved experiments by a symmetry-based spectral self-combination procedure. With this new method the symmetry of cross-peaks along the horizontal F1=0 axis allows a success spectral data self-combination after reversing the F1 dimension. Signal-to noise ratio gains of 41% can be achieved without sacrificing resolution or spectral quality.

7.7 Tilt 45

Tilt 45 and Homonuclear *J*-resolved 2D-NMR spectroscopy (Not available in Mnova Lite)**:**

Homonuclear *J*-resolved two-dimensional NMR spectroscopy is used to generate one frequency dimension representing chemical shifts and another with coupling constant information. This technique is most frequently used with homonuclear J-resolved spectra where the effect of couplings in the f2 dimension is not required.

The sequence:

© 2024 MESTRELAB RESEARCH

$$d1 \cdots 90^{o} \cdots \frac{t_1}{2} \cdots 180^{o} \cdots \frac{t_1}{2} \cdots t_2$$

(where *d*1 is for relaxation and $t_1/2$ and t_2 are the two time dimensions for the two-dimensional experiment) uses the 180º pulse to refocus chemical shifts during t_1.

In a *J*-resolved spectrum, the ^{1}H frequencies are found on the horizontal axis (f2) with the normal 1D spectrum displayed at the top while the vertical axis (f1) contains only proton-proton coupled multiplets, each centered about a zero frequency point. Then, the 2D *J*-resolved spectrum may be 'tilted 45º' (a projection at an angle of 45º) along a horizontal line through the center of the matrix, so that all the lines of each multiplet line up in the f1 dimension. Each row of the 2D matrix is shifted by an amount proportional to its distance from the middle of the spectrum. If all the individual spectra are now added together, the result is a ^{1}H decoupled NMR spectrum which is normally shown above the 2D spectrum.

Finally, the user could clean up the spectrum by symmetrizing it about the f1 = 0 Hz row. As it is explained in the previous chapter, this consists of comparing the intensities in the spectra equally spaced above and below the f1 = 0 Hz row and taking the smallest value.

This command is available by following the menu 'Processing/More Processing/Tilt 45'.

7.8 Reducing t1 Noise

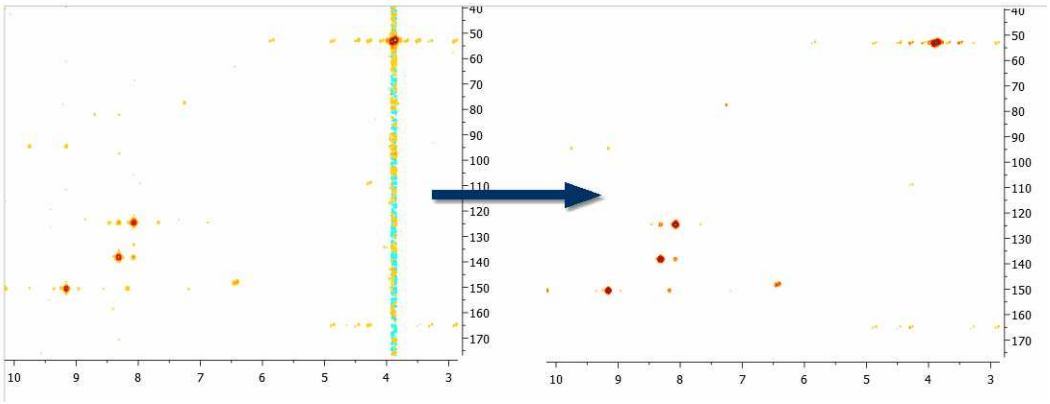
Reduce t_1 noise (Not available in Mnova Lite)**:**

t_1 noise is a particularly troublesome type of artifact which appears as streams of signals or ridges. The ridges (which are associated with strong signals in a contour plot), run parallel to the f1 axis and are found at their chemical shifts along the f2 axis. They can seriously interfere with the observation of real cross peaks from the signals.

The t1 command allows the user to clean up the spectrum along the f1 dimension by a factor proportional to the r.m.s. noise of that trace. It takes advantage of the fact that t1 noise is normally associated with the intense peaks which can be reduced without a significant loss of information.

The algorithm for t1-noise reduction has been designed to work with HSQC-like spectra.

This command is available by following the menu 'Processing/More Processing/Reduce t_1 noise'.



© 2024 MESTRELAB RESEARCH

7.9 Covariance NMR

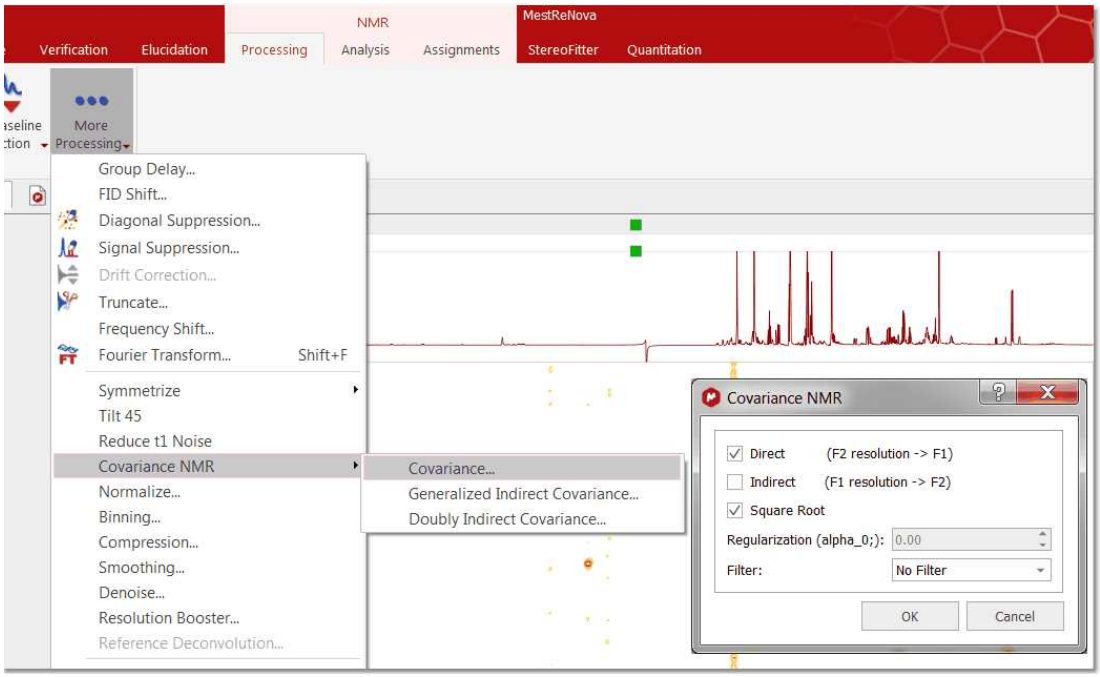
(Not available in Mnova Lite)

Resolution and sensitivity are two key factors in NMR spectroscopy. In the case of 2D NMR, the resolution of direct dimension (f2) depends, among other things, on the number of acquired complex points whilst the resolution of the indirect dimension (f1) is directly proportional to the number of increments (or number of acquired FIDs). In general, it could be said that the resolution along the direct dimension comes *for free* in a sense that increasing the number of data points does not augment the acquisition time of the experiment significantly. However, increasing the number of t1 data points (increments) has a direct impact in the length of the experiment as can be seen from the Total acquisition time for a 2D NMR spectrum:

$$T = n*N1*Tav$$

Where **n** is the number of scans per t1 increment, **N1** is the number of T1 increments and **Tav** is the average length of one scan. This usually means the resolution of the indirect dimension f1 is kept lower than that of f2.

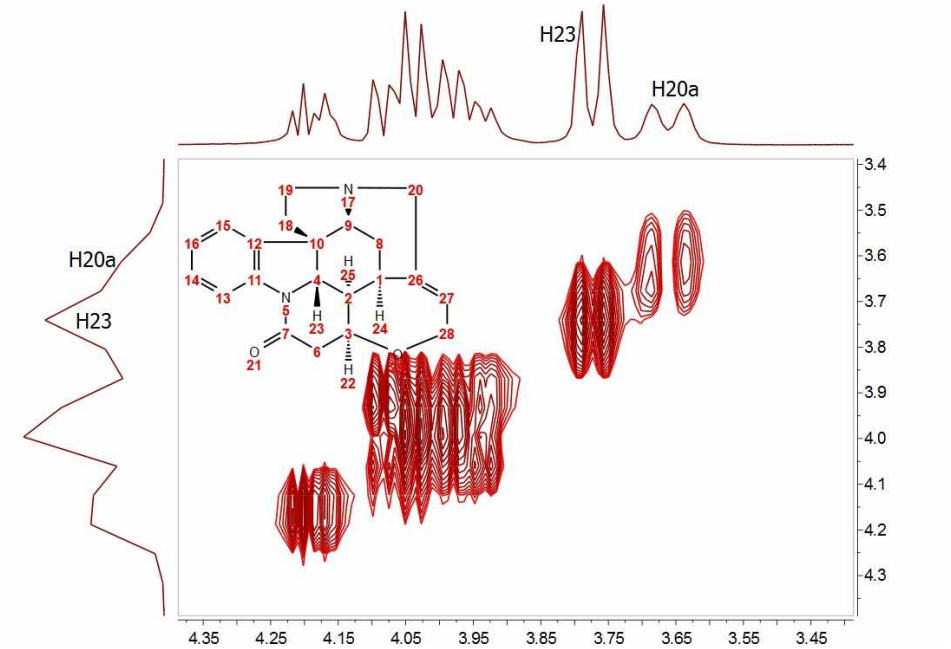
You can easily apply the Covariance NMR tool just by following the menu 'Processing/More Processing/Covariance NMR/Covariance'. This will display the 'Covariance NMR' dialog box which will allows you to select the 'Regularization Factor', the 'Filter' and an 'Indirect Covariance NMR'. For further information about the CoNMR options, please check this paper: JBiomolNMR (2007) 38, 73–77



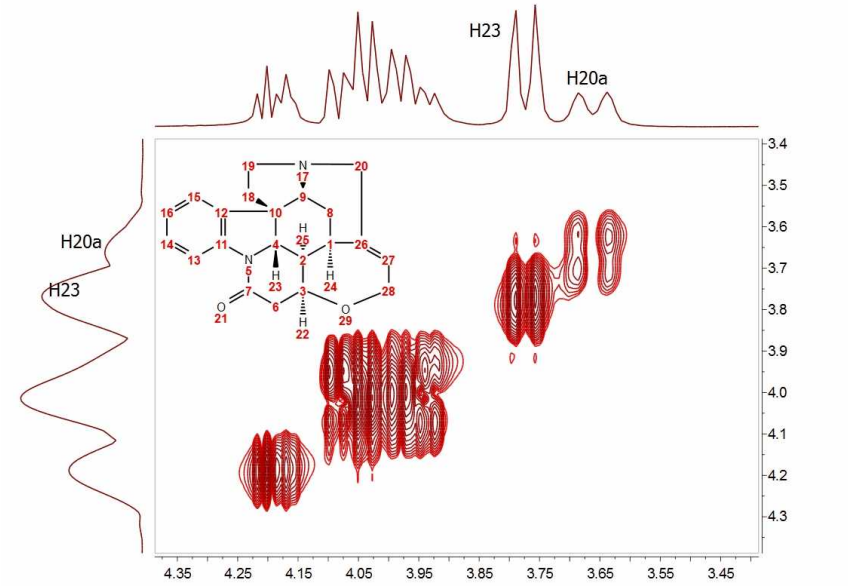
For example, let's consider the COSY spectrum (magnitude mode) of Strychnine which has been acquired with 1024 data points in the direct dimension and with 128 t1 increments. It's clearly appreciated that the resolution along F2 is much higher than the one along F1.

© 2024 MESTRELAB RESEARCH

You can easily see that doublets corresponding to protons H20a and H23 are resolved in F2 but not in F1.



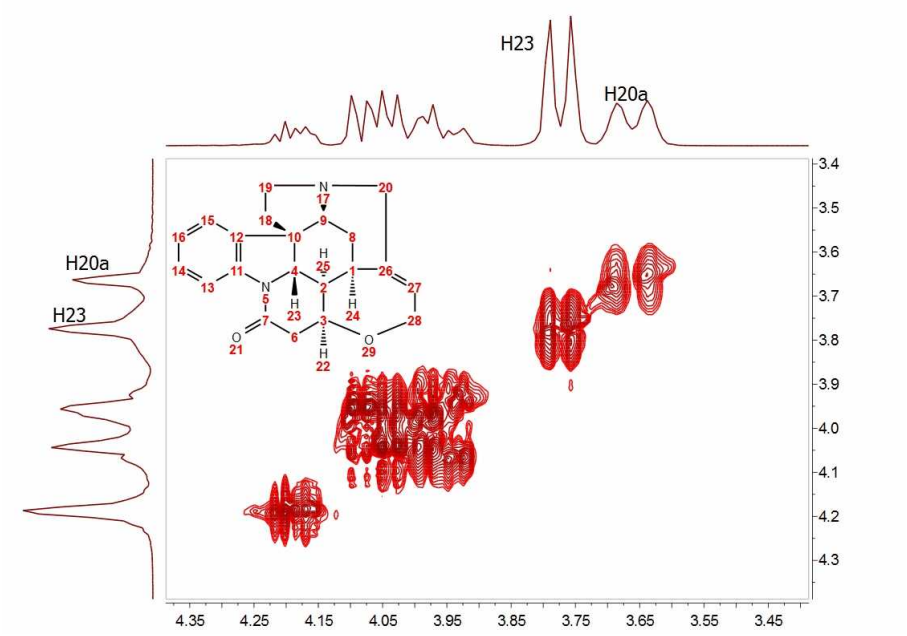
How could this be improved? We could try to extrapolate the FID (somehow) along the columns (F1) to a higher number of points (e.g. 1024). A well known technique is simply to add zeros, a process called **zero-filling** which basically is equivalent to a kind of interpolation in the frequency domain. For example, in this particular case we could try to extrapolate the FID along t1) from 128 to 1024 points in order to match the number of points along f2. The figure below shows the results:



© 2024 MESTRELAB RESEARCH

It can be observed now that the resolution along f1 is slightly higher than in the previous case but we cannot still appreciate the inner structure of the multiplets (e.g. H20a and H23). Zero Filling is certainly a good technique to improve resolution but of course, it cannot invent new information from where it does not exist. In this case we have zero filled from 128 to 1024 data points (e.g. 4 fold). In theory, zero filling by at least a factor of two is highly recommended because it enforces causality but beyond that, the gain in resolution is purely cosmetic. We might get more data point per hertz, but no new information is achieved as it's shown in the figure above.

Is there a better way to extrapolate the FID? Yes, and the answer is very evident: **Linear Prediction**. In few words, Forward Linear Prediction uses the information contained in the acquired FID to predict new data points so that we are artificially extending the FID in a more natural way than with Zero Filling. Of course, we cannot create new information with this process but the resulting spectrum will look better. This is illustrated in the next figure:

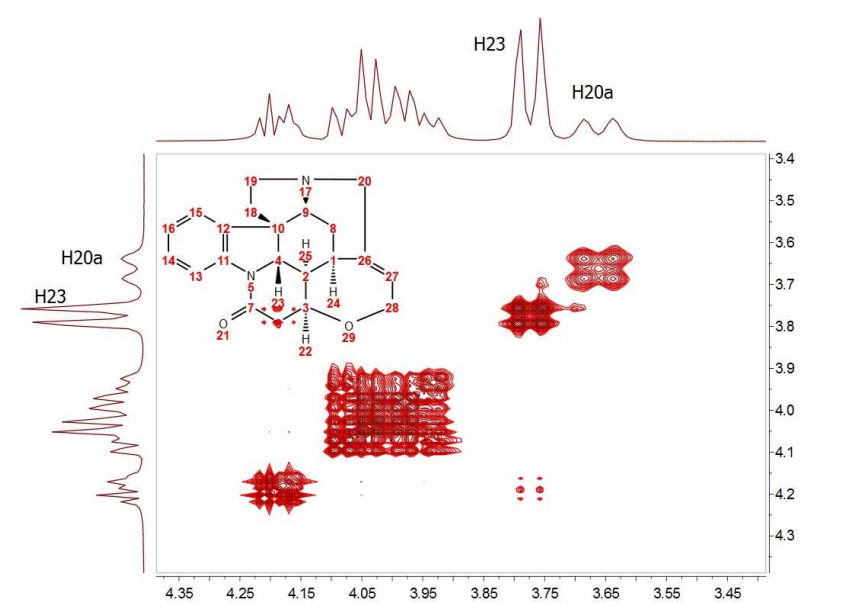


In this case, we have extended the t1-FID from 128 to 512 data points and then zero-filled up to 1024. Now we can see that the f1-lines are narrower but the couplings cannot be resolved yet.

In recent years there has been a great interest in the development of new methods for the time-efficient and processing of 2D (nD in general) NMR data. One of the more exciting methods is the so-called Covariance NMR, a technique developed by Brüschweiler et al. In fact, there are several types of Covariance NMR: Direct, Indirect Covariance NMR (there is a third method, Unsymmetrical Indirect Covariance which can be considered as a subtype of Indirect Covariance NMR). In this manual we will cover only the first type, Direct Covariance NMR leaving the other 2 types for future tutorials.

Before going any further with Covariance NMR, let's see the results of applying this technique to the same spectrum. This is what we get:

© 2024 MESTRELAB RESEARCH



At first glance, this result looks like a kind of magic: Now the splitting of H20a and H23 are clearly resolved in both dimensions. Actually, the resolution along F1 is virtually analogous to that of the F2 dimension.

How did we arrive to such a good result? From an intuitive stand point, what we have attained here was a transfer of the resolution of F2 to the F1 dimension. In other words, we have applied a mathematical process which takes advantage of the higher spectral resolution in the F2 dimension to transfer it to the F1 one.

Mathematically, direct Covariance NMR is extremely straightforward as defined by the following equation:

$$\mathbf{C = (F^T F)} \quad (1)$$

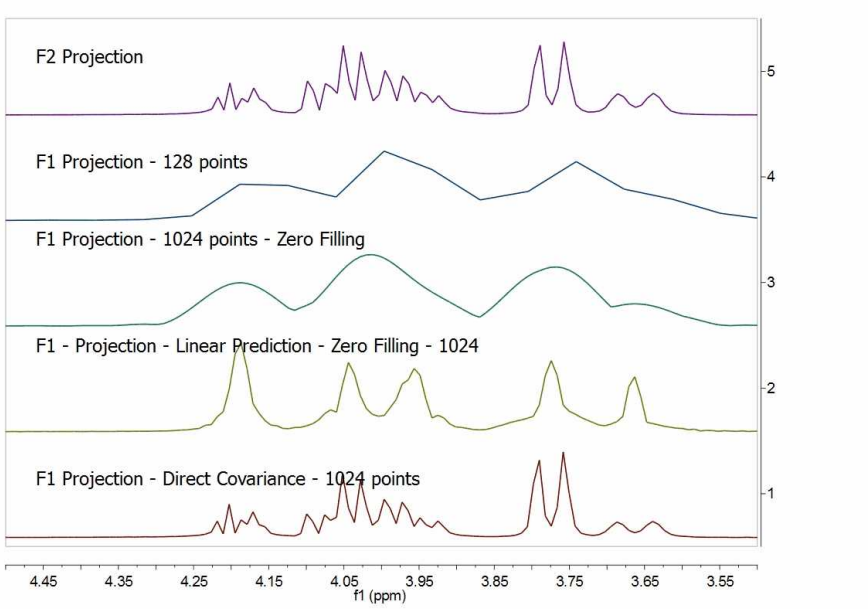
Where C is the symmetric covariance matrix, F is the real part of the regular 2D FT spectrum and F^T its transpose (NOTE: direct covariance NMR can also be applied in the mixed frequency-time domain, that's, when the spectrum has been transformed along F2. In this case, a second FT will not be required – neither apodization or phase correction along the indirect dimension).

In order to approximate the intensities of the covariance spectrum to those of the idealized 2D FT spectrum, the square root of C should be taken. Root squaring may also suppress false correlations that many be present in $F^T F$ due to resonance overlaps. Taking the square root of C matrix is in practice done using standard linear algebra methods (in short, diagonalizing the matrix and then reconstruct $C^{1/2}$ using eigenvectors and square root of eigenvalues).

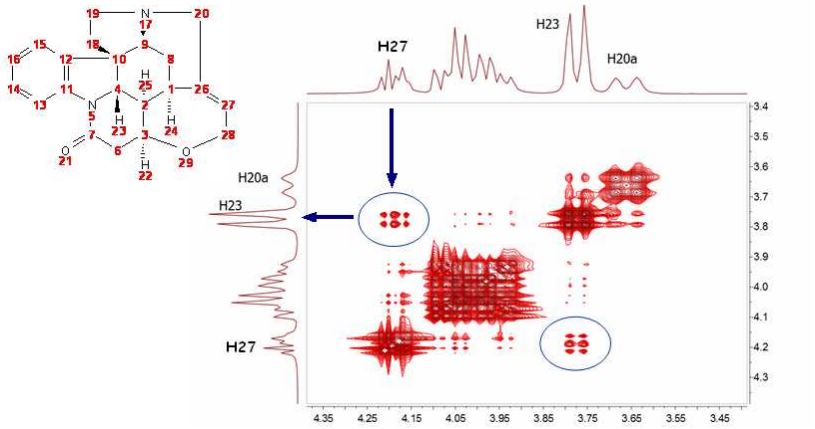
So direct Covariance NMR is able to produce a 2D spectrum in which the resolution in both dimensions is determined by that resolution of the spectrum in the direct dimension.

Let´s see a stacked plot representation with the 1D projections obtained from the different processing methods described here (Zero-Filling, Linear Prediction and Direct Covariance NMR). You can see in the picture below the power of the Covariance NMR method.

© 2024 MESTRELAB RESEARCH

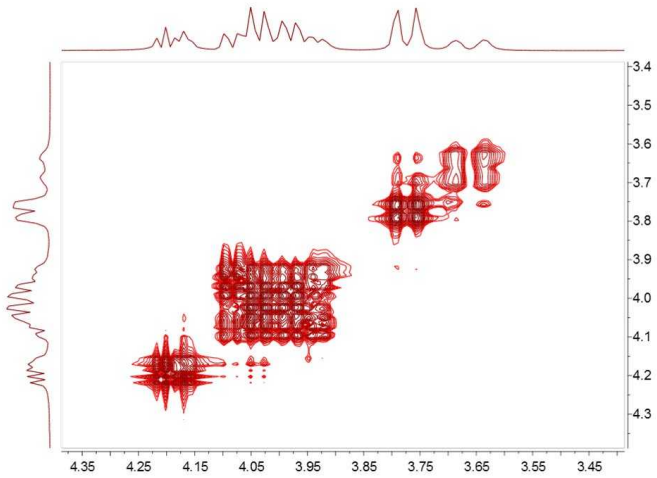


Covariance NMR spectroscopy provides maximal resolution along the indirect dimension, but when the number of acquired data points is too small, the covariances exhibit poor statistics that manifest themselves as spurious cross-peaks. For example, in this case we can see some unexpected cross peaks (e.g correlation H27 and H23 which in principle should not appear in a GCOSY spectrum, but rather in a TOCSY spectrum). Some other artifacts might also arise when protons of different spin systems are coupled to resonances with overlapping proton multiplets.



Mnova includes a novel filter which addresses this situation. This filter combines the standard 2D FFT spectrum with the CoNMR version in such a way that the resulting spectrum, while keeping the high spectral resolution along F1, it's free of artefacts:

© 2024 MESTRELAB RESEARCH

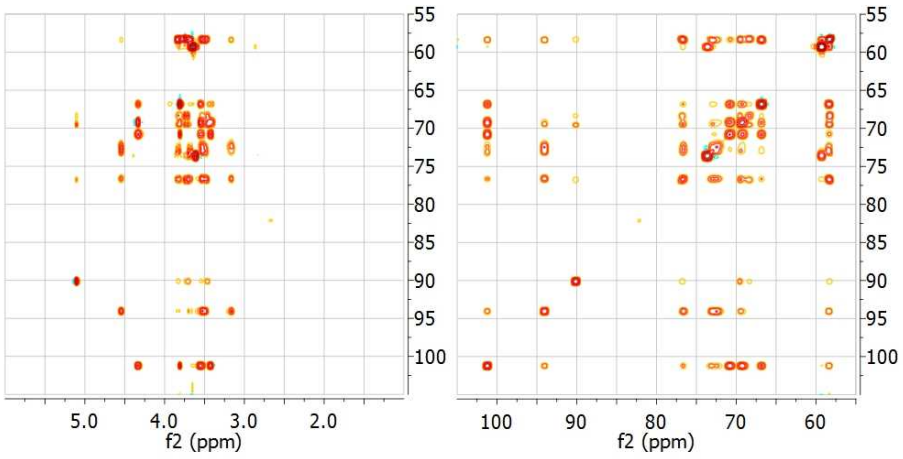


INDIRECT COVARIANCE NMR

Interestingly, if we have a heteronuclear spectrum, we can transfer the correlation information from the indirect dimension to the direct one by simply changing the order in which the multiplication is carried out:

$$C_{indirect} = (F \cdot F^T)^{1/2}$$

For example, the spectrum below shows the HSQC-TOCSY spectrum of sucrose (left) and its resulting indirect covariance counterpart (right) which contains essentially the same spin-connectivity information as a 13C-13C TOCSY with direct 13C detection:



The advantage is obvious: Indirect Covariance NMR yields a 13C-13C TOCSY correlation without having to detect the 13C nucleus. In other words, it brings in a sensitivity increase of 8 over a 13C

© 2024 MESTRELAB RESEARCH

detected experiment. Of course, in indirect covariance NMR spectroscopy, the spectral resolution along both frequency axes is determined by the sampling along the evolution t1 time.

UNSYMMETRICAL COVARIANCE NMR

You can also carry out **unsymmetrical Covariance NMR** with Mnova. However, as opposed to direct/indirect covariance NMR where one needs to deal with just one single spectrum, unsymmetrical NMR processing is probably not as intuitive as it's necessary to use 2 independent spectra and we have decided to implement it using a very open and flexible approach, that is, by using the spectral arithmetic module.

There two ways to apply an 'Unsymmetrical CoNMR' with Mnova:
1. Using the Arithmetic feature
2. **Using the Generalized Indirect Covariance NMR**

As you can see above, Covariance NMR is all about matrices operations. When you use direct/indirect CoNMR you simply multiply the spectrum matrix by its transpose where the order of the multiplication depends upon the CoNMR technique (direct vs indirect). The same concept applies to unsymmetrical CoNMR where you just need to multiply one spectrum by the transpose of another one.

Let´s see an example to apply an unsymmetrical CoNMR by using the **'Arithmetic'** feature of Mnova, assuming that matrix A corresponds to an HSQC spectrum and matrix B corresponds to a COSY (or TOCSY) spectrum, unsymmetrical CoNMR can be achieved by the following matrix operation:

C = A * TRANS(B)

Where TRANS denotes matrix transposition.

It's important to bear in mind that with any algebraic matrix operation, it's necessary that matrices A & B are compatible according to their row/column dimensions.

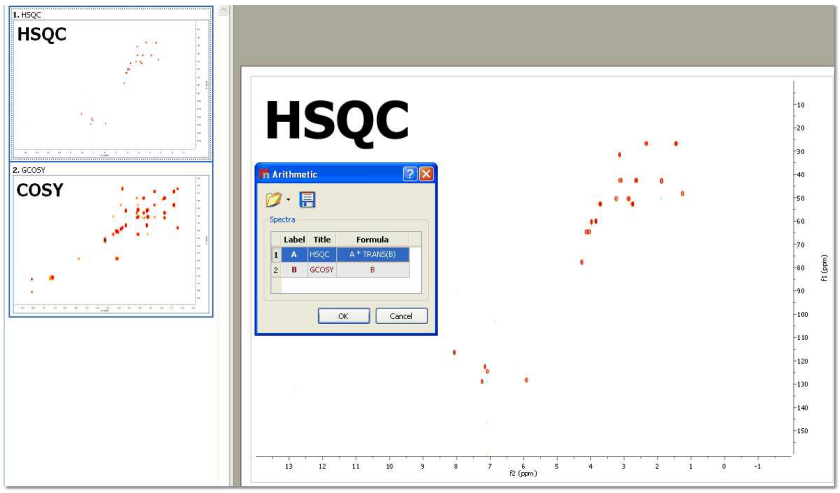
To carry out unsymmetrical CoNMR, just open the 2 individual spectra in Mnova, process then as usual (making sure that the sizes are compatible) and finally go to Processing/Arithmetic to enter the desire equation (e.g. A * TRANS(B))

Let´s see step by step, how to generate a HSQC-COSY from separate HSQC and COSY spectra with Mnova

1. Import the HSQC and COSY spectra into Mnova

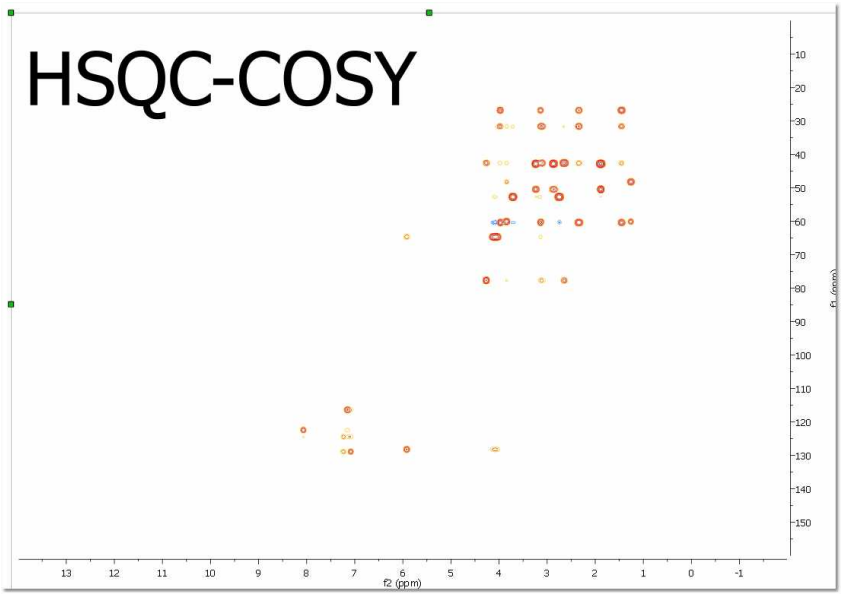
2. Select both spectra in the page navigator and follow the menu 'Analysis/More Tools/Arithmetic'

© 2024 MESTRELAB RESEARCH



Finally enter the equation as depicted above "A*TRANS(B)".In this case A corresponds with the HSQC spectrum whereas B is the COSY experiment. TRANS means the transpose operation.

Once the operation has been applied, click on the fit to highest intensity button  . You should get something like this:



Generalized Indirect Covariance NMR (GIC)

Multidimensional nuclear magnetic resonance (NMR) experiments measure spin-spin correlations,which provide important information about bond connectivities and molecular structure. However, direct observation of certain kinds of correlations can be every time-consuming due to limitations in sensitivity and resolution.

Covariance NMR derives correlations between spins via the calculation of a symmetric covariance matrix, from which a matrix-square root produces a spectrum with enhanced resolution. The covariance concept has also been adopted to the reconstruction of non symmetric spectra from pairs of 2D spectra that have a frequency dimension in common. Since the unsymmetric covariance NMR procedure lacks

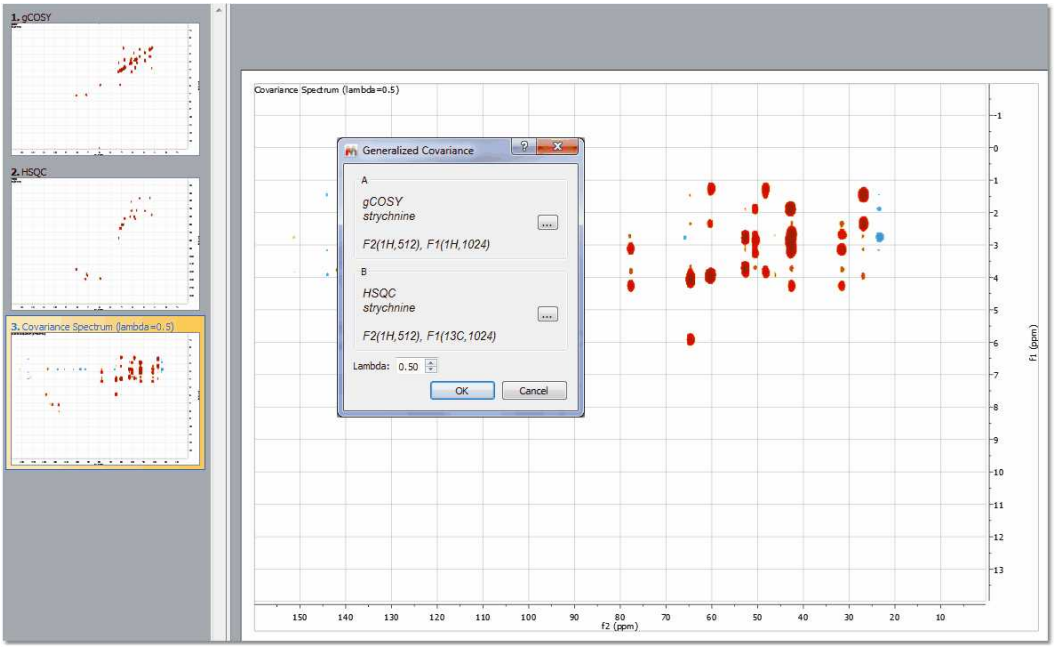
© 2024 MESTRELAB RESEARCH

the matrix square root step, it does not suppress relay effects and thereby may generate false positive signals due to chemical shift degeneracy. This generalized covariance formalism permits the construction of unsymmetric covariance NMR spectra subjected to arbitrary matrix functions, such as the square root, with improved spectral properties.

This method is basically the same as the above one (Arithmetic) but using a more rigorous mathematic formalism which allows to minimize the number of false positive peaks, because now the user will be able to do the square of the two matrix product (or any other values of the lambda parameter; ie: 0.25)

To use this method, just load your datasets and follow the menu 'Processing/More Processing/Covariance NMR/Generalized Indirect Covariance'. Finally select the spectra that you want to use and enter the value for the lambda parameter.

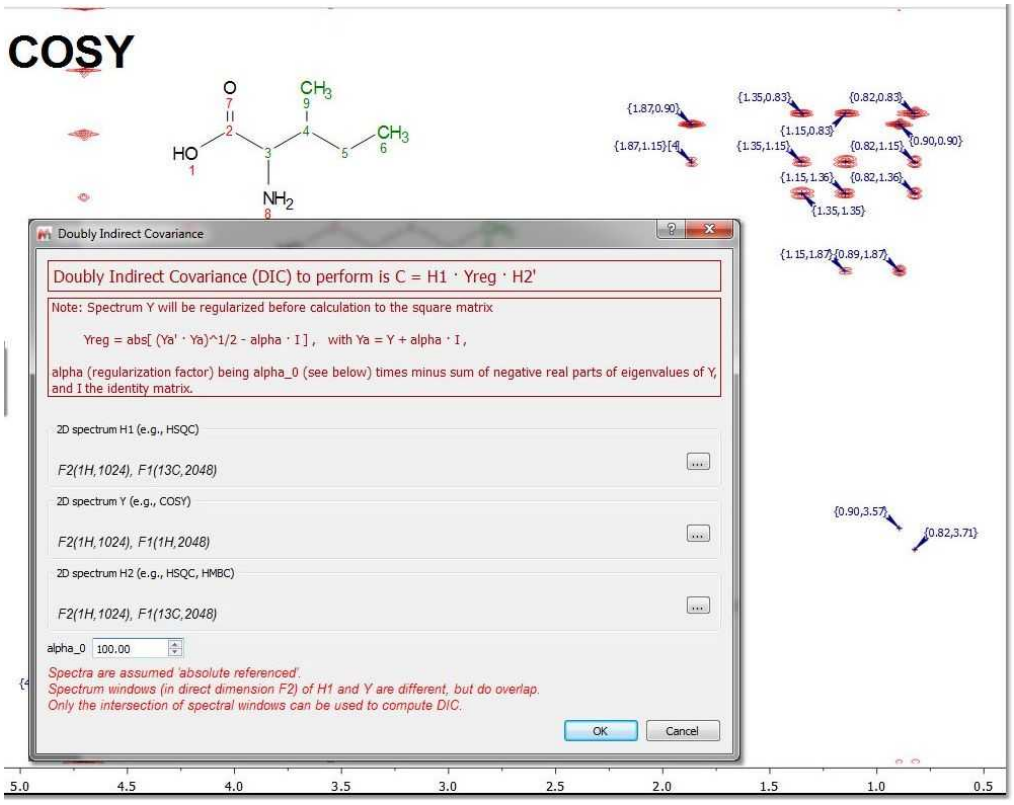
Please bear in mind that both spectra must have the same number of points in F2 to apply GIC



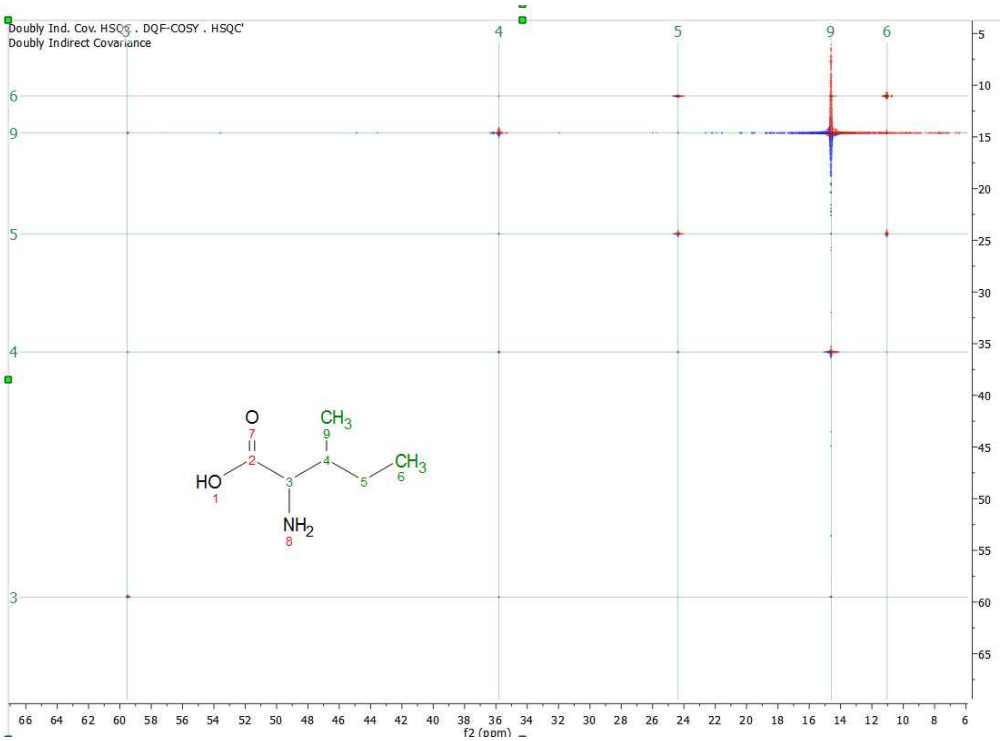
Doubly Covariance NMR

The Double Covariance NMR spectroscopy, is extremely useful for structure elucidation as resulting spectrum is actually a carbon-connectivity map that can be directly analyzed with basic graph theory to obtain the skeletal structure of a molecule or even of individual mixture components or their fragments.

In the example shown below, we have a COSY and a HSQC loaded in the the same document.



After having followed the menu 'Processing/More Processing/Covariance NMR/Doubly Indirect Covariance', we will get a synthetic spectrum (HSQC,HMBC) with the carbon-connectivity map:



For further information about CoNMR, please read our blog:

© 2024 MESTRELAB RESEARCH

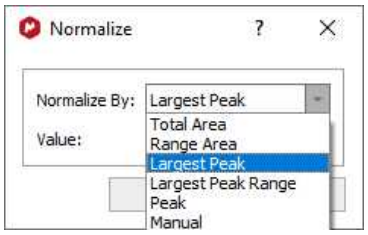
http://nmr-analysis.blogspot.com/2008/10/introduction-to-covariance-nmr.html
http://nmr-analysis.blogspot.com/2008/11/indirect-covariance-nmr-fast-square.html
http://nmr-analysis.blogspot.com/2008/11/removal-of-artifacts-in-direct.html

An this review: http://www.ebyte.it/library/docs/nmr14/2014_NMR_Cobas_Covariance.pdf
and this paper: JBiomolNMR (2007) 38, 73–77

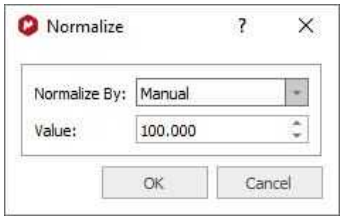
For further information about Doubly Indirect CoNMR: *J.Am.Chem.Soc*. 2010, 132,16922

7.10 Normalization

You can normalize the intensity of the spectrum by using this tool located in the 'More Processing Menu'. There are six different methods: total area, largest peak, largest peak range, a desired peak, a peak range or the 'manual normalization':



- Total Area: Set the total area of each spectra to a specified value. Note you can use Cut or Blind Regions tools to exclude regions (such as residual water peaks).
- Largest Peak: Set the height of the highest peaks to the specified value. Again you can use the Cut or Blind Regions tools to exclude some peaks.
- Largest Peak Range: Set the height of the highest peaks within a range (default: the current zoom in region) to the specified value.
- Peak: Set the height of the specified ppm point to a specified value.
- Peak Range: Set the sum-integral of a specified range (default: the current zoom in region) to a specified value.
- Manual: Multiply all points of the spectrum by the specified value. (100 in the example below):





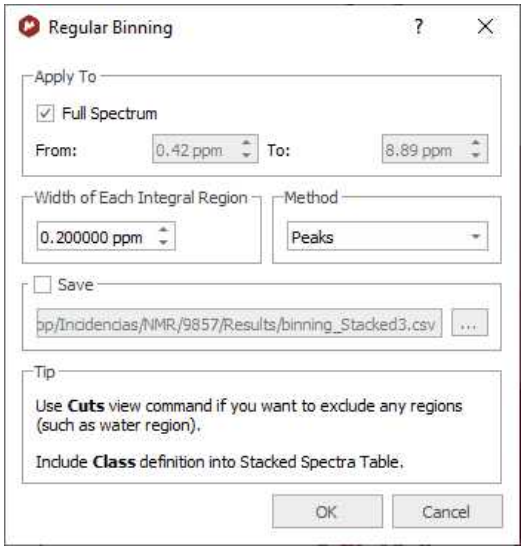
This tool is extremely useful when the user wants to stack spectra with different relative intensities and also after applying a binning.

Please bear in mind that peaks, integrals and multiplets will be scaled after a normalization.

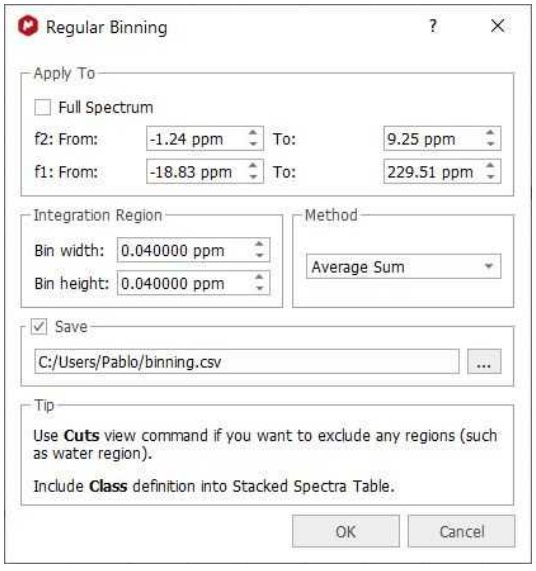
7.11 Binning

Spectral binning is a widely-used technique where the spectrum is subdivided into several regions (bins), and the total area within each bin is used as an abstracted representation of the original spectrum. The area encapsulated by a bin would ideally capture all of the area associated with a given resonance across all spectra in the dataset, thereby mitigating the effect of minor peak shift and line width variations for a compound across samples.

This command is available by following the menu 'Processing/More Processing/Binning'. Then, the 'Binning' dialog box will open and the user can choose the desired limits of the spectrum to apply the binning (or select Full Spectrum). The user can also select the width of each integral region. A typical 64k point NMR spectrum would be reduced using bin widths of 0.04 ppm, resulting in ~250 bin integral values.



You can also bin 2D datasets:



© 2024 MESTRELAB RESEARCH

The 'Sum' method will sum all the points of the bucket, the 'Average Sum' will divide the sum by the number of points on the bin, the 'Center' method will only return the value found in the middle of the bin. For example if the bin has 5 points it will return the value for 3rd point. The 'Peaks' method will use GSD for the peak picking.

There will be an option to save the matrix as CSV. The file generated is the same as the one obtained when saving as "Script: NMR CSV Matrix (Transposed).

Binning (also called bucketing) is a tool used in the multivariate analysis of NMR-based metabonomics data to address the NMR peak misalignment issue. In NMR metabonomics, small variations in the resonance position of the individual peaks caused by experimental and instrumental variations can adversely impact PCA results.

Fixed-width binning (usually at 0.04 ppm) is commonly used to alleviate the impact of the peak misalignment by averaging up the data points falling inside the bin width. However, since it drastically reduces the data resolution, it makes it more difficult to interpret the PCA results such as identifying the changed metabolites from the loadings plot.

The goal of binning a spectrum is to produce a new spectrum in which each new spectral data point corresponds to the integral of a bucket with a given width (e.g. 0.04 ppm). In other words, this is simply a data reduction operation analogous to bucketing integration. Once the spectrum has been binned, you can export it in ASCII format in order to use it for further PCA analysis.

It is often convenient to use the Normalize processing command to normalize the spectrum after applying a binning.

See also here an practical example of this feature

7.12 Compression

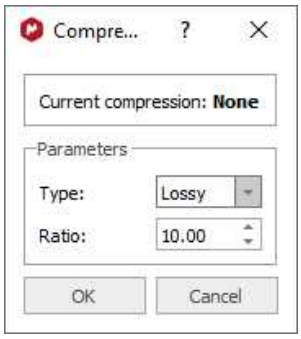
Compression (Not available in Mnova Lite)**:**

A typical 2D experiment could be as large as 64Mb, whereas the final size of a processed 4D spectrum reaches easily the gigabyte range, resulting in a considerable burden on the data storage and backup systems and in low processing efficiency. Furthermore, these data must be transmitted and stored on computer networks. It is clear that advances in technology for transmission or storage are not sufficient to solve this problem. These considerations clearly raise the issue of efficient data compression.

Mnova incorporates a fast in-house developed wavelet-based compression algorithm, which is generally applicable to 2D NMR data sets (obtaining high compression rates; up to 800:1). We have tested this compression methodology with some examples of 2D spectra focusing on keeping the NMR signal information intact either on cases of qualitative purposes of signal assignment or on quantitative analysis as commonly required for solution structure calculations derived from nuclear Overhauser effects.

This command is available by following the menu 'Processing/More Processing/Compression'. Then an 'Open' dialog will be shown:

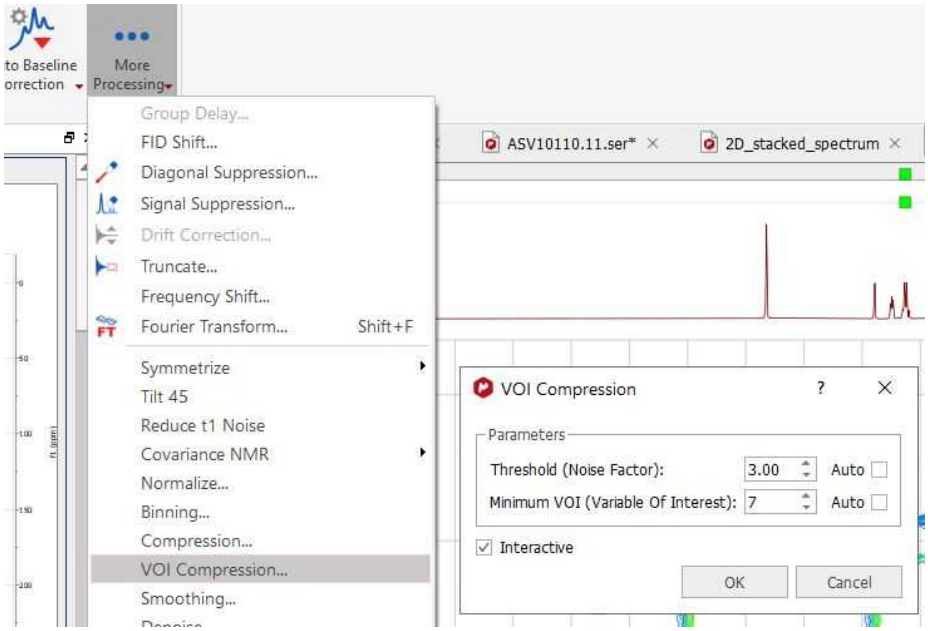
© 2024 MESTRELAB RESEARCH



The user can select the Compression Ratio or select 'Type:Lossless'. Lossless data compression allows the exact original data to be reconstructed from the compressed data.

7.13 VOI Compression

This compression algorithm is useful for 2D NMR datasets in order to get a denoised spectra:



The VOI Compression widget has the following options:

- Threshold (Noise Factor): it is a decimal value between [0.1, 10.0] that represents the factor that multiplies the noise value of the spectrum (calculated internally by the program). The automatic value is set to 3.0.
- Minimum VOI (Variable of Interest): it is an integer between [1, 100] that represents the minimum number of connected points (surrounding) to considered a point as a valid peak. The automatic value is calculated as the median of the peaks' half width.
- Interactive: if this check box is activated, then the VOI compression in applied any time that the Threshold (and/or Minimum VOI) changes in the widget

The feature is available in the 'Processing/Layout Template' and also from the PCA data preparation.

For further information about this compression algorithm, please check this paper (DOI: 10.1039/C7CC09891J)

© 2024 MESTRELAB RESEARCH

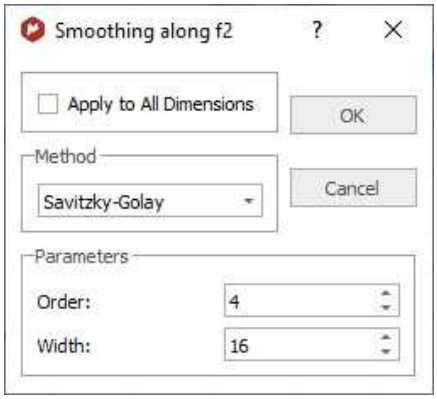
7.14 Smoothing

Smoothing (Not available in Mnova Lite)**:**

The signal to noise ratio (S/N) of a spectrum can be enhanced by smoothing (or filtering) techniques. This technique is used to reduce the effect of noise on a spectrum and possibly reveal signals embedded in, or distorted by, noise. Noise contains rapid fluctuations which are are generally faster than the rates of change of genuine signals. Smoothing applies a low-pass filter to the spectral data to remove these rapid fluctuations while having minimal effect on signals.

Mnova incorporates some of the more used signal smoothing algorithms.

This command is available by following the menu 'Processing/More Processing/Smoothing'. Then, a dialog box will open:



The user can select the smoothing algorithm (Moving Average Filter, Whittaker Smoother, Savitzky-Golay or Wavelets) in the Method scroll bar. The user can also apply the smoothing to All Dimensions (by ticking the applicable box).

'Moving average algorithm' : The simpler software technique for smoothing signals consisting of equidistant points is the moving average. An array of raw (noisy) data (y_1, y_2, …, y_N) can be converted to a new array of smoothed data. The "smoothed point" $(y_k)_s$ is the average of an odd number of consecutive 2n+1 (n = 1, 2, 3, ..) points of the raw data y_{k-n}, y_{k-n+1}, …, y_{k-1}, y_k, y_{k+1}, …, y_{k+n-1}, y_{k+n}, i.e.

$$(y_k)_s = \sum_{i=-n}^{i=n} \frac{y_{k+i}}{2n+1}$$

The odd number 2n+1 is usually named filter width (or span in Mnova). The greater the filter width the more intense the smoothing effect.

The moving average algorithm is particularly damaging when the filter passes through peaks which are narrow compared to the filter width.

The user can select the desired filter width (Span) in the dialog box.

Whittaker Smoother: A smoothing algorithm is proposed by Whittaker in1923. If y is a series of m data points and z is the smooth series which should approximate y, we minimize:

© 2024 MESTRELAB RESEARCH

$$Q_2 = \sum_{i=1}^{m}(y_i - z_i)^2 + \lambda \sum_{i=2}^{m}(z_i - z_{i-1})^2$$

The first term measures the fit of z to y, the second term is a so-called penalty: it discourages changes in z. The influence of the penalty is tuned by the parameter λ; the larger a λ is chosen, the smoother z will be, at the cost of a worse fit to the data.

The user can select the Smooth Factor in the dialog box or let Mnova detect the best one automatically.

'Savitzky-Golay algorithm' : A much better procedure than simply averaging points is to perform a least squares fit of a small set of consecutive data points to a polynomial and take the calculated central point of the fitted polynomial curve as the new smoothed data point.

Savitzky and Golay showed that a set of integers (A_{-n}, $A_{-(n-1)}$ …, A_{n-1}, A_n) could be derived and used as weighting coefficients to carry out the smoothing operation. The use of these weighting coefficients, known as convolution integers, turns out to be exactly equivalent to fitting the data to a polynomial, as just described, and it is computationally more effective and much faster. Therefore, the smoothed data point $(y_k)_S$ by the Savitzky-Golay algorithm is given by the following equation:

$$(y_k)_s = \frac{\sum_{i=-n}^{n} A_i y_{k+i}}{\sum_{i=-n}^{n} A_i}$$

Sets of convolution integers, instead of the smoothed signal, can be used to obtain directly, instead, its 1^{st}, 2^{nd}, …, m^{th} order derivative, therefore the Savitzky-Golay algorithm is very useful for calculation of the derivatives of noisy signals consisting of discrete and equidistant points.

The smoothing effect of the Savitzky-Golay algorithm is not as aggressive as in the case of the moving average and the loss and/or distortion to vital information is comparatively limited. However, it should be stressed that both algorithms are "lossy", i.e. part of the original information is lost or distorted. This type of smoothing has only cosmetic value.

The user can select the 'order' and the 'Width'.

Wavelets: Wavelet thresholding is the basis of wavelet based noise reduction. For a function *f* with Gaussian noise

$$y_i = f(t_i) + \sigma\varepsilon_i \cdots (i \in \mathrm{N})$$

this means that the function *f* is restored.

Hard thresholding is a simple “keep or kill” selection. All wavelet coefficients below a threshold λ are zeroed.

© 2024 MESTRELAB RESEARCH

$$c_{j,k}^{hard} = \begin{cases} 0, & |d_{j,k}| \le \lambda \\ c_{j,k} & |c_{j,k}| > \lambda \end{cases}$$

Soft thresholding shrinks the coefficients towards zero:

$$c_{j,k}^{soft} = \begin{cases} c_{j,k} - \lambda, & c_{j,k} > \lambda \\ 0, & |c_{j.k}| \le \lambda \\ c_{j,k} + \lambda & c_{j,k} < -\lambda \end{cases}$$

The most important step is now a proper choice of the threshold λ.

A *universal threshold* consists of:

$$\lambda = \frac{\sigma\sqrt{2\log n}}{\sqrt{n}}$$

where *n* is the sample size and σ the scale of the noise on a standard deviation scale.

The overall procedure of noise suppression consists of a wavelet transform (WT), which yields the wavelet coefficients *cj*;*k,* followed by thresholding of these coefficients and by an inverse wavelet transform (IWT), which restores the original spectrum.

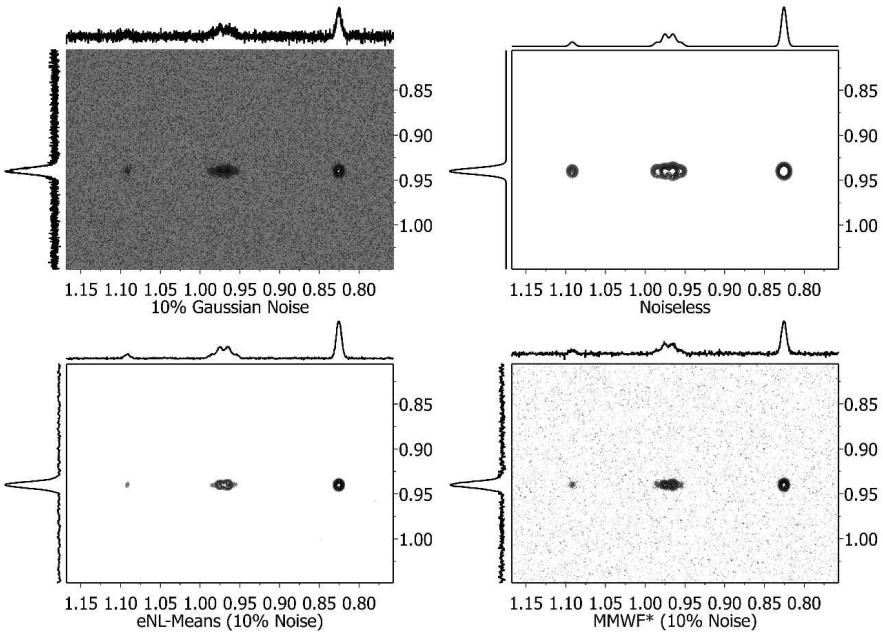
The user can select the scale (σ) and the fraction threshold (λ%) or also the soft or the Universal Threshold (by ticking the applicable box).

7.15 Denoise

In any experimental technique, the distinction between signals and noise is a real factor, and this is particularly the case with NMR because of its low inherent sensitivity. The application of the so-called 'spatial filters' has been proposed for the efficient denoising of images as well as for 1D and 2D NMR data sets.

Mnova includes a number of those filters aimed at reducing the noise level while maintaining the original spectral resolution.

© 2024 MESTRELAB RESEARCH

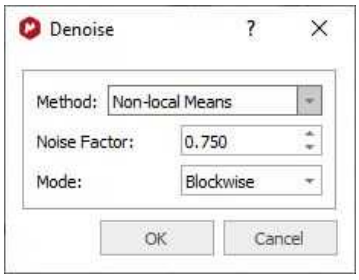


Comparison of the MMWF and eNL-means filter with a synthetic 2D spectrum 10% Gaussian noise (top-left). Top-right spectrum is the original noiseless spectrum whilst the two spectra at the bottom of the figure are the denoised spectra using MMWF* (bottom-right) and eNL-Means (bottom-left)*

Enhanced Non-Local Means

This is an algorithm based on the Non-Local Means denoised procedure which exploits the property that many structural similarities are present in different parts of the spectrum and that are not necessarily located in a local neighborhood. This algorithm is complemented by the application of a hybrid median algorithm used to remove potential spikes in the resulting NL-means denoised algorithm.

The **blocksize** is the default mode as it gives the highest denoising power. As a drawback, it is much slower than the other two modes (pixelwise and fast pixelwise).



Total Variation: denoising algorithm based on Total Variation by Chambolle's projection. Futher information about this algorithm can be found here.

© 2024 MESTRELAB RESEARCH

7.16 Resolution Booster

Resolution Booster (Not available in Mnova Lite)**:**

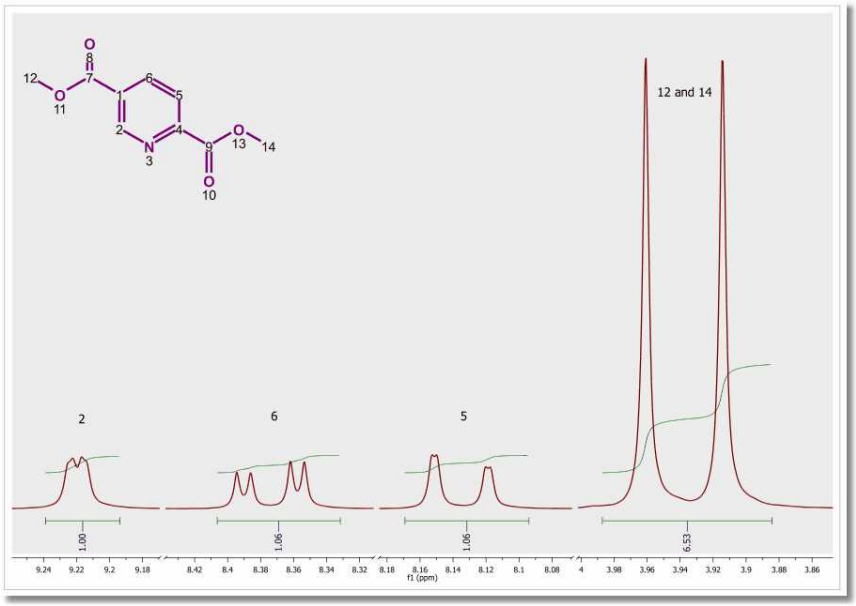
Resolution is a key concept in high resolution NMRand considerable effort (e.g. shimming) is usually devoted to ensure optimum resolution. High spectral resolution is important for the measurement of NMR parameters, especially for signal intensities, chemical shifts, and coupling constants. However, in many areas of high-resolution NMR the observed resonance lines are broadened in some undesirable way that may complicate, if not preclude, the accurate analysis of e.g. scalar couplings. Moreover, you can directly measure accurate values of *J* only when the splitting is much larger than the linewidth. The classical solution to the line broadening problem, other than using higher magnetic fields and assuming proper shimming, is multiplication of the FID by a resolution-enhancement function. More sophisticated procedures involve nonlinear data-processing methods such as the much-discussed maximum entropy method. The Filter Diagonalization Method (FDM) and Reference Deconvolution are other well known processing procedures that can be used for resolution enhancement.

The most frequently used methods for resolution enhancement are those which involve appropriate mathematical treatment of the FID (weighting). For example, one popular, routinely used weighting function is the Lorentzian/Gaussian transformation. Other well-known weighting functions include the TRAF, convolution difference, and sine-bell functions. Most of these methods have in common that they alter the FID such that its beginning is deemphasized, relative to the latter parts. Although these methods can improve resolution significantly, caution must be taken because information can be destroyed or artefacts created by extreme enhancement, such as that which results in large negative lobes on both sides of a signal.

The **Resolution Booster** is a new frequency domain post processing method which yields a considerable resolution enhancement with a fast calculation, while avoiding the negative artefacts of the other resolution enhancement functions.

Real Example:

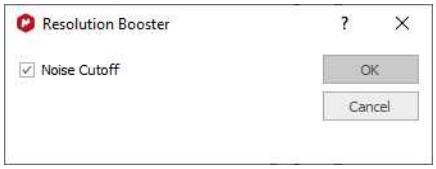
In order to illustrate the power of our Resolution Booster method, we will use as an example the spectrum of ***dimethyl pyridine-2,5-dicarboxylat***e acquired at 250 MHz:



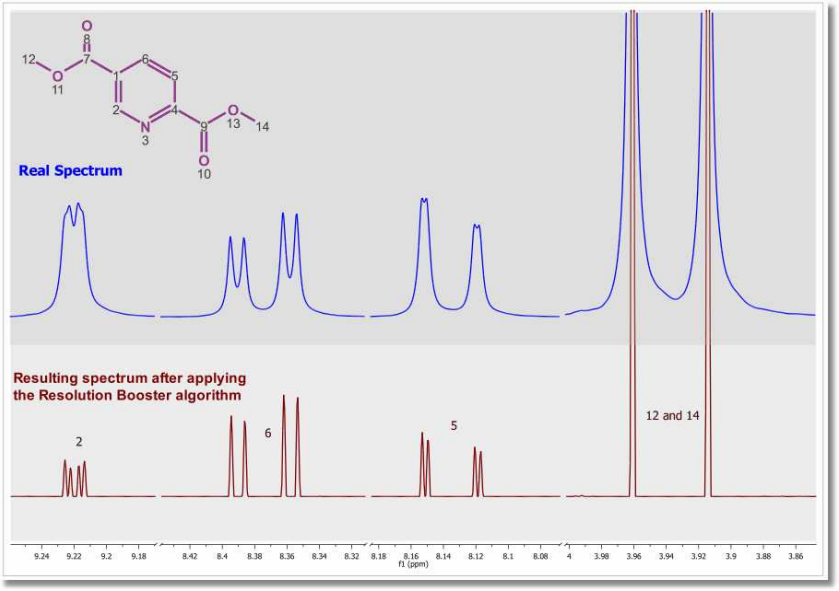
This spectrum has been processed without applying any weighting function and the resolution is **0.13 Hz/pt**. If we look at the signals corresponding to proton 2 in the structure, we can appreciate a small

splitting due to 4 bond coupling with proton 6. Proton 6 shows a large splitting due to 3 bond coupling with proton 5 and a small splitting due to the 4 bond coupling with proton 2. Proton 5 appears as a double doublet because of the 3 bond coupling with proton 6 and a small 5 bond coupling with proton 2. The latter is barely appreciated in the figure because of the lack of resolution. In fact, the same splitting should show in proton 2 but this can not be seen at this resolution level. It is important to remember that you can measure couplings constants accurately when the splitting is much larger than the line width.

Following the menu 'Processing/More Processing/Resolution Booster' will display the 'Resolution Booster' dialog box, where the user will be able to select a noise cutoff option:



In the picture below you will be able to see the result of applying the **Resolution Booster** method:



In this case, resolution has been increased by **~230%** making possible the calculation of the weak, long range coupling constants.



Note:

It is important to notice that this procedure may change relative intensities. On isolated lines, in principle, it is approximately proportional to the second derivative which, when all lines have the same line width (as they often do) is proportional to the line height. However, broad lines can get suppressed and unresolved humps and shoulders get resolved (+) but their intensities and, to some extent, positions can not be trusted (-).

Please bear in mind that we can not integrate the spectrum after having applied Resolution Booster as this algorithm is sensitive to the signals linewidths. If all the lines in the spectrum would have exactly the

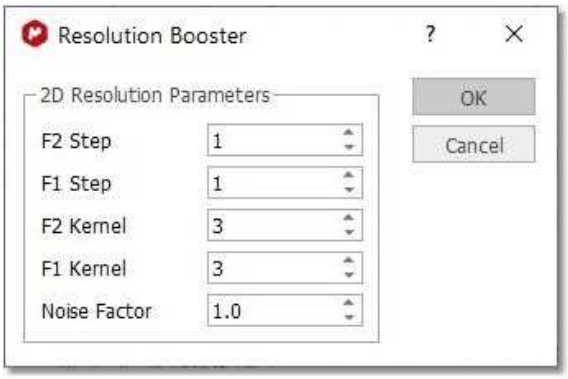
© 2024 MESTRELAB RESEARCH

same line width, Resolution Booster could also be considered a valid tool for qNMR analysys. But in real life, this is never true.

Thus, RB should be considered as a Resolution Enhancement tool which you can use for any of the following purposes:

- to measure chemical shifts & coupling constants more accurately
- to reveal signals which could otherwise be hidden and badly resolved because of spectral overlap

The Resolution Booster can also be used with 2D-NMR spectra (even with phase sensitive experiments):



See also:
http://nmr-analysis.blogspot.com/2007/11/resolution-booster.html
http://nmr-analysis.blogspot.com/2007/11/selective-resoluton-booster.html
http://nmr-analysis.blogspot.com/2007/12/introducing-2d-resolution-booster-rb.html

7.17 ElPulpo Resolution Enhancement

Spectroscopic techniques, particularly NMR, frequently encounter the challenge of overlapping peaks. Thus, the need to enhance the resolution of such spectra has been the subject of extensive research for decades. In NMR, the pursuit of higher resolution began with hardware advancements, notably the development of sophisticated shimming systems. These systems were designed to improve magnetic field homogeneity and stability, as well as the stability of the radio frequency (RF) sources used. However, these improvements are inherently limited by the natural linewidths of the resonance peaks of the samples, which are predominantly dictated by relaxation effects and molecular mobility. The latter is often insufficient to fully average out dipolar interactions and other inhomogeneities.

With the advent of computers in NMR technology, digitized spectra became the norm, and a persistent drive emerged to enhance resolution post-acquisition through computational algorithms. Over the decades, numerous algorithmic strategies have been proposed, yet the consensus in the scientific community suggests that the potential for further enhancements still exists.

In this context, we introduce "**ElPulpo**", a novel **resolution enhancement algorithm** specifically designed for 1D NMR spectra. ElPulpo distinguishes itself by its computational agility and its streamlined approach, requiring only one key parameter: the **'Reference Linewidth'**. Notably, a good default value of this parameter can be pre-estimated by means of another algorithm that we have used for years and integrated with ElPulpo.

Very importantly, ElPulpo was meticulously designed with the following requirements in mind:

a) It respects the intrinsic quantitative nature of NMR spectra, upholding its compatibility with quantitative NMR (qNMR) standards. Regardless of how ElPulpo affects the lineshapes of the

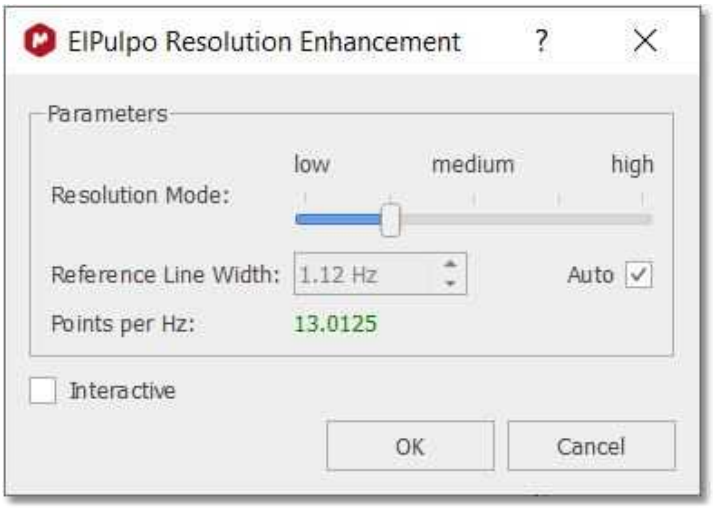
spectral peaks, it does not alter their integrals —both absolute and relative—affirming its suitability for qNMR analysis.

b) ElPulpo works in the frequency domain, applying a number of evaluation steps. All these steps are rigorously (mathematically) linear and therefore fully compatible with NMR spectra of mixtures, no matter how complicated.

c) ElPulpo keeps under control the propagation of experimental noise. In mode 1 (see below) it effectively attenuates noise by approximately 50%. In modes 2 and 3, the noise propagation factor is around 0.8 and 1.2, respectively. Even with the higher modes that most aggressively reduce the linewidths of spectral peaks, the noise propagation factor is kept below approximately 3.5.

d) ElPulpo operates both on the real and imaginary parts of the spectrum and, by its mathematical nature, maintains the Hilbert-transform relationship between them. In practice, this means that it is fully compatible with spectral phasing practices, both manual and automatic. Spectra can be phased or re-phased either before or after the application of ElPulpo, a property that is often very handy.

ElPulpo is available through the menu "Processing/More Processing…/ElPulpo Resolution Enhancement" and requires two fundamental parameters:

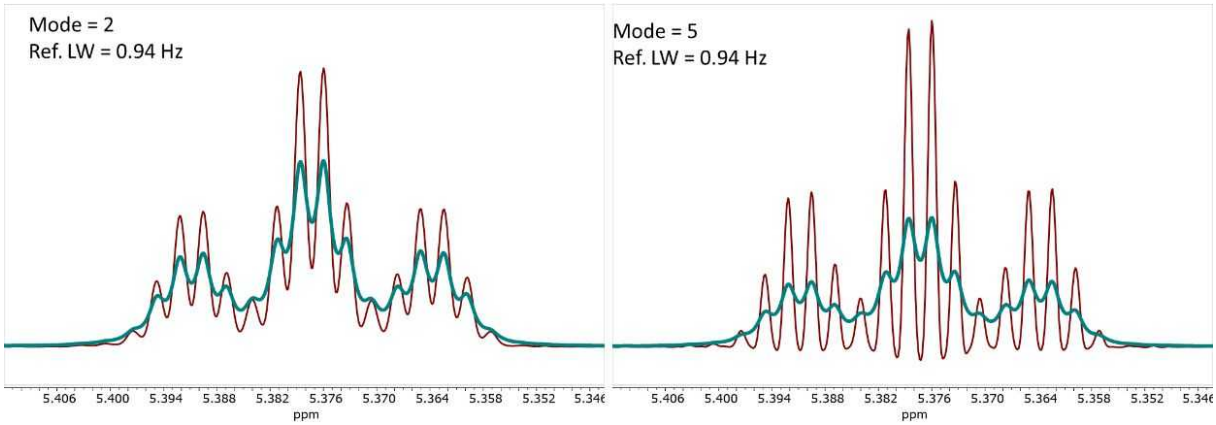


Resolution Mode: This parameter balances resolution enhancement against noise effects in the algorithm's operation. Lower modes (e.g., 1, 2) prioritize signal integrity, improving resolution conservatively while minimizing noise propagation. They are preferred when maintaining a high signal-to-noise ratio (SNR) is important.

Higher modes (e.g. 4, 5) lean towards more aggressive resolution enhancement, suited for scenarios where resolution is paramount, albeit with decreased noise tolerance. This trade-off allows users to adapt the algorithm's performance to specific analytical needs. The default value is mode 2.

The figure below illustrates the impact of employing two distinct modes. On the left side, the original, unprocessed spectrum is displayed (cyan curve), overlaid with the spectrum processed by ElPulpo in mode 2 (red line). On the right side, the same spectrum is presented, this time processed using mode 5. It's important to note that ElPulpo is engineered to preserve peak integrals; as it narrows line widths for enhanced resolution, it correspondingly increases peak heights to maintain integral constancy.

© 2024 MESTRELAB RESEARCH

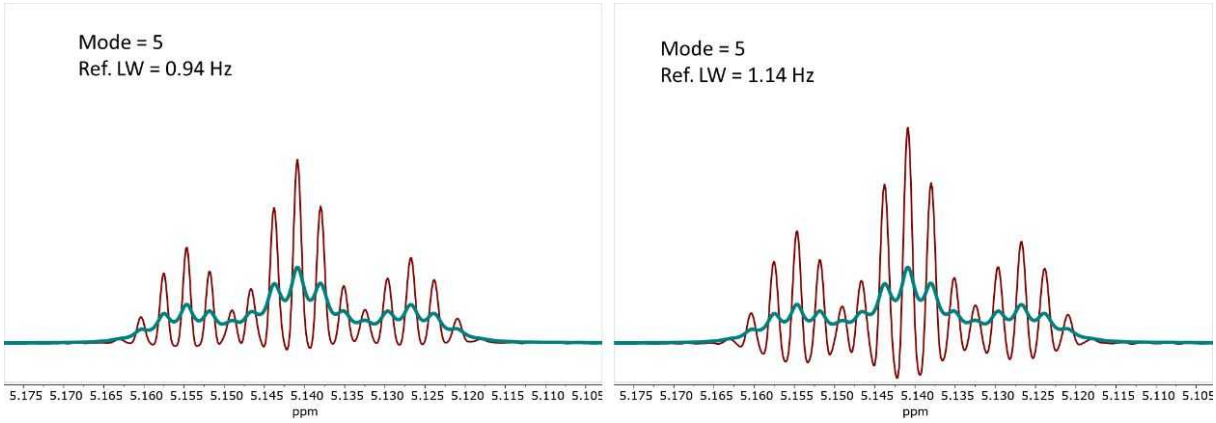


Reference Line Width: This parameter is pivotal for the algorithm's performance. The ideal value for the Reference Line Width should match the actual line width of the peaks in the spectrum. However, given the variability in peak widths across a spectrum, the algorithm requires a compromise value. The software facilitates this by offering an automatic setting for this parameter. It calculates the average line width of all peaks in the spectrum and then adjusts this average by a factor, currently set to 0.8.

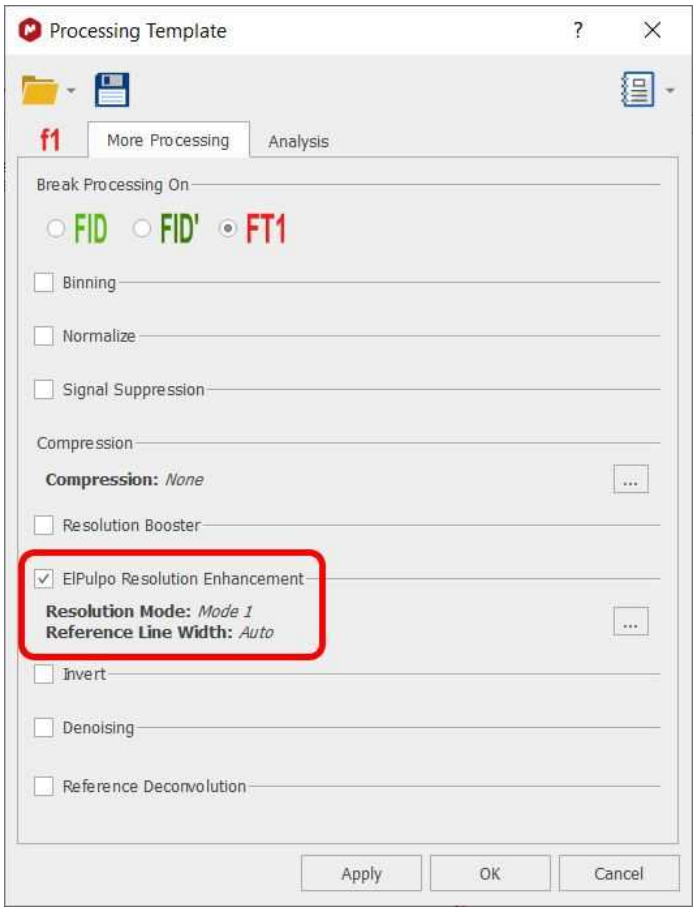
It is important to understand the behavior of the algorithm in response to adjustments in the Reference Line Width (refLW): When refLW is small, its influence on the peak diminishes, tending towards zero, minimally affecting the peak. Conversely, increasing refLW initially leads to peak narrowing, but exceeding a certain point introduces negative side lobes.

Optimal peak refinement occurs when refLW matches the original line width (originalLW) of the peaks, avoiding negative lobes. If refLW is larger than originalLW, further narrowing of the central peak is accompanied by the emergence of negative lobes. On the other hand, when refLW is less than originalLW, narrowing diminishes and no lobes are formed.

The figure below demonstrates the influence of different Reference Line Width settings in ElPulpo processing. Again, the cyan curve depicts the original spectrum, while the red curve represents the spectrum processed by ElPulpo. On the left, a Reference Line Width of 0.94, approximating the actual linewidth of the spectrum, is used. On the right, a slightly larger Reference Line Width of 1.14 Hz is applied. This adjustment leads to some excursions of the peaks into the negative domain, a direct consequence of the increased line width parameter.



This feature can also be applied from the 'Processing Template':



See also this video: https://youtu.be/gvfg24K6beU

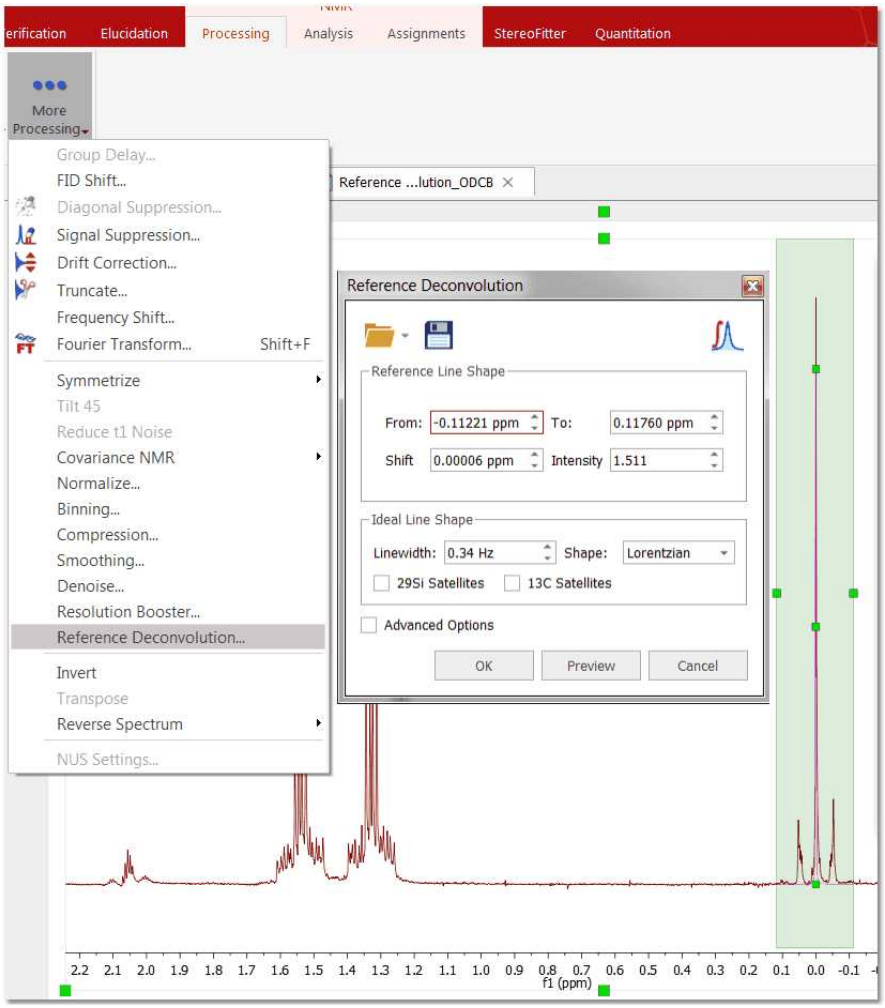
7.18 Reference Deconvolution

Reference Deconvolution is a signal processing method to remove experimental effects that broaden lines. Reference deconvolution is a technique which uses the shape of a single resonance line to measure the actual frequency distribution produced by the local magnetic field in homogeneity and then deconvolves that distribution from the whole spectrum. It is a simple linear process which requires no prior knowledge of the number of lines, their intensities, or their relaxation characteristics. No fitting procedures are used.

This ability to generate sharp line spectra is very important for DOSY measurements, improving t1 noise in 2D spectra, qNMR (through reducing truncation effects), and we can expect it to also add robustness to Verification, metabolomics, and in many more real-life situations.

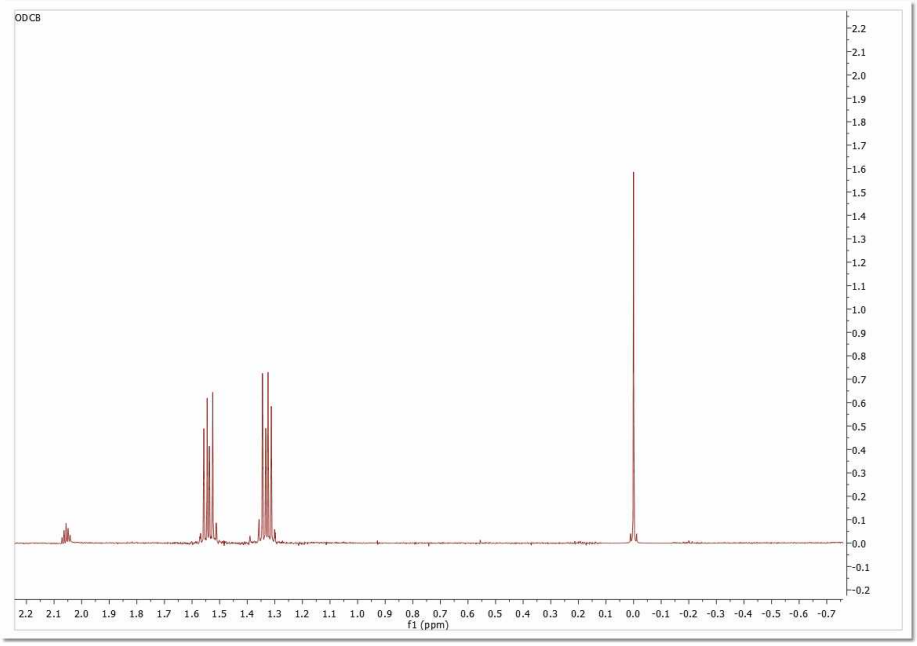
Here you can see an example of the NMR spectrum which has been folded several times to optimize digitization. To correct the severe lineshape problems and spinning sidebands, we will apply 'reference deconvolution' by following the menu 'Processing/More Processing/Reference Deconvolution'. After that you will notice that the mouse cursor will change to an integral curve; the next step will be to click&drag over the region of interest (which must be always a singlet) to display the Reference Deconvolution dialog box, from where additional settings can be applied:

© 2024 MESTRELAB RESEARCH

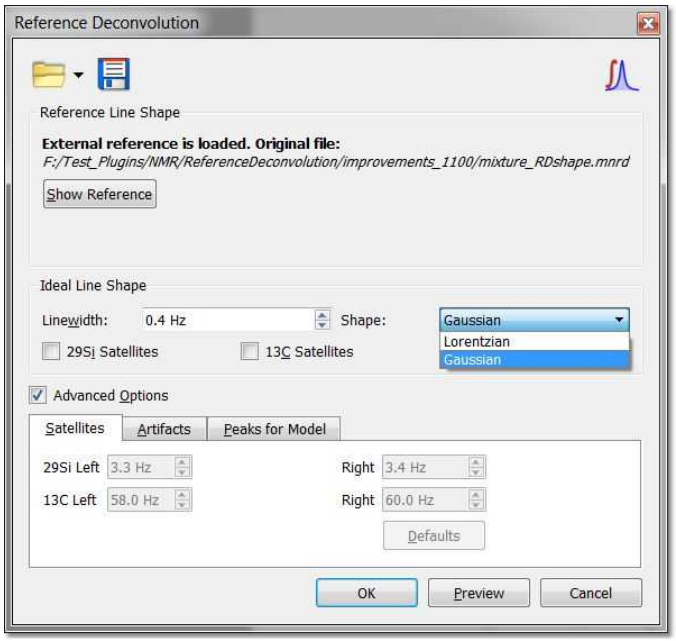


After having clicked on the OK button, you will get the result:

© 2024 MESTRELAB RESEARCH



Checking the 'Advanced' box will display additional tabs with settings for the 13C and 28Si satellites; to minimize the artifacts and to select the peaks of interest for the reference deconvolution model:



You can choose the ideal line shape (Gaussian or Lorentzian) from the dialog above.

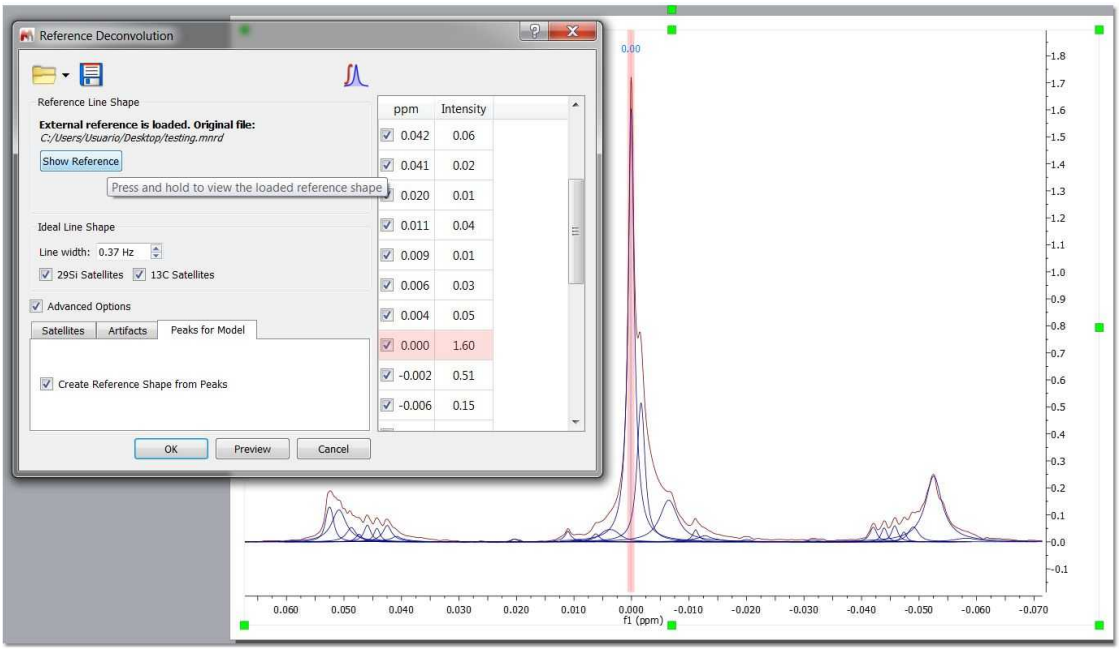
The model settings can be saved to be used with other datasets by clicking on the 'Load/Save' buttons.Saving the model will store:

1. The experimental shape between specified limits.
2. The peaks found by peak picking in the same region, including info if the peaks are selected or deselected by the user.

© 2024 MESTRELAB RESEARCH

3. The parameter which shows if user want to use experimental shape or the synthetic shape from the peaks.

Loading a settings file and clicking on the "Show Reference" button, will display all the GSD peaks in the reference region (the non-selected ones will appear in grey). You can switch to the experimental shape (by unchecking the option "Create shape from peaks"):



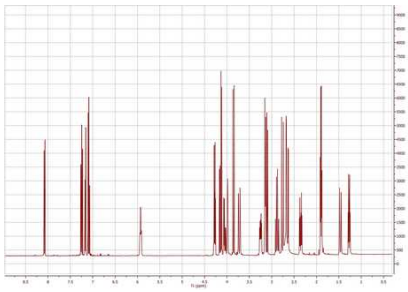
See also this blog post

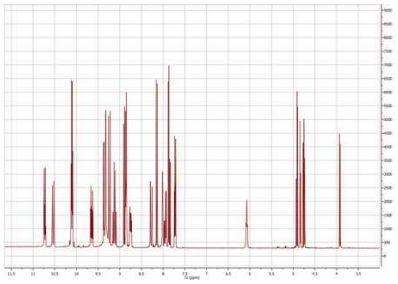
7.19 Inverting, Transposing and Reversing Spectra

Invert: The command 'Processing/Invert' multiplies all spectrum data points by -1. It is equivalent to applying a zero order phase correction of 180º It is very useful for DEPT spectra.

Transpose (Not available in Mnova Lite)**:** This command allows the user to transpose (by interchanging the rows and columns of the matrix) a spectrum for more convenient representation. This command is available by following the menu 'Processing/Transpose'.

Reverse Spectrum: The commands ('Processing/More Processing/Reverse Spectrum') on this submenu allow you to reverse the spectrum along **F1**, **F2**, (horizontally or vertically) or **both axes** to obtain a more convenient representation.



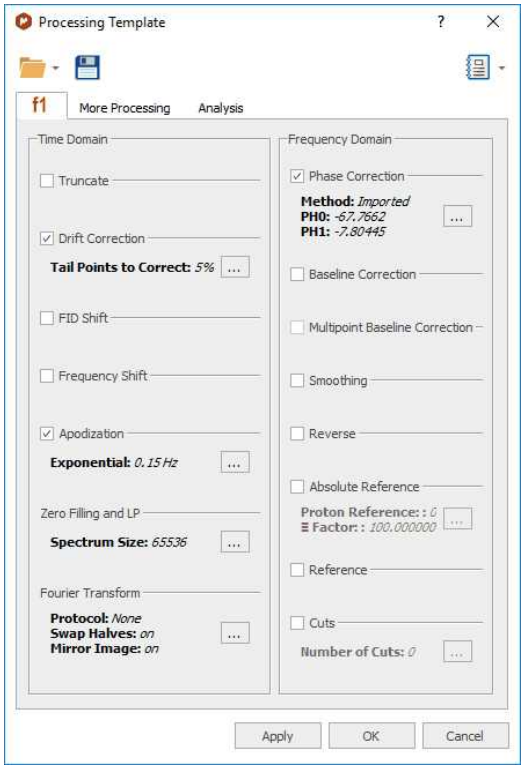


7.20 Processing Templates

Mnova includes a powerful and flexible feature which allows the user to easily automate the full processing of both 1D & 2D NMR data sets. This is ideal for the batch processing of spectra of the same type, and can also incorporate analysis operations.

For example, let's say you have acquired 20 proton spectra which you want to process using the same processing operations. The procedure is:

1. Load one spectrum and process it as desired.
2. Go to ***Processing/Processing Template***. The following dialog appears:



This dialog includes all the processing and analysis features implemented in Mnova. You can choose the required options by selecting the check boxes and, if necessary, specify options by clicking the button ...; next to the corresponding command.

3. Once you are happy with the result, save the script file to a Mnova Processing file (*.mnp) by clicking on the save button in the dialog box.

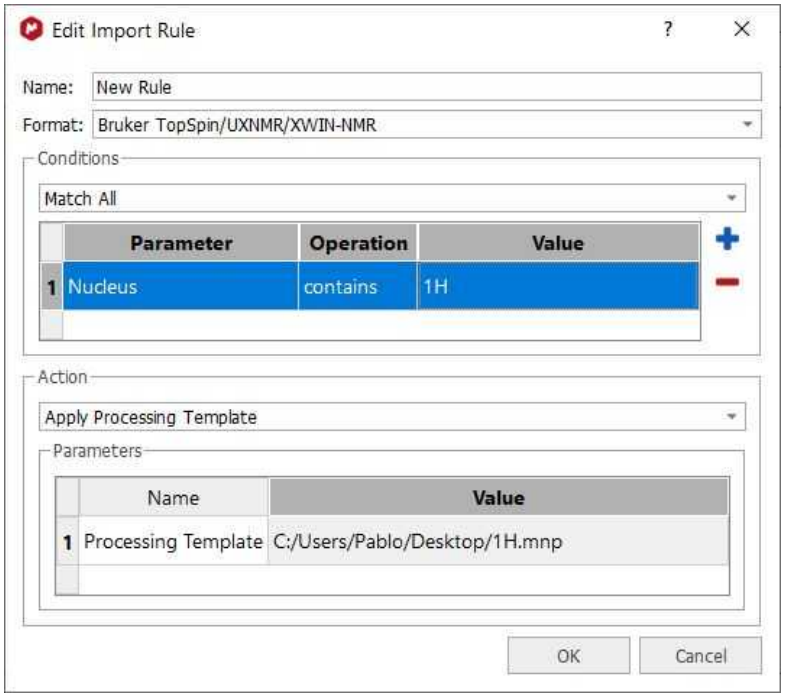
4. You can now apply this processing script file to any other spectrum/spectra by selecting the spectra you wish to process following this procedure (one or several spectra), and then opening the previously saved file by using the open command in the Processing Template dialog box.

© 2024 MESTRELAB RESEARCH

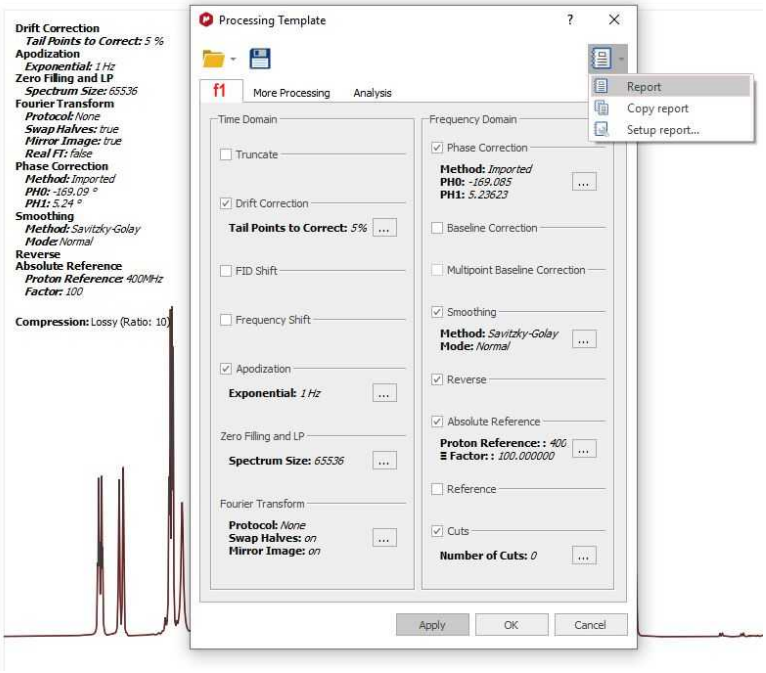
A few helpful tips ...

1. If you want to apply a processing script to all the spectra opened in one document, first select all the spectra in all the pages.
2. Next you can simply go to ***Processing/Recent Processing Templates*** and select the appropriate processing file script.
3. Remember that only spectra acquired using the same conditions should be processed using a processing script file. For example, if you have acquired a spectrum using a sequential mode and another one using simultaneous quadrature detection, you will get erroneous results.

You can set a processing template by default from the 'File/Preferences/Import' dialog:



You can report into Mnova the processing template you have created, just by clicking on the 'Report' button. From the scroll down menu, you will be able to 'copy the report to clipboard' and to customize it:



Use the script below to run a saved processing template:

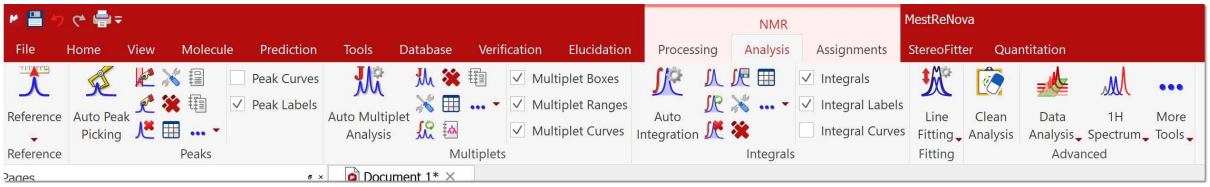
```
//<GUI menuname="ApplyProcessingTemplate"shortcut="Ctrl+2"tooltip="Apply a saved processing tem
function ApplyProcessingTemplate()
{
var spec = new NMRSpectrum( nmr.activeSpectrum() );
var processing = new String;
if (!spec.isValid())
return;
processing = "C:\\ProcessingTemplate.mnp";
nmr.processSpectrum(spec, processing);
spec.process();
mainWindow.activeWindow().update();
}
```


© 2024 MESTRELAB RESEARCH

Part VIII

8 Analysis Tools

This section will give you an outline of the features already implemented, and which will have full or at least partial capabilities in this version:



Chemical Shift Referencing
Peak Picking
Integration
Multiplet Analysis
Molecular Structure
Assignments
Prediction of NMR Spectra

The 'Clean Analysis' tool will delete all analysis features (peaks, integrals, multiplets) for the selected spectra as well as all assignments of the molecules linked with that spectrum.

8.1 Chemical Shift Referencing

In NMR, the frequencies are usually measured relative to a frequency standard. As these frequencies are strictly measured relative to a frequency standard proportional to the magnetic field, it is convenient to define the chemical shift as:

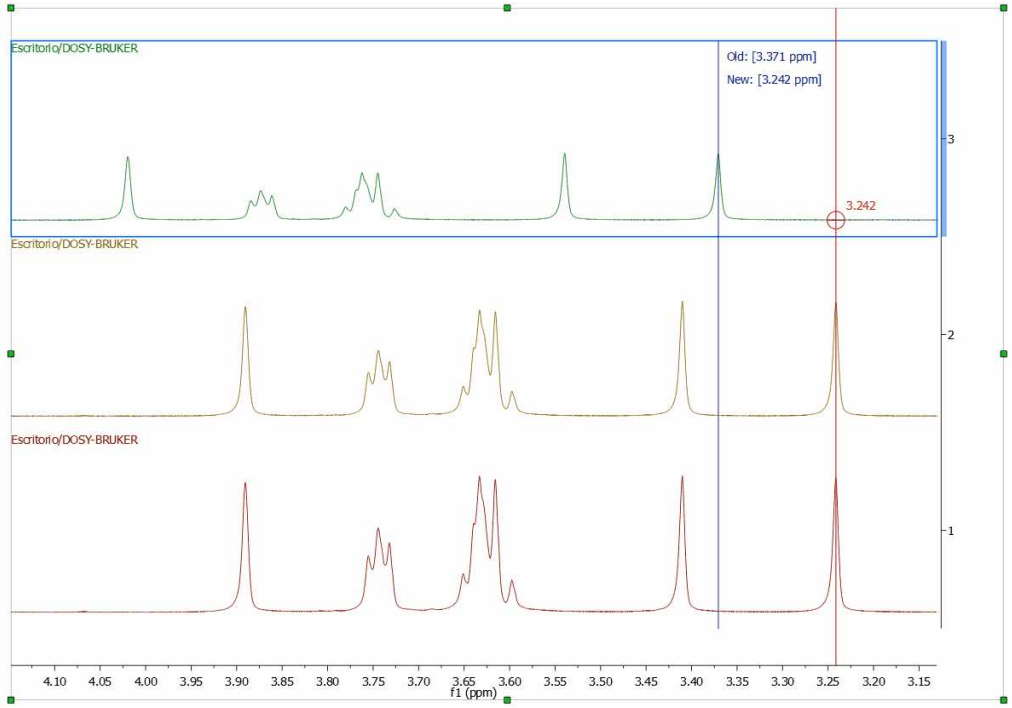
$$\delta = 10^6 \cdot \frac{(\nu - \nu_{ref})}{\nu_{ref}} = 10^6 \cdot \frac{\sigma_{ref} - \sigma}{1 - \sigma_{ref}} \approx 10^6 \cdot (\sigma_{ref} - \sigma)$$

Thus, in order to establish a chemical shift scale, it is necessary to choose some substance as a reference and define its chemical shift. In non-aqueous solvents, the most used reference substance is **TMS** (tetramethysilane) which is defined to have a chemical shift of **0.0 ppm**. For biomolecules, slightly different compounds (e.g. TSP, $(CH_3)_2SiCD_3CD_2CO_2Na$, Sodium-salt of trimethylsilyl-propionic acid) are used, since TMS is not soluble in water.

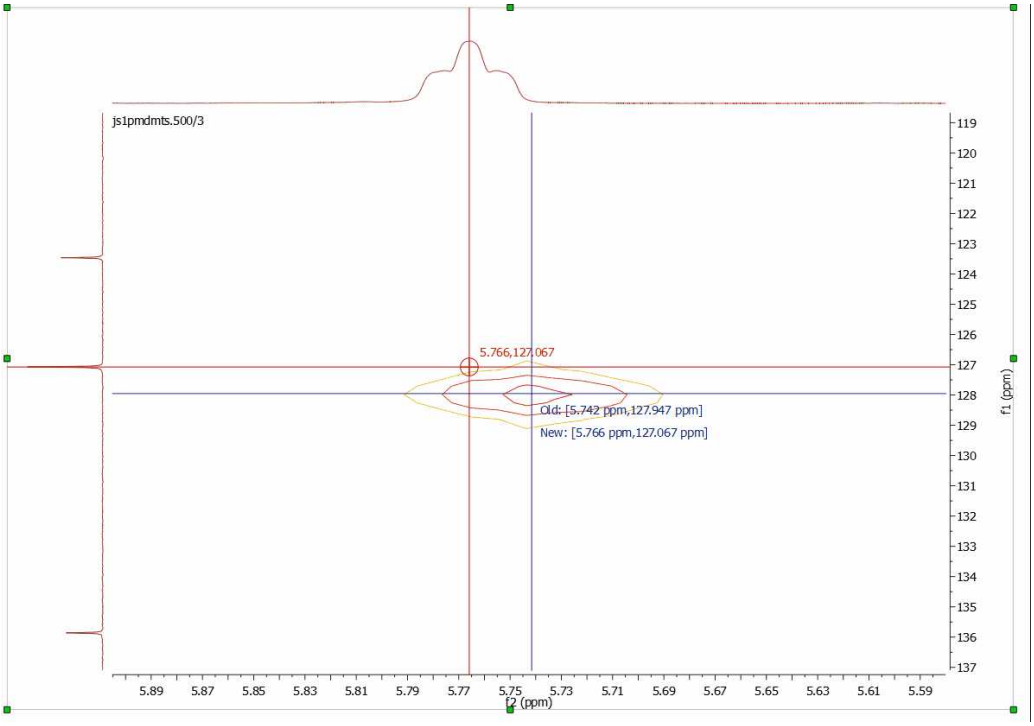
There are two way to reference your spectra, one graphically and another by using a dialog box.

The **Graphic Reference** (R as shortcut) will allow you to reference your spectra just with two clicks. Once click to select the position that you want to reference and another click to select the new chemical shift. This feature will be very useful to reference stacked spectra (for example):

© 2024 MESTRELAB RESEARCH



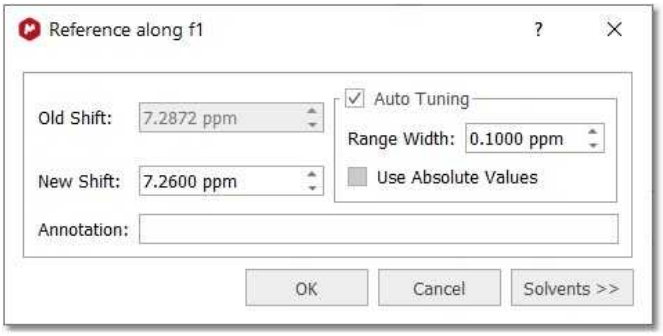
you can also use this tool to reference the 2D-NMR spectra directly with the 1D external trace. To do it just press the 'R' key, next click on the peak that you want to reference and finally click again on the new chemical shift (matching with the peaks of the external traces):



You can also calibrate your spectrum by selecting ***Analysis/Reference/References*** (L shortcut) on the main menu or by clicking on the 

toolbar button and then following this procedure:

1. Zoom-in on the spectrum region containing the reference peak
2. Select the peak you want to be a reference point. You will notice that the mouse cursor automatically *jumps* to the peaks maxima. To select a peak at an arbitrary position which is not a local extreme (e.g. the shoulder of a peak), press **SHIFT** key, move the mouse to the desired position and click. The **following** dialog box appears:

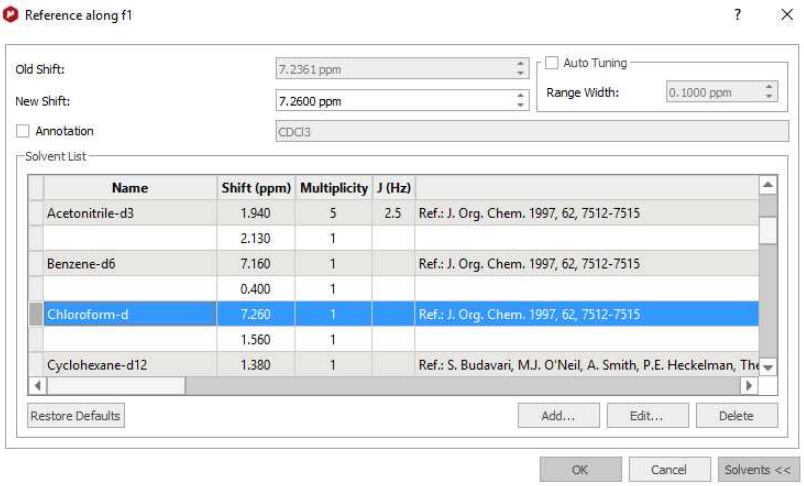


You will be able to type any annotation after having referenced your signal.

The 'Auto Tuning' option finds the maximum peak inside a limit and references it. This is very useful for example if you need to reference automatically a large amount of metabonomic spectra, where the solvent peak could slightly change its shift with the pH of the sample. Check the "'use absolute values" to take into account also negative peaks for the reference.

You can type the new value for that peak or you can click on the 

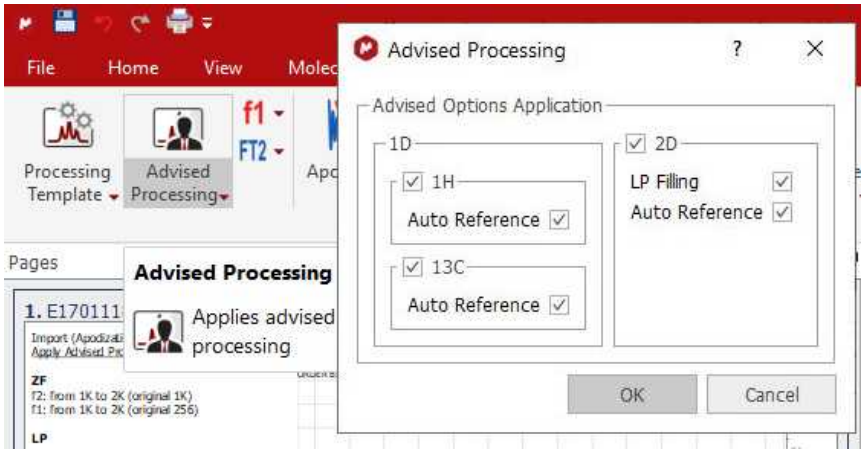
button to get a list of solvent chemical shifts.



You can add new entries or edit the list of standard reference chemical shifts by using the buttons at the bottom of the dialog box.

There is also an 'Advised processing' feature which will apply a suggested processing with the option to automatic reference the spectra:

© 2024 MESTRELAB RESEARCH



8.1.1 Absolute Reference of x-Nuclei experiments

According to IUPAC, using a suitable secondary reference compound is no longer recommended. The unified scale is now the preferred method of referencing all spectra.

For example; in the past, the middle peak of the CDCl3 1:1:1 triplet at 77.0 ppm was often used to reference 13C-NMR spectra; but now the recommended method for 13C spectral referencing is the following:

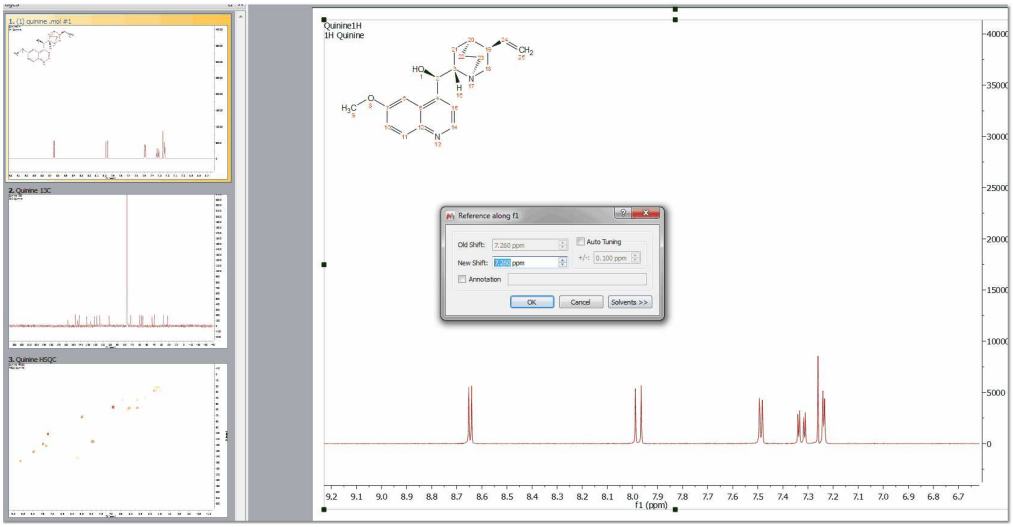
1. Obtain a 1H spectrum of the same compound, and set 0 ppm using the TMS resonance.
2. Determine ν_{TMS}^{obs} TMS from the 1H spectrum.
3. To determine the absolute frequency of the 0 ppm position in the 13C spectrum, use:

$$\nu_C = \nu_{TMS}^{obs} \frac{\Xi_C}{100} \quad , \text{ where } \Xi_C = 25.145020$$

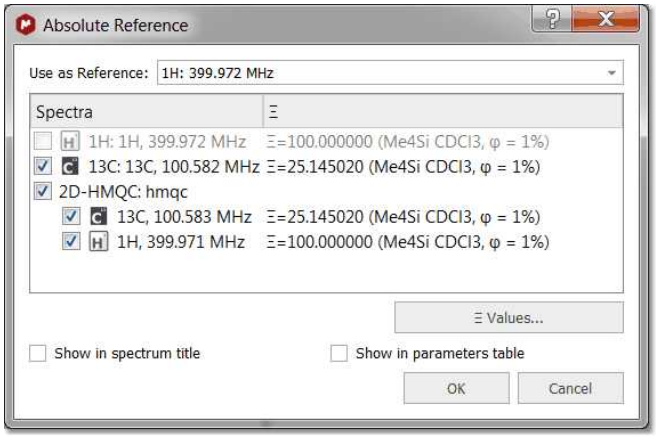
4. Finally use ν_c as shown below to reference the 13C spectrum.

Let´s see how you can use the Absolute Reference in Mnova:

1st: Load into Mnova for example a 1H, 13C and HSQC experiments and reference the solvent in the 1H dataset:

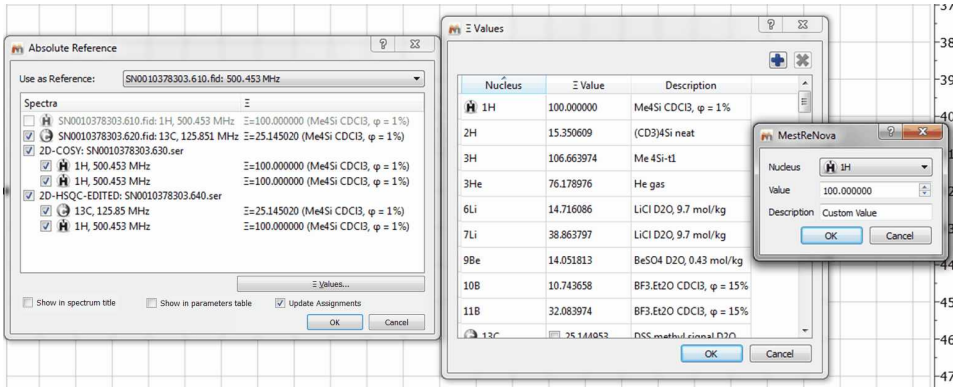


2nd: Select 'Absolute Reference' from the 'Reference' scroll down menu of the 'Analysis ribbon, to display the applicable dialog box. Once there, check the boxes of the experiments that you want to reference



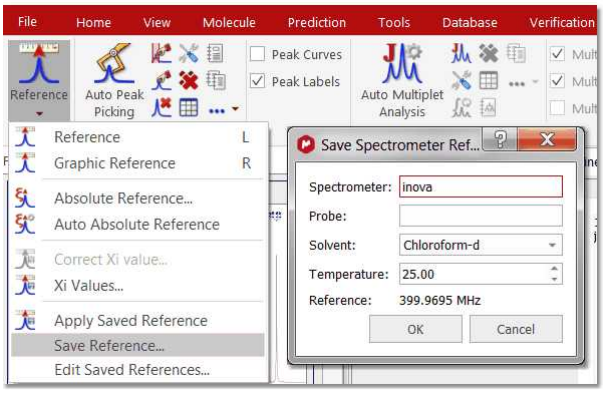
If the document contains assignments, check the applicable box to update them.

The user can add any custom Xi value for any nucleus, using the "Xi values" table:

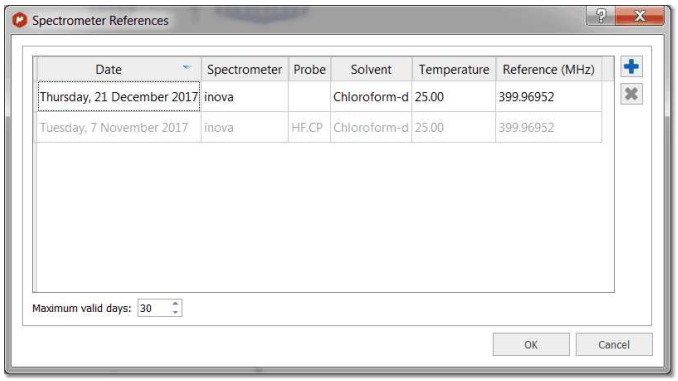


© 2024 MESTRELAB RESEARCH

Select 'Save Reference' from the 'Reference' scroll down menu to save the Absolute Reference that you have applied:



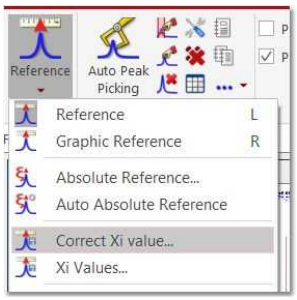
This will be useful for subsequent 1H spectra on the same spectrometer and in the same solvent.The user will be able to use this saved value to reference his/her 1H spectra. Since this value may change slowly with time, the user must be able to update the stored value at a later time (by selecting 'Edit Saved References'). By default the saved values will be valid for 30 days.



Selecting 'Auto Absolute Reference' will reference automatically the spectra (according to the know references from the list above).

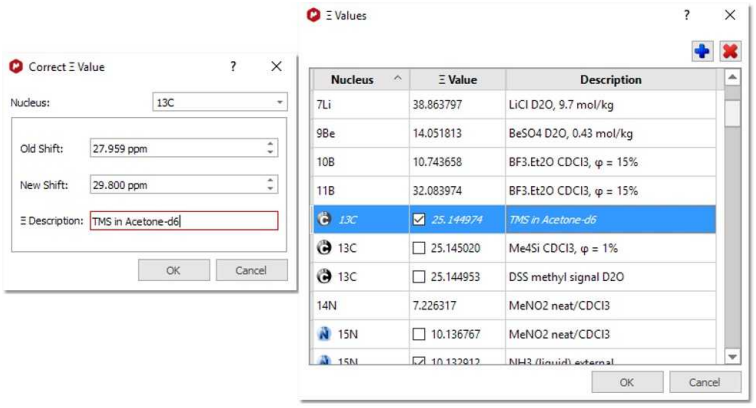
The user can select to automatically apply the known references when importing by following the menu 'File/Preferences/NMR/Import',

Once you have applied the 'Absolute Reference', you could correct the Xi values (if you know that they are wrong) by following the menu 'Analysis/Reference/Correct Xi value':



© 2024 MESTRELAB RESEARCH

After that, you will only need to select a peak graphically and enter the new shift.

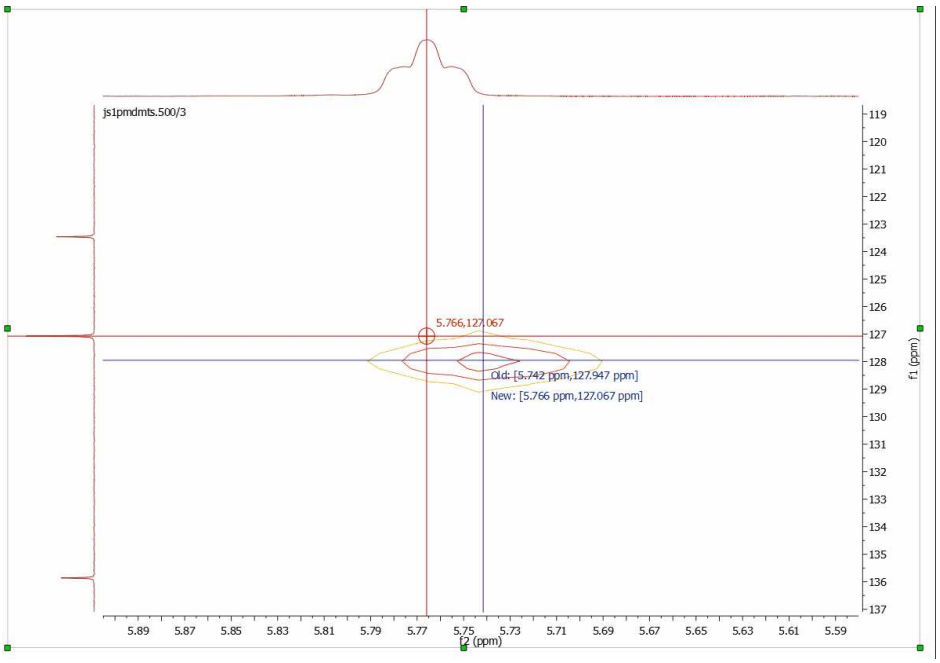


Then the new Xi value gets recalculated, added automatically to the list of Xi and the spectrum is shifted to the new shift.

See also this [blog post]

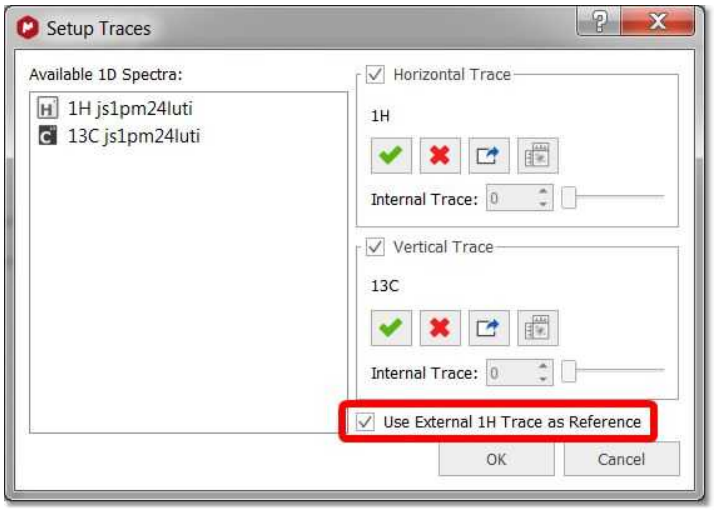
8.1.2 Reference of 2D-NMR spectra with the 1D external trace

Press the 'R' key (or follow the menu 'Analysis/Reference/Graphic Reference') to activate the **Graphic Reference** feature. Next click on the peak that you want to reference and finally click again on the new chemical shift (matching with the peaks of the external traces):



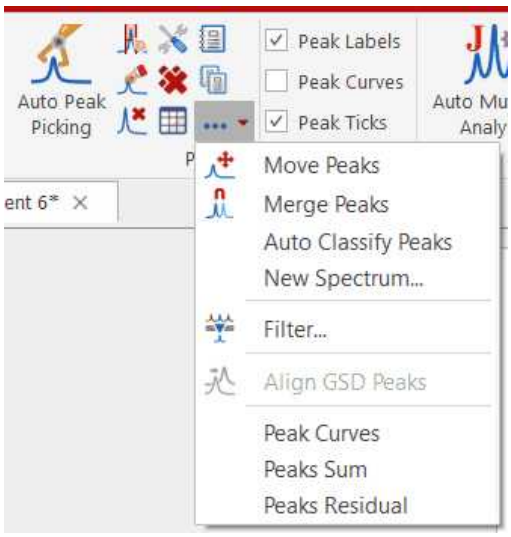
To apply an automatic referencing, right click over one of the traces and select 'Setup' to display the 'Setup Traces' dialog box. Once there click on the 'Use External 1H Trace as Reference' box':

© 2024 MESTRELAB RESEARCH



8.2 Peak Picking

Mnova implements a series of peak picking algorithms for 1D and 2D. These algorithms can be accessed via the menu item 'Analysis/Peak Picking'.



From here, you will be allowed you to use **Automatic** Peak Picking, **Manual** Peak Picking (Manual Threshold), **Peak by Peak** Peak Picking, Move Peaks, Auto Classify Peaks (it will classify peaks as compound, impurity, solvent, artifact, etc by using the molecular structure of the document), create a new synthesized spectrum, to show or not show peaks on screen, to delete all peaks or delete peaks one by one, to set Peak Picking Options and to report the peak list, etc.



NOTE

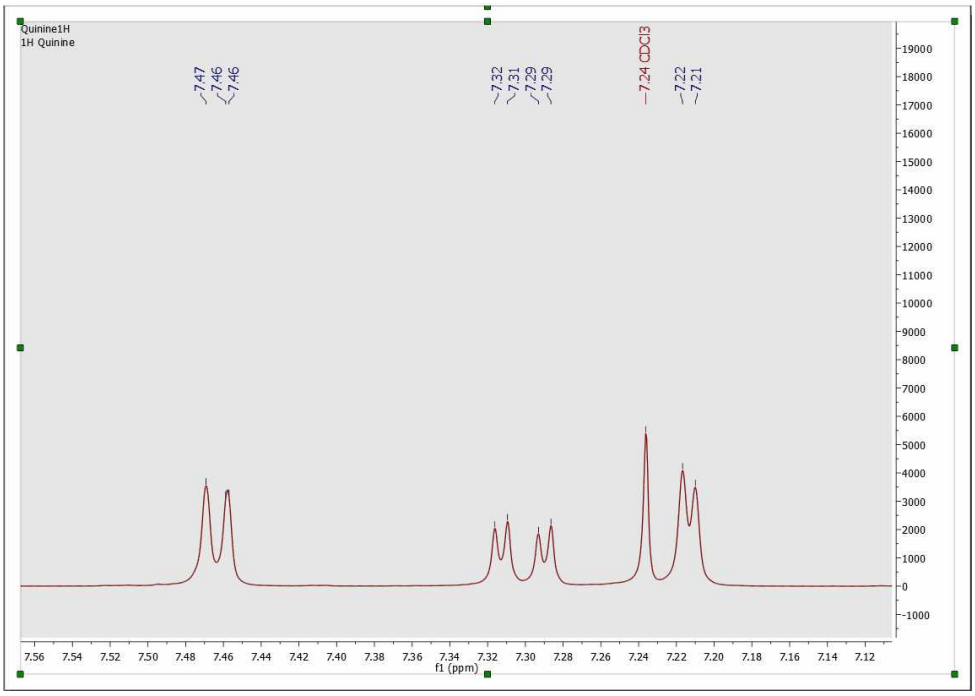
You can move the peaks labels up and down just by clicking and dragging them.

© 2024 MESTRELAB RESEARCH

Automatic Peak Picking

The Automatic Peak Picking option can be launched by simply clicking on the **'Peak Picking'** icon on the toolbar. This algorithm will pick all the peaks in the spectrum, and, by default, show **them** on screen with automatic recognition of the solvent lines. This algorithm can be applied indistinctively to 1D and 2D spectra.

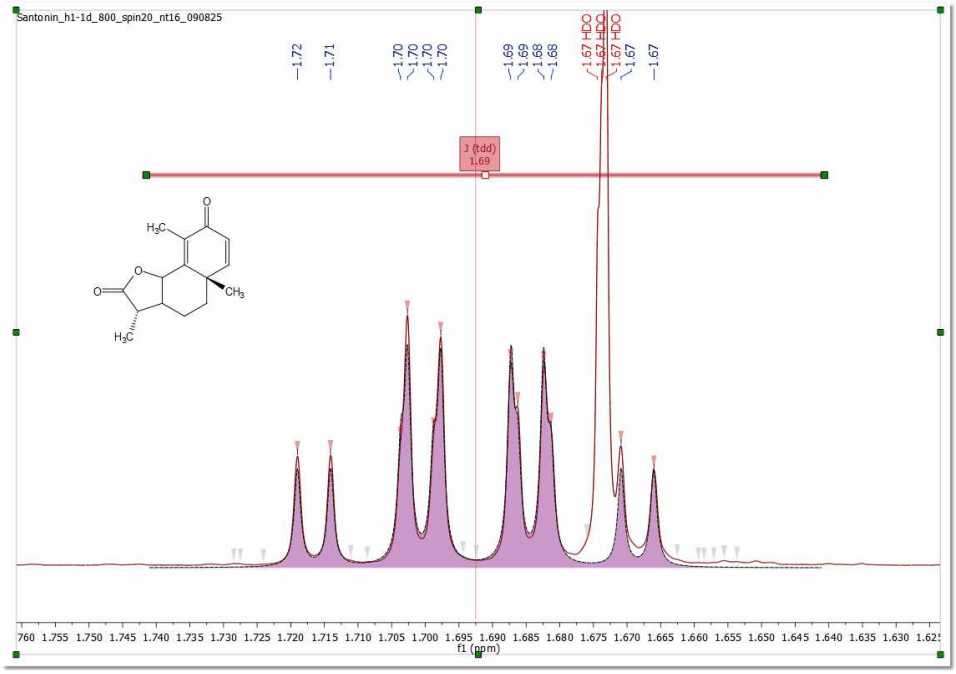
This is a major change to Mnova NMR functionality. The default automatic Peak Picking algorithm is now based on **GSD**, rendering enhanced resolution, identification of overlapped peaks and also including autodetection of solvent and impurities (this algorithm is slower than the Standard, so if you are not interested in the deconvolution, you may prefer to use the Standard algorithm. See options below)



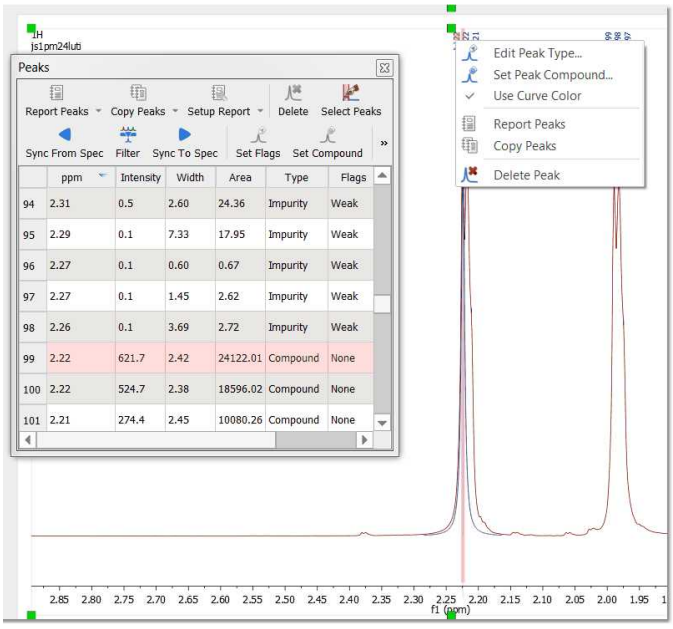
You can see above an example of the autoediting capabilities in the new algorithm, which automatically identifies solvents (as well as impurities, reference peaks, etc).

You can see below another example of the enhanced resolution achieved by the new Peak picking algorithm, now capable of identifying shoulders on large peaks as smaller peaks and of labelling and include them in multiplet analysis.

© 2024 MESTRELAB RESEARCH

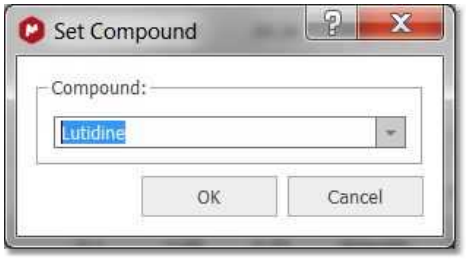


Peak curves are now active objects which can be interacted with, and three-way highlighting of peaks table, peak labels and the peak themselves has been implemented. For example, double clicking on any peak label will display the 'Peaks Table', hovering the mouse over the peak label (or on any table) will highlight the peak in the spectrum and allow the user to interact directly with that peak. Clicking on any peak of the spectrum will automatically **scroll up/down the table** to the position of currently highlighted peak in the spectrum. **Right clicking** on any peak, will allow you to change the **'Peak Type'** from the 'Peaks context' menu (and to set peak compound, use curve color, report, copy and delete peaks).

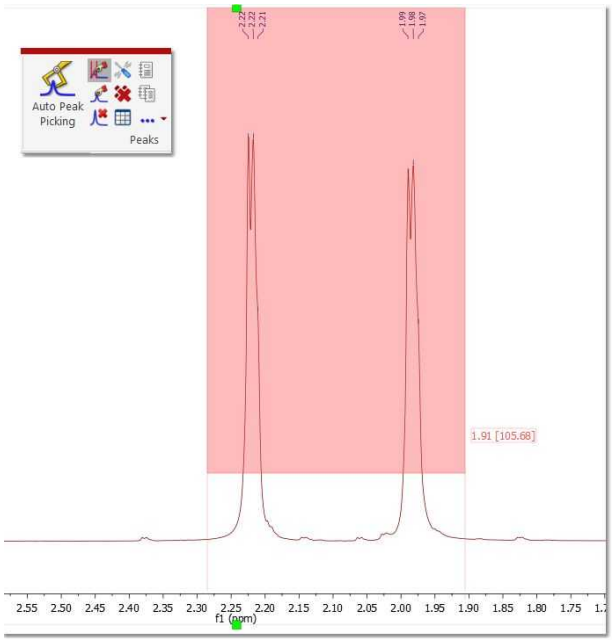


	ppm	Intensity	Width	Area	Type	Flags
94	2.31	0.5	2.60	24.36	Impurity	Weak
95	2.29	0.1	7.33	17.95	Impurity	Weak
96	2.27	0.1	0.60	0.67	Impurity	Weak
97	2.27	0.1	1.45	2.62	Impurity	Weak
98	2.26	0.1	3.69	2.72	Impurity	Weak
99	2.22	621.7	2.42	24122.01	Compound	None
100	2.22	524.7	2.38	18596.02	Compound	None
101	2.21	274.4	2.45	10080.26	Compound	None

If your document contains more than one compounds, you will be able to select the applicable compound for each peak by right clicking on the peak label (or by selecting the peak(s) from the Peaks Table) and selecting 'Set Peak Compound':



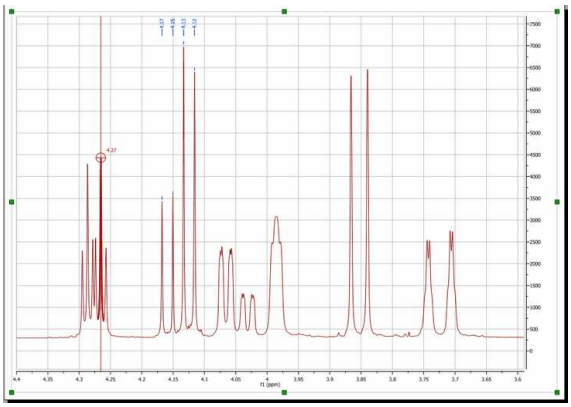
Manual Threshold (shortcut: k)
The Manual Peak Picking allows the user to set an area of the spectrum where the software will carry out the Peak Picking, by simply left clicking and dragging the mouse over the area.



Peak by Peak (shortcut: Ctrl+k or Cmd+k in Mac)

The Peak by Peak option will allow you to hover the mouse over specific peaks of interest and to only select those for the peak picking output, by just left clicking on each one at a time. The procedure is very simple: just point to the peak you want to pick so that it is highlighted and then left click on the mouse.

© 2024 MESTRELAB RESEARCH

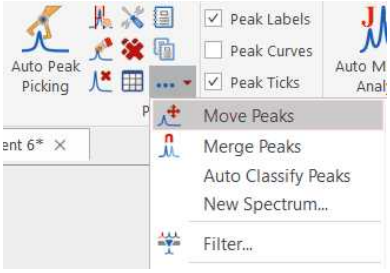


Note that, by default, Mnova will find the peak top by using parabolic interpolation. To pick a peak at an arbitrary position (e.g. not at a local extreme), press <Shift> and click.

NOTE (useful for 2D Homonuclear spectra)

Normal Peak by Peak mode is activated by clicking Ctrl+K only once. Double 'Peak by Peak mod'e is activated by clicking Ctrl+K a second time. The tool gets deactivated after clicking Ctrl+K a third time. In double mode, the peak by peak tool adds a peak at the 2 positions, either when the cursor is pointing at lowfield or highfield peak.

Mnova also allows the user to **move a peak label** to a new location, by using the 'Move Peaks' tool under the Peak Picking scroll down menu.

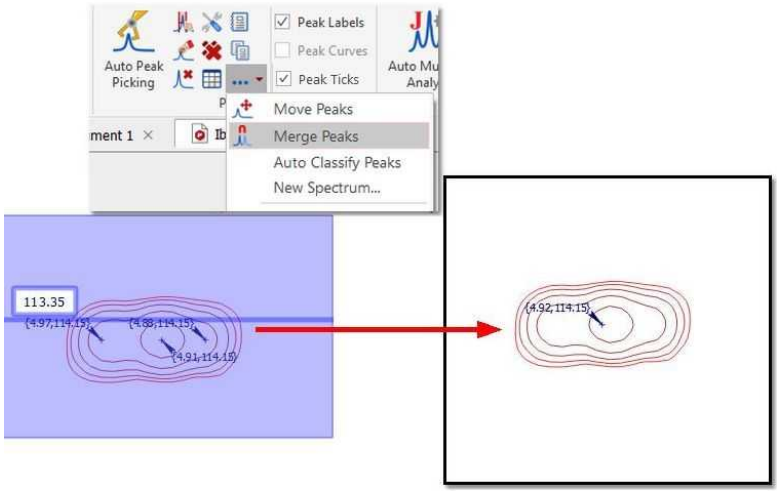


Note: When a peak is moved its Annotation information remains unchanged, although other info such as peak widths and intensities are updated.

Merge Peaks

Use this mode to merge more than one peak labels into a new one:

© 2024 MESTRELAB RESEARCH

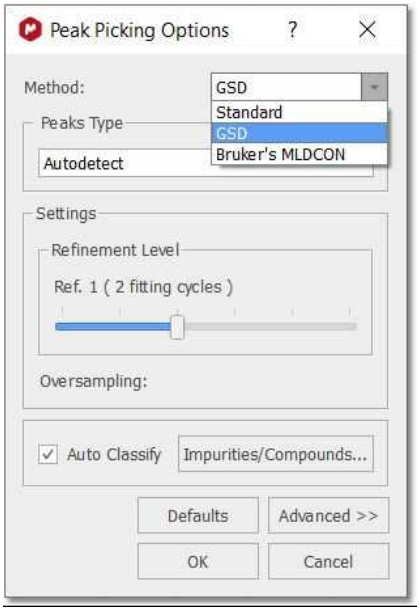


Once you have finished with the 'Peak Picking', select the ‘Report’ option in the Peak Picking menu. Select 'Copy Peaks' to copy the report to the clipboard. Choose the peak report template for the 'Peak List' just by selecting 'Setup' in the 'Report Special' scroll down menu of the 'Peaks Table'.

The peak list of a spectrum can be exported via File/Save As and selecting "Script: NMR 1D Peak List". It includes values of ppm and intensity for each peak. Follow the menu 'File/Save As/Script: NMR Peaks Table' to export the peaks in a table (only the fields set as 'Visible' in Setup Table get exported).

8.2.1 Peak Picking Options

Mnova has three Peak Picking algorithms: GSD (by default, with global spectral deconvolution and slower than the Std), Standard (without deconvolution, faster than GSD) and Bruker´s MLDCON (for Bruker datasets):



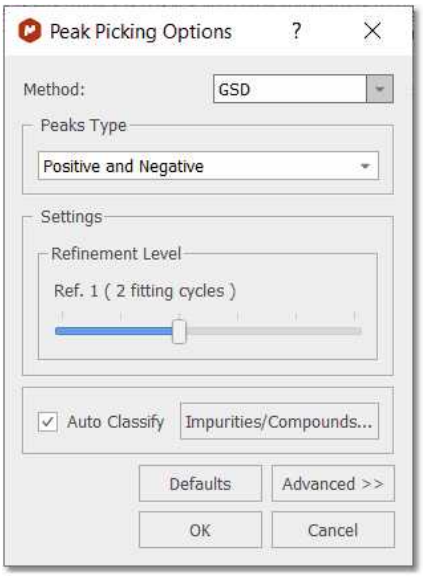
You can select the Peaks Type from the scroll down menu:

© 2024 MESTRELAB RESEARCH



The Autodetect mode will let Mnova to decide regarding the experiment type, so if for example you have a 2D NMR spectrum acquired in magnitude mode, Mnova will only search for positive peaks. However, if you have a DEPT dataset, it will look for both positive and negative peaks. The user can also force the search to only positive, only negative or both type of peaks.

GSD Peak Picking options:
The Peak Picking based on GSD (Global Spectral Deconvolution) will have these options:



GSD has different operational levels, ranging from a very fast but inaccurate method to slower but much more accurate levels. In addition, GSD makes it possible the automatic recognition of solvent peaks, impurities, etc. We recommend to use a GSD level of 1 (flash) or 2.

From this menu we can set the Refinement Level (number of cycles) and the 'Auto Classify' of the GSD peaks. Clicking on the 'Defaults' button will restore the default settings from the registry.

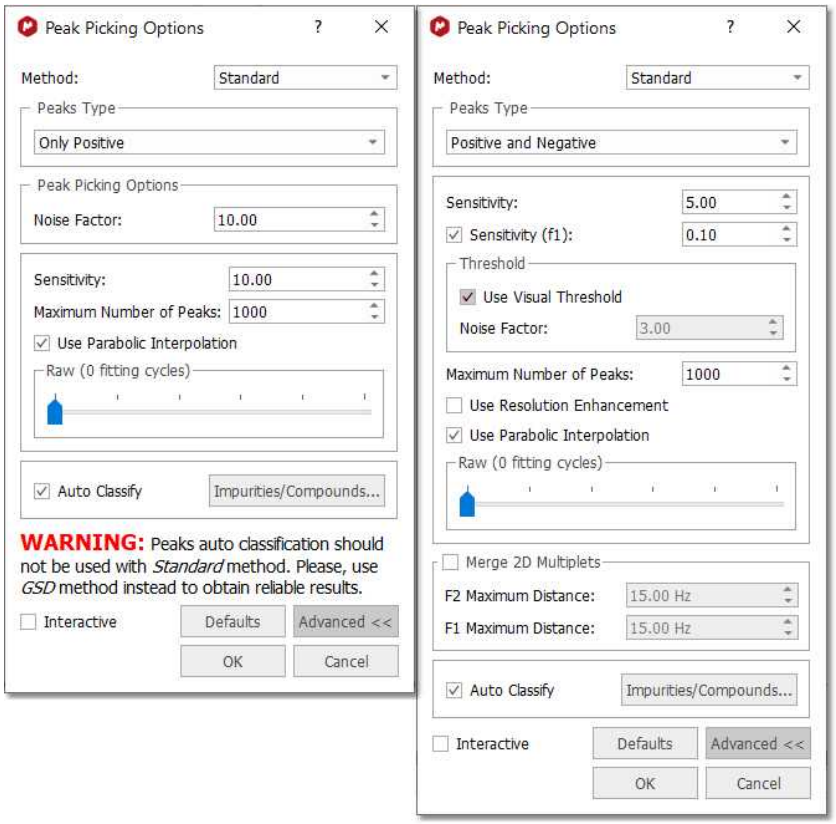
The 'Refinement Level' will be the number of GSD fitting cycles applied over the spectrum. For example if you select 2 cycles, that means that the program will apply a GSD to your original spectrum and then another to the deconvoluted spectrum. GSD has different operational levels, ranging from a very fast but inaccurate method to slower but much more accurate levels. We recommend to use a GSD level of 1 (flash) or 2.

'Auto Classify': the program will try to identify the type of each signal of the spectrum (compound, solvent, impurity, etc). This information will appear in the 'Type' column of the Peaks table. This feature should be used only with GSD (and not with the Standard method).

© 2024 MESTRELAB RESEARCH

Clicking on the 'Advanced' button will allow you to select the 'Quantitative GSD' algorithm which generates less residuals in the fitting and better area values.

Standard Peak Picking options:
This algorithm is much faster than GSD, but it does not include deconvolution.



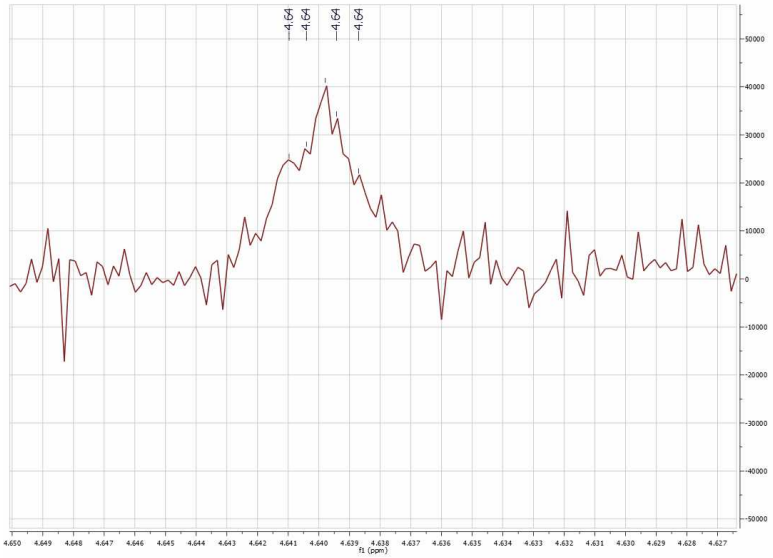
A series of options can be controlled on the 'Peak Picking Options' dialog box, which can be accessed via the Peak Picking scroll menu. The user can choose the 'Noise Factor' (with visual threshold for 2D) and the 'peaks type'. Clicking on the 'Advanced' button, will allow you to change the 'sensitivity' factor (the higher the value, the less peaks will be picked), maximum number of peaks, whether to 'use parabolic interpolation', merging options for 2D multiplets. All these options can be evaluated interactively, so that their results can be viewed on screen in **real time**, by making sure the **'Interactive'** box is selected.

- **Sensitivity:** it is basically a kind of smoothing parameter. For example, in noisy spectra, large values of this parameter will smooth the spectrum in the background so that less peaks will be detected. By default, this value will be 1 which means that the program will try to find the maximum number of peaks in the spectrum. This is the recommended value for routine 1D spectra.

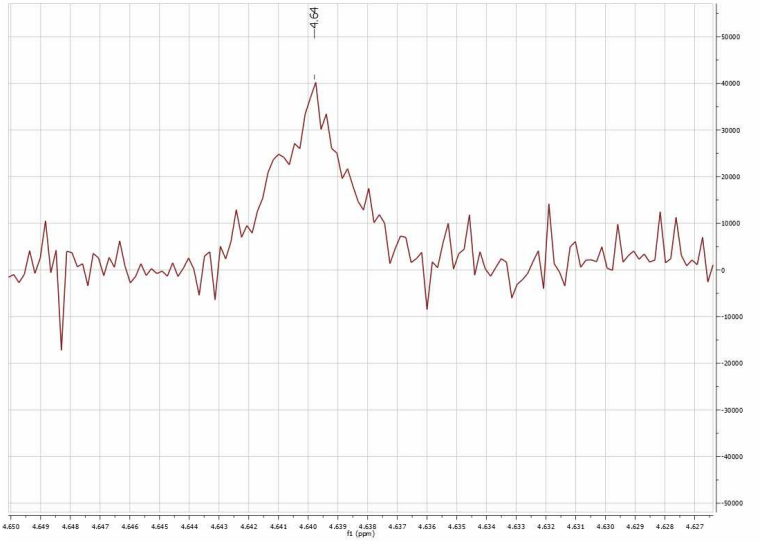
The sensitivity factor is indeed being used by the program and its main application is to avoid the detection of too many peaks in noise spectra. As we said above, this option can be considered as a smoothing parameter which is somehow applied before the peak picking algorithm is applied. For instance, if you have a very noise peak with a lot of local extrema caused by the random fluctuations of the noise, the peak picking algorithm could find just too many peaks which do not correspond to real peaks, just noise local maxima/minima. In order to avoid that situation, the sensitivity factor can be increased in such a way that the peak is smoothed on the fly and ideally, only the real peak would be detected.

© 2024 MESTRELAB RESEARCH

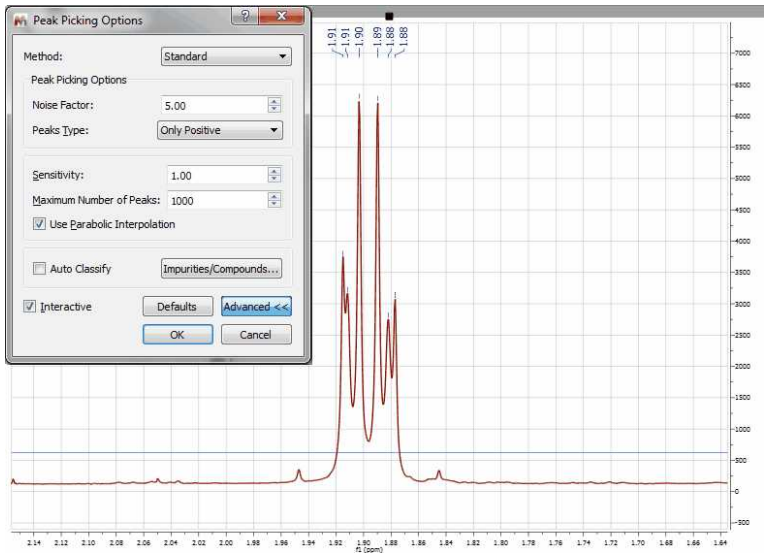
For example, this is what happens with a very low value for the sensitivity in a noisy spectrum:



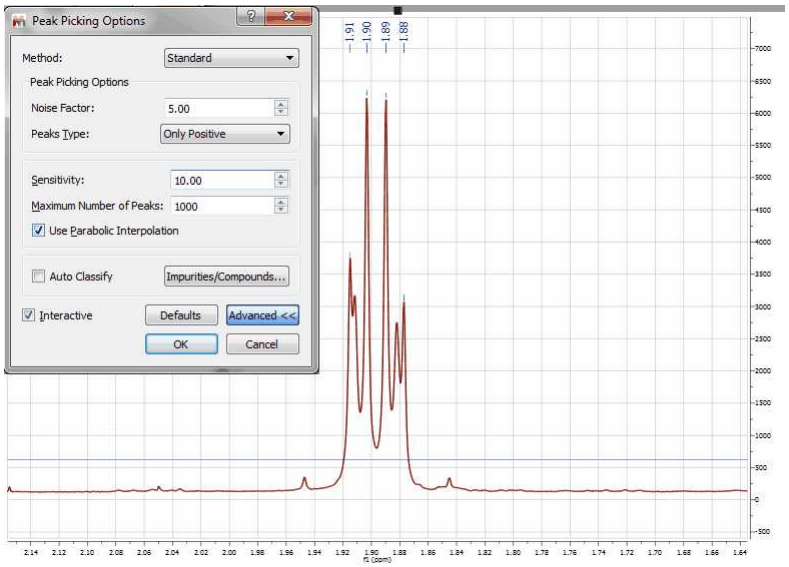
And this is the result when a higher value is used:



It is shown below, how this sensitivity factor is being used by the program by opening a 1H-NMR spectrum of strychnine. First you will see the result with sensitivity = 1 (BTW, it's important to realize that the sensitivity factor can be lower than 1. The lower the value, the higher the resolving power of the algorithm):



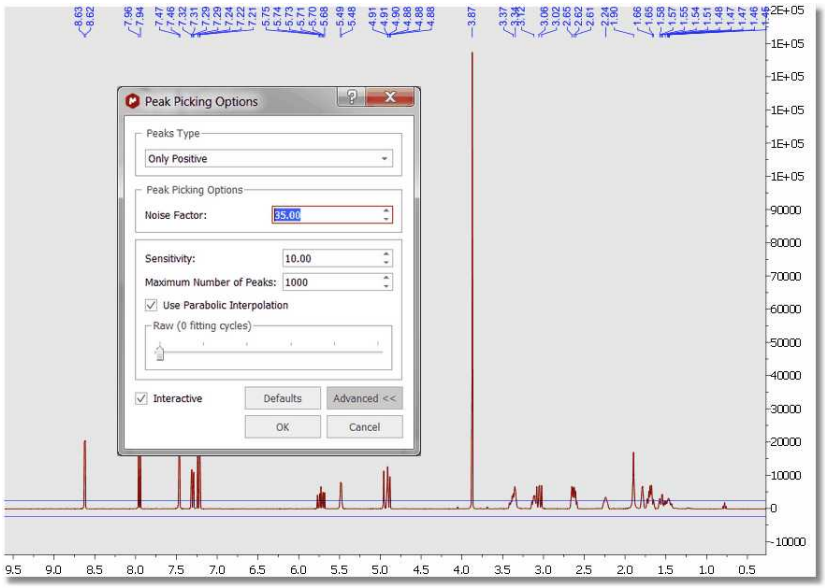
So you can appreciate that with sensitivity =1 all peaks have been detected. Now let's increase the value to 10. This is the result:



You can see that only 4 peaks have been detected whilst 2 less resolved peaks are missing now.

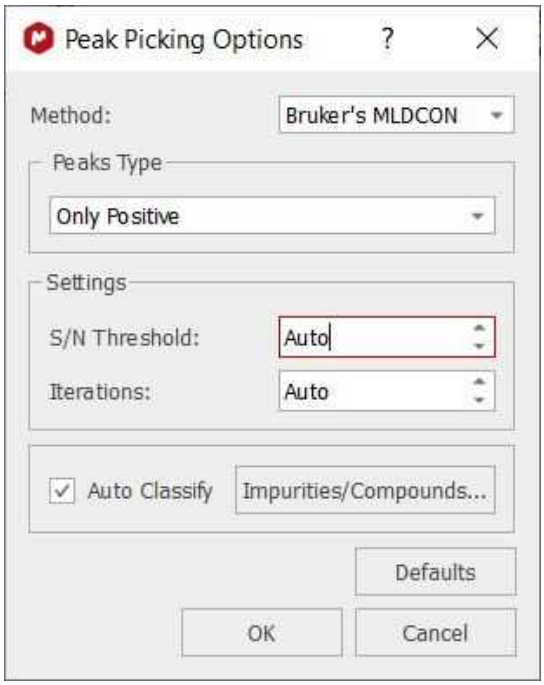
- **Noise Factor:** it is a kind of intensity threshold. The program will automatically calculate the noise value of the spectrum. This noise value is then multiplied by the noise factor. After running the peak picking algorithm, peaks smaller than the noise value multiplied by the noise factor will be rejected. The program shows this vertical threshold as two horizontal lines in the spectrum, as you can see in the picture below:

© 2024 MESTRELAB RESEARCH



Bruker's MLDCON

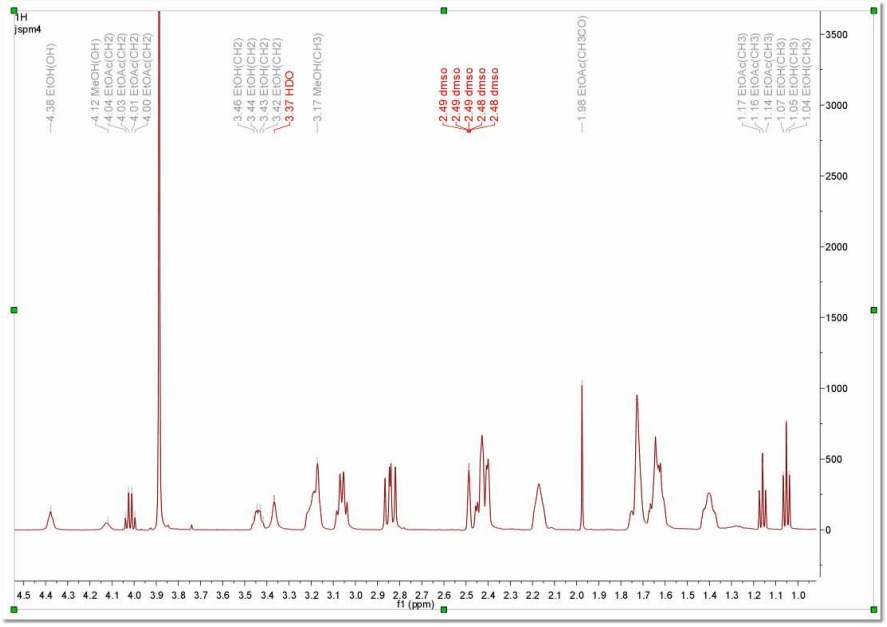
This algorithm can be used for any Bruker 1D spectra (positive and negative peaks) with a baseline correction applied. It requires to have a Java virtual machine installed. You can select the signal to noise threshold and the number of iterations.:



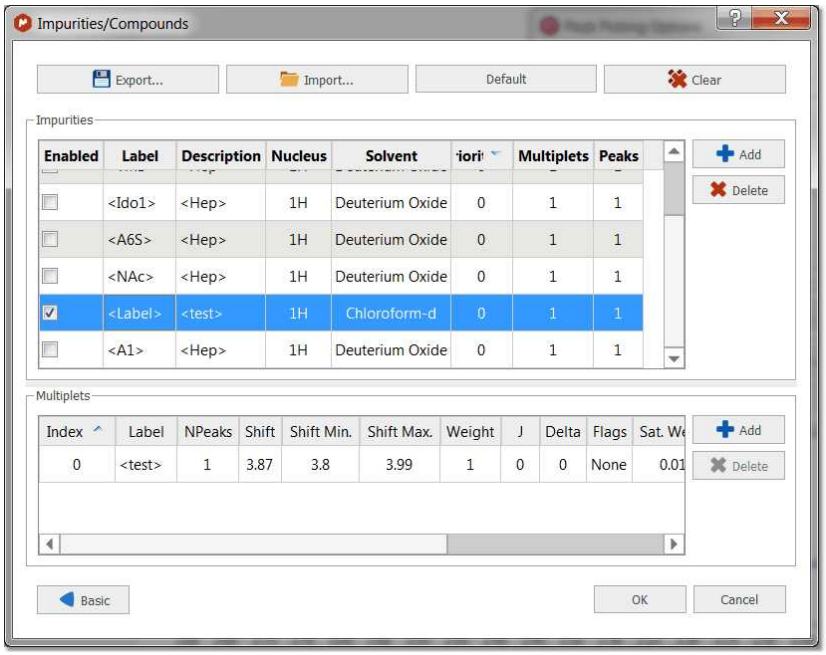
8.2.1.1 Impurities Auto detection

This novel algorithm will be used to detect the solvents as impurities in your samples.

The first version of the algorithm will detect MeOH, EtOH, iPrOH and EtOAc in deuterated DMSO. Here you can see an example containing several solvents.



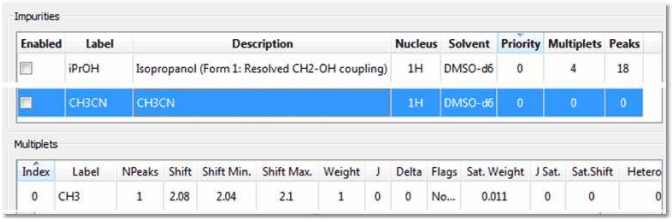
In the Peak Picking options, you can select what solvents you want to be detected and you will be able to edit this table and to include additional solvents:



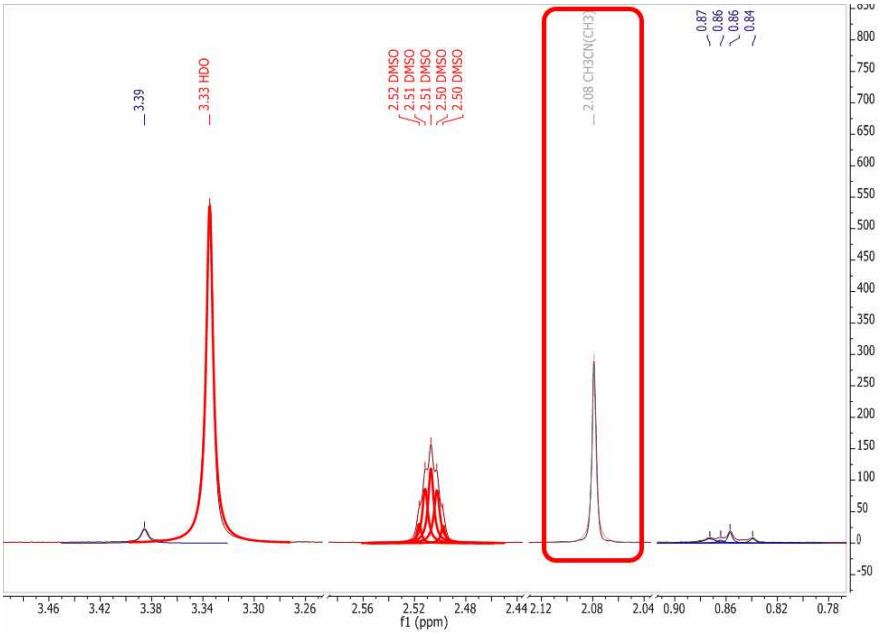
Clicking on the Advanced button, will allow you to add further impurities or to edit the existing ones:

For example let´s add CH3CN as a new solvent impurity. As CH3CN only has one multiplet visible in the 1H spectrum (the Me group), you can enter for the label CH3 and 1 for the number of peaks (Npeaks). For the base chemical shift, we have used a value of 2.08 and a minimum and maximum shift values of 2.04 and 2.10 respectively. This is the interval in which the CH3 resonance will be searched for.

© 2024 MESTRELAB RESEARCH



This is it! Now, when a Pick Picking with Auto Classify option enabled will be run, the CH3CN peak will be automatically detected, as illustrated in the spectrum below:



8.2.2 GSD (Global Spectral Deconvolution)

1H NMR is for sure the most powerful technique for structure elucidation, especially for small organic molecules. Typically, an organic chemist uses the chemical shift, coupling constants and integration information contained in an 1H-NMR spectrum to either verify or elucidate an unknown compound. Of course, it's quite common that a simple 1H-NMR spectrum is not enough to unambiguously confirm a structure and thus other NMR experiments (e.g. 13C-NMR, HSQC, COSY, etc) are used to get more structural information.

Nevertheless, we have often found that many organic chemists do not always try to get the most out of 1H-NMR spectra (which is the cheapest experiment), in particular when some multiplets are complex to interpret (due to the strong coupling) or when peaks overlap prevents valuable information to be detected in some multiplets.

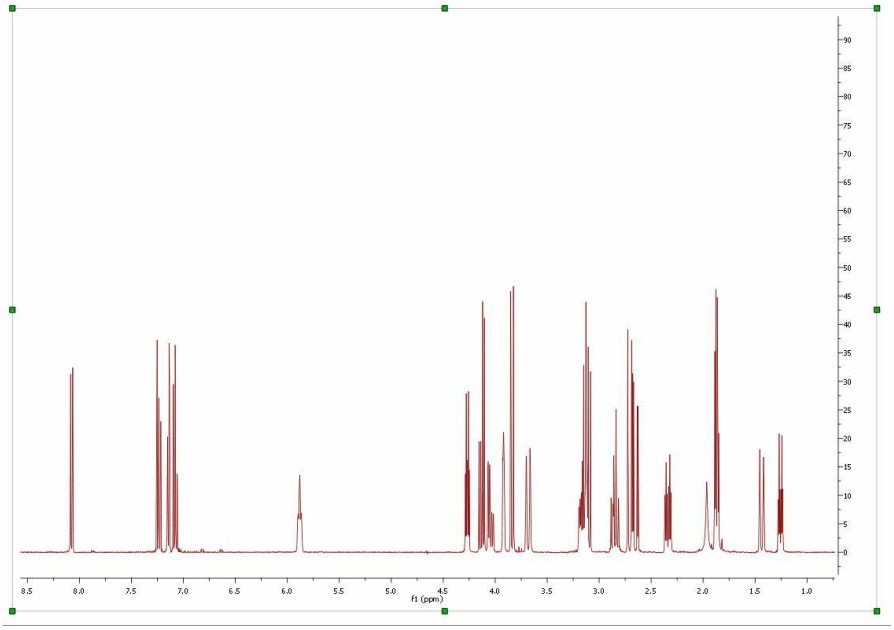
As it is well known, there are two principal factors limiting the resolution power in a spectrum. First, we have the natural line width limitation imposed by the T2 (spin-spin relaxation). For example, if T2 is about 1 second, the peak linewidth at half height cannot be less than 0.32 Hz (remember, line width at half height = 1 / (pi * T2) = 1 / 3.1415 = 0.32) no matter how powerful is our NMR instrument or the field homogeneity. On the other hand, there are instrumental shortcomings (e.g. spatial uniformity of the applied magnetic field, etc).

© 2024 MESTRELAB RESEARCH

Nonetheless, there is an additional limiting factor, and whose importance is generally underestimated which has to do with the generally large number of transitions in 1H-NMR spectra. In short, the peaks we can observe in a 1H-NMR are just a small fraction of the actual transition resonances which are not observable because of the limited digital resolution. In fact, every peak in an 1H-NMR spectrum is basically an envelope of a large number of transitions and its shape is dominated by the coupling pattern of the spin system. Even in molecules of modest size the number of distinct peaks is tens to thousands times smaller than that of quantum transitions.

These problems have been the motivation of the development of a brand new peak analysis algorithm, the so-called GSD (Global Spectral Deconvolution); which applies automatically a deconvolution of the whole spectrum in a few seconds and does not require that the user set a starting point for the fitting of each multiplet.

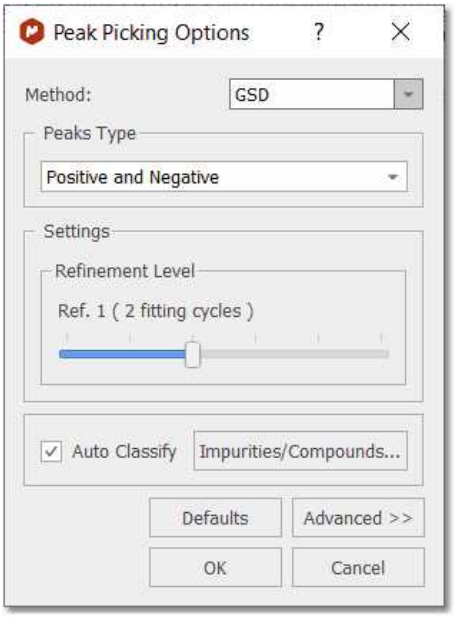
Let´s see the GSD in action. Just open a 1H-NMR spectrum:



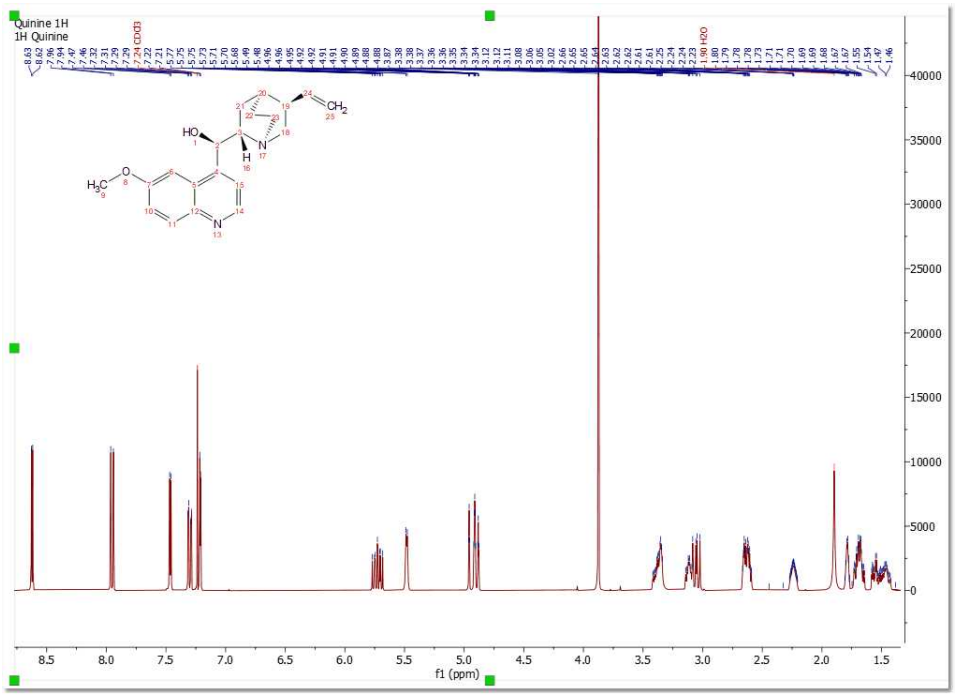
Select GSD as the Peak Picking method (in the Peak Picking Options) and apply a Peak Picking which will to carry out the deconvolution of the whole spectrum.

See also: "Using GSD for multiplets analysis"

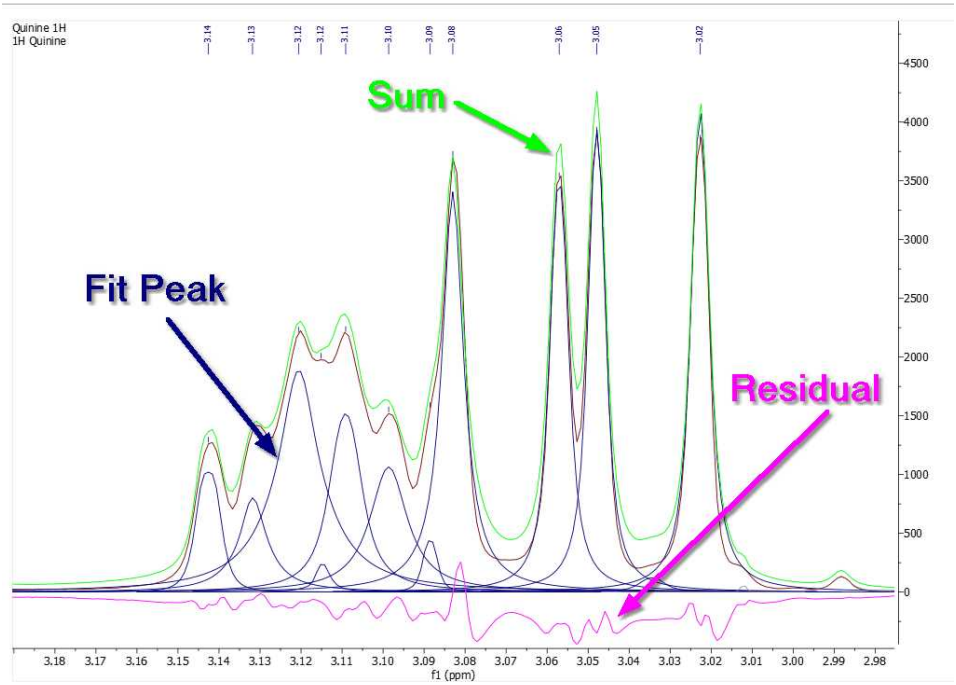
© 2024 MESTRELAB RESEARCH



Automatic multiplet deconvolution will be applied to the whole spectrum without asking the user to select multiplets, specify number of lines and their approximate locations, etc… **Everything is automatic!** (Ideally, data would be zero filled to 64K points and line broadening functions would not be used.).



Let´s expand an area to see how the algorithm works:

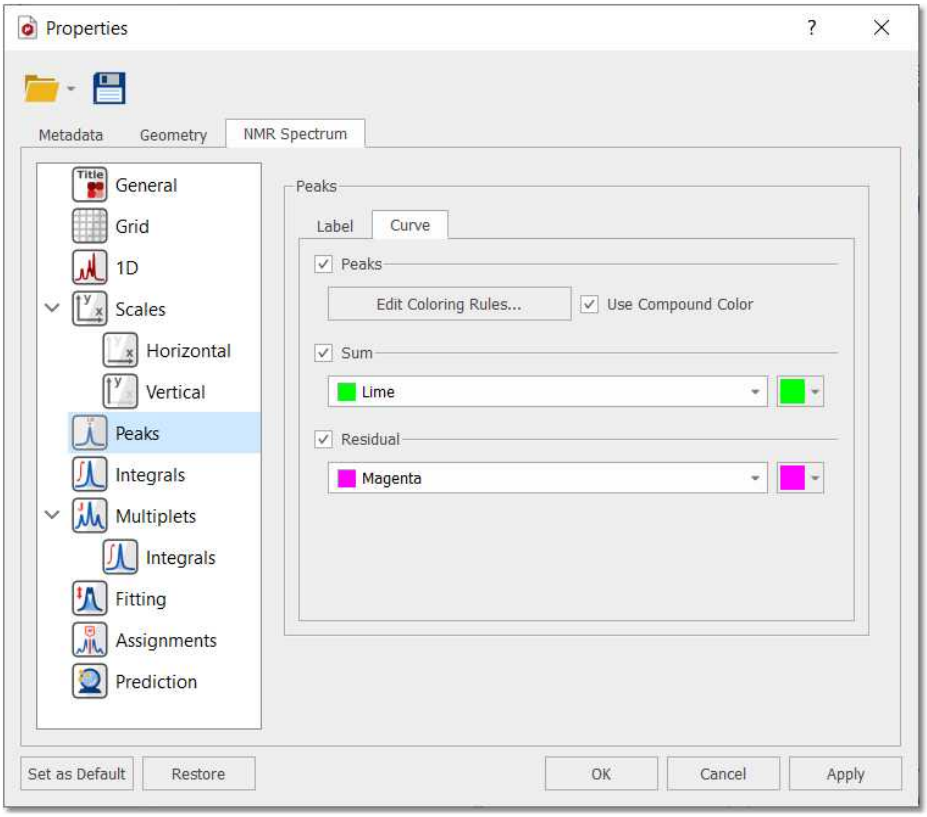


As you can see in the picture above you will obtain a blue line to show the fit peaks, a magenta line to show the residue and lime line to show the sum.

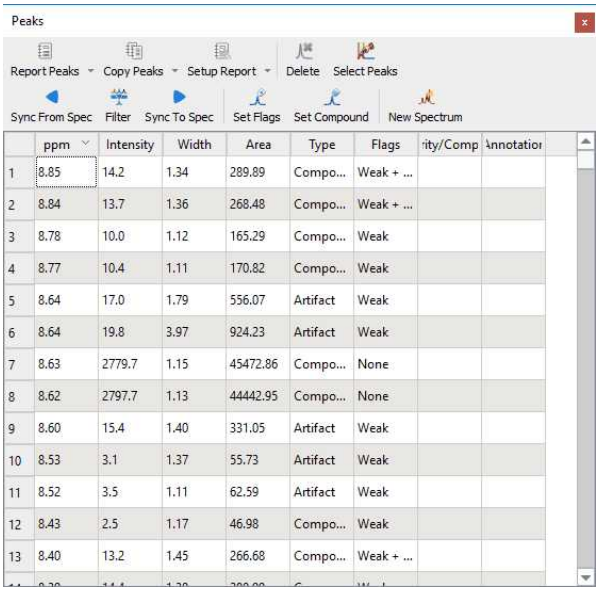
Note, that you can display Sum, Fitting curves and Residuals peaks by right clicking on the spectrum

© 2024 MESTRELAB RESEARCH

and selecting Properties (to check the applicable boxes under the Peaks/Curve tab).



It's very important to realize that the result of GSD is just a list of peaks, but each peak is fully characterized (chemical shift, Peak Width, Height, Phase, Area, etc). To see the list of the GSD peaks just follow the menu 'View/Tables/Peaks' (or right click on any toolbar and select 'Tables/Peaks'):

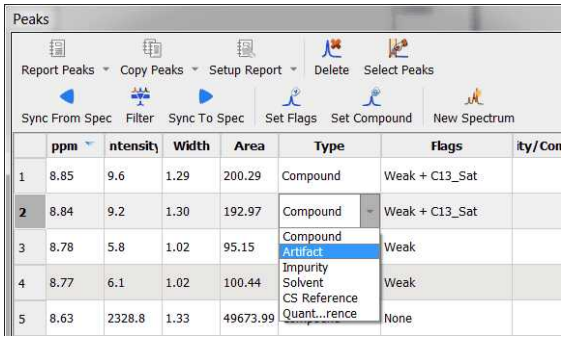


	ppm	Intensity	Width	Area	Type	Flags	ity/Comp	nnotatio
1	8.85	14.2	1.34	289.89	Compo...	Weak + ...		
2	8.84	13.7	1.36	268.48	Compo...	Weak + ...		
3	8.78	10.0	1.12	165.29	Compo...	Weak		
4	8.77	10.4	1.11	170.82	Compo...	Weak		
5	8.64	17.0	1.79	556.07	Artifact	Weak		
6	8.64	19.8	3.97	924.23	Artifact	Weak		
7	8.63	2779.7	1.15	45472.86	Compo...	None		
8	8.62	2797.7	1.13	44442.95	Compo...	None		
9	8.60	15.4	1.40	331.05	Artifact	Weak		
10	8.53	3.1	1.37	55.73	Artifact	Weak		
11	8.52	3.5	1.11	62.59	Artifact	Weak		
12	8.43	2.5	1.17	46.98	Compo...	Weak		
13	8.40	13.2	1.45	266.68	Compo...	Weak + ...		

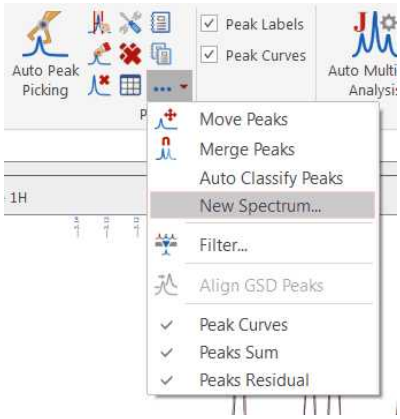
This table contains information about the chemical shift, width, height, Area, Type and Flags.

© 2024 MESTRELAB RESEARCH

Double click on the applicable cell (or on the **'Set Flags'** button) to manually edit the 'Type' and the 'Flags' table. To change the flag to several peaks at the same time just hold down the Ctrl/Cmd key and click.

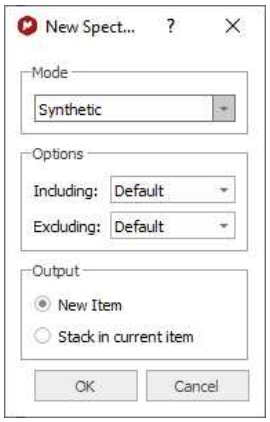


Once you have edited your GSD spectrum, you can generate a new spectrum with the fit peaks, just by selecting 'New Spectrum' in the Peak Picking scroll down menu:



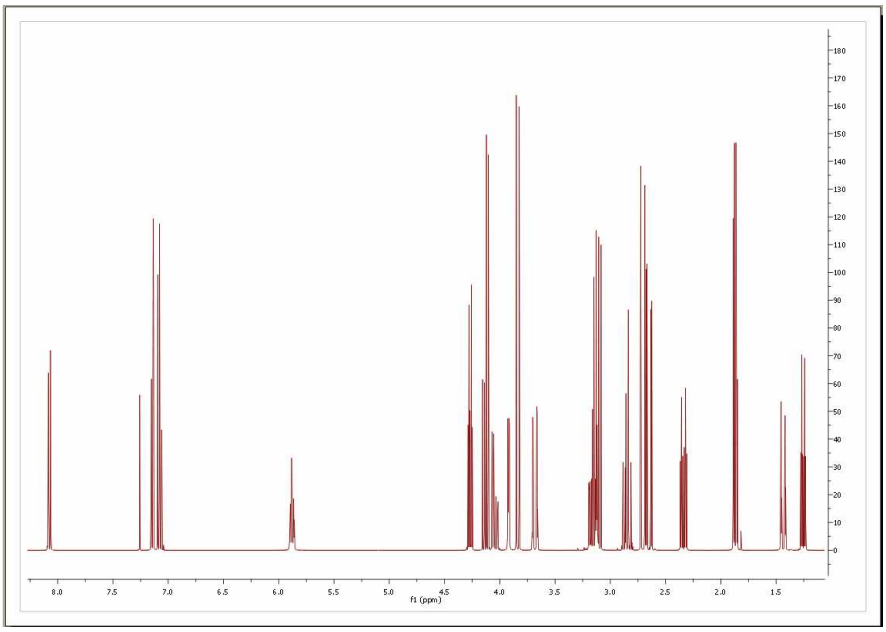
This will display a dialog box from where you will be able to select the mode of the new spectrum (Synthetic, Sticks, Uniform Line Width or Proportional Line Width). From here you can also excludes the peaks with a specific 'Type or Flag' and you can get the spectrum a new item or stacked with the original one.

We will generate a new spectrum with a proportional Line Width of 0.3, including only the peaks marked as compound (without artifacts, solvents, impurities, etc.)

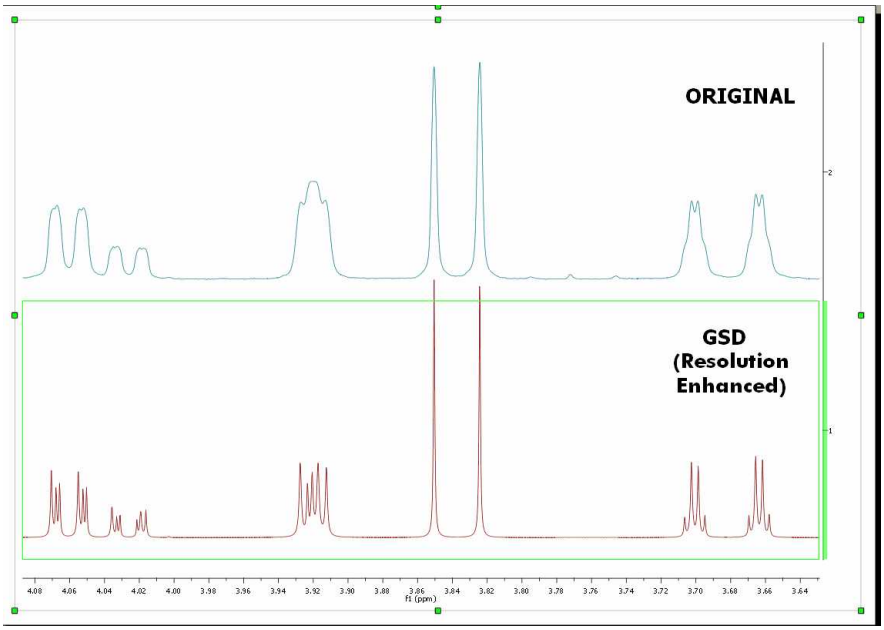


© 2024 MESTRELAB RESEARCH

Here is the result of the New spectrum from GSD:



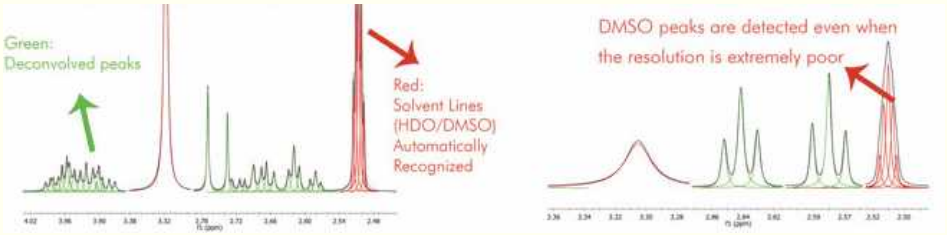
You will see in the picture below an expanded region of the original spectrum and the GSD one in stacked one. As you can see, now you will be able to measure the coupling constants more accurately, as only the compound peaks are displayed and they are displayed at an artificially increased higher resolution



Follow the menu 'Tools/Export ASCII/GSD Spectra' to export the GSD peaks as ASCII.

© 2024 MESTRELAB RESEARCH

Please bear in mind that the 'Auto Classify Peaks' feature incorporates a **'Solvent Detection'** tool which is an **Expert System** that exploits the resolution enhancement power of GSD and applies a scoring system which analyses multiplet patterns, 13C satellites, line widths and chemical shifts to decide whether a given line corresponds with a solvent resonance.

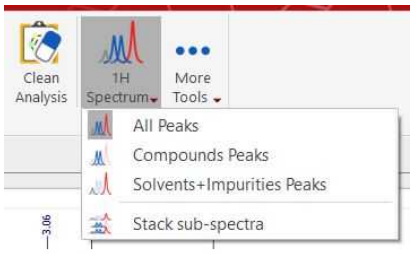


You can display the 1D spectrum with 'All Peaks' (default view) and also to decompose the spectrum into 3-sub-spectra:

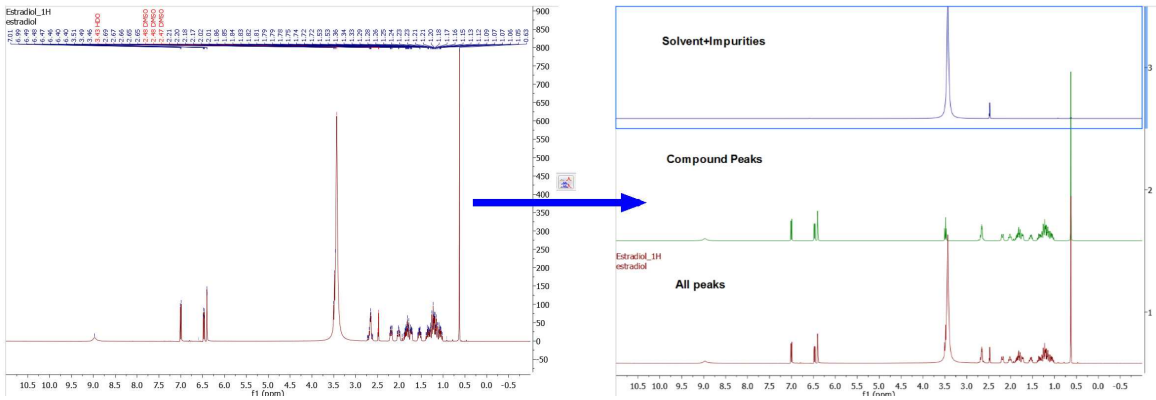
1. Containing only the 'compound peaks'.

2. Containing only 'solvent+impurity' peaks.

3. A stack plot with the different views (all, compounds and solvent+impurities):



Here you can find an example of the stacked sub-spectra view obtained from a 1H-NMR spectrum:



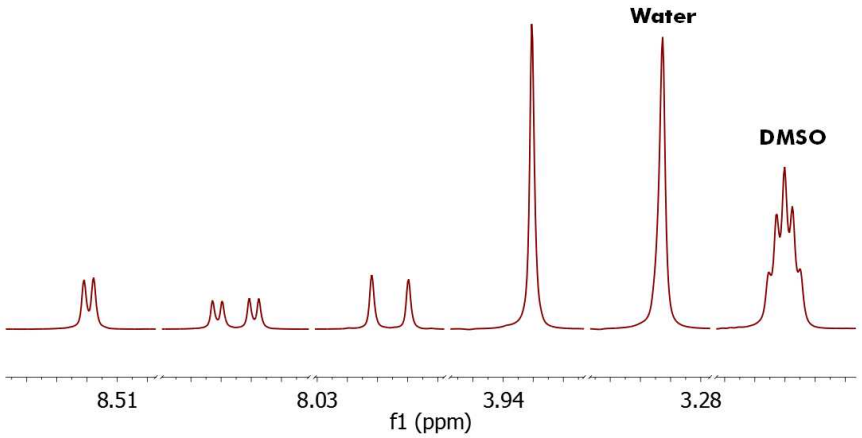
Improving GSD with Line Fitting

© 2024 MESTRELAB RESEARCH

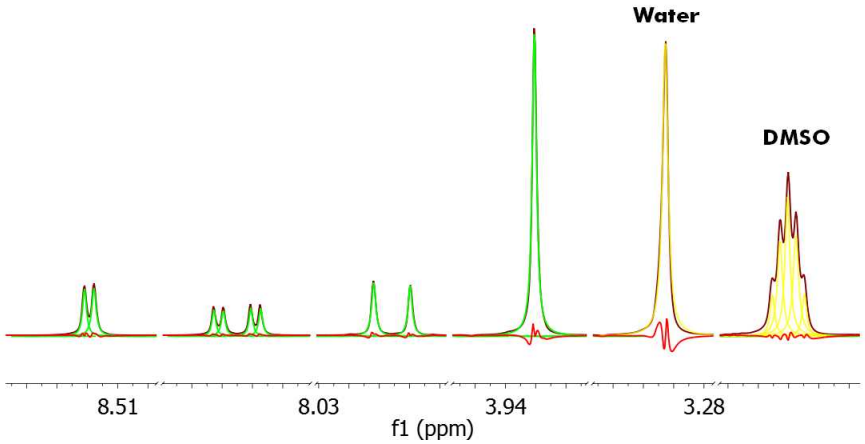
After the GSD peak picking, allow the user to define a line fitting region, and it uses all the GSD-peaks in that region as the starting peaks. The user changes the peaks and then re-fit the peaks as usual, until satisfied. The Peaks table gets automatically updated every time a peak is modified in the Line Fitting panel.

8.2.2.1 What else can I do with GSD?

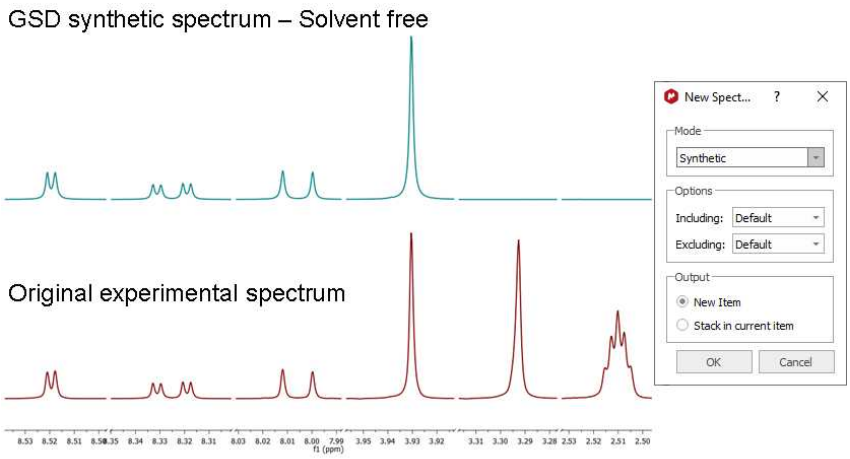
As you have seen above, from the GSD list of peaks you could create a mocked spectrum with enhanced resolution; but in addition you can use GSD for example to **remove solvent lines**. Consider the following spectrum containing solvent peaks (DMSO/Water):



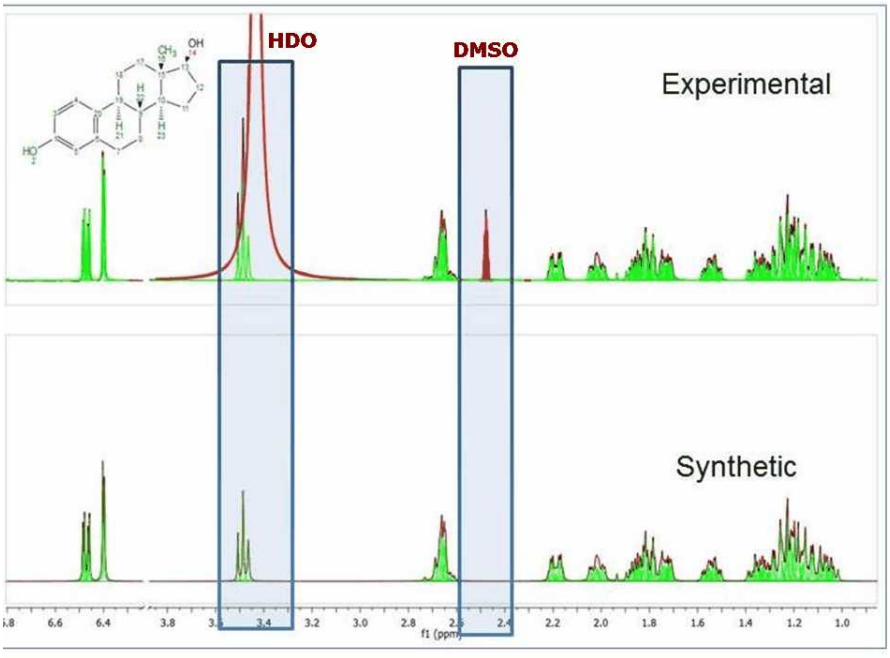
To remove the undesired peaks, just run a Peak Picking with GSD and with the 'Auto Classify Peaks' option enabled, in order to autodetect the solvent peaks (in yellow in the figure below):



And finally we create a new synthetic spectrum ***without*** the solvent lines:



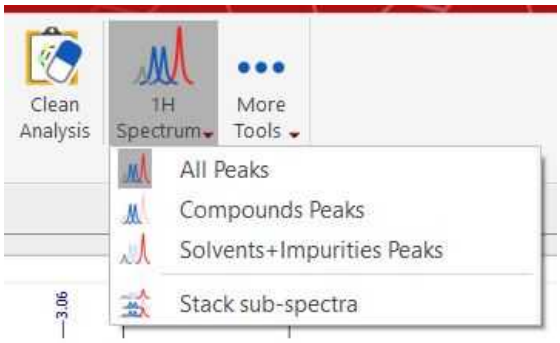
Here you can see another example where the solvent and compound signals are overlapped. Mnova´s autoedit successfully separates a triplet signal sitting on the HDO signal. The approach of generating dark regions used by other software products would miss the triplet at 3.5 ppm and therefore any verification efforts would fail.



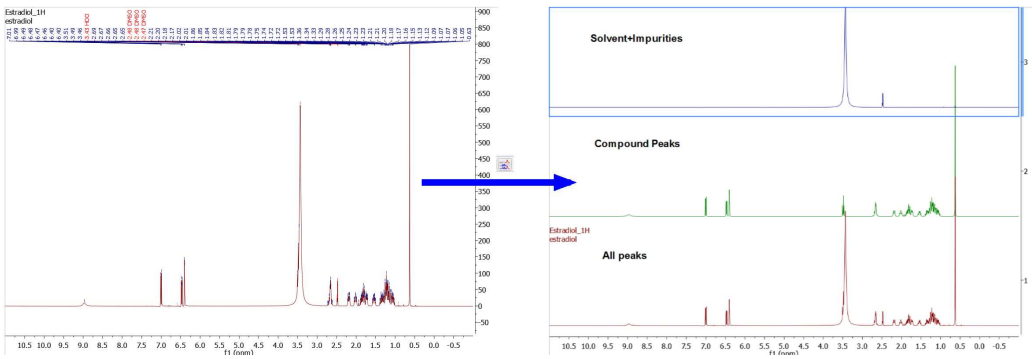
You can display the 1D spectrum with 'All Peaks' (default view) and also to decompose the spectrum into 3-sub-spectra:

1. Containing only the 'compound peaks'.
2. Containing only 'solvent+impurity' peaks.
3. A stack plot with the different views (all, compounds and solvent+impurities):

© 2024 MESTRELAB RESEARCH



Here you can find an example of the stacked sub-spectra view obtained from a 1H-NMR spectrum:

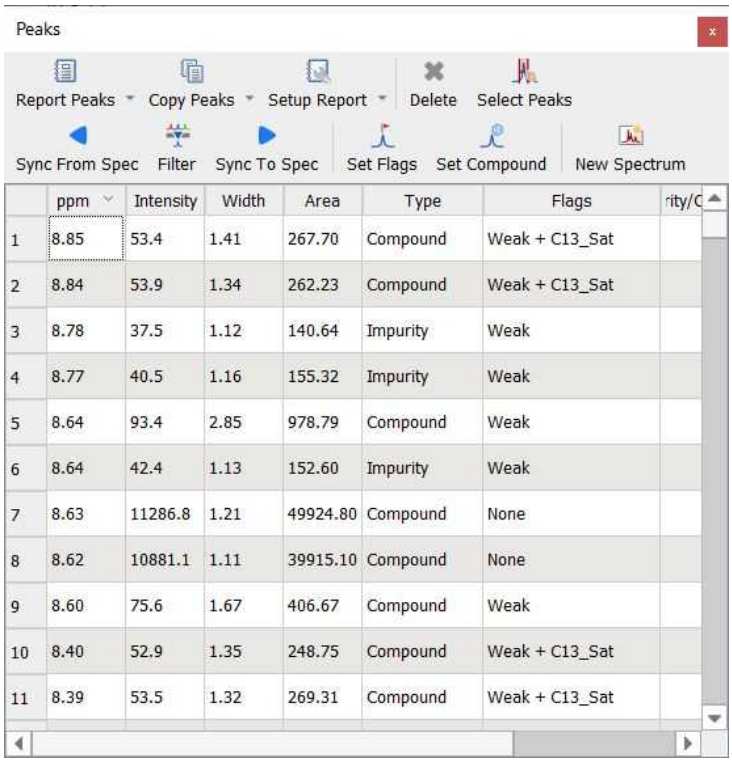


8.2.3 Peaks Table and Reports

Peak Table: The user will obtain the peak table by following the menu 'View/Tables/Peaks'. Clicking on any peak of the spectrum will automatically scroll up/down the table to the position of currently highlighted peak in the spectrum.

Please note that **hovering** the mouse over the peak label will **highlight** the applicable **peak** shape in the spectrum. The same effect you will obtain if you hover the applicable cell in the Peaks Table. Double clicking on a row in Peaks table will *zoom in* that peak in the spectrum. Move the peaks labels up and down just by clicking and dragging them.

© 2024 MESTRELAB RESEARCH



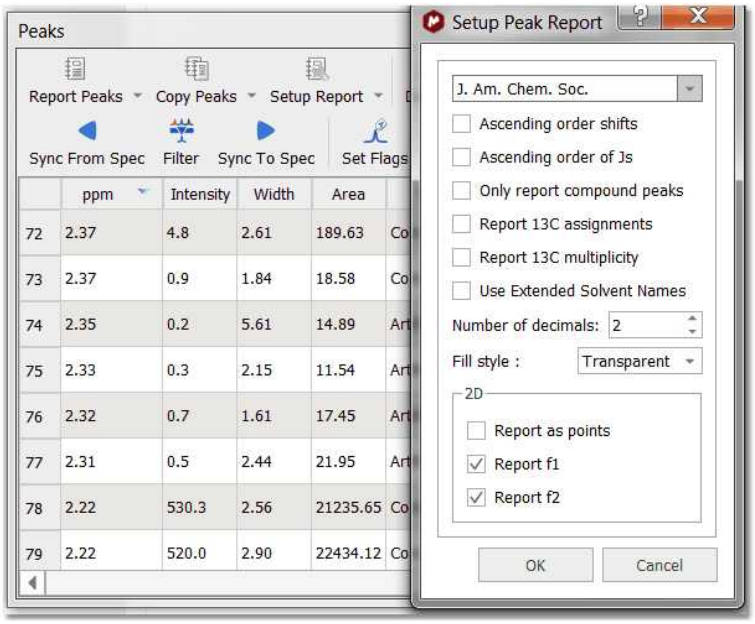
	ppm	Intensity	Width	Area	Type	Flags	rity/C
1	8.85	53.4	1.41	267.70	Compound	Weak + C13_Sat	
2	8.84	53.9	1.34	262.23	Compound	Weak + C13_Sat	
3	8.78	37.5	1.12	140.64	Impurity	Weak	
4	8.77	40.5	1.16	155.32	Impurity	Weak	
5	8.64	93.4	2.85	978.79	Compound	Weak	
6	8.64	42.4	1.13	152.60	Impurity	Weak	
7	8.63	11286.8	1.21	49924.80	Compound	None	
8	8.62	10881.1	1.11	39915.10	Compound	None	
9	8.60	75.6	1.67	406.67	Compound	Weak	
10	8.40	52.9	1.35	248.75	Compound	Weak + C13_Sat	
11	8.39	53.5	1.32	269.31	Compound	Weak + C13_Sat	

The Peaks table displays the list of peaks, including the following information: chemical shift in ppm and Hz, Intensity (height), Width (in Hz, at half maximum of the peak -FWHM), Type (compound, solvent, impurity, etc), Flags, Multiplicity (for the carbons) and 'Annotations'.
The user can report the Peak table directly on the spectrum by clicking on the **'Report Table'** icon under the 'Report Peaks' scroll down menu . To delete an undesired peak, click on the **'Delete'** icon of the Peak List, or select 'Delete Manually' on the Peak Picking scroll bar icon:



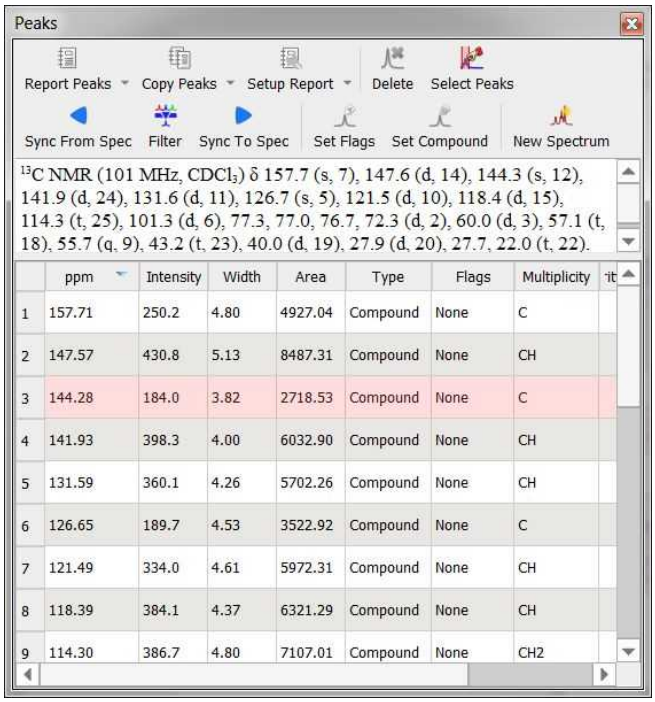
By default, peak frequencies are listed directly on the spectrum but they can also be accessed via the *Peak Picking table* and also as a **'Peak List'** by clicking on the **'Report Peaks'** icon. The user will be able to select the 'Peak Report' Template between several journal formats by selecting 'Setup Report' in the 'Peaks table'.

© 2024 MESTRELAB RESEARCH



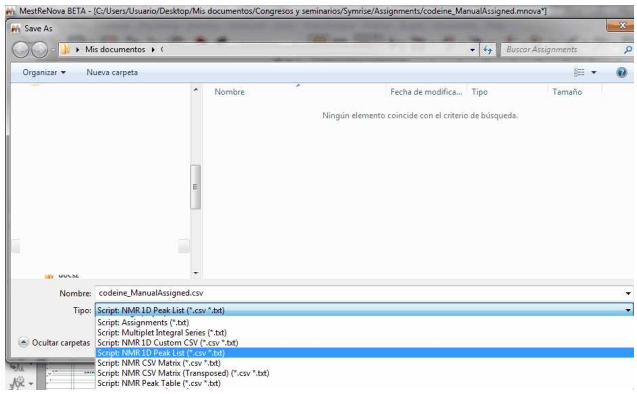
From here you can select the order of the report (ascending or descending), the option to only report the compounds peaks, to report the 13C assignment and to select the number of decimals.
For 2D experiments, you will have the choice to report a list of F1, F2 peaks or both. This option will be remembered when creating a reporting template.

After making assignments to the 13C peaks the multiplicity gets automatically filled in Peaks table. This multiplicity information can be included in the Peaks Report by selecting it in Setup Report.

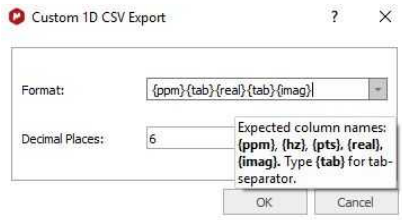


The user will be able to copy the **'Peak List'** report to the clipboard by selecting 'Copy Peaks' on the 'Peaks Table'.

The peak list can also be exported as an ASCII file by following the menu 'File/Save as' and selecting Script: NMR 1D Peak List (*.csv, *.txt):

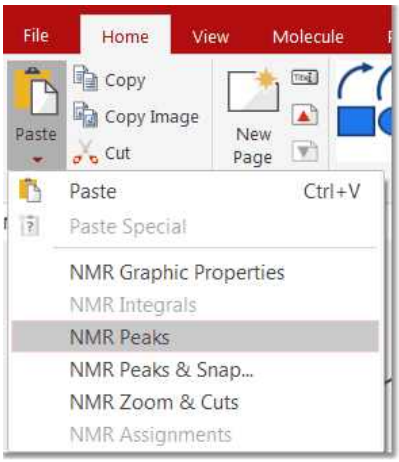


You can export NMR spectra via 'File/Save As/NMR Custom CSV File' with options to export PPM+Re+Im, Hz+Re, PPM+Re or Hz+Re+Im, either from right to left or from left to right.



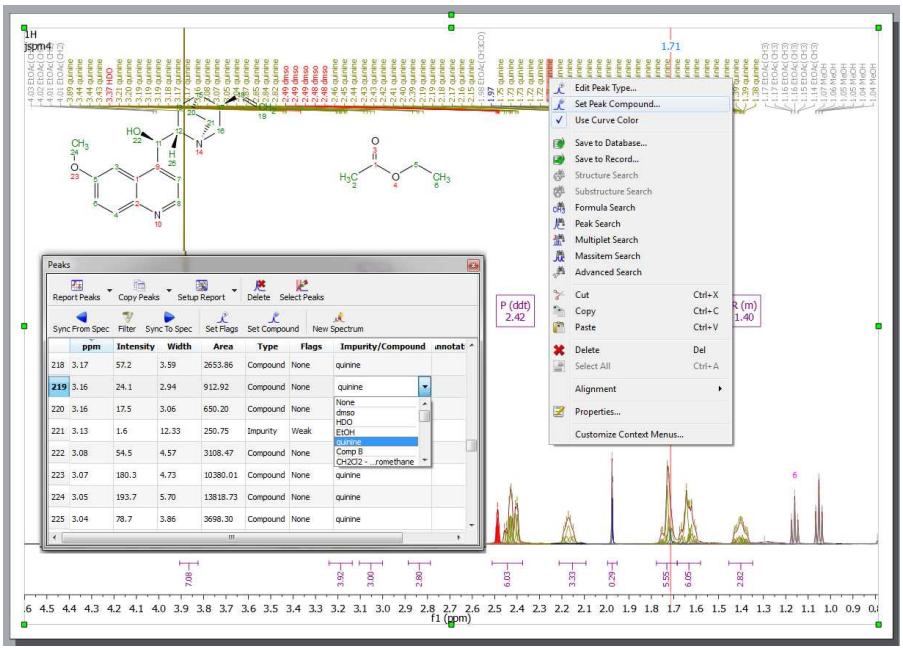
Follow the menu 'File/Save As/Script: NMR Peaks Table' to export the peaks in a table (only the fields set as 'Visible' in 'Setup Table' will be exported).

To copy&paste the peaks from one spectrum to another, just press 'Ctrl/Cmd+C' in the original spectrum and then follow the menu 'Home/Paste Properties/Paste NMR Peaks in the other spectrum (or spectra, if you have previously selected them in the page navigator):

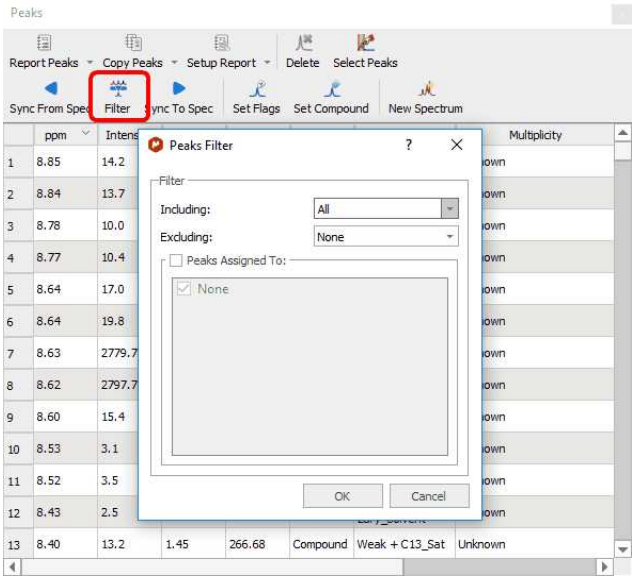


If your document contains more than one compounds, you will be able to select the compound for each peak from the Peaks Table (by clicking on the 'Set Compound' button or by double clicking on the 'Impurity/Compound' cell). The same effect will be obtained by right clicking on the Peak label and selecting ‘Set Peak Compound’:

© 2024 MESTRELAB RESEARCH



You can apply some filters to show for example only Compounds Peaks. If the spectrum contains peaks belonging to more than one compound, you can show only the peaks assigned to one of them (by using the 'Filter' button):

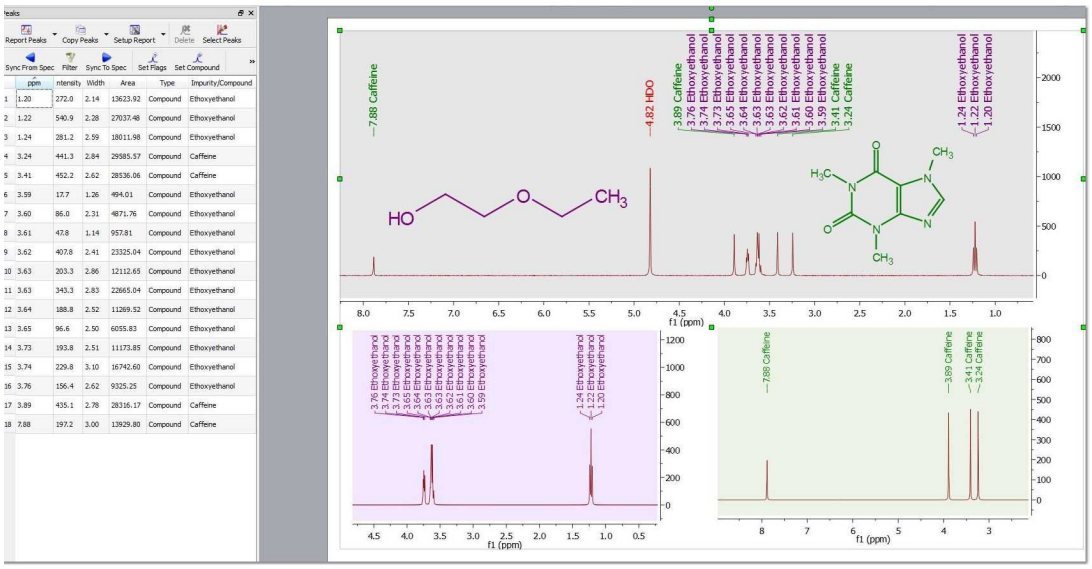


You can synchronize the type of the peaks in the table (i.e: only Compounds peaks) with the peaks showed in the spectrum (by using the button 'Sync from Item') or viceversa, synchronize the spectrum peaks with the peaks showed in the table ('Sync to Item' button).

The 'New spectrum' button 

will generate a synthetic spectrum from the selected peaks in the table or for all the peaks shown in the table if there is no selection. Here you can see an example, showing a mixture of two compounds on top and the synthetic spectra (generated with the 'New Spectrum' feature) of each compound at the bottom:

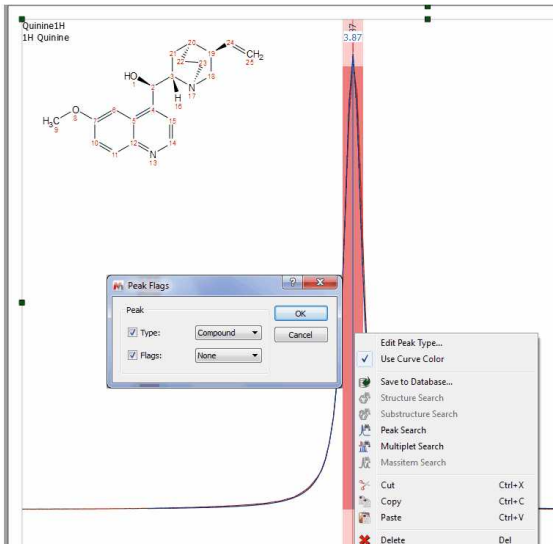
© 2024 MESTRELAB RESEARCH



Select 'Auto Classify Peaks' in the Peak Picking scroll down menu to edit automatically the Type and Flags of each signal:

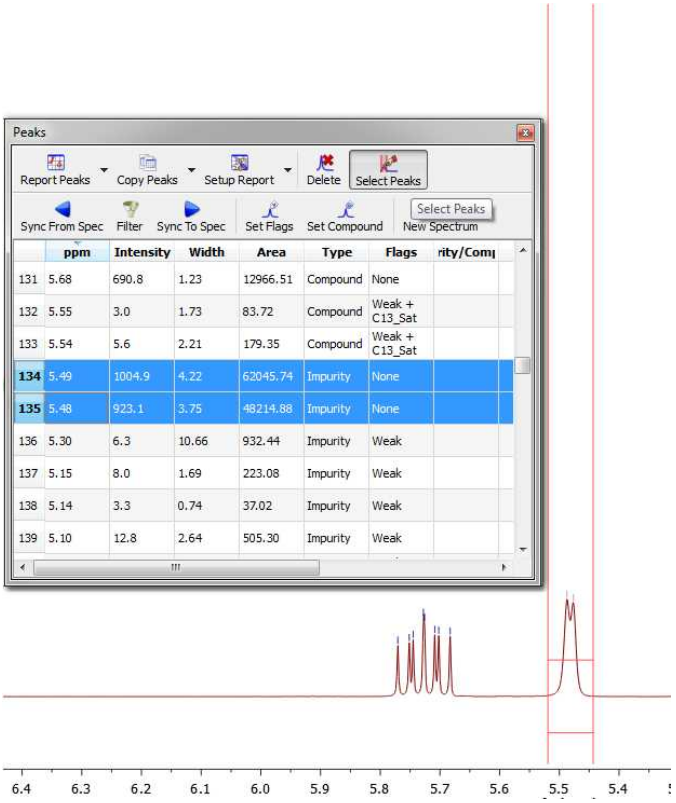
You can also manually edit the 'Type' and the 'Flags' table just by double clicking on the applicable cell.

Right click on any peak and select 'Edit Peak Type' to change the Peak type:

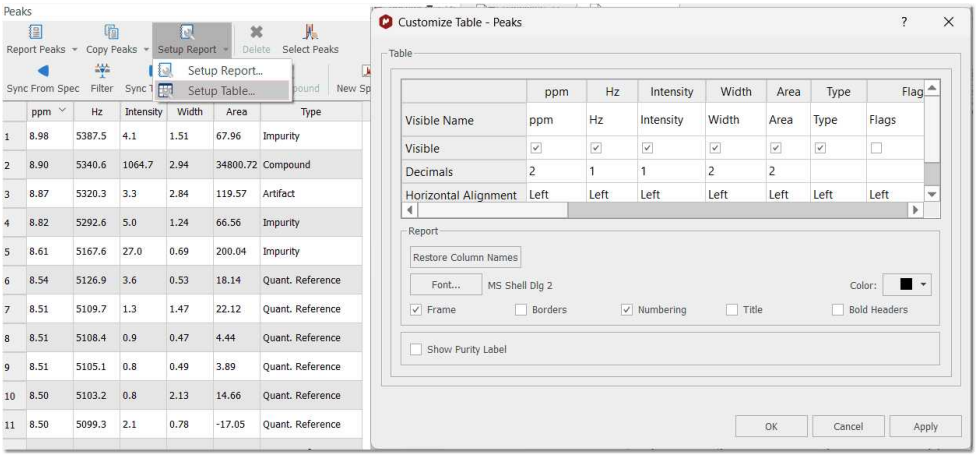


The user will be able to highlight in the table any desired peak just by clicking on the 'Select Peaks' button and then selecting the peaks in the spectrum (by clicking and dragging); then the user will be able to modified their flags (or Type) by clicking on the 'Set Flags' button. In the example below, we have changed the type of two peaks (from compound to impurity):

© 2024 MESTRELAB RESEARCH



You can customize the appearance of the table by clicking on the 'Setup Table' button:



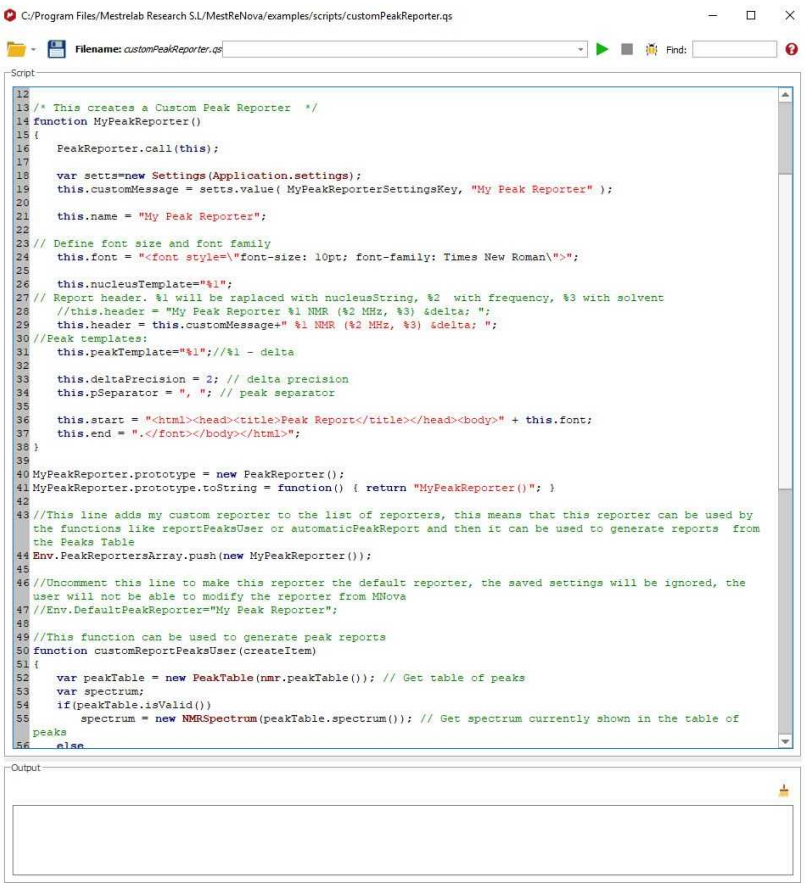
Purity *(as a ratio of Compounds Vs (Impurity+Artifacts) peaks)* can be displayed in Peaks table by selecting the checkbox "Show Purity Label" in Setup Table (disabled by default).

To export the peaks in a table, follow the menu 'File/Save As/Script: NMR Peaks Table'; (only the fields set as 'Visible' in Setup Table get exported).

See also Tables.

Customizing Peak Reports

You can add additional report templates by creating new scripts. You will find an example of a script (customPeakReporter.qs) with a different 'Peak Report Template' in the 'examples/scripts' folder of Mnova. To see how to customize this script, take a look at the 'Multiplet Analysis' chapter. The concept will be the same.




```
/* This creates a Custom Peak Reporter  */
function MyPeakReporter()
{
    PeakReporter.call(this);

    var setts=new Settings(Application.settings);
    this.customMessage = setts.value( MyPeakReporterSettingsKey, "My Peak Reporter" );

    this.name = "My Peak Reporter";

// Define font size and font family
    this.font = "<font style=\"font-size: 10pt; font-family: Times New Roman\">";

    this.nucleusTemplate="%1";
// Report header. %1 will be raplaced with nucleusString, %2  with frequency, %3 with solvent
    //this.header = "My Peak Reporter %1 NMR (%2 MHz, %3) &delta; ";
    this.header = this.customMessage+" %1 NMR (%2 MHz, %3) &delta; ";
//Peak templates:
    this.peakTemplate="%1";//%1 - delta

    this.deltaPrecision = 2; // delta precision
    this.pSeparator = ", "; // peak separator

    this.start = "<html><head><title>Peak Report</title></head><body>" + this.font;
    this.end = ".</font></body></html>";
}

MyPeakReporter.prototype = new PeakReporter();
MyPeakReporter.prototype.toString = function() { return "MyPeakReporter()"; }

//This line adds my custom reporter to the list of reporters, this means that this reporter can be used by
the functions like reportPeaksUser or automaticPeakReport and then it can be used to generate reports  from
the Peaks Table
Env.PeakReportersArray.push(new MyPeakReporter());

//Uncomment this line to make this reporter the default reporter, the saved settings will be ignored, the
user will not be able to modify the reporter from MNova
//Env.DefaultPeakReporter="My Peak Reporter";

//This function can be used to generate peak reports
function customReportPeaksUser(createItem)
{
    var peakTable = new PeakTable(nmr.peakTable()); // Get table of peaks
    var spectrum;
    if(peakTable.isValid())
        spectrum = new NMRSpectrum(peakTable.spectrum()); // Get spectrum currently shown in the table of
peaks
    else
```

For 2D. you can modify the 'PeakReporter.qs' script (which is located in the scripts folder of Mnova) by just changing the **false** with **true** in this line:

```
//Report f1 value in 2D spectrum

 this.reportf1In2D=false;
```

with: `this.reportf1In2D=true;`

and you will get a report like this:

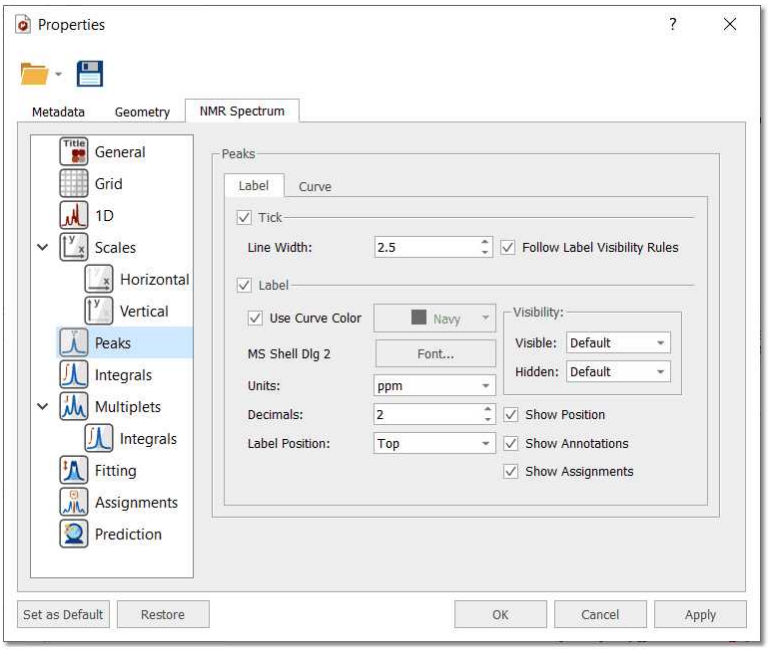
NMR (400 MHz, CDCl3) δ (8.62 147.34), (7.97 131.35), (7.94 131.37), (7.46 118.14), (7.32 121.23), (7.28 121.24), (7.21 101.03), (5.48 71.99), (4.91 114.03), (3.87 55.34), (3.11 59.66), (3.05 56.77), (2.65 56.82), (2.62 42.87), (1.78 27.53), (1.54 21.65).

© 2024 MESTRELAB RESEARCH

See also:
Peak Picking Properties

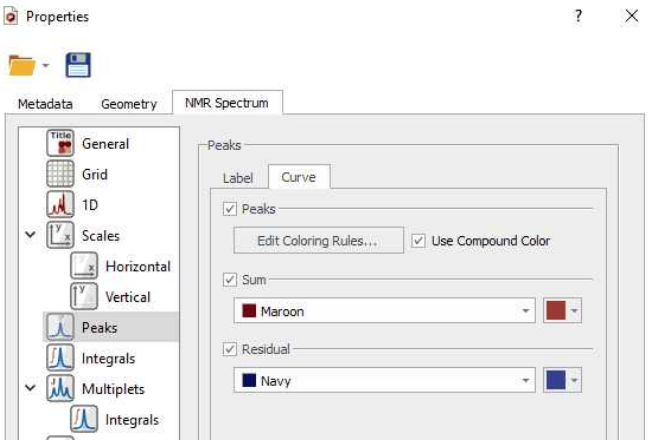
8.2.4 Peak Picking Properties

The user can modify the properties of the **Peak Picking** by following the menu 'Home/Properties/Peaks', by right clicking the left mouse and selecting 'Properties/Peaks' from the *pop-up* menu or by using the shortcut Alt+Enter.

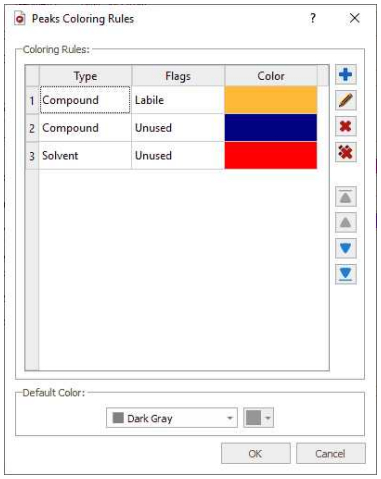


Then, the user can display or hide the peaks (according the peak type by selecting the applicable option in the scroll down menus) and to show/hide the Position, Annotations, Ticks or Assignments by checking (or unchecking) the applicable check box. You can select the color, the font and the units (ppm or Hz) of the 'peak picking label'; the number of decimal figures to be shown on screen and the position of the 'peak labels'.

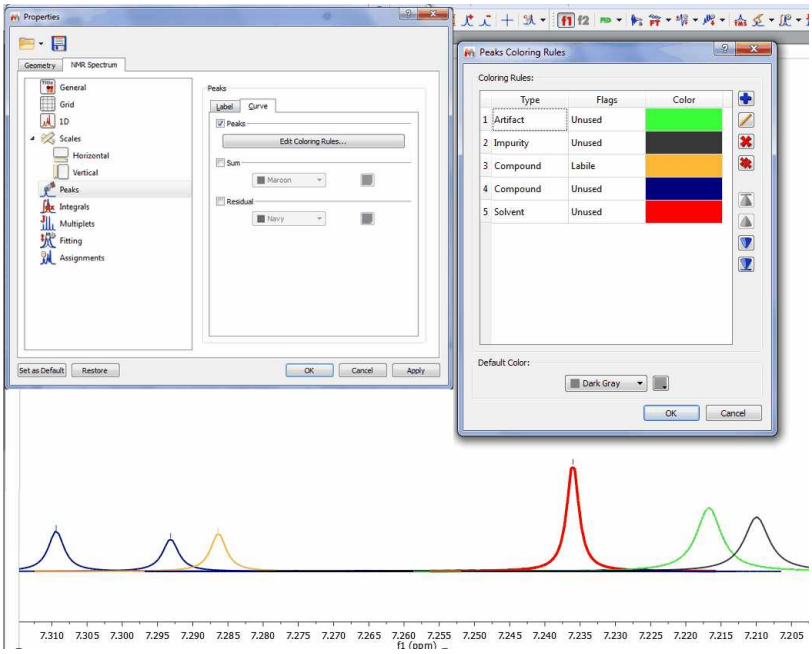
Clicking on the 'Curve' tab, you will be able to customize the color of peak curves (Peaks, Sum or Residual):



Clicking on the 'Edit Coloring Rules', will display a dialog box to allow you to create different colours rules depending on the peak type and flag:



Let´s see an example with different peak curve colours:



8.3 Integration

Spectral integration can provide invaluable quantitative information about a molecule as the integral of a spectrum indicates the number of nuclei which contribute to a given line (or set of lines).

Mnova implements a series of integration algorithms for both 1D and 2D data sets. These algorithms can be accessed via the menu item 'Analysis/Integration':

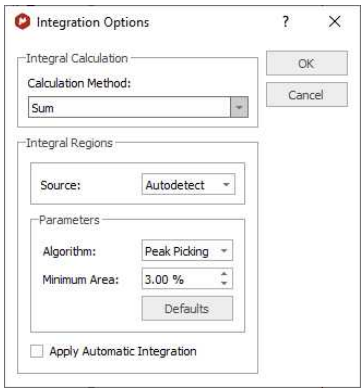
© 2024 MESTRELAB RESEARCH



Autodetect Integration

1D spectra

The program automatically determines the regions containing signals. The algorithm uses the following **options**:



- **Calculation Method:** you can select between 'Sum' (classical integration, faster than 'Peaks' but without deconvolution), 'Edited Sum' or 'Peaks' (it takes into account only the integrals of the GSD spectrum and because of the deconvolution applied, it is slower than the 'Sum' algorithm). In the Peaks calculation method, you can select what peaks will be taken into account for the integration (for example, only compounds peaks and not solvent or impurity peaks, etc...)

Edited Sum: in this integration method, the values from Sum method are corrected using a correction factor:

Sum(Ps_i)/Sum(P_i): where "Ps_i" are the selected peaks (by default, including Compound peaks and excluding Hidden, C13_Sat and Rotational); "P_i" are all peaks in the integration range.
Int (Edited Sum) = Int (Sum)* (Sum(Ps_i)/Sum(P_i))

For further information about the edited Sum algorithm, please check this paper:
http://pubs.acs.org/doi/abs/10.1021/acs.analchem.5b04911

- **Algorithm:** you can select between the regular algorithm which is called 'Derivative' and the new algorithm based in the 'Peak Picking'.

- Derivative: you will find the options described below:
Sensitivity: This value controls how small peaks can be detected. It is a kind of smoothing parameter so that large values will filter out noisy signals. Peaks of small intensity will also be discarded in the automatic integration. Reduce this value if some small signals of interest are not detected.

© 2024 MESTRELAB RESEARCH

Merging distance: This parameter is used to control the separation of integrals. Integral regions separated by less than this value will be merged into a single one

Minimum Area: Integrals with a value smaller than a given percentage of the largest integral will be discarded

- Peak Picking algorithm**:** this integral algorithm is based in the peak picking analysis. The user will be able to set the maximum value of the coupling constants (**J Max**), the **Peak Width Factor** and also the **Minimum Area** of an integral (integrals with a value smaller than a given percentage of the largest integral will be discarded).

Bear in mind that this algorithm is based on the peak picking results. If no peak picking has been applied, the program applies one on the fly automatically but the best way to proceed will be by first applying a peak picking to make sure that all the real peaks have been detected and, more importantly, artifacts and very small peaks (e.g. impurities) have not.

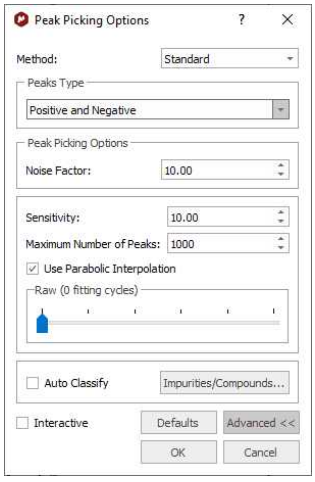
So as always, it will be a good idea to apply a baseline correction, followed by peak picking – In this case, it's important to ensure that the noise factor is right.

Once you're happy with the peak picking results, it's time to apply integration algorithm. The critical parameter is the 'peak width factor'; the larger this parameter, the wider will be the integral segments.

2D spectra

Automatic integration of 2D spectra is based on the 2D peak picking algorithm. If no peak picking has been applied when the automatic integration command is issued, **Mnova** will automatically apply a 2D peak picking on the background and then, for every peak detected, it will find the applicable volumes.
Thus, the options that control the 2D peak picking procedure also apply to the 2D automatic integration algorithm.

A series of Automatic Peak Picking Options can be controlled on the 'Peak Picking Options' dialog box, which can be accessed via the Peak Picking scroll menu (see the 'Peak Picking' section of this guide). The user can choose the noise factor (the higher the factor the less peaks will be picked), the peak type, whether to use parabolic interpolation, merging options for 2D multiplets, maximum number of peaks, etc. All these options can be evaluated interactively, so that their results can be viewed on screen in **real time**, by making sure the **'Interactive'** box is selected.

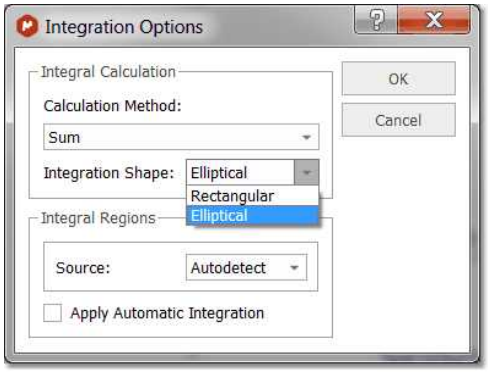


Peak volume integration in Mnova is measured as the sum of all digital intensities within the integration area (footprint). This footprint can be of 2 shapes: rectangular or ellipsoid footprints. In low resolution spectra with no significant fine structure, the ellipsoids footprints fit well, whereas higher resolution

© 2024 MESTRELAB RESEARCH

spectra tend to show rectangular patterns according to the projections of the correlated individual multiplets.

The default setting is with ellipsoids. But you can change it to rectangular in the integration options.



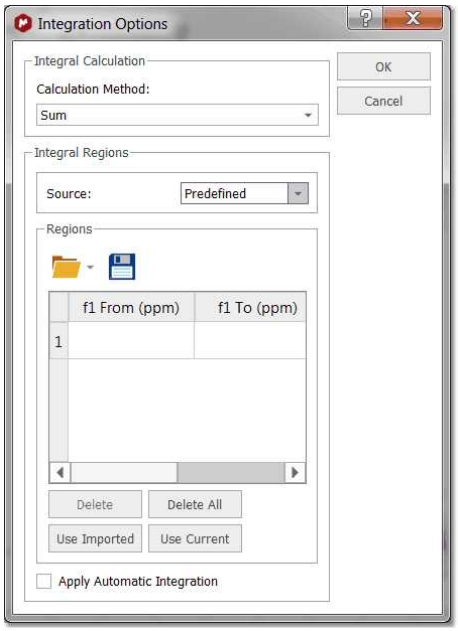
By the way, you can also manually change the size of a footprint by click-and-drag on one of the edges of the footprint.

See Integral Properties

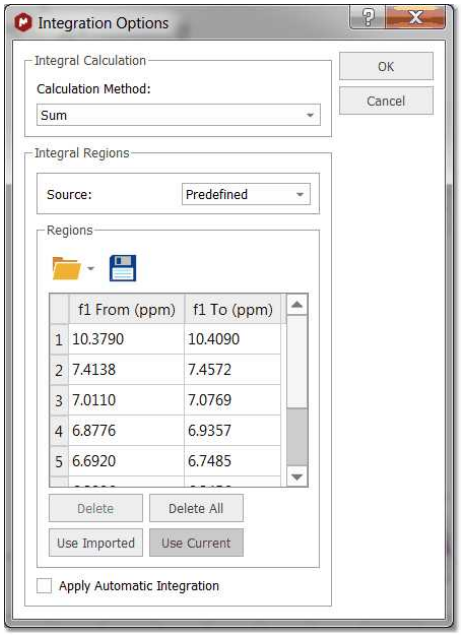
Predefined Regions Integration

The user will be able to predefine the integral ranges to automate the integral analysis. Just go through the following procedure:

1. Open the 'Integral Option' dialog box (by clicking on 'Options' in the 'Integration' scroll down menu) and select the Method: **'Predefined Regions'** as is shown in the picture below:



2. This will display a new dialog box, where the user will be able to select the desired ranges to apply the integration. If you have already integrated the spectrum you can click on the 'Use Current' button to import the integral ranges:



Clicking on OK, will apply the integrations to the current spectrum and keep the regions to apply further integrations to additional spectra by just clicking on 'Predefined Regions' in the Integral menu:

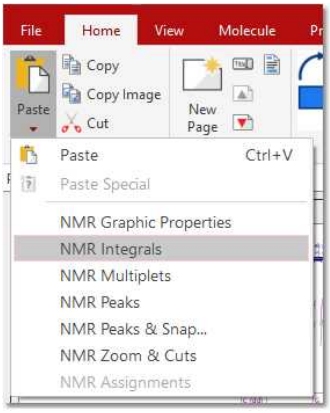


The user will be able to save these regions for later uses, by clicking on the 'Save Integral Regions' icon which will be loaded by clicking on the 'Load Integral Regions' icon. To save the integral regions, follow the menu 'File/Save as' and select 'MestReNova Integral Regions'.

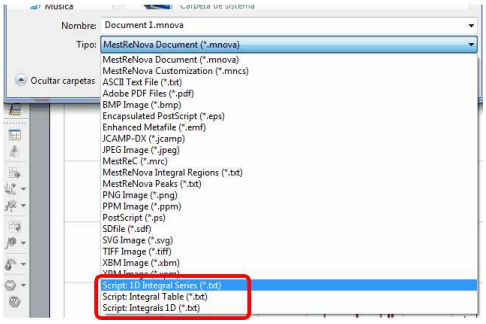
You will also be able to use the original Bruker integrals by using the 'Use Imported' button. Please bear in mind that if you import a Bruker file with MNova, the integrals will appear automatically, however if you open and old Mnova-document with Bruker data then this button is the way to use Bruker ranges. With the 'Use Imported' button you will be able to reload the original Bruker integrals.

To copy&paste the spectral regions from one spectrum to another, just press 'Ctrl/Cmd+C' in the original spectrum and then follow the menu 'Home/Paste/NMR Integrals' in the other spectrum (or spectra, if you have previously selected them in the page navigator):

© 2024 MESTRELAB RESEARCH



The integral list can also be exported as an ASCII file by following the menu 'File/Save as' and selecting 'Script: Integrals 1D (*.txt)' (or 2D depending on the dataset). You can also export the integral series and tables:



Manual Integration (shortcut: I)

First, select integration mode by clicking on the Manual integration command as depicted below:



.

- Next, click and drag with the mouse to select the area to be integrated
- The first integral will be normalized to 1.0. All following integrals will be referenced relative to the first integral.

Follow the menu 'File/Save as' and select 'MestReNova Integrals (*.txt)' to save the integrals list as a 'text file'

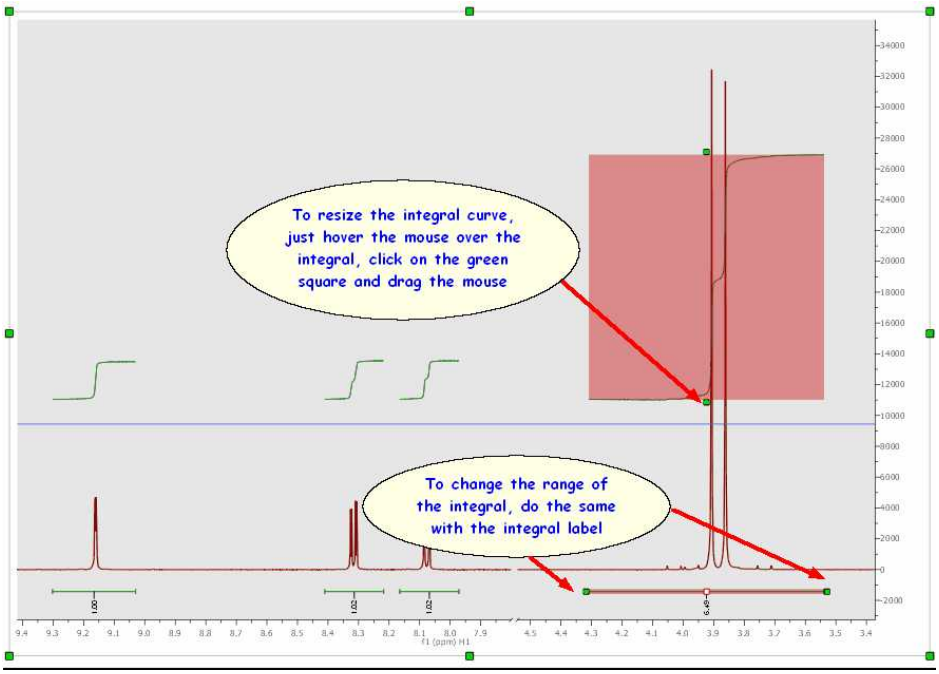
Integral Settings:

All the integral curves are mouse-sensitive and they respond to usual mouse operations. If you want to move up or down all the integrals, just click and drag (with the left mouse button) over anyone of the

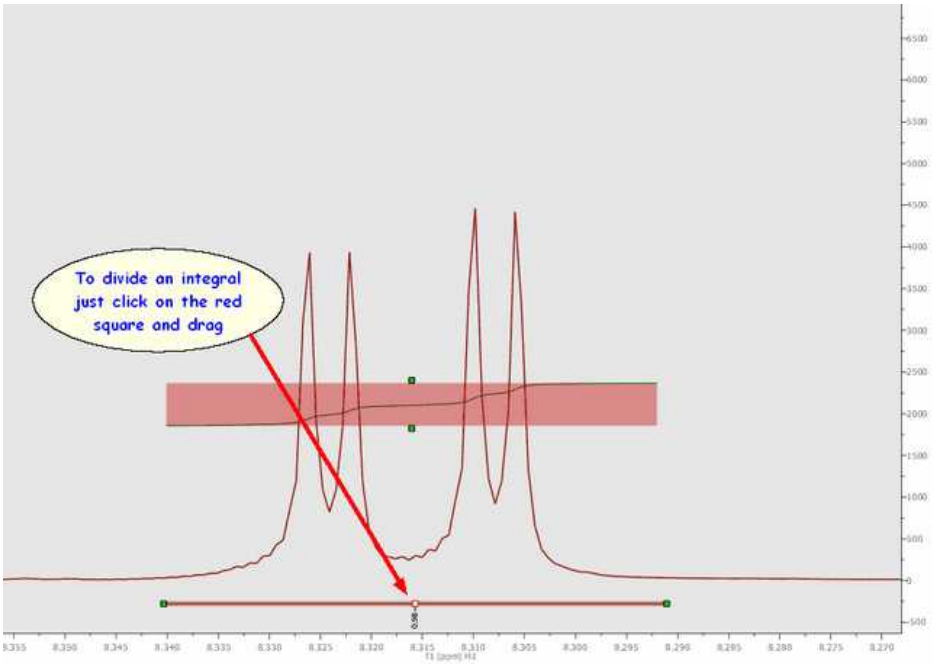
© 2024 MESTRELAB RESEARCH

integrals (notice that hovering the mouse over the integral will highlight it in red). If you keep pressed the SHIFT key at the same time, the height of the integral curves will be changed.

The same effect will be obtained if you hover the mouse over integral curve, click on one of the green squares and drag the mouse up or down. If you want to change the range of the integral, do the same with the integral label (notice that once you hover the mouse over the green squares of the integral curve or label, the mouse pointer turns to vertical or horizontal arrows, respectively).

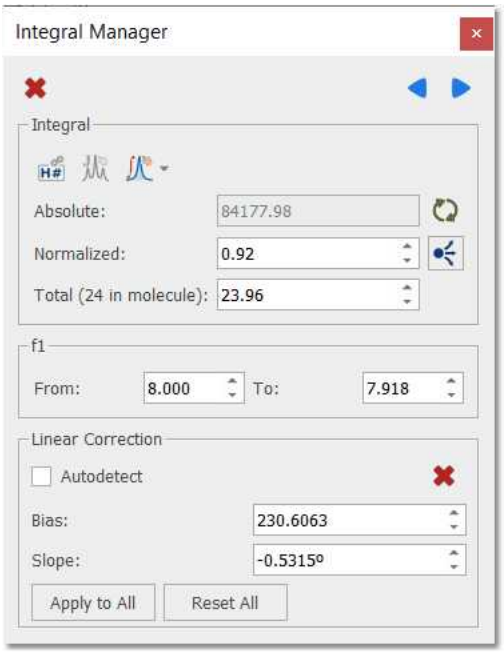


Cutting Integrals: to divide any integral just by clicking on the red square of the integral label and dragging the mouse:

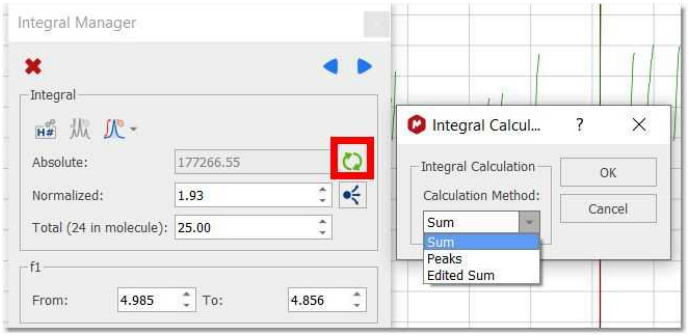


© 2024 MESTRELAB RESEARCH

Integration Manager (shortcut: Shift+I): The integral editor will be displayed by double clicking on any integral curve. From there, you can change the integral range, normalize the integral to a specific integral, navigate through them and also to reference integrals to the total integral value for the spectrum.



You can manually add integrals and multiplets using different integration calculation methods (Sum, Edited Sum and Peaks). To avoid misunderstandings in the integration results, you can track the method used in each integral/multiplet. This will be displayed in the Integral/Multiplet Manager, where there is an option to recalculate each integral/multiplet with ability to choose the integration method in each case.



A similar option can be found under the Analysis/Integrals menu.



Please note that the 'Integral Manager' dialog is **modeless**, so you will be able to use any other tool (i.e. zooming) whilst you are applying the analysis.

You can resize the limits of the integral by using the arrow buttons  or entering the desired value on the applicable edit box:

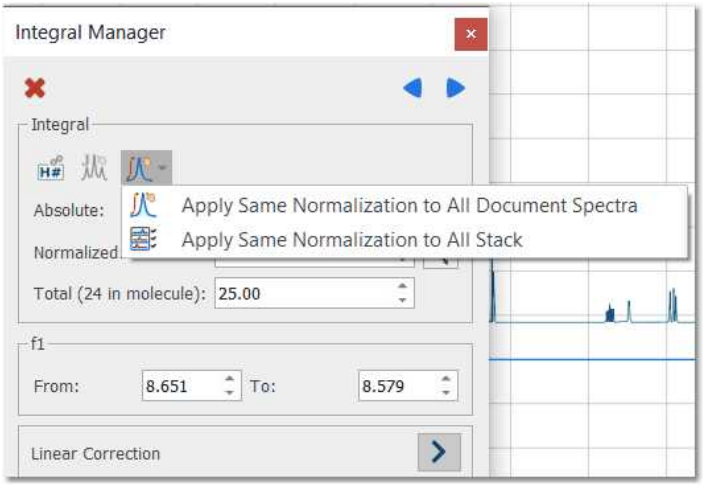


Bear in mind that you are able to navigate over the integrals by using the **'Previous or Next'** icons.

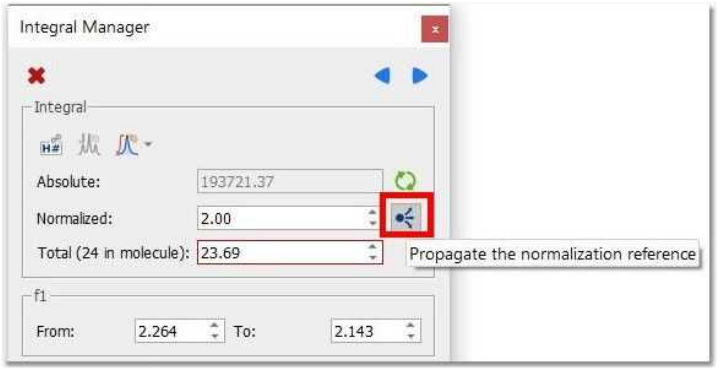
If you want to resize the height of the integral curve, just hover the mouse over one of the integral curves and hold down SHIFT, click on left mouse button and drag up or down (to increase or decrease the height).

If you want to normalize the integrals, just overwrite the desired value on the 'Reference edit box' (on the Integrals Editor') and press OK, all integrals will update with reference to the chosen one.
The user can also delete an integral one by one by clicking on the 'Delete' button.

You can apply the same integral normalization to all the spectra (in the current document) or to all the traces of a stack plot by selecting the applicable option, as you can see below. This is very useful if you need for example to determine relaxation times from a series of 1D spectra, to be able to compare the integral values between the different traces.



You can propagate the normalization reference of any signal to all the traces in a 1D or 2D stack by using this command:



Linear Correction: Linear correction is basically a straight line built connecting the left and right boundaries of every integral in such a way that the final area will include only the integral above that

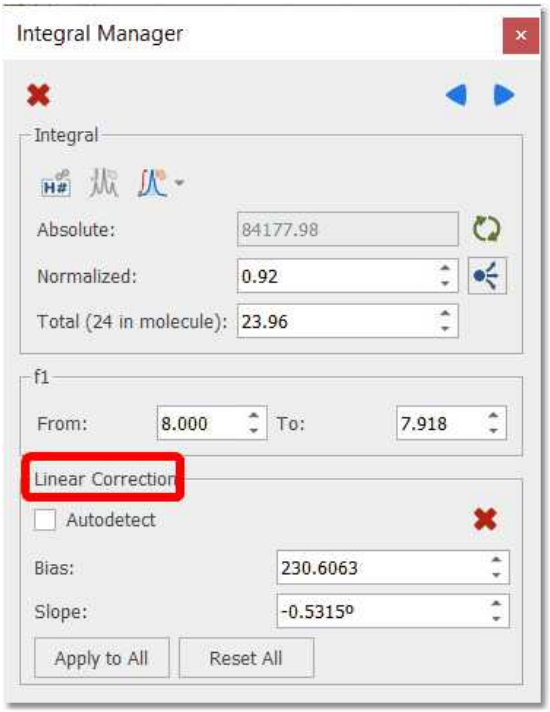
© 2024 MESTRELAB RESEARCH

line. In other words, if A is the total area under the peak(s) and B is the area under that straight line, the reported integral R will be:

R = A – B

Mnova has an option to automatically calculate this straight line. In order this to work properly, it's recommended to have a good digital resolution (e.g. by applying ZF), as the program utilizes 3 points at both edges of the integral to build the straight line.

If you want to apply an integral correction or rather, a linear baseline correction just on the integral region, just adjust the *Bias* and *Slope* parameters or check the Autodetect box:



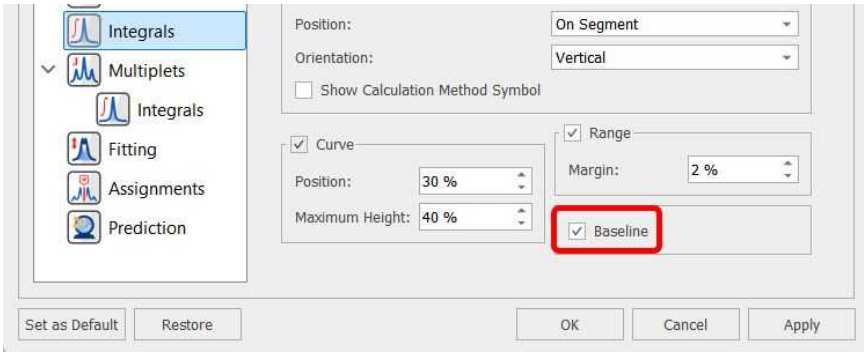
Use the controls for *Bias* and *Slope* correction as follows:

- Adjust the *Bias* parameter until the initial (left) part of the integral is flat.
- Adjust the *Slope* parameter to flatten the top (right) part of the integral curve.

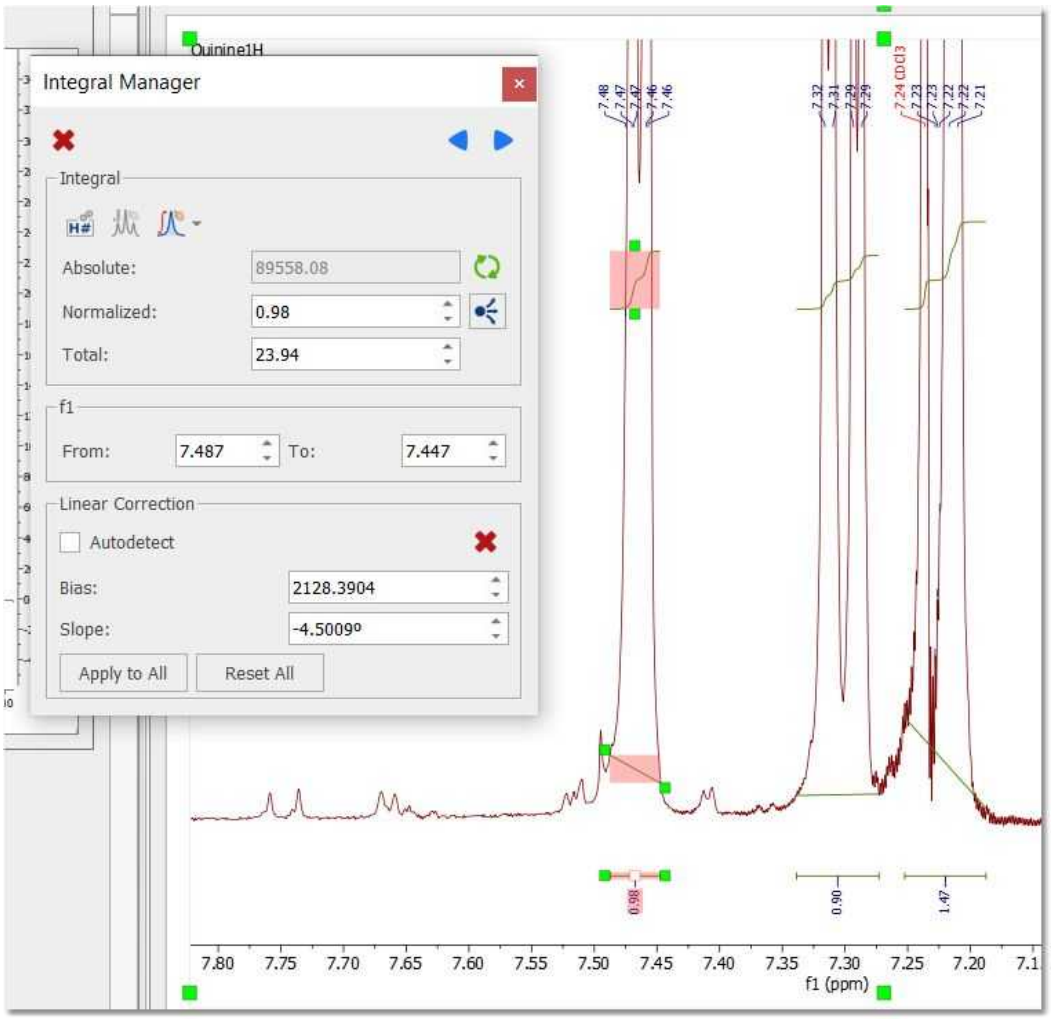
The *Auto* box computes the *Bias* and *Slope* values automatically.
The button *Apply to All* is used to correct all the integrals simultaneously.

To display the baseline of the linear correction applied, please check this box from the 'integral properties':

© 2024 MESTRELAB RESEARCH



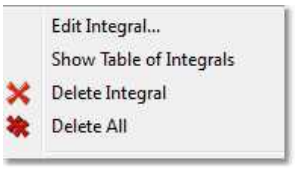
The display format is the same as the integral curve and segment, and has two handlers that can be moved vertically in order to modify the bias and slope of the baseline.



We strongly recommend to correct the baseline prior integration using any of the baseline correction algorithms.

Likewise, if you press the right mouse button on an integral, you will get a pop-up menu with the following options:

© 2024 MESTRELAB RESEARCH



Edit Integral: displays the Integration Manager dialog box.
Show Table of Integrals: to show the integrals table
Delete Integral: deletes the current integral.
Delete All: deletes all integrals.

Autodetect Nuclides Count: this command will be very useful to count the nuclides present in any spectrum.

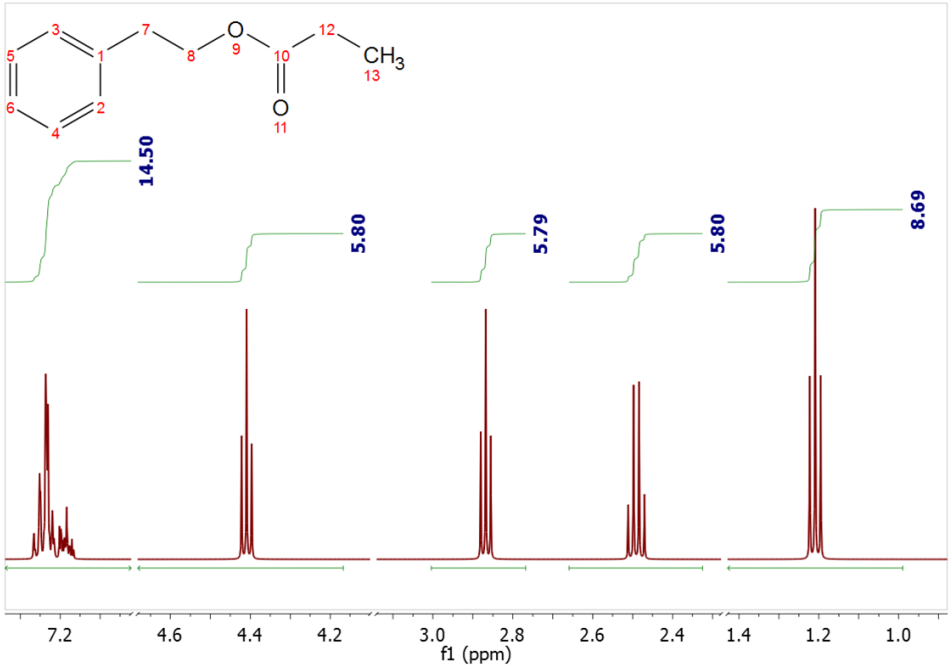
Integration only gives information on the relative number of different nuclides. From a practical stand point, first the absolute integral values are computed and then the integrals are normalized (referenced).

For example, if we know that a given integral corresponds to a CH3 group, we can set it to a value of 3 so that all other integrals will be scaled accordingly. Note that these values are not exact integers and need to be rounded to the nearest integer to obtain the significant value. But that means that the reference should have correct intensity to start with, which is not guaranteed.

Our goal is to automate this process, that is, starting from the absolute integral values, automatically select the reference group and scale the remaining values in order to obtain the correct number of nuclides.

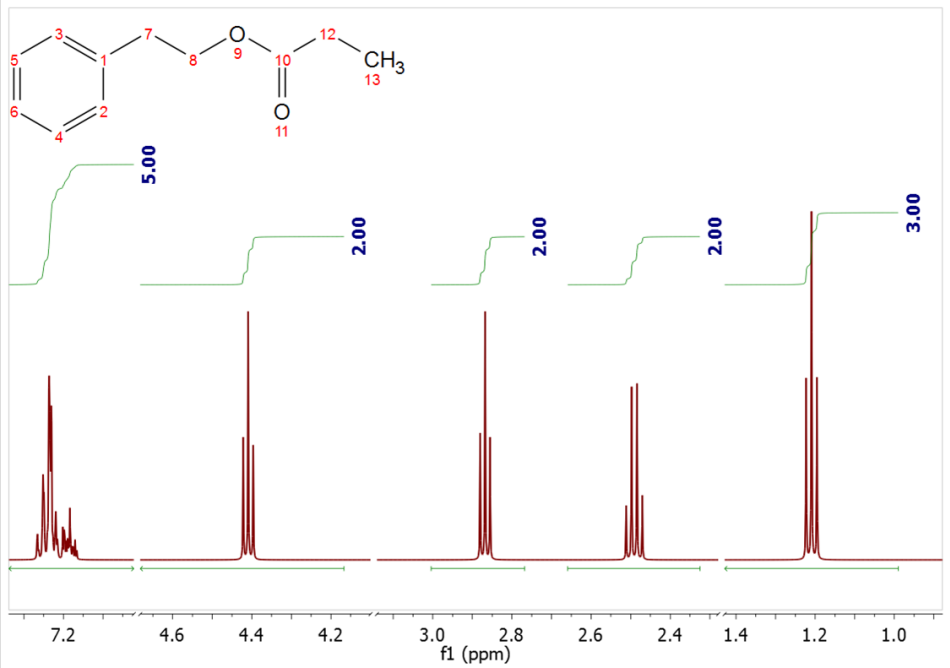
It is a simple algorithm to auto normalize the 1H NMR integrals without prior-knowledge. It will be useful especially for auto batch processing using the scripts of Mnova.

Starting from this situation:



We can arrive to this one after having applied the algorithm:

© 2024 MESTRELAB RESEARCH

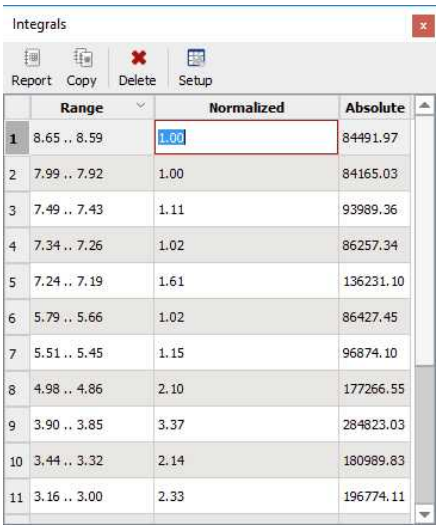


See also:
Integral Properties

8.3.1 Integrals Table

The Integrals Table (by following the menu: 'View/Tables and then select: Integrals') includes information about the integral range (in ppm) and the normalized and absolute values of each integral. In this table the user can delete a desired integral by clicking on the **'Delete'** icon . The user can also normalize the integrals by double clicking on the normalized value of the integral which the user would like to set (in the red square on the capture below). Once it is highlighted, the user has to overwrite the desired value, press return, and all integrals will update with reference to the chosen one.

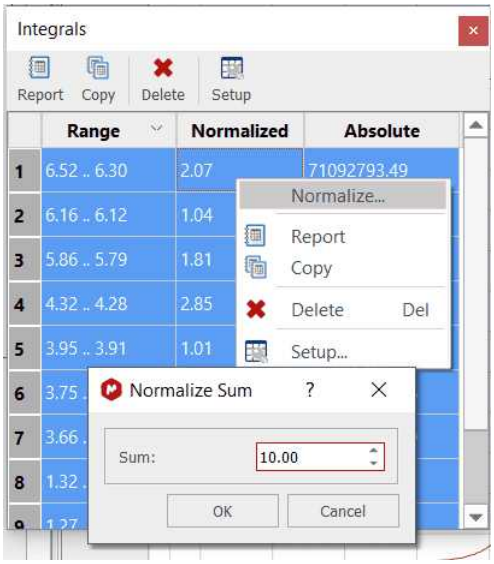
This method is an alternative way to normalize integrals. The user can obtain the same result by double clicking directly on the integral curve on the spectrum, as we have explained in the chapter above.



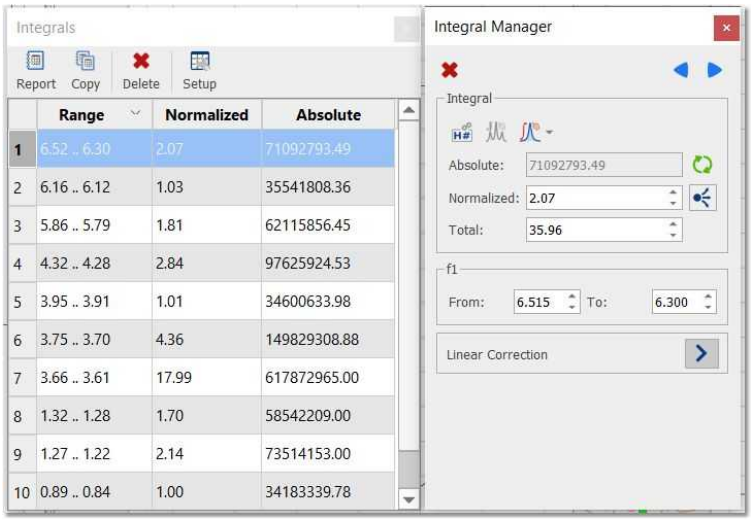
	Range	Normalized	Absolute
1	8.65 .. 8.59	1.00	84491.97
2	7.99 .. 7.92	1.00	84165.03
3	7.49 .. 7.43	1.11	93989.36
4	7.34 .. 7.26	1.02	86257.34
5	7.24 .. 7.19	1.61	136231.10
6	5.79 .. 5.66	1.02	86427.45
7	5.51 .. 5.45	1.15	96874.10
8	4.98 .. 4.86	2.10	177266.55
9	3.90 .. 3.85	3.37	284823.03
10	3.44 .. 3.32	2.14	180989.83
11	3.16 .. 3.00	2.33	196774.11

© 2024 MESTRELAB RESEARCH

You can normalize on the sum of several integrals, by highlighting the applicable integrals (from the Integrals table) and right clicking and selecting "Normalize" from the context menu. Finally type the total value to normalize:



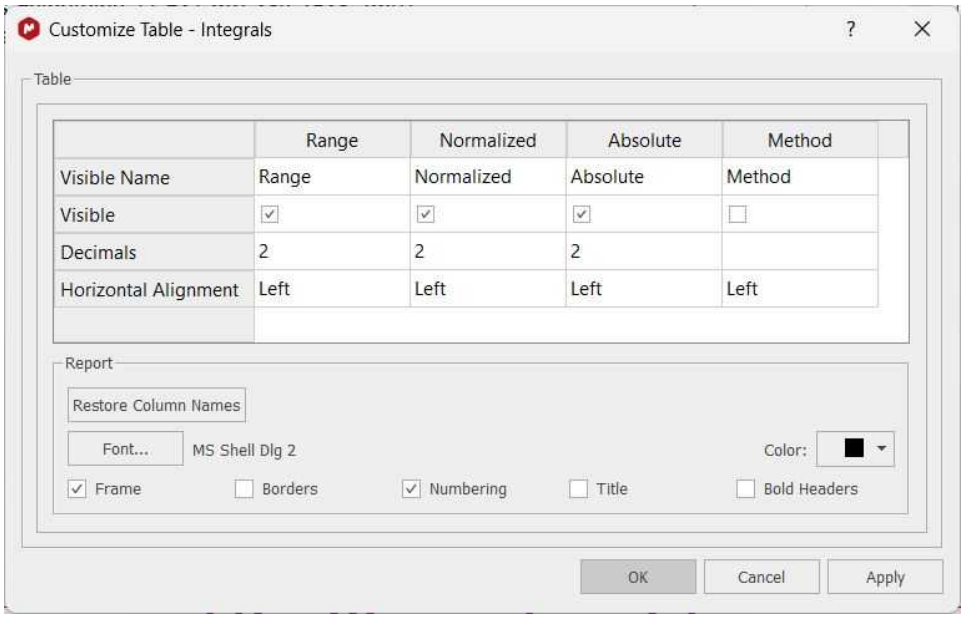
Double clicking on any cell of the 'range' or 'absolute' columns will display the 'Integral Manager', which will allow you to normalize or change the integral range:



You can also display the integral calculation method (Peaks, Sum or Edited Sum) by clicking on the 'Setup' button.

Click on the Setup button to customize the table:

© 2024 MESTRELAB RESEARCH



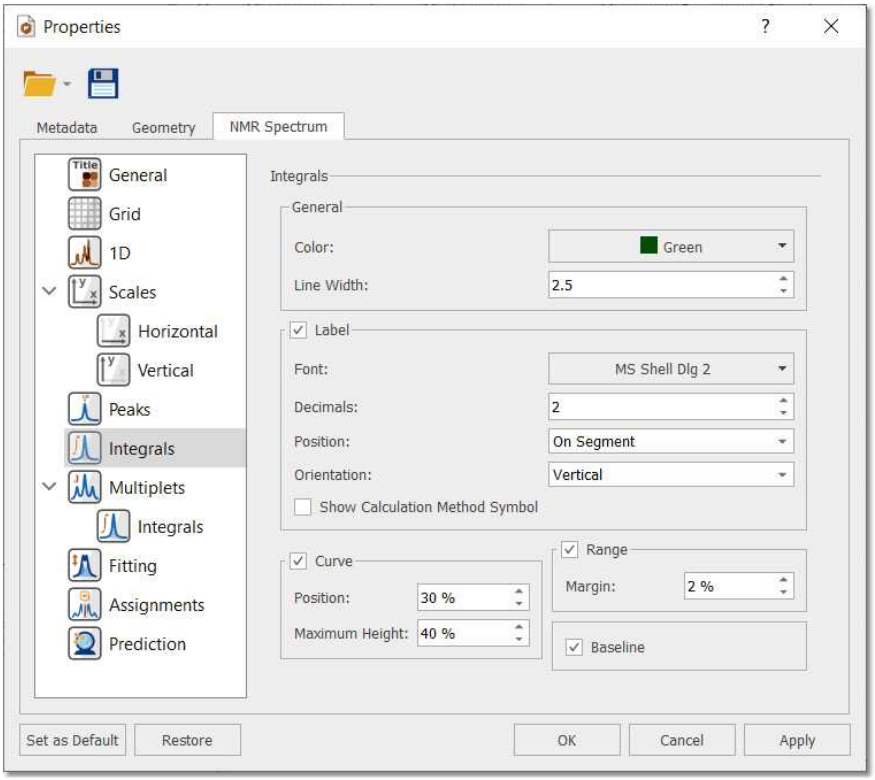
8.3.2 Integral Properties

The user can modify the properties of the integrals by following the menu 'Home/Properties/Integrals' or by double clicking the left mouse (or pressing the right mouse) button on the spectrum display and selecting *Properties/Integrals* from the *pop-up* menu.

The options are different in 1D and 2D-NMR:

1D Spectra

© 2024 MESTRELAB RESEARCH



Integrals: Check/Uncheck this option if you want to display/hide the integrals (labels + curves)

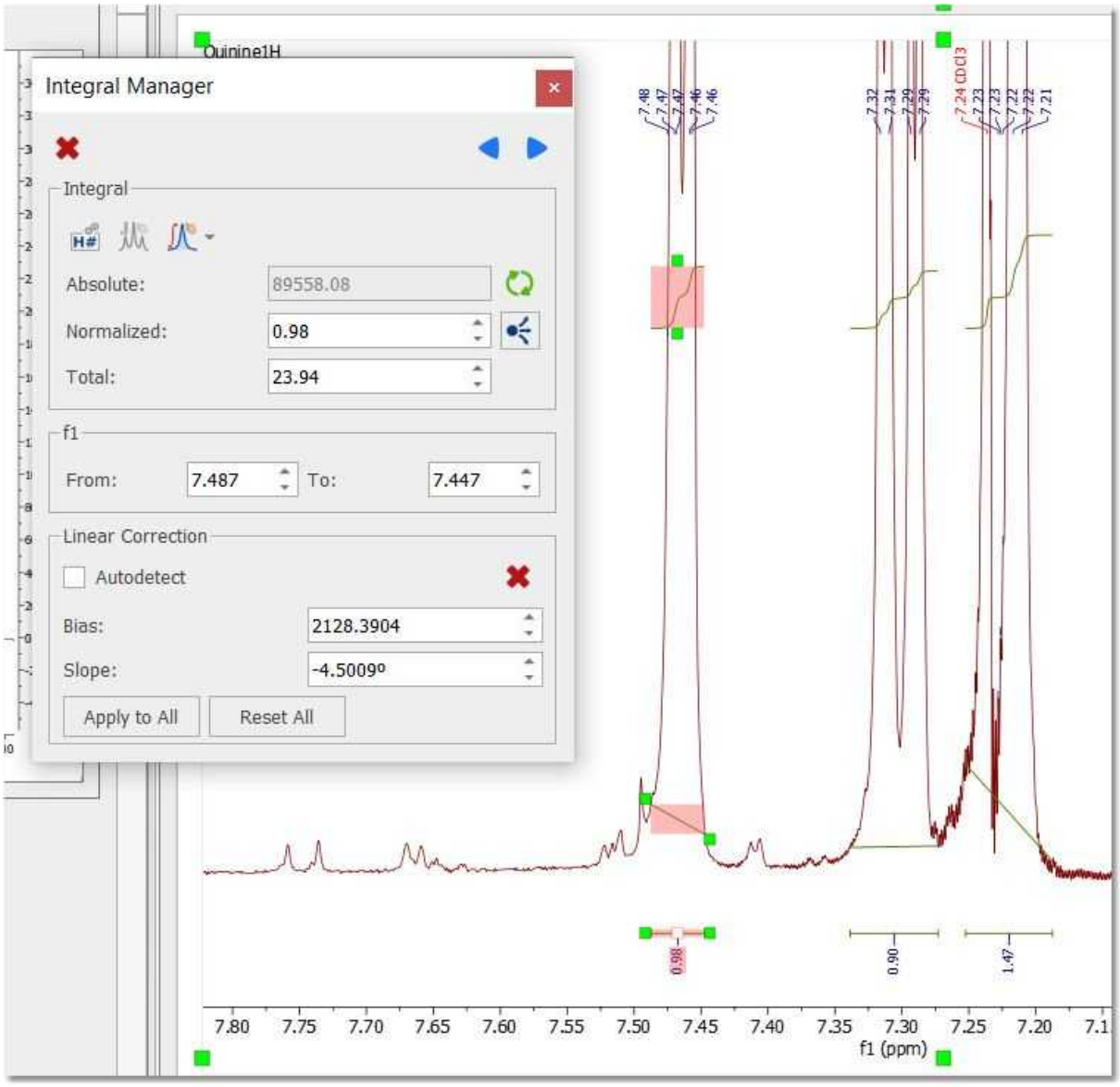
Label: Check/Uncheck this option if you want to display/hide the integral labels
Font: Select the font type
Decimals: Select the number of decimal figures to be shown on screen
Position: Use this option to show the integral label on the curve or on the integral segment
Margin: distance (in % with respect to the spectrum item height) from the top
Orientation: vertical/horizontal
Show Calculation Method: it will display and S (Sum); E (Edited Sum) or P (Peaks) in the integral label; depending on the integral calculation method used.

Curve: Check/uncheck this option to show/hide the integral curves

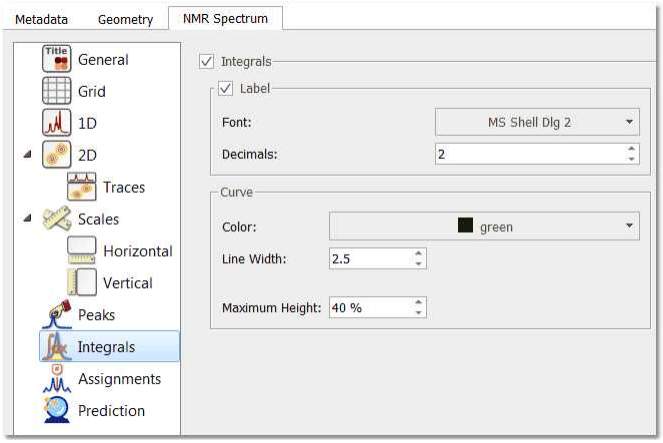
Color: Select the curve color
Line Width: to select the width of the line of the integral curve
Position: distance (in % with respect to the spectrum item height) from the top
Maximum height of the integral curve.

Baseline: If the option 'Baseline' is checked, the 'integrals baselines' will be displayed. The display format is the same as the integral curve and segment, and has two handlers that can be moved vertically in order to modify the bias and slope of the baseline.

© 2024 MESTRELAB RESEARCH



2D Spectra



© 2024 MESTRELAB RESEARCH

Integrals: Check/Uncheck this option if you want to display/hide the integrals (labels + curves)
Label: Check/Uncheck this option if you want to display/hide the integral labels
Color: Select the integral label color
Font: Select the font type
Decimals: Select the number of decimal figures to be shown on screen
Curve: Check/Uncheck this option to show/hide the integral curves
Color: Select the integral color
Line Width: Select the with of the line of integral curve

8.4 Multiplets analysis

(Not available in Mnova Lite)
You will find the quinine datasets under the 'File/Help/Download Example datasets' menu

Mnova incorporates a powerful multiplet analyzer. The user will carry out an automatic multiplet analysis by clicking on its respective icon, placed on the analysis toolbar .

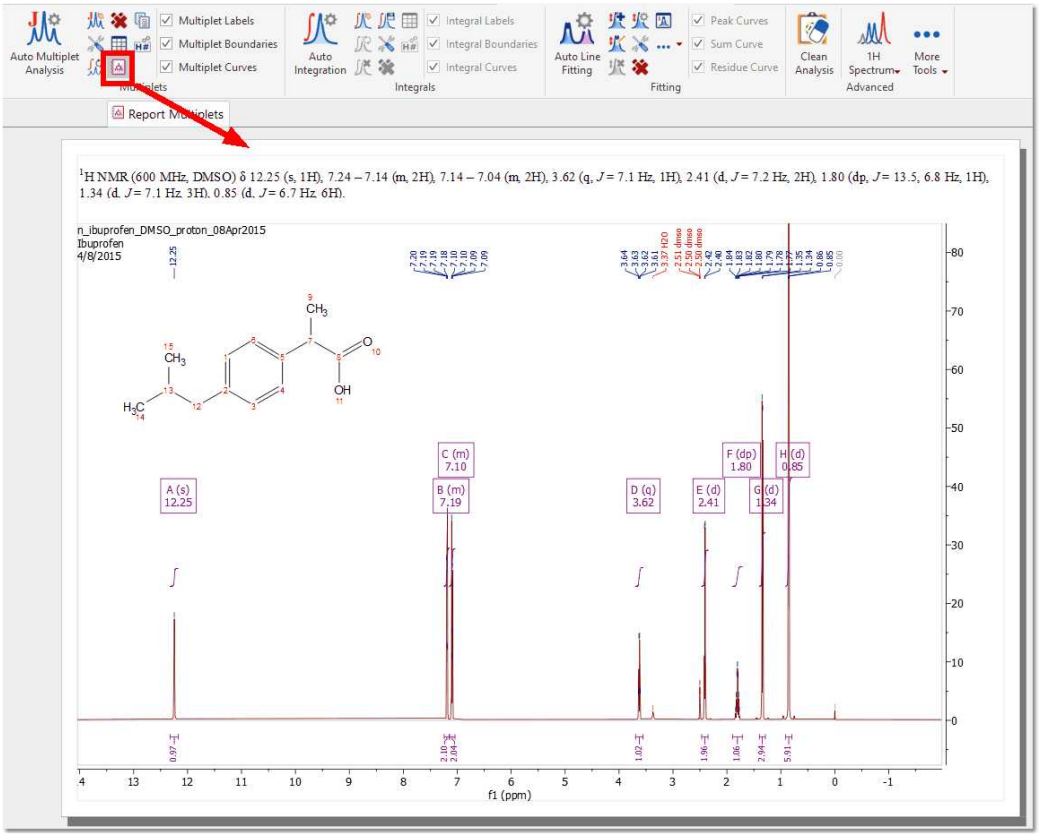


Bear in mind that when you select an automatic multiplet analysis, Mnova will take into account the Multiplet Analysis options
See also this chapter about using GSD for multiplets analysis.

The user can also apply a manual integration followed by an automatic multiplet analysis (to obtain the same ranges in both cases), but please bear in mind that **integrals and multiplets are two independent lists**, so you will be able to have different ranges in both. In that case where the multiplets are made from integrals, MNova visualizes only multiplet integrals.

In the picture below, you will see the result of having applied an automatic multiplet analysis and selected **'Report Multiplets'**

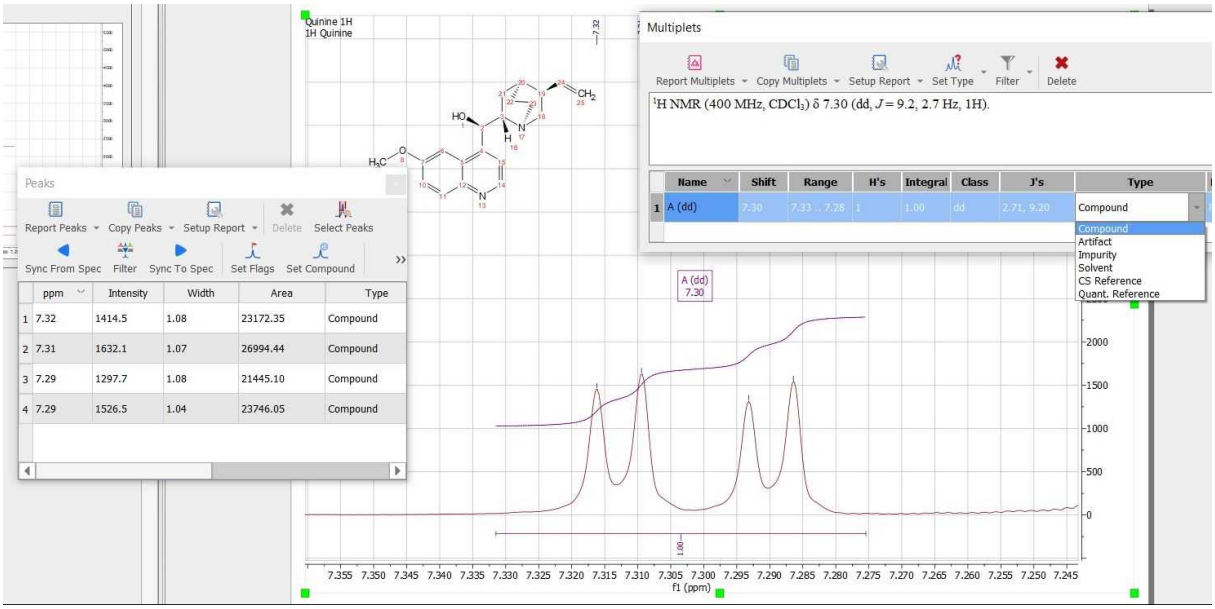
© 2024 MESTRELAB RESEARCH



Manual Multiplet Analysis

Another valid workflow would be to press the 'J' key to activate the manual multiplet analysis and click&drag on the regions that you want to analyze. You will not need to use the integration at all.

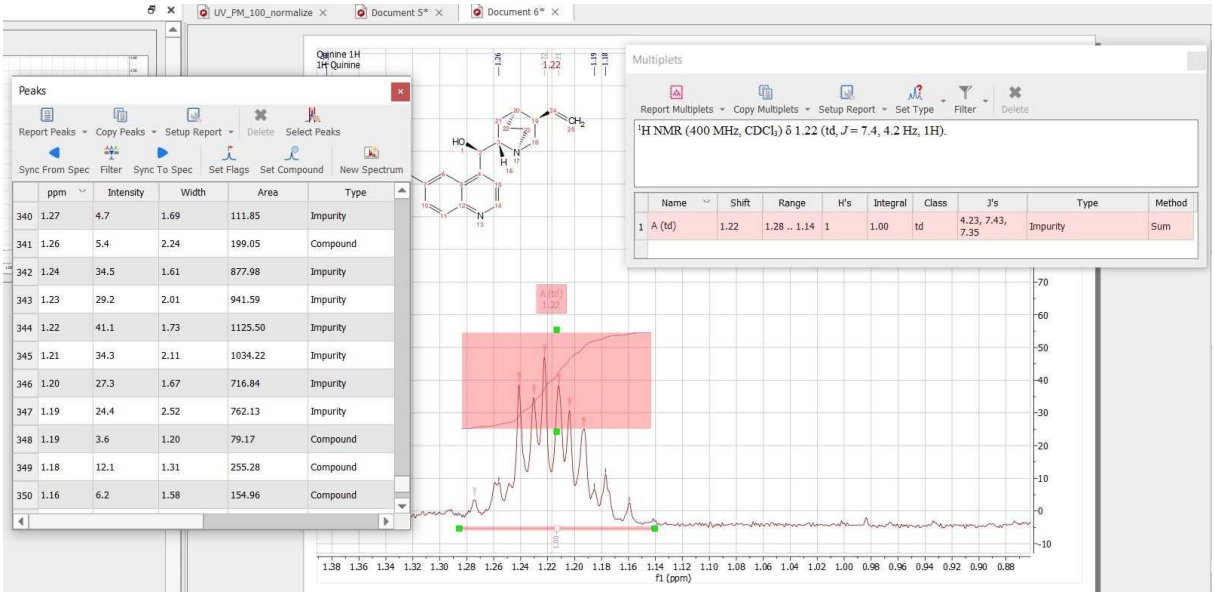
You can create non-compound multiplets (with artifact, impurity, solvent, CS Reference or Quant. Reference peaks) using the manual multiplet analysis. Please bear in mind that the automatic multiplet analysis will always return "multiplet compound" types.



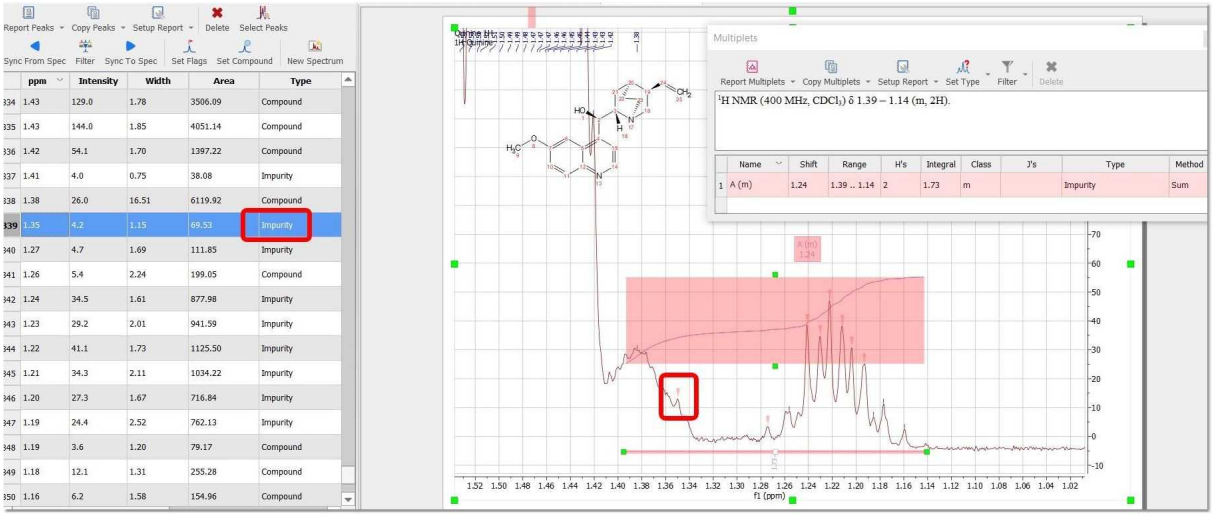
© 2024 MESTRELAB RESEARCH

If there are no peaks, a peak picking will be done and Mnova will assign the multiplet type according to the largest peak type on the selected region:

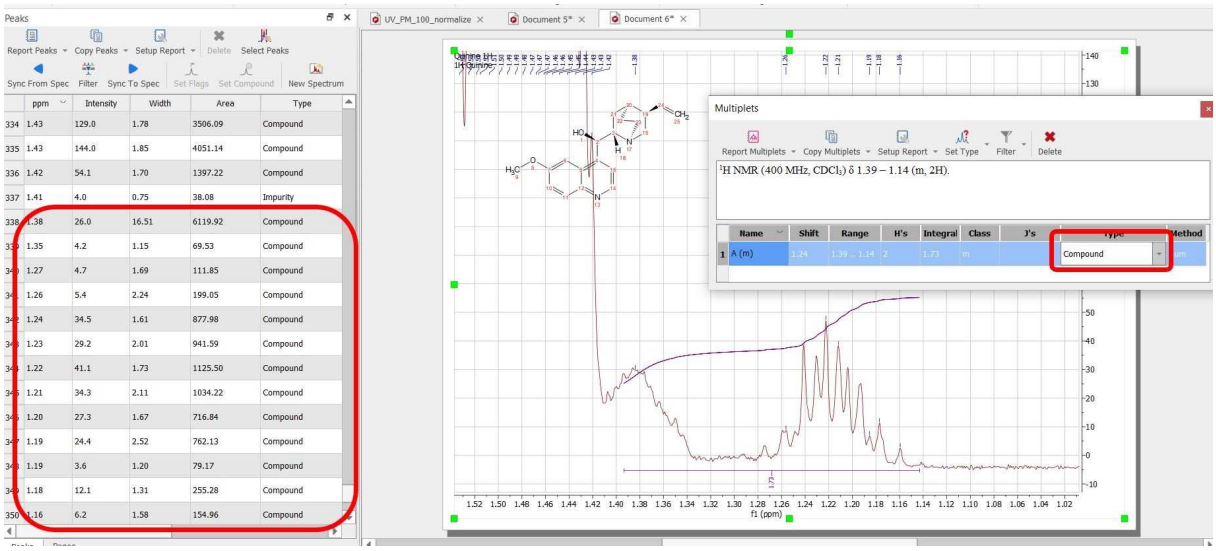
If there are already peaks in the region, the peak with the largest area will be used as the main type, so the multiplet will have that type and it will only include the peaks belonging to that type in the region. In the example below, the biggest peak is an impurity peak, so the multiplet will only include impurity peaks (and not the smaller compound peaks):



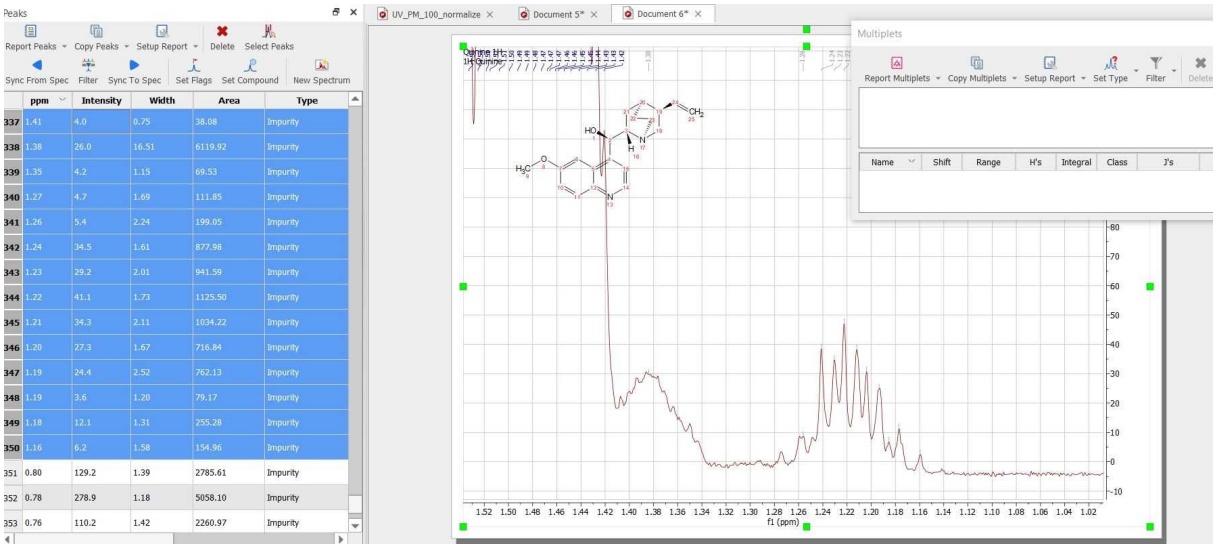
When editing the multiplet range, all the peaks of the same type as the currently edited multiplet which are not already assigned to the multiplet, will become part of the edited multiplet. In the example below, we increased the size of the multiplet to include a compound and an impurity peak, but only the impurity was added:



When changing the multiplet type, all the peaks belonging to the multiplet will be changed to that type. In the example below, we changed the multiplet peak to compound and all the peak types were updated:



If you change all the peak types of a particular multiplet, Mnova will remove that multiplet (as there will not be peak types matching with the Multiplet type). In the example below, we had a multiplet with compounds peaks, after having changed all the peaks to impurity, the multiplet was gone:

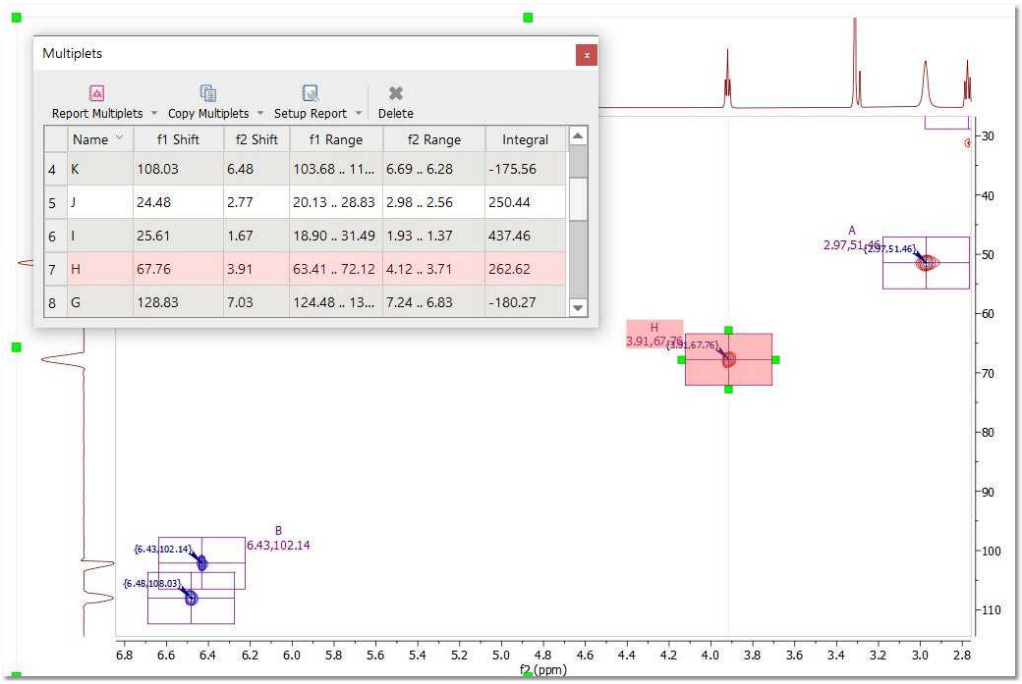


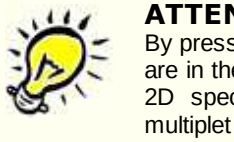
If you want to show the integral curves of your multiplets, just check the applicable box on the ribbon (or on the Multiplets Properties)

You can export the multiplet integrals list by following the menu: 'File/Save As:/Script: Multiplet Integral' or 'Script: Multiplet Integrals Series' if you work with arrayed spectra.

You can also apply a multiplet analysis in a 2D-NMR spectrum, as you can see below:

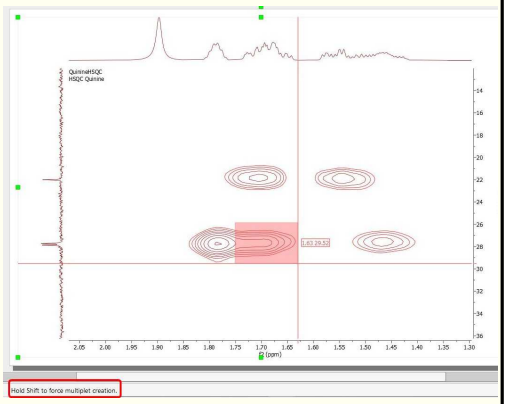
© 2024 MESTRELAB RESEARCH





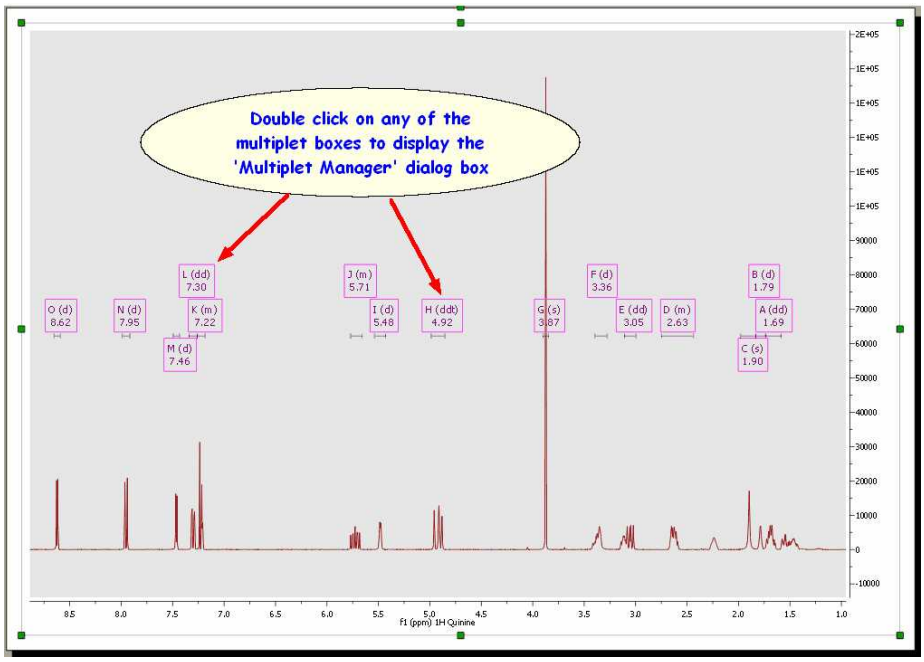
ATTENTION

By pressing and holding the Shift key while you are in the manual multiplet analysis mode for a 2D spectrum, it forces the creation of the multiplet (even if no peaks were found using the current peak picking settings); a single peak is searched from the center of the region and if none is found, a new peak will be created (at the center of the region with a fixed width of 3 points).The status bar will show a message informing about this behavior.

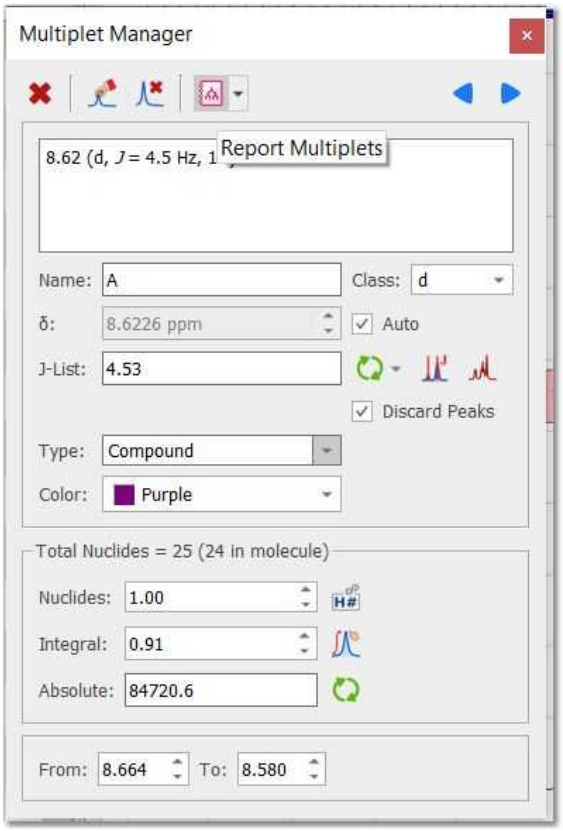


8.4.1 Multiplet Manager

The user will be able to set the multiplet parameters by double clicking on the magenta square (shortcut: Shift+J) containing the multiplet information.



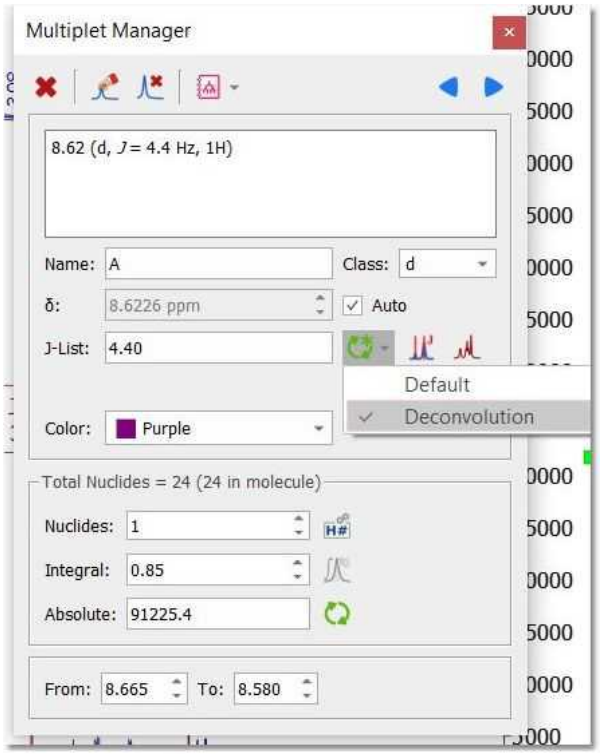
This will display the 'Multiplet Manager' dialog box, where you will be able to modify the multiplet limits, the type (compound, solvent, impurity, artifact, reference...) as well as to normalize the values or report the multiplets just by clicking on the applicable button .



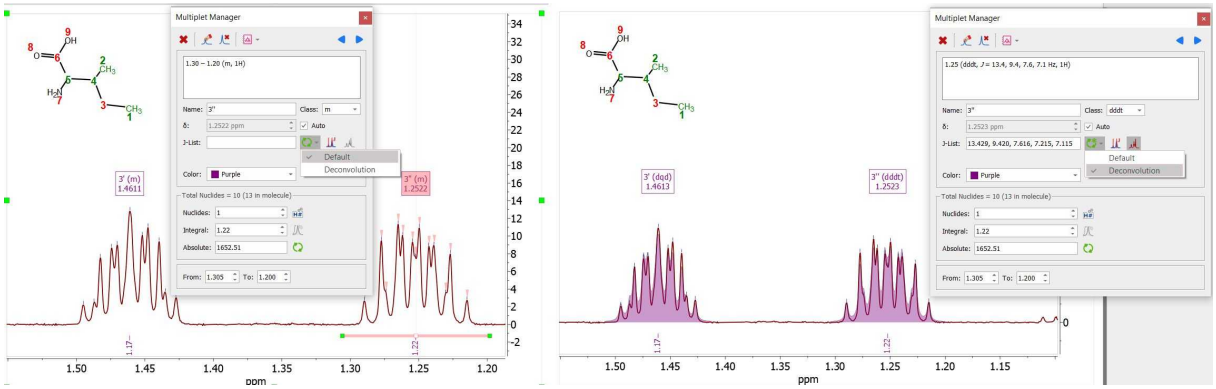
© 2024 MESTRELAB RESEARCH

Since Mnova 14.3.0 there is a new method to do first order multiplet analysis by deconvolution, to calculate the multiplicity of 1D NMR signals as well as the coupling constants. It can provide the list of scalar coupling constants from complex multiplet structures and has been implemented as an alternative analyser of multiplets when the multiplet structure is not identified (resulting to "m") by the default Mnova analyser

The deconvolution method is available from the Multiplet manager dialog, under the option "recalculate J list". Clicking on this button will display a dialog with two options:

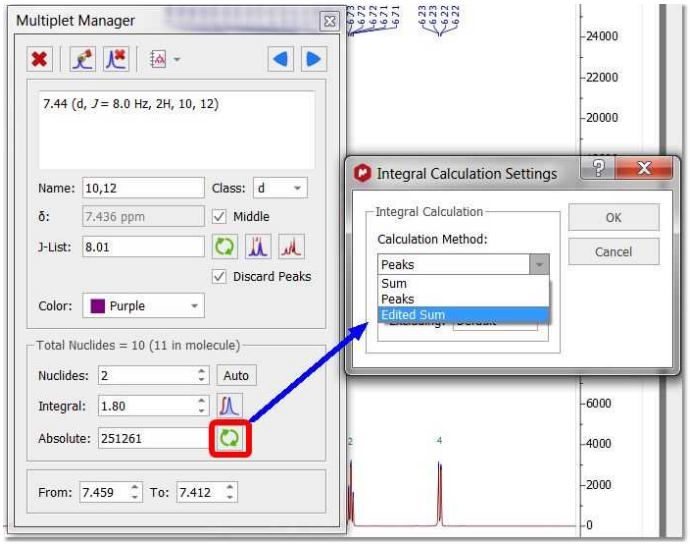


Here you can find an example where the deconvolution method was able to resolve two multiplets which were undefined by the default method:



You can manually add multiplets using different integration calculation methods (Sum, Edited Sum and Peaks). To avoid misunderstandings in the integration results, you can track the method used in each multiplet. This will be displayed in the Multiplet Manager, where there is an option to recalculate each integral/multiplet with ability to choose the integration method in each case.

© 2024 MESTRELAB RESEARCH



A similar option can be found under the Analysis/Multiplets menu.

From the multiplet manager, you will be able to change the name, class (you can add new classes to the drop down menu just by typing the name and pushing Enter key), the coupling constant tolerance, limit and the color of the multiplet box. Please bear in mind that if the class of the multiplet is called 'mx' (being x any letter); the multiplet will be reported as a chemical shift instead of the multiplet range.

Here you can see the difference:



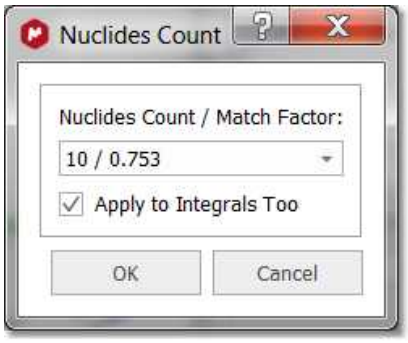
The number of hydrogens (Nuclides) will be rounded (up or down) by default with respect to the multiplet integral value (which in this case corresponds with the area under the multiplet range, not with the area under the integral curve).

Bear in mind that, integrals and multiplets are two independent lists, so you can change the nuclides (or multiplet) value, without affecting to the integration.

Click on the applicable icon  of the 'Multiplet Manager' dialog box to import the integral normalization values.

Clicking on the 'Auto' button  of the 'Multiplet Manager' dialog box will display the 'Nuclides Count dialog box'. From this window, you will be able to select the total number of nuclides of the spectrum (nine hydrogens in the example below) and apply it to the spectrum.

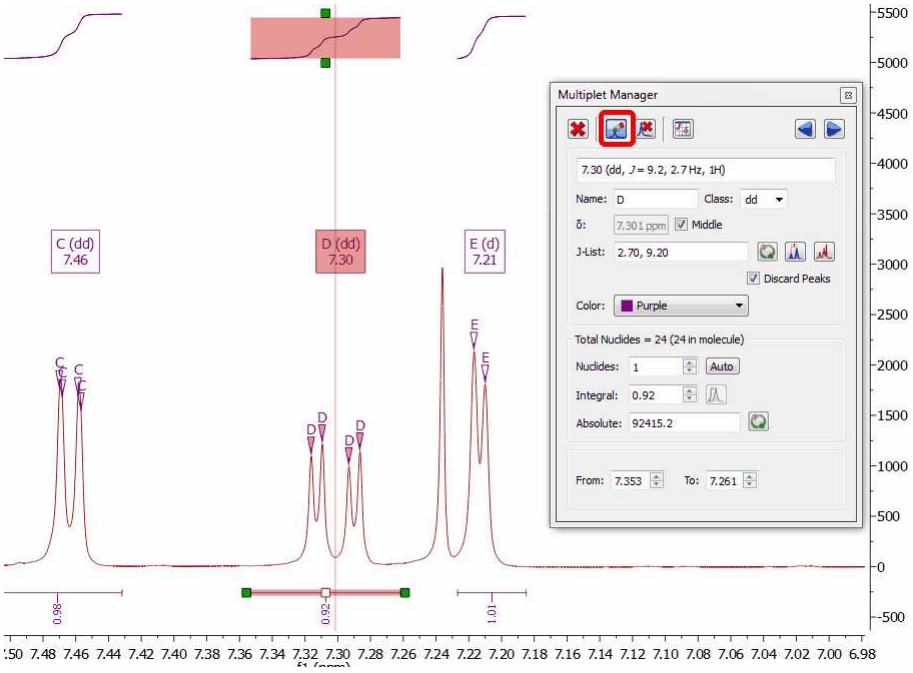
© 2024 MESTRELAB RESEARCH



From the multiplet manager dialog box, you can delete a multiplet by clicking on the 'Delete' button or navigate through the multiplets by using the 'Previous' or 'Next' buttons

From this dialog box, you can also "add multiplet peak" (if you pick a peak twice with this feature, the peak will be removed) or delete undesired peaks by using the applicable icons.

Once you click on this icon , you will see the applicable label above each peak of the multiplet, indicating the name of the multiplet:

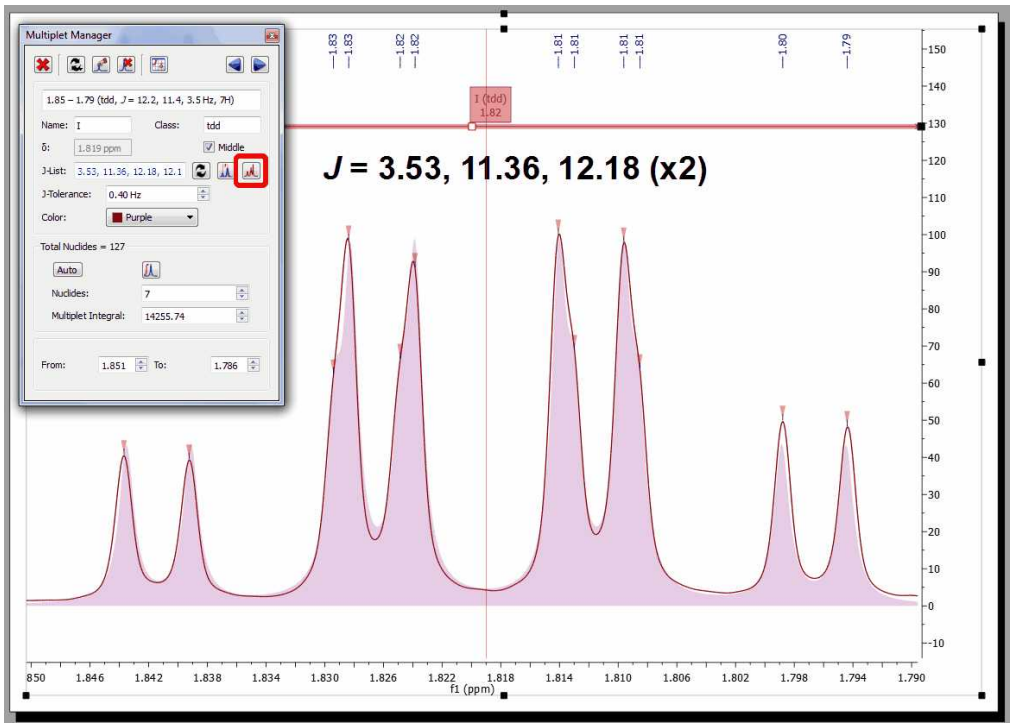




Please note that the 'Multiplet Manager' dialog is **modeless**, so you will be able to use any other tool (i.e. zooming) whilst you are applying the analysis.

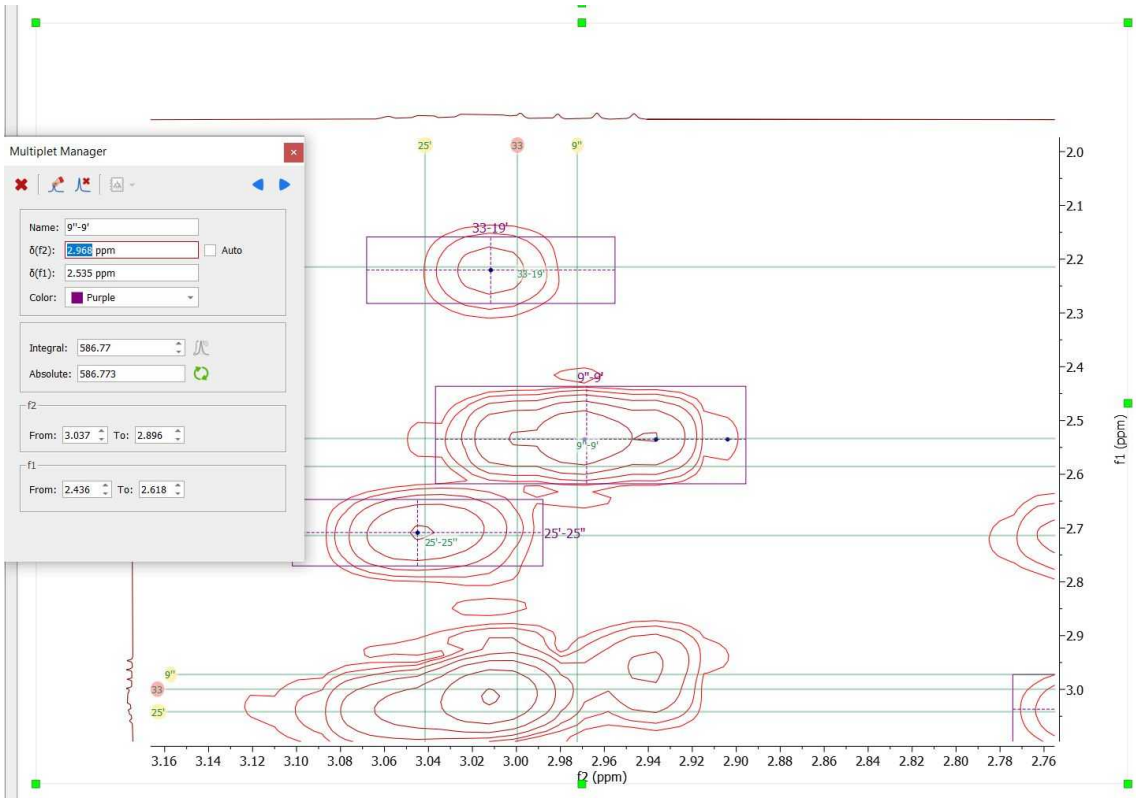
Clicking on this button will allow you to select two points in the spectrum and the difference between them in Hz will be added to the J list.

Mnova offers the possibility of displaying the corresponding synthetic multiplet built using these calculated *J* couplings. This is a useful tool to check whether the values calculated by Mnova using first order rules actually match the experimental multiplet. In order to show the synthetic multiplet, just check the button highlighted in red in the figure below. The program will automatically create a synthetic multiplet with a light red color as background.



Double clicking on a 2D multiplet box will also display the 'Multiplet Manager' dialog box:

© 2024 MESTRELAB RESEARCH



By default the 'Auto' box is checked; in this case the center of the multiplet depends on the multiplet peaks, and the horizontal and vertical shifts appear as disabled in the widget. If you uncheck the box, you can edit the horizontal and vertical multiplet shifts (very useful for overcrowded regions). The multiplet "center" will be updated according to these values.

8.4.2 Using GSD for multiplets analysis

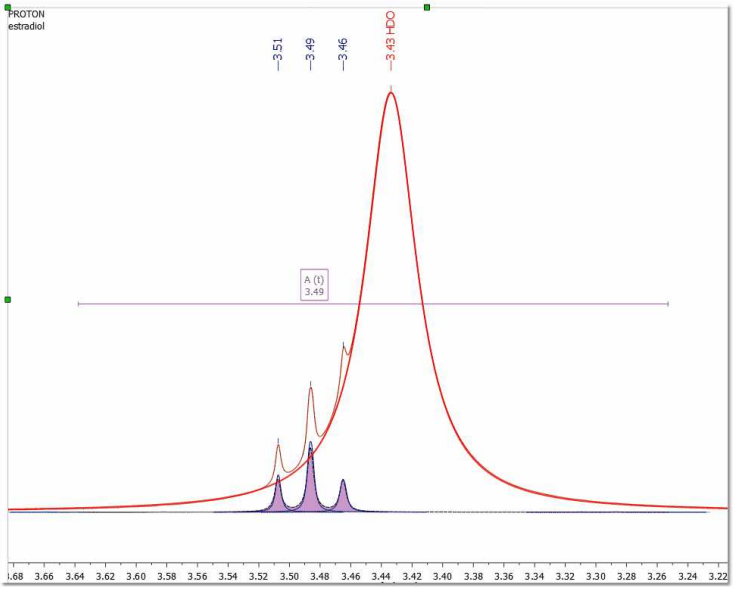
Exploiting the power of GSD for an improved Multiplet Analysis

By default Mnova uses Global Spectral Deconvolution (GSD) algorithm for automatic peak picking and multiplet analysis.

Multiplet Analysis benefit directly from the exploitation of GSD capabilities to carry out the automatic analysis, with the enhanced peak picking capabilities resulting in much more reliable automatic multiplicity identification and labeling.

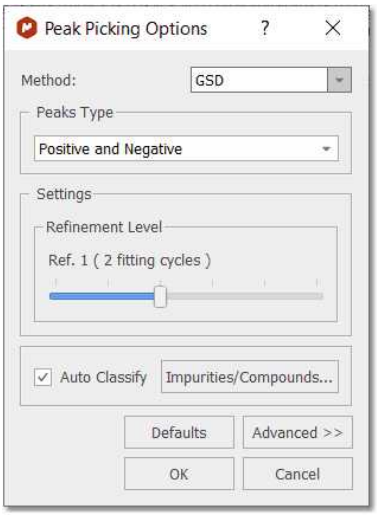
Here you can see an example of a triplet which is hidden under the solvent signal:

© 2024 MESTRELAB RESEARCH



In order to use this algorithm, make sure that you have the following default options in Mnova.

1 – Defining the GSD level
GSD has different operational levels, ranging from a very fast but inaccurate method to slower but much more accurate levels. In addition, GSD allows the automatic recognition of solvent peaks, impurities, etc. We recommend to use a GSD level of 1 (flash) or 2. This level can be set from Peak Picking/Options as depicted below:

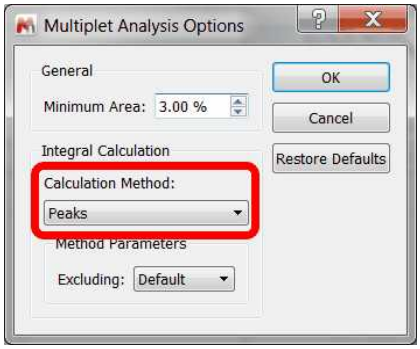


Also, make sure that the Auto Classify option is checked.

From now on Mnova will use GSD when automatic peak picking algorithm is issued (which is the default setting).

2 – Set the Automatic Integration method to GSD
Finally, for Mnova to be able to detect the multiplets regions automatically using GSD, it's necessary to define the **'peaks'** as integral calculation method under the 'multiplet options' (this should be the default setting).

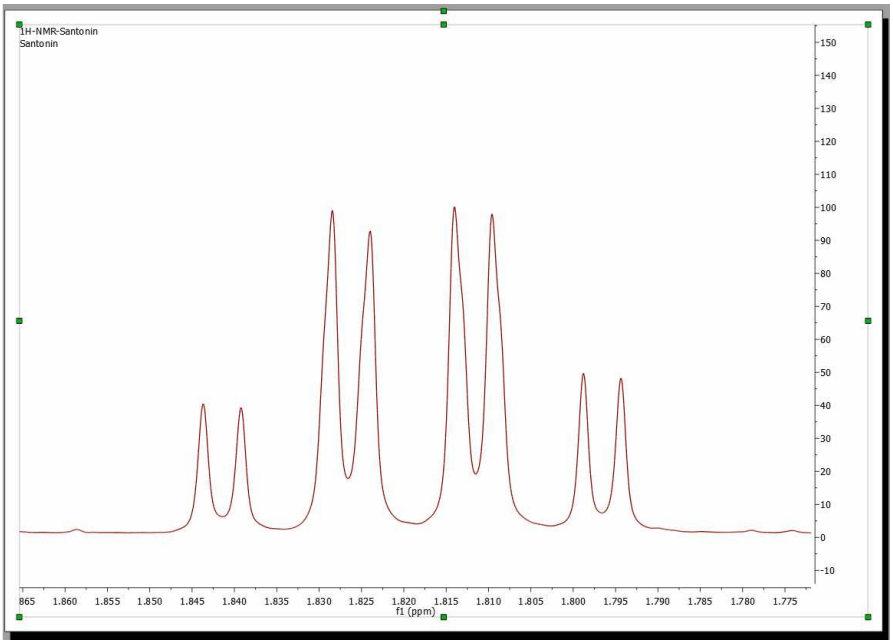
© 2024 MESTRELAB RESEARCH



First Order Multiplet Analysis with GSD with Resolution Enhancement

(click here to download the spectrum used)

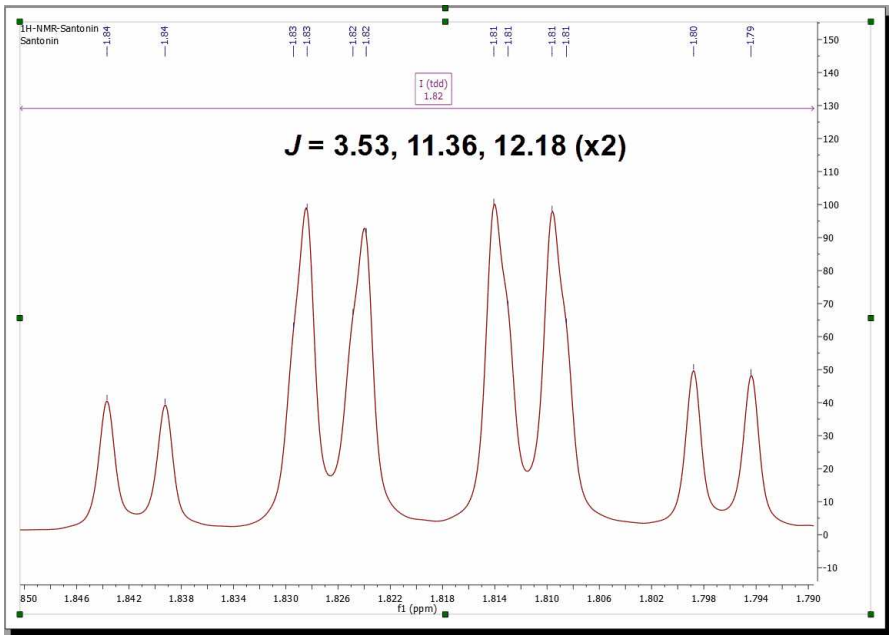
Consider the following multiplet:



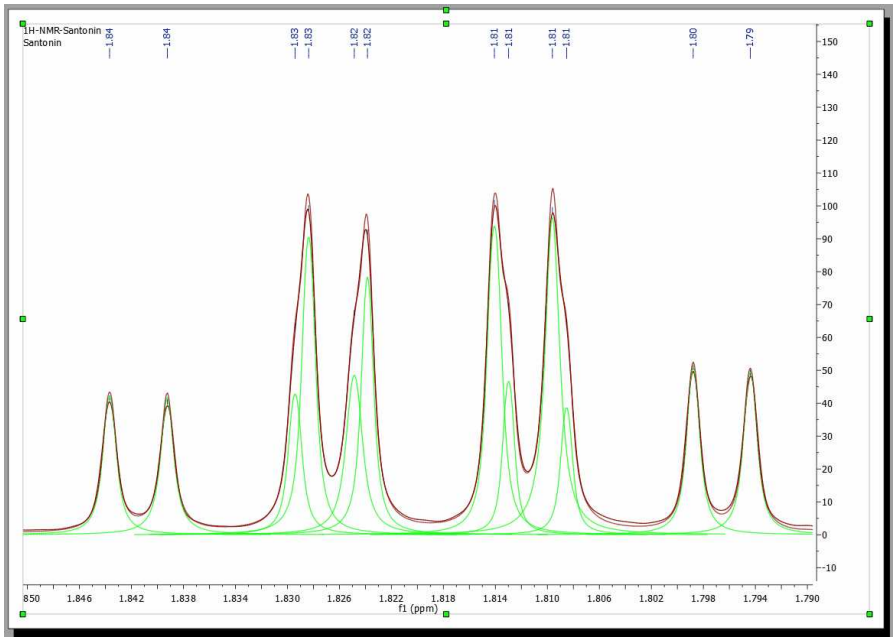
At first sight, this looks like a doublet quadruplet (J = 12.2 x 3, 3.6 Hz) and in fact, this is what some NMR software packages would report if this multiplet were analyzed using based on traditional peak picking algorithms.

Now Mnova Multiplet Analysis is armed with GSD, making the analysis of NMR spectra a more efficient process. Assuming that all Mnova options have been set properly in order to use GSD for multiplet analysis, this is what Mnova would report for this multiplet:

© 2024 MESTRELAB RESEARCH



As it can be noticed, Mnova now finds an extra coupling of 11.4 Hz. If we take a closer look at the fine structure of the multiplet once GSD has been applied, we can observe that the 4 peaks in the center of the multiplet are actually split into 8 peaks as illustrated in the figure below:



8.4.3 Setting multiplets

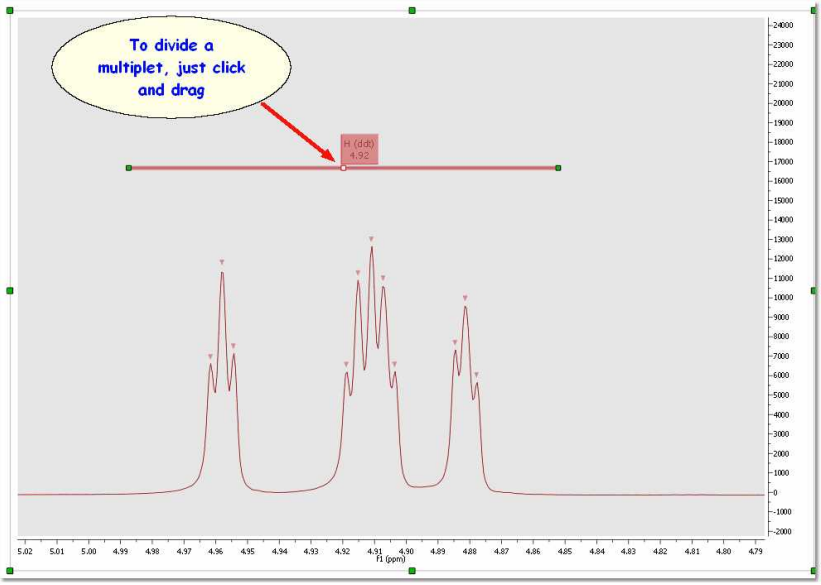
All the multiplet boxes are mouse-sensitive and they respond to usual mouse operations. If you want to move up or down all the multiplet boxes, just click and drag (with the left mouse button) over anyone of the boxes (notice that hovering the mouse over the box will highlight it in red).

To change the range of the multiplets, hover the mouse over the multiplet box, click on one of the green squares and drag the mouse to the left or right (notice that once you hover the mouse over the green squares of the multiplet label, the mouse pointer turns to horizontal arrow).

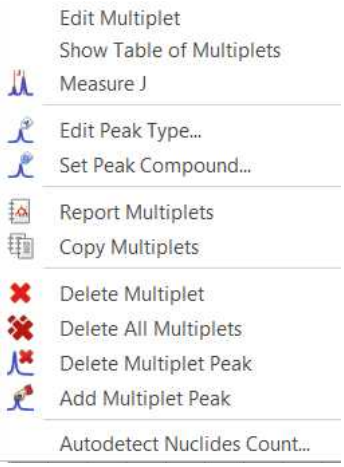
© 2024 MESTRELAB RESEARCH



Cutting Multiplets: to divide any multiplet just by clicking on the red square of the multiplet label and dragging the mouse:



Right clicking on the multiplet box, will display the applicable context menu:

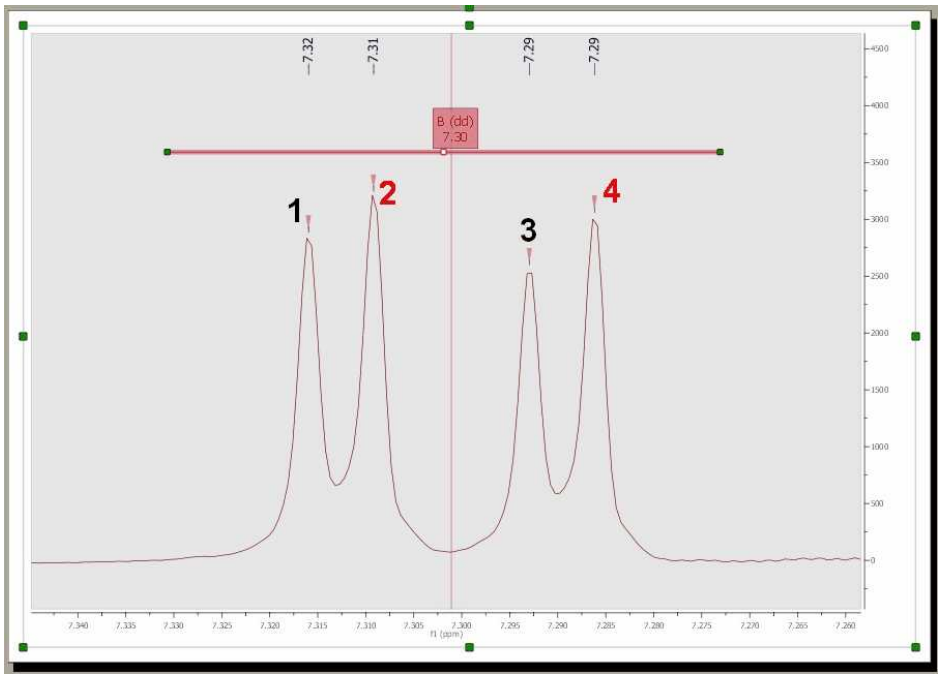


From here you will be able to display the 'Multiplet Manager' or the 'Table of Multiplets'. You can also change the 'Peaks Type' (from compound to impurity, solvent, artifact, etc) or to assign the peaks inside the multiplet to a specific compound. You can generate a 'multiplet report', copy the multiplets to the clipboard, delete and add multiplet peaks, etc as you can see in the picture above.

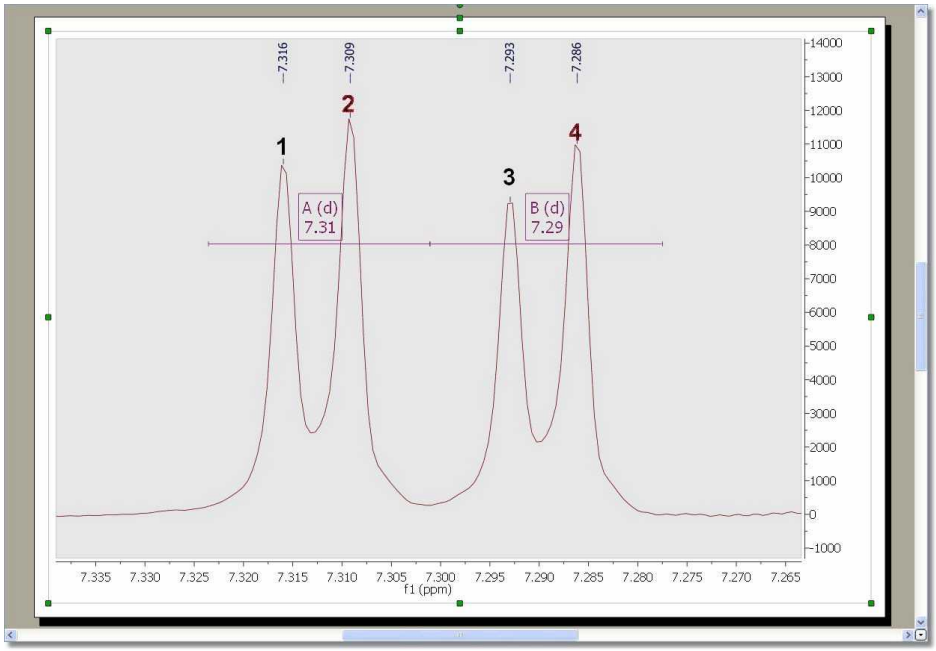
© 2024 MESTRELAB RESEARCH

8.4.4 How to resolve overlapped multiplets?

It is really easy to resolve overlapped multiplets. Imagine the below overlapped doublets:



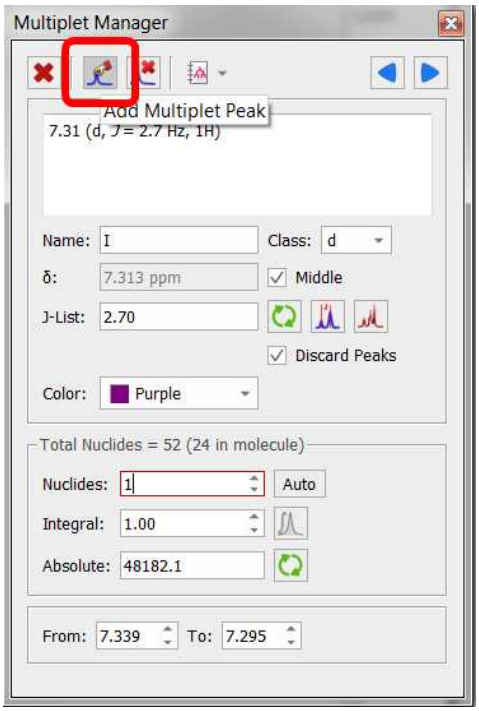
where the peaks 1 and 3 correspond to one doublet and the other peaks (2 and 4) to the second doublet. First of all, divide the multiplet just by clicking on the red square of the multiplet label (to obtain two overlapped multiplets):



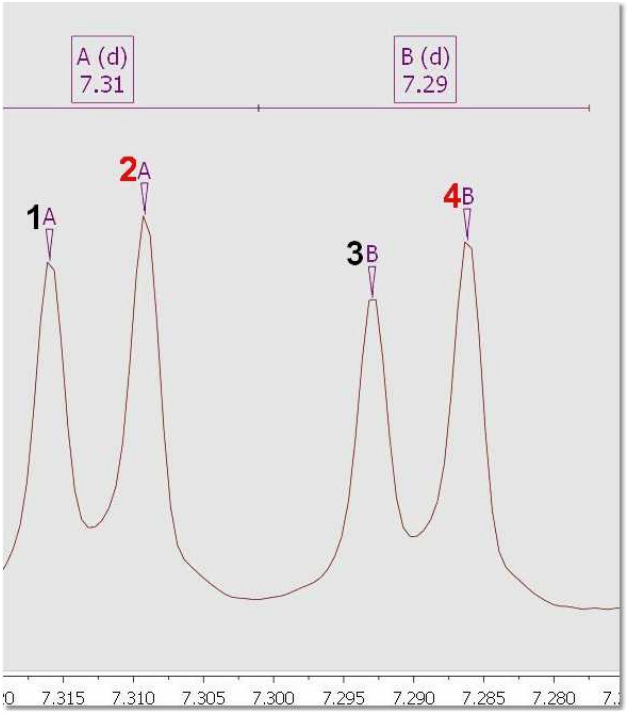
Next, double click on any of the multiplet boxes to launch the 'Multiplet Manager' dialog box. Once there, click on the 'Add multiplet peak' button (red square in the picture below). With this tool you can also

© 2024 MESTRELAB RESEARCH

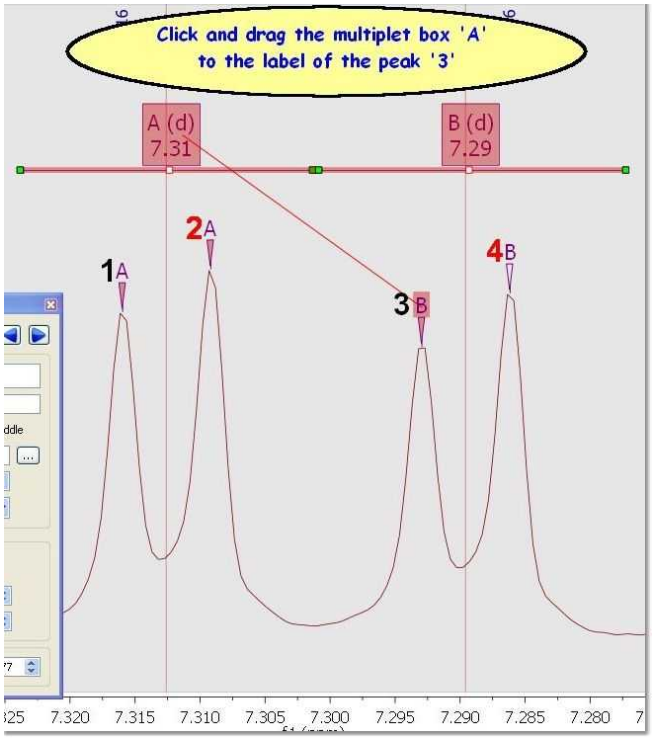
remove peaks from the multiplet, by picking over a previously picked peak. Use this button  to delete undesired peaks.



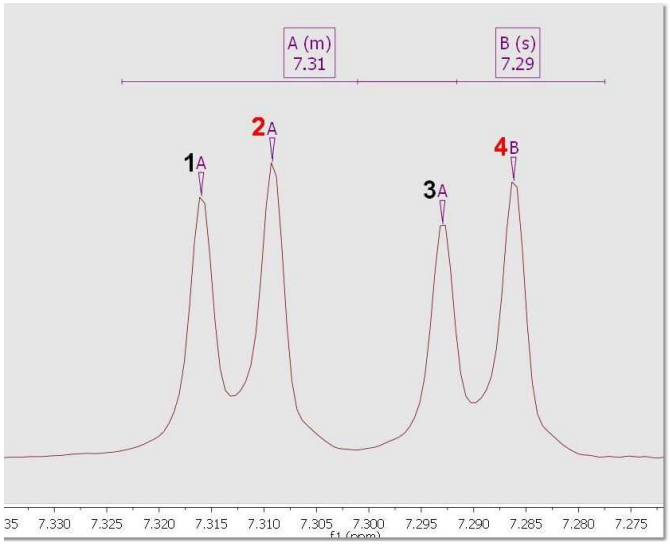
This will add a peak label with the multiplet name (A and B) over each peak, as you can see below:



Finally, hover the mouse over the multiplet box A, click and drag to the the label (**B**) of the peak **3**, or vice versa, hover the mouse over the label of the peak 3, click and drag it to the multiplet box A (as it is shown in the picture below):

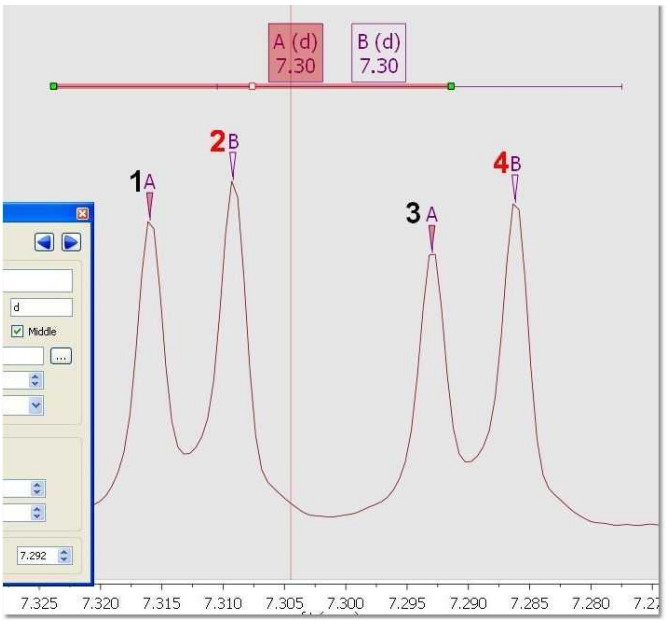


After that, the peak **3** will be a part of the multiplet **A;** (at this moment the multiplet A contains the peaks 1, 2 and 3):



Finally, do the same procedure with the peak **2** and the multiplet box **B** (click and drag the peak label to the multiplet box or vice versa), in order to include the peak 2 into the multiplet B:

© 2024 MESTRELAB RESEARCH

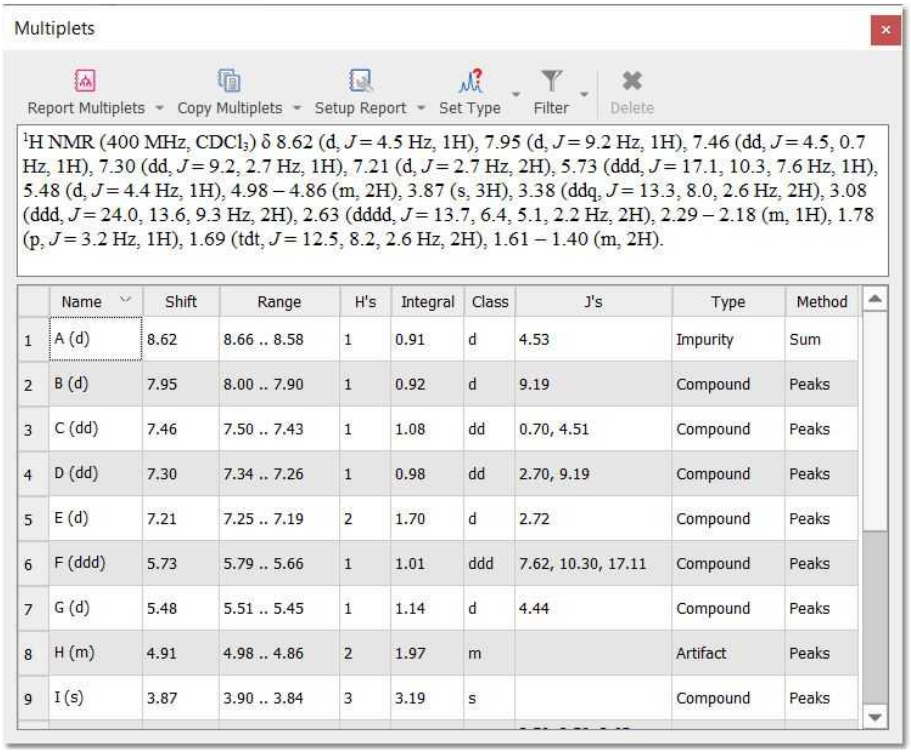


As you can see in the picture above the multiplet A contains the peaks 1 and 3 and the multiplet B contains the peaks 2 and 4.

8.4.5 Multiplets Table and Reports

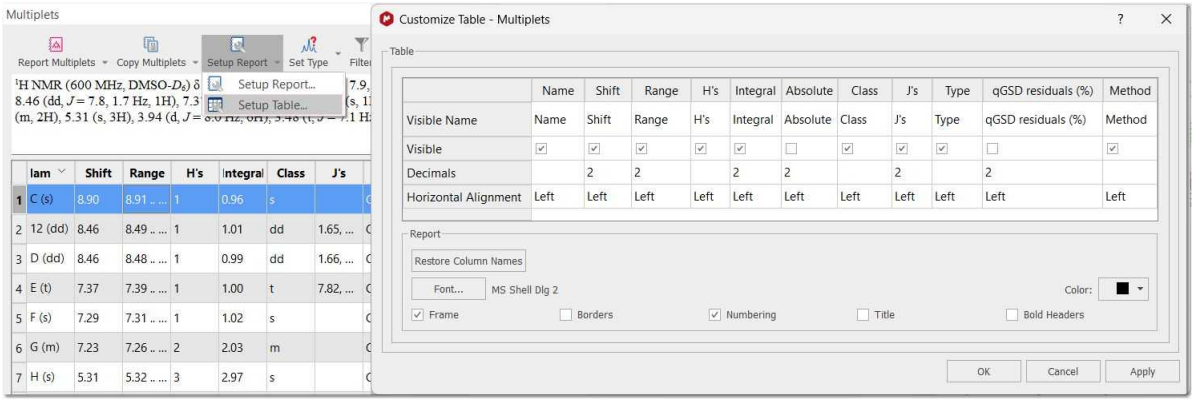
The user will be able to get the 'Multiplets' table by following the menu 'View/Tables'. The 'Multiplets' table includes information about the name (by default Mnova will have named the multiplets in alphabetical order, but the user is able to change this order in the 'Multiplet Manager'), the chemical shift (ppm), the range (ppm), number of hydrogens, integral value, class, coupling constant values (Hz), type of multiplet (which can be changed from here) and integral calculation method.

© 2024 MESTRELAB RESEARCH

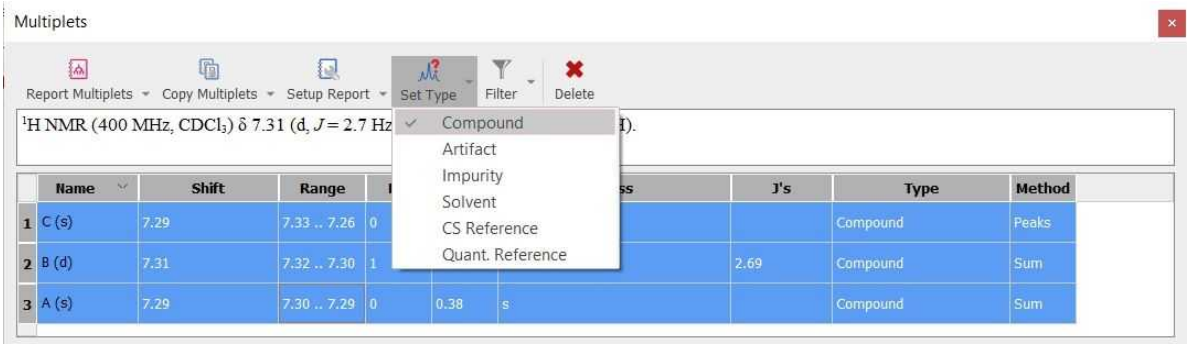


	Name	Shift	Range	H's	Integral	Class	J's	Type	Method
1	A (d)	8.62	8.66 .. 8.58	1	0.91	d	4.53	Impurity	Sum
2	B (d)	7.95	8.00 .. 7.90	1	0.92	d	9.19	Compound	Peaks
3	C (dd)	7.46	7.50 .. 7.43	1	1.08	dd	0.70, 4.51	Compound	Peaks
4	D (dd)	7.30	7.34 .. 7.26	1	0.98	dd	2.70, 9.19	Compound	Peaks
5	E (d)	7.21	7.25 .. 7.19	2	1.70	d	2.72	Compound	Peaks
6	F (ddd)	5.73	5.79 .. 5.66	1	1.01	ddd	7.62, 10.30, 17.11	Compound	Peaks
7	G (d)	5.48	5.51 .. 5.45	1	1.14	d	4.44	Compound	Peaks
8	H (m)	4.91	4.98 .. 4.86	2	1.97	m		Artifact	Peaks
9	I (s)	3.87	3.90 .. 3.84	3	3.19	s		Compound	Peaks

You can customize the multiplets table by selecting 'Setup table' in the Setup Report scroll down menu.

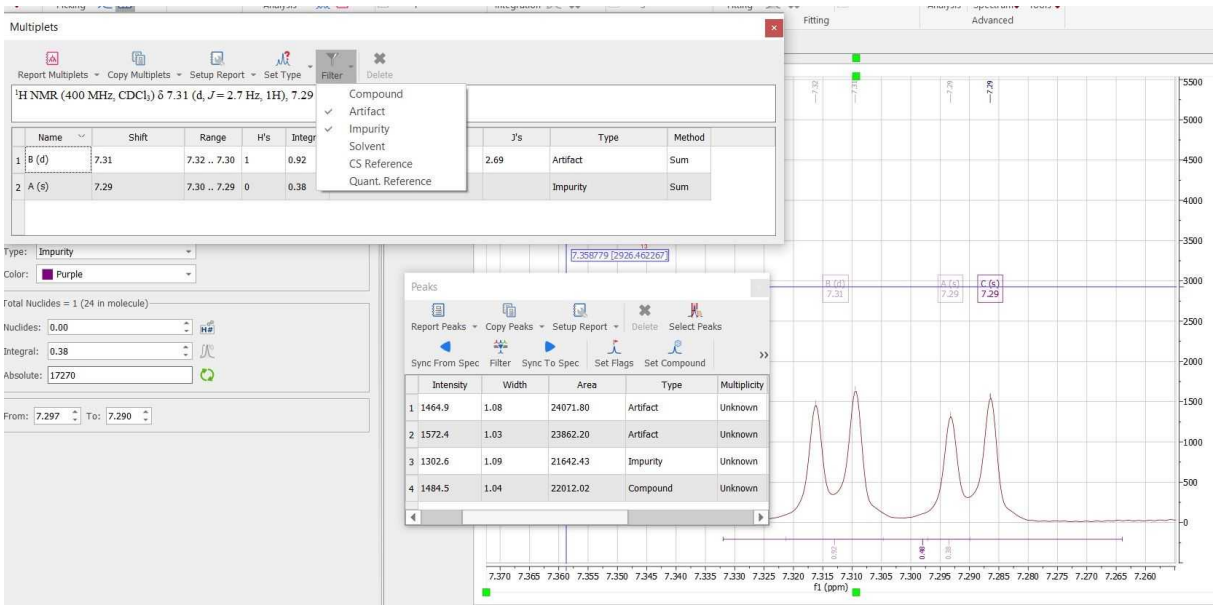


Click on the 'Set Type' button to change the type of all the selected multiplets in the table:



© 2024 MESTRELAB RESEARCH

Click on the Filter button to choose in a dropdown which types of multiplets you want to display in the table. In the example below, we are displaying only impurities and artifact multiplets (but not compounds multiplets):



Double clicking on any cell of the multiplet table will display the 'Multiplet Manager', which will allow you to edit the applicable multiplet information.

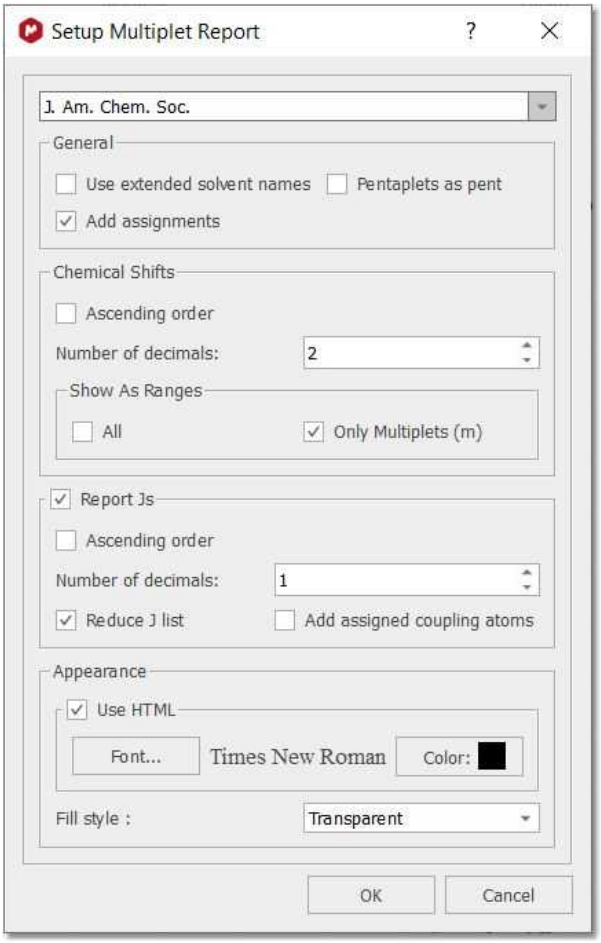
The user can paste the 'Multiplets' table on the spectrum by clicking on the **'Report table'** option (under the 'Report Multiplets scroll down menu) and also can delete a multiplet from this table by using the **'Delete'** icon  when the undesired multiplet is highlighted.

Finally, the user will be able to obtain the multiplet report list by clicking on the **'Report Multiplets'** button.

The same result will be obtained by right clicking on the multiplet box and selecting 'Report Multiplets' or by selecting 'Report Multiplets' in the 'Multiplet Analysis' ribbon.

How can I select the Journal template for my multiplet reports?

The format of the Multiplet Report can be selected just by selecting 'Setup Report' on the Multiplet Table. This will display the 'Multiplet Report' dialog box with a scroll down menu, which will allow the user to select the desired template (JACS, Royal Society of Chemistry, Angewandte or any other Custom Report), to use extended solvent names, peantaplets as *pent* or to include the assignments in the report.



You can also sort the multiplets in ascending order and select the number of decimals for the chemical shift values.

From here you can also report your multiplets as ranges or even combine the report of the multiplets (some as ranges and other as chemical shifts). To do that, please rename the class of the multiplet (from the multiplet manager dialog box) as 'mx' (being x any letter) and this specific multiplet will be reported as a chemical shift instead of the multiplet range.

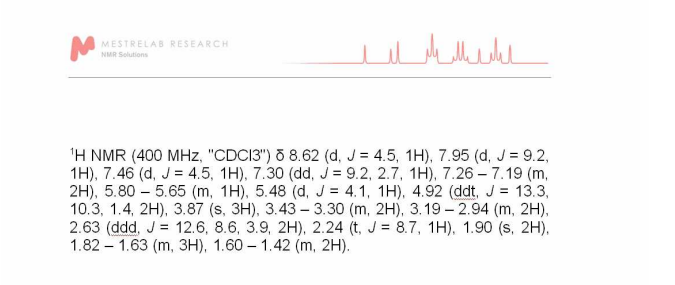
You can report the *J* coupling values in ascending order with the desired number of decimals. Uncheck the option 'Reduce J List' to show all degenerated coupling constants and check the 'Add assigned coupling atoms' if you want to include them in the report. Please note that if the matched coupling constant reported in the assignment table has a value lower than half of the digital resolution, the coupling partner info will not be added to the multiplet report. In the cases when more than one J is found in the assignments, the coupling atom will not be added.

You can report the multiplets as plain text or with HTML format.

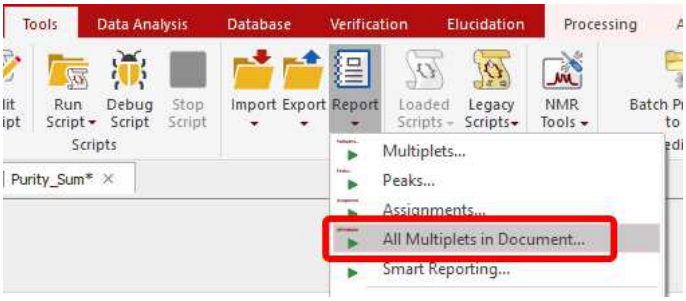
The user will be able to copy the multiplet report to the clipboard by selecting 'Copy Multiplets' on the Multiplets table.

And then paste the multiplet report on another document just by using Ctrl+V or Cmd+V.

© 2024 MESTRELAB RESEARCH



Follow the menu 'Tools/Report/All Multiplets in Document' to generate multiplet reports in each spectral page of a document.

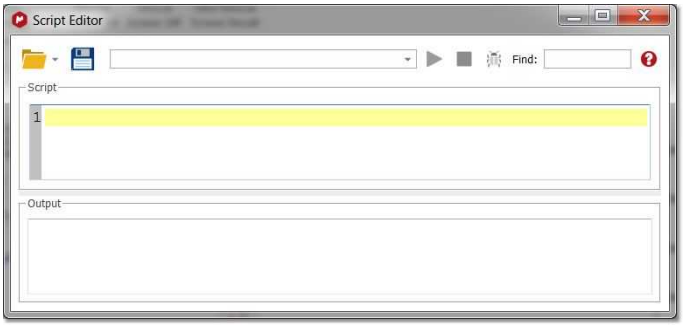


How can I create a new 'Multiplet Report' format?

The user will be able to change or create his own multiplet report templates; by following the menu 'Script/Edit Script'.

You can see below how to add a new multiplet reporter to the Mnova GUI.

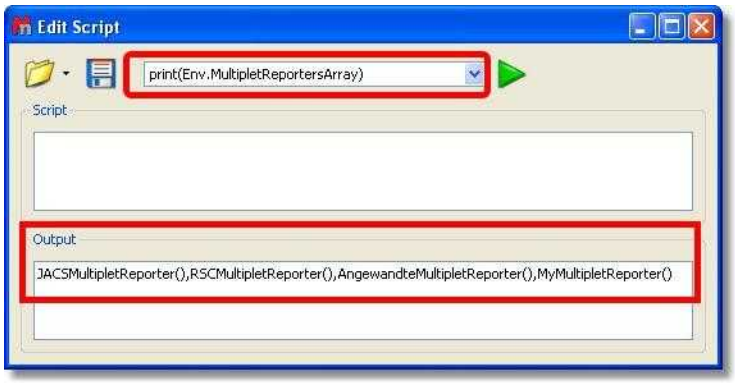
1. Follow the menu 'Tools/Edit Script' to launch the 'Script editor'.



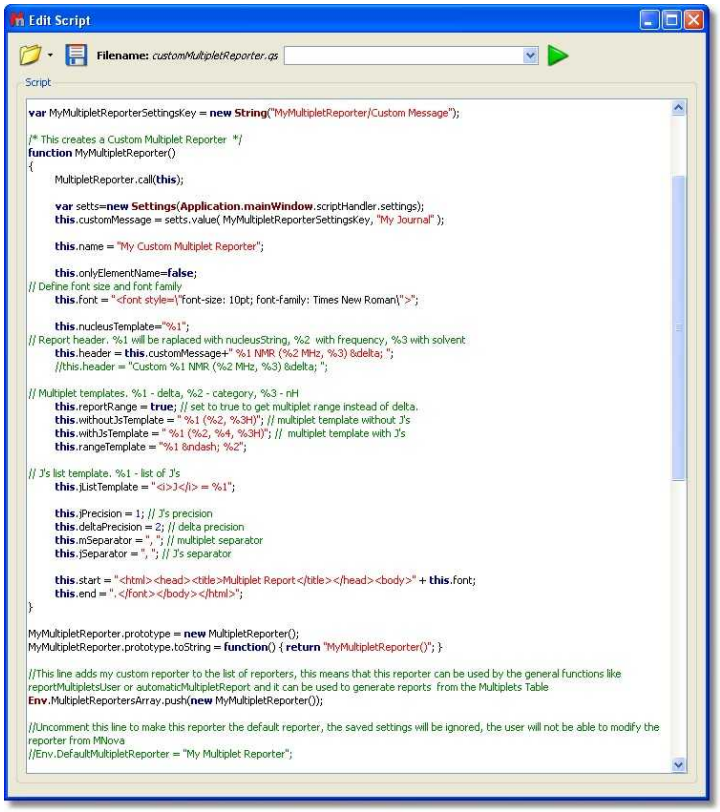
2. Write your own script or edit the example 'customMultipletReporter.qs' under the script folder of Mnova (Mnova\examples\scripts). Do not forget to include the below sentence in the script; in order to add the reporter to the program:

```
Env.MultipletReportersArray.push(new MyMultipletReporter());
```

We have created a variable in the scripts which saves a list of the known reporters. To see the list, just type in the editor:
print(Env.MultipletReportersArray)

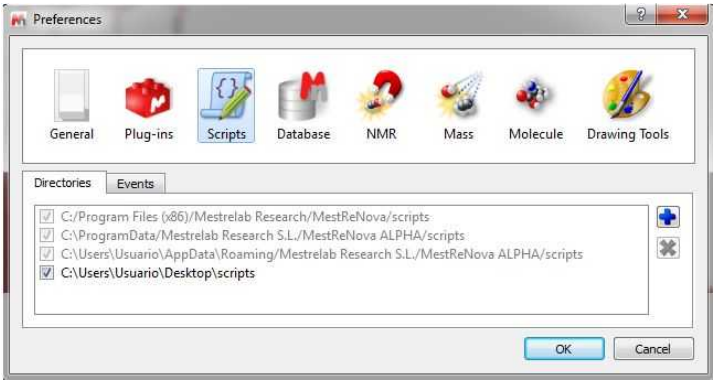


See below how to edit this script.



3. Save the resulting script (for example 'My Multiplet Reporter') in a directory where Mnova will be able to find it and restart Mnova. To add new script directories follow the menu 'File/Preferences/Scripts/Directories':

© 2024 MESTRELAB RESEARCH



4. The script will be automatically added to the Mnova GUI (for example, to the 'Multiplet Table/Report Special/Setup' menu). This will allow you to select the 'Multiplet Report' format for your analysis. Of course, you can add as many different scripts as you need.



Please bear in mind that the multiplet format that you select on this dialog box, will be applied always when you select the 'Multiplet Report' option; however you can select any other formats for individual reports (without changing the default one) just by following the menu 'Tools/Report/Multiplets' and selecting the desired format for that report.

Let's see how to create a new multiplet reporter script from the example 'customMultipletReporter.qs' (stored at the 'example/scripts' folder of Mnova) :

```
/**************************************************************************************************
************
Copyright (C) 2009 Mestrelab Research S.L. All rights reserved.

This file is part of the MNova scripting toolkit.

Authorized users of MNova Software may use this file freely, but this file is provided AS
IS
with NO WARRANTY OF ANY KIND, INCLUDING THE WARRANTY OF DESIGN, MERCHANTABILITY AND FITNESS
FOR A PARTICULAR PURPOSE.
**************************************************************************************************
**********/

var MyMultipletReporterSettingsKey = new String("MyMultipletReporter/Custom Message");

/* This creates a Custom Multiplet Reporter  */
function MyMultipletReporter()
{
       MultipletReporter.call(this);
```

```
	var setts=new Settings(Application.mainWindow.scriptHandler.settings);
	this.customMessage = setts.value( MyMultipletReporterSettingsKey, "My Multiplet
Reporter" );

	this.name = "My Multiplet Reporter";

	this.onlyElementName=false;
// Define font size and font family
	this.font = "<font style=\"font-size: 10pt; font-family: Times New Roman\">";

	this.nucleusTemplate="%1";
// Report header. %1 will be raplaced with nucleusString, %2  with frequency, %3 with
solvent
	this.header = this.customMessage+" %1 NMR (%2 MHz, %3) &delta; ";
	//this.header = "Custom %1 NMR (%2 MHz, %3) &delta; ";

// Multiplet templates. %1 - delta, %2 - category, %3 - nH
	this.reportRange = true; // set to true to get multiplet range instead of delta.
	this.withoutJsTemplate = " %1 (%2, %3H)"; // multiplet template without J's
	this.withJsTemplate = " %1 (%2, %4, %3H)"; //  multiplet template with J's
	this.rangeTemplate = "%1 &ndash; %2";

// J's list template. %1 - list of J's
	this.jListTemplate = "<i>J</i> = %1";

	this.jPrecision = 1; // J's precision
	this.deltaPrecision = 2; // delta precision
	this.mSeparator = ", "; // multiplet separator
	this.jSeparator = ", "; // J's separator

	this.start = "<html><head><title>Multiplet Report</title></head><body>" + this.font;
	this.end = ".</font></body></html>";
}

MyMultipletReporter.prototype = new MultipletReporter();
MyMultipletReporter.prototype.toString = function() { return "MyMultipletReporter()"; }

//This line adds my custom reporter to the list of reporters, this means that this reporter
can be used by the general functions like reportMultipletsUser or automaticMultipletReport
and it can be used to generate reports  from the Multiplets Table
Env.MultipletReportersArray.push(new MyMultipletReporter());

//Uncomment this line to make this reporter the default reporter, the saved settings will
be ignored, the user will not be able to modify the reporter from MNova
//Env.DefaultMultipletReporter = "My Multiplet Reporter";


//This function can be used to generate multiplet reports
function customReportMultipletsUser(createItem)
{
	var multipletTable = new MultipletTable(nmr.multipletTable()); // Get table of
multiplets
	var spectrum;
	if(multipletTable.isValid())
		spectrum = new NMRSpectrum(multipletTable.spectrum()); // Get spectrum
currently shown in the table of multiplets
	else
		spectrum = new NMRSpectrum(nmr.activeSpectrum());
```


© 2024 MESTRELAB RESEARCH

```
        if( spectrum.isValid() )
        {
                var reporter = MultipletReporter.getReporterByName("My Multiplet Reporter");
                return reporter.report(spectrum, createItem);
        }
        else
                return undefined;
}

//Uncomment this line to use the previous function to report multiplets instead of the
default one
//Env.ReportMultipletsUser = customReportMultipletsUser;

//Function to setup my reporter
function customReportMultipletsUserSetup()
{
        var rep = MultipletReporter.getReporterByName("My Multiplet Reporter");

        var diag = new Dialog();
        diag.title = "Custom Setup";

        var lEdit = new LineEdit();
        lEdit.text = rep.customMessage;
        diag.add(lEdit);

        if(diag.exec())
        {
                rep.customMessage = lEdit.text;
                settings.setValue( MyMultipletReporterSettingsKey, rep.customMessage );
        }
}

//Uncomment this line to use the previous function to setup the report multiplets
//Env.ReportMultipletsUserSetup = customReportMultipletsUserSetup;
```

Firstly, we are going to change the name of the 'Multiplet Reporter' and the message which appears at the beginning of the Multiplet Report (customMessage) to 'My Report' and 'My Journal' respectively' (see the text below highlighted in yellow)

```
/* This creates a Custom Multiplet Reporter  */
function MyMultipletReporter()
{
      MultipletReporter.call(this);

      var setts=new
Settings(Application.mainWindow.scriptHandler.settings);
      this.customMessage = setts.value( MyMultipletReporterSettingsKey,
"My Journal" );

      this.name = "My Report";

      this.onlyElementName=false;
```

this.onlyElementName=false; changing false with true, we will obtain only the element name without the atomic mass (For example: H, C instead of ^{1}H, ^{13}C).

© 2024 MESTRELAB RESEARCH

The function: this.font = "<font style=\"font-size: 10pt; font-family: Times New Roman\">"; will define the font size and the font family of the multiplet report.

We are going to change the font of the multiplet report to 14pt and Arial:

```
// Define font size and font family
      this.font = "<font style=\"font-size: 14pt; font-family: Arial\">";
```

The line: this.header = this.customMessage+ "%1 NMR (%2 MHz, %3) δ "; is used to print the header of the report, where %1 will be the nucleus (H or C), %2 the frequency of the spectrometer (in MHz), and %3 the solvent, followed by a delta symbol (δ). For example: "^{1}H NMR (500 MHz, $CDCl_3$) δ".
Let´s modify the header of the report by putting the 'delta' symbol in italic:

```
// Report header. %1 will be replaced with nucleusString, %2  with
frequency, %3 with solvent
      this.header = this.customMessage+" %1 NMR (%2 MHz, %3)
<i>&delta;</i> ";
```

We can also modify the way to report the multiplet infomration

```
// Multiplet templates. %1 - delta, %2 - category, %3 - nH
      this.reportRange = true; // set to true to get multiplet range
instead of delta.
      this.withoutJsTemplate = " %1 (%2, %3H)"; // multiplet template
without J's
      this.withJsTemplate = " %1 (%2, %4, %3H)"; //  multiplet template
with J's
      this.rangeTemplate = "%1 &ndash; %2";
```

The sentence: this.reportRange = true is used to obtain the multiplet range instead of the chemical shift.

The functions: this.withoutJsTemplate = " %1 (%2, %3H)" and this.withJsTemplate = " %1 (%2, %4, %3H)" are used to customize the appearance of the multiplet report by changing the positions of %1, %2, %3H, or %4 (where, %1 means: chemical shift; %2 means: type of multiplet (s, d, t, etc); %3H means: number of hydrogens and %4 means the coupling constant value). As you can see, the first line shows a multiplet without coupling constants, (while the last line shows a multiplet with coupling constants).

So, if you need to obtain something like this (japanese format):

1H NMR (300 MHz, Solvent) δ ppm 6.43-6.22 (1 H, m), 3.17 (1 H, q, J = 7.15 Hz) etc…

You should modify both lines, as you can see below:

```
this.withoutJsTemplate = %1 (%3H, %2)";
this.withJsTemplate = " %1 (%3H, %2, %4 Hz)";
```

If you prefer to obtain something like this:

© 2024 MESTRELAB RESEARCH

1H NMR (300 MHz, Solvent) δ ppm 1.23 (d, *J* = 1.2 Hz, 3 H), etc…

Just replace the original lines with:

```
this.withoutJsTemplate = %1 (%2, %3H)";
this.withJsTemplate = " %1 (%2, %4 Hz, %3H)";
```

You can also customize the coupling constant format:

```
// J's list template. %1 - list of J's
      this.jListTemplate = "<i>J</i> = %1";

      this.jPrecision = 1; // J's precision
      this.deltaPrecision = 2; // delta precision
      this.mSeparator = ", "; // multiplet separator
      this.jSeparator = ", "; // J's separator

      this.start = "<html><head><title>Multiplet
Report</title></head><body>" + this.font;
      this.end = ".</font></body></html>";
}
```

To obtain the coupling constant symbol in normal instead of *'italic'*, just modify the script by removing the italic format (J). If you prefer to obtain it in "**bold**" just type:

```
this.jListTemplate =  "<b>J</b> = %1";
```

To add a superscript to get for example: ^3J:

```
this.jListTemplate = "<sup>3</sup><i>J</i> = %1";
```

The following paragraph will be used to customize the appearance of the coupling constants list:

The second line is used for the precision of the coupling constants values, the next will be used for the precision of the chemical shift and the remaining two lines will print the separation (coma, colon, dots, etc.) between the multiplets and the coupling constants values.

If you need to obtain the coupling constants in descending order, replace 'true' with 'false' in the following line:

```
var jList = new JList(multiplet.jList());
jList.sort(true);
```

To obtain the multiplet range in ascending order, just replace the rangeMin with rangeMax in the below line of the script:

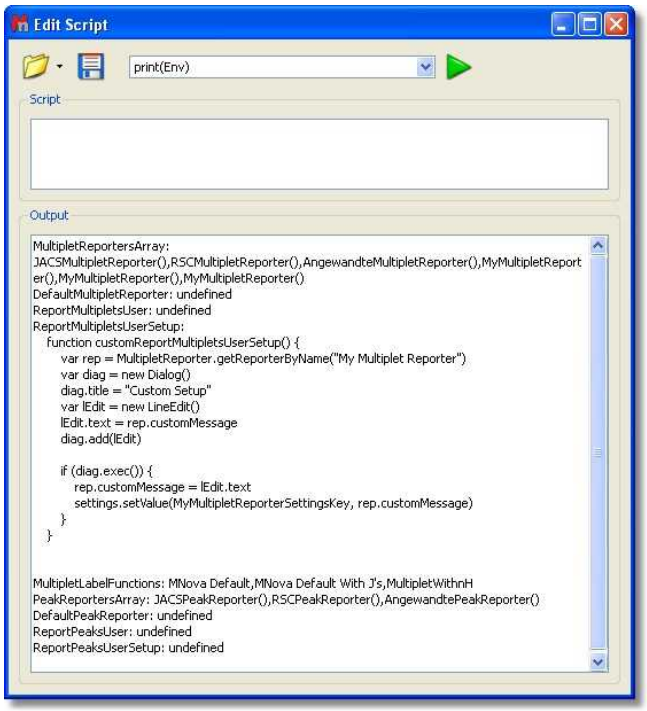
```
shiftStr = this.rangeTemplate.argDec(multiplet.rangeMax, 0, 'f',
this.deltaPrecision).argDec(multiplet.rangeMin, 0, 'f', this.deltaPrecision);
```

You can select a multiplet report format as the default one, without allowing the user to modify it, just by removing the two slash symbols (before this sentence: `Env.DefaultMultipletReporter = "My Multiplet Reporter";`). Please bear in mind that if you do that, you will not obtain any dialog box when you follow the Menu 'Multiplets Table/Report Special/Setup'.

```
//Uncomment this line to make this reporter the default reporter, the
saved settings will be ignored, the user will not be able to modify the
reporter from MNova
Env.DefaultMultipletReporter = "My Multiplet Reporter";
```

© 2024 MESTRELAB RESEARCH

Indeed, the variable **'Env'** contains several environment variables which can be used to customize the Mnova behaviour. Typing `print(Env)` in the Script editor, you will get the list of the available variables. In the example below, we have only defined the 'Setup' of the 'Multiplet Reports'. All the remaining variables are 'undefined'.



You can define the *'DefaultMultipletReport'* variable by uncomment this line in the script:
```
Env.DefaultMultipletReporter = "My Multiplet Reporter";
```

Doing that, the user will not be able to modify the multiplet report.

You can define the *'ReportMultipletUser'* variable by uncomment this line of the script:
```
Env.ReportMultipletsUser = customReportMultipletsUser;
```

Defining this variable, you will have a format for the 'multiplet report' and the 'copy special' and another different (the default one) for the report which appears on top of the 'multiplets table'.

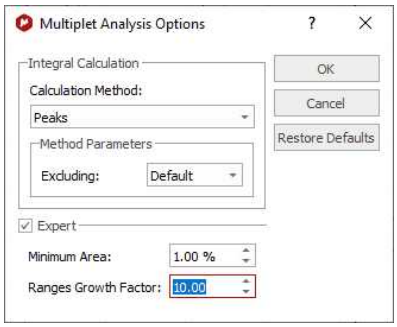
The '*ReportMultipletUserSetup'* is the variable defined in the menu 'Multiplet Table/Report Special/Setup' for the 'Multiplet Report'.

The 'MultipletLabelFunctions' are different options (showed in the 'Multiplet Properties' dialog box) which allow the user to customize the information which appears in the multiplet boxes.

8.4.6 Multiplet Analysis options

Follwing the menu 'Analysis/Multiplet Analysis/Options' will display the applicable dialog box:

© 2024 MESTRELAB RESEARCH



From here you can select the minimum area and the integration calculation method (Peaks, which uses GSD, Sum, which is the classical integration method and 'Edited Sum' which uses the traditional integration and the peak classification from GSD, discarding impurities and solvents from the area calculation) and also to select what kind of peaks will be take into account for the multiplet analysis. By default **only compounds peaks** will be, so the solvent peaks will not affect to the multiplet analysis (very useful for example if a solvent signal overlaps with a compound peak).

When running automatic multiplet analysis using Sum or Edited Sum methods the multiplet ranges will not overlap.

The 'Ranges Growth Factor' field will allow you to tune the multiplet range.

8.4.7 Multiplets Properties

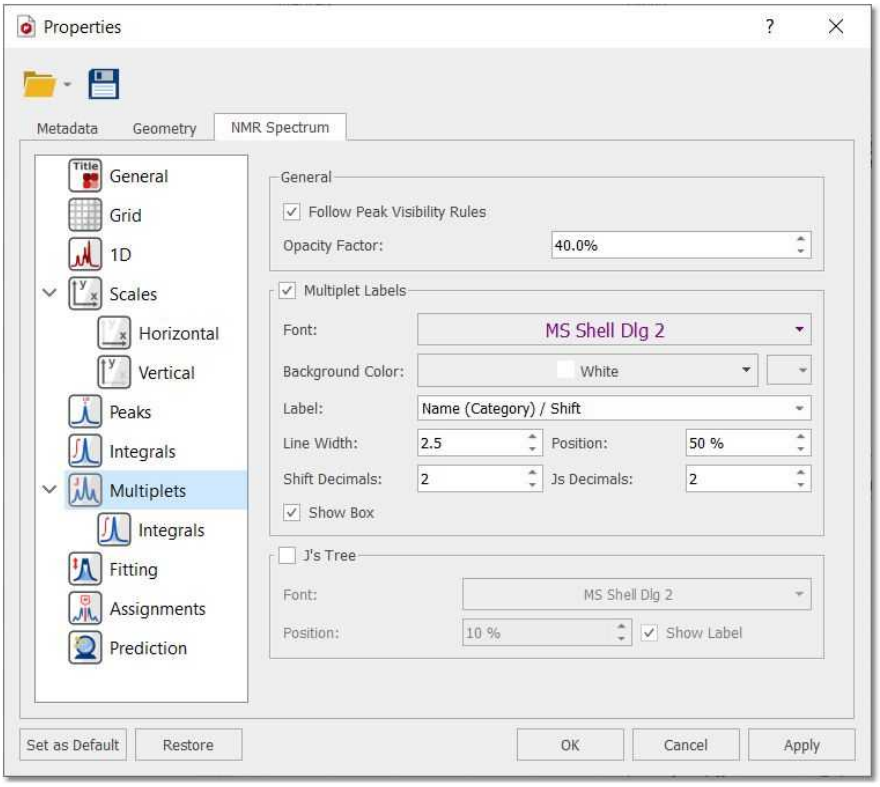
The user can modify the properties of the **Multiplet Analysis** by following the menu 'Home/Properties/Multiplet Analysis' or by double clicking the left mouse (or pressing the right mouse) button on the spectrum display and selecting *Properties/Multiplets* from the *pop-up* menu.

If the spectrum contains peaks belonging to more than one compound, we can show only the peaks assigned to one of them by filtering the desired peaks in the Peaks Table. Checking the 'Follow Peak Visibility rules' box will allow you to show only the multiplet boxes applicable to those visible peaks.

You will find an option to select the opacity factor for the non-compound multiplets (for the labels, integrals, j-trees and ranges) making them less visible than compound ones.

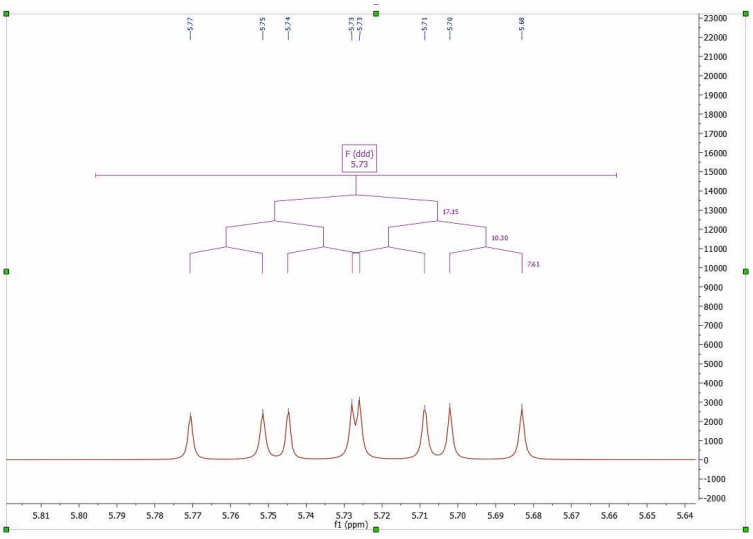
You can also select the color, line width, decimals for the shifts and coupling constants, the font and the margin of the 'Multiplet' boxes:

© 2024 MESTRELAB RESEARCH



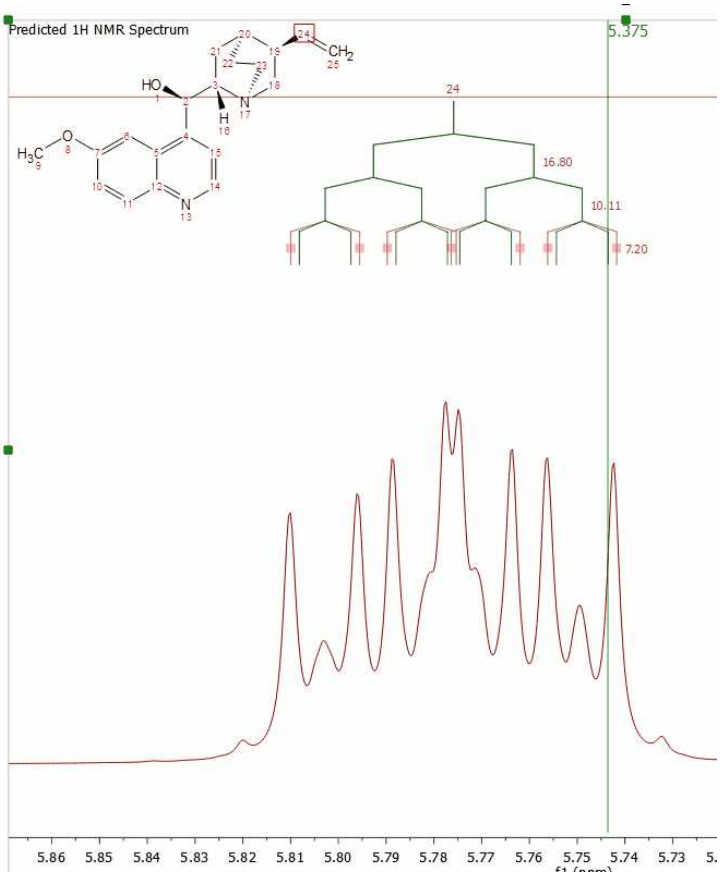
You can also hide the multiplet square box by unchecking the applicable box.

The user will be able to display the coupling constant tree just by checking the applicable option. Here you can see an example:

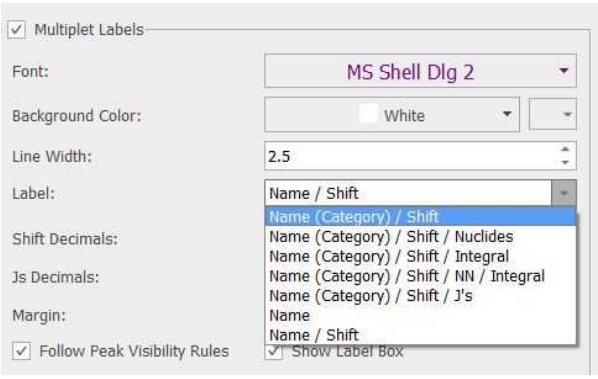


These objects are mouse sensitive in the case of predicted spectra, making possible the graphical edition of both the chemical shift and couplings by using drag & drop.

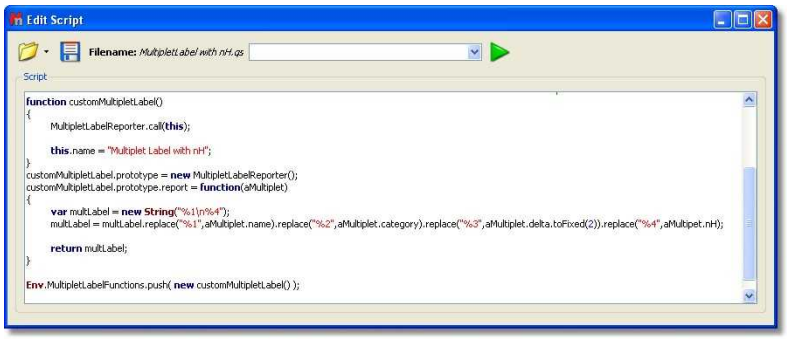
© 2024 MESTRELAB RESEARCH



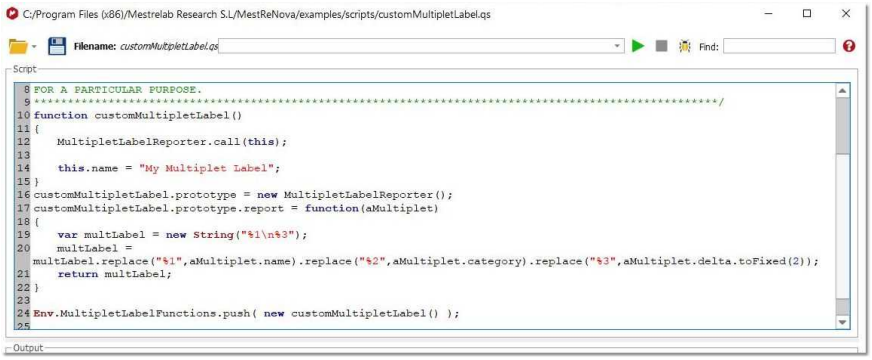
You can modify the information of the multiplet label by selecting the desired option in the scroll down menu:



You can also add new labels by using the scripting feature. You will see below a script to show multiplet labels with the number of Hydrogens.



You will find another example (CustomMultipletLabel.qs) in the 'Examples/Scripts' folder of Mnova:

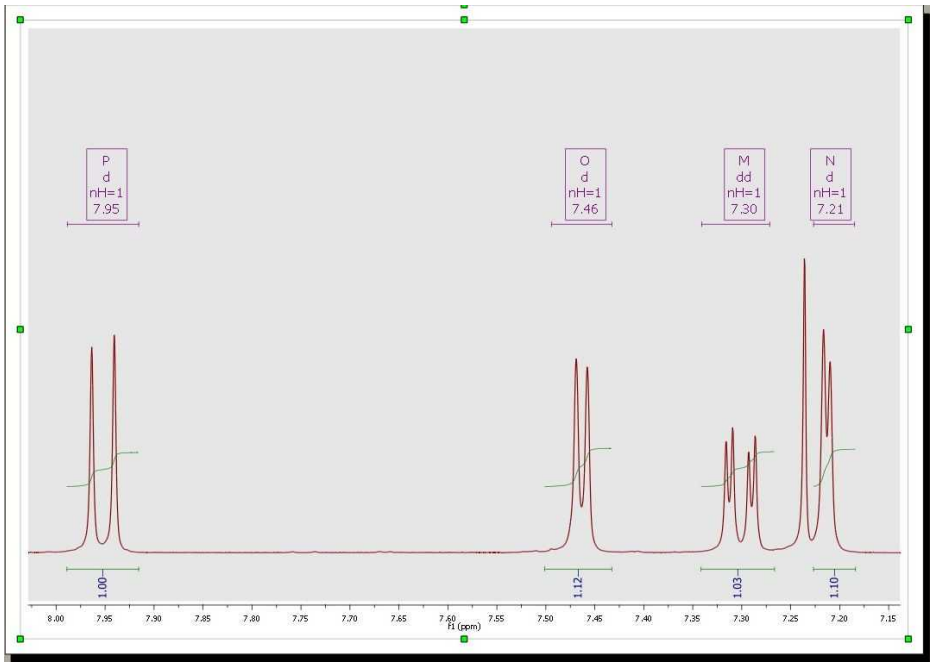


You will only need to save this script in a folder where Mnova can find it ('File/Preferences/Scripting/Scripts Directories) and after having restarted the software, you will get the new entry in the 'Multiplet Label' scroll down menu of the 'Properties' dialog box.

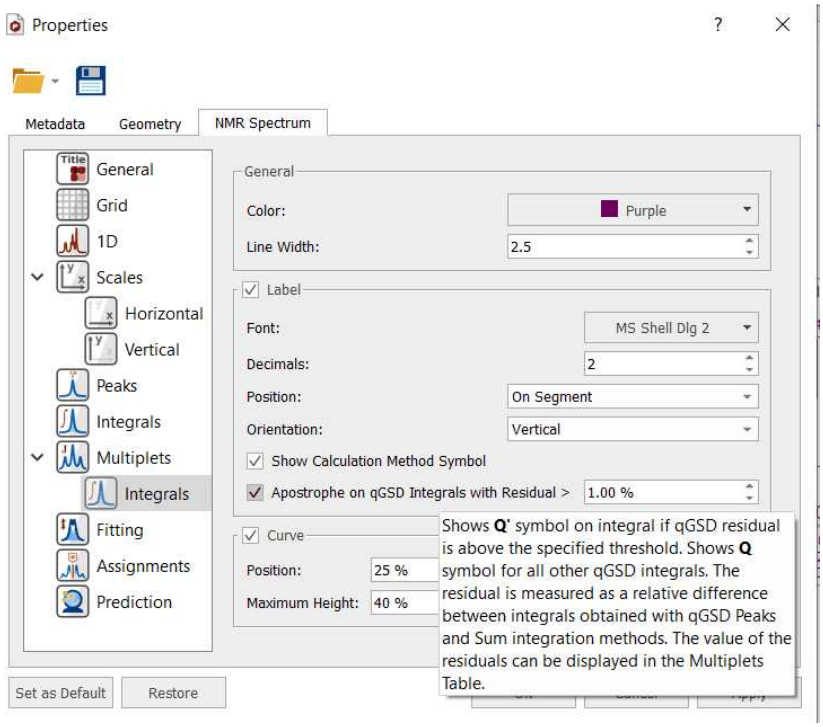
You can see in the Scripts chapter, all the multiplet properties which you can include in the 'multiplet label'.

Adding the above script to the Mnova scripts directories, you will obtain your multiplet labels as you can see in the picture below:

© 2024 MESTRELAB RESEARCH

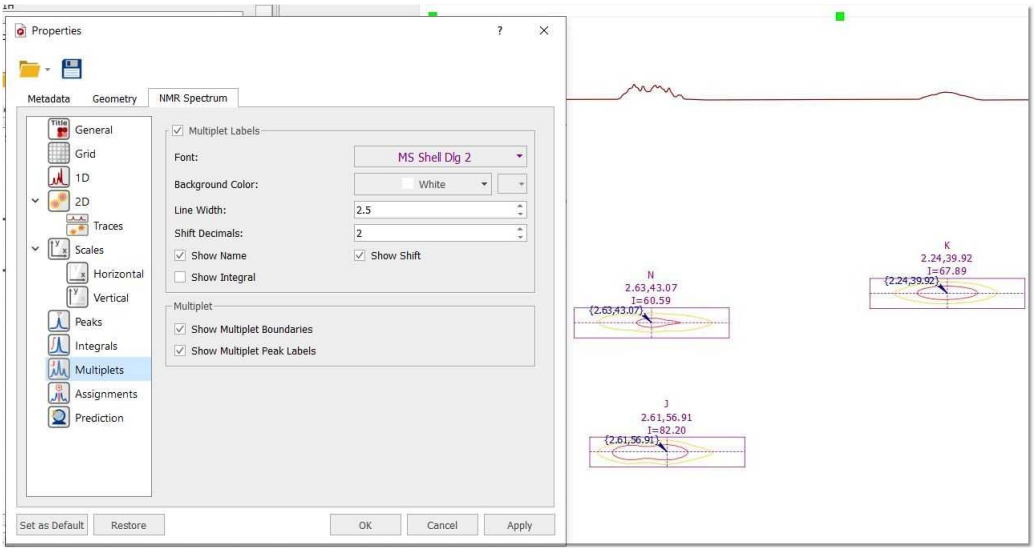


The user can also show the Multiplet Integrals Curves and customize the appearance. Please bear in mind that integrals and multiplets are two different list, so you can select what integrals you want to show (the 'pure integrals' or the integrals existing under the multiplet ranges):



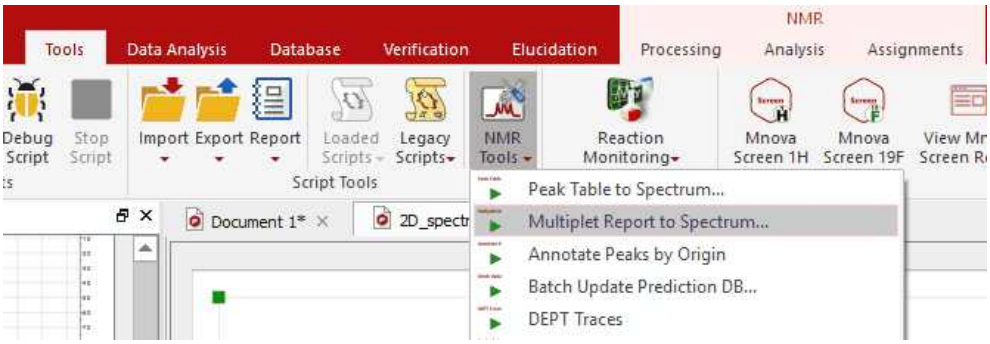
From here, you can also display the integral calculation method and also to label those integrals with a residual bigger than a value.

If you are working with a 2D dataset, the multiplets properties window will be similar and you will have the capability to change the font, color, line width, decimals, show name, integrals and shift values and multiplets and peak labels.



8.4.8 Importing spectral data from literature to synthesize a spectrum

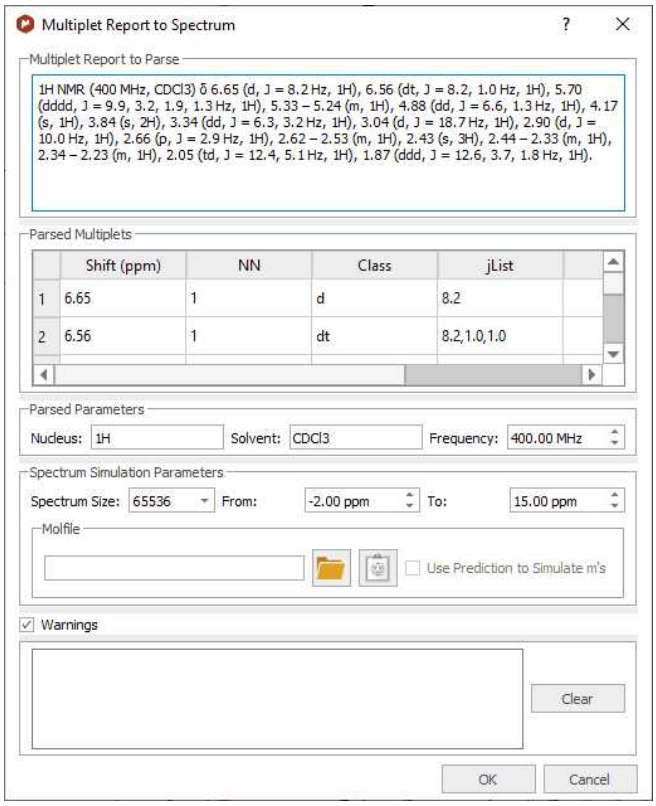
To synthesize a spectrum from a multiplet report (copied from a paper); you will only need to copy to clipboard the multiplet report and follow the menu 'Tools/NMR Tools/Multiplet Report to Spectrum':



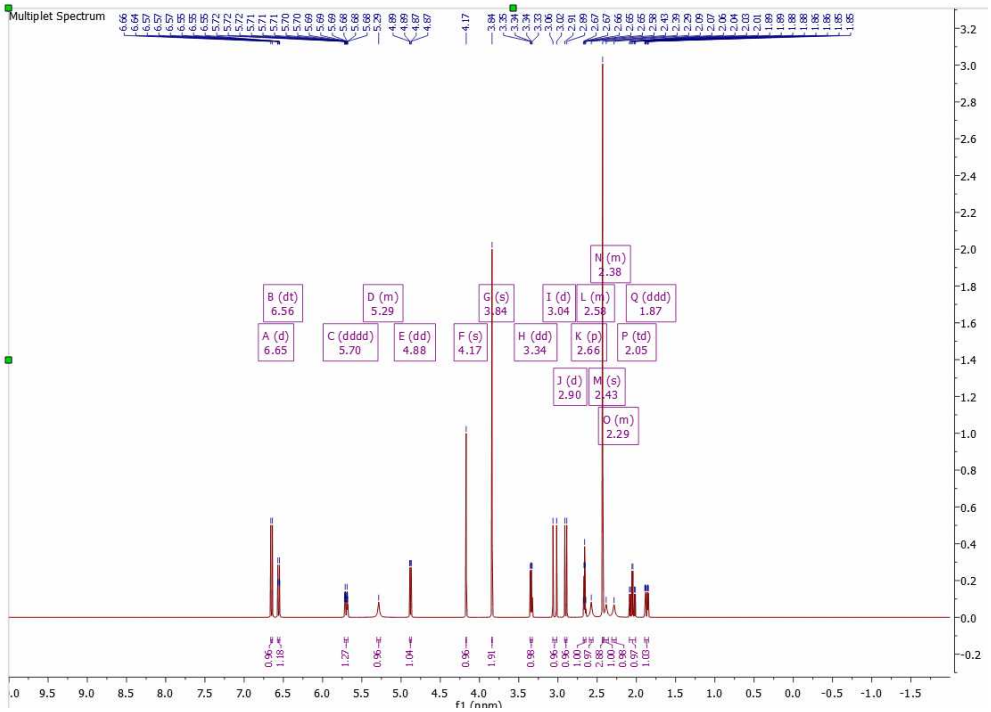
This will display a dialog box to paste your multiplet report. For example, let´s paste the report below:

1H NMR (400 MHz, CDCl3) δ 6.65 (d, J = 8.2 Hz, 1H), 6.56 (dt, J = 8.2, 1.0 Hz, 1H), 5.70 (dddd, J = 9.9, 3.2, 1.9, 1.3 Hz, 1H), 5.33 – 5.24 (m, 1H), 4.88 (dd, J = 6.6, 1.3 Hz, 1H), 4.17 (s, 1H), 3.84 (s, 2H), 3.34 (dd, J = 6.3, 3.2 Hz, 1H), 3.04 (d, J = 18.7 Hz, 1H), 2.90 (d, J = 10.0 Hz, 1H), 2.66 (p, J = 2.9 Hz, 1H), 2.62 – 2.53 (m, 1H), 2.43 (s, 3H), 2.44 – 2.33 (m, 1H), 2.34 – 2.23 (m, 1H), 2.05 (td, J = 12.4, 5.1 Hz, 1H), 1.87 (ddd, J = 12.6, 3.7, 1.8 Hz, 1H).

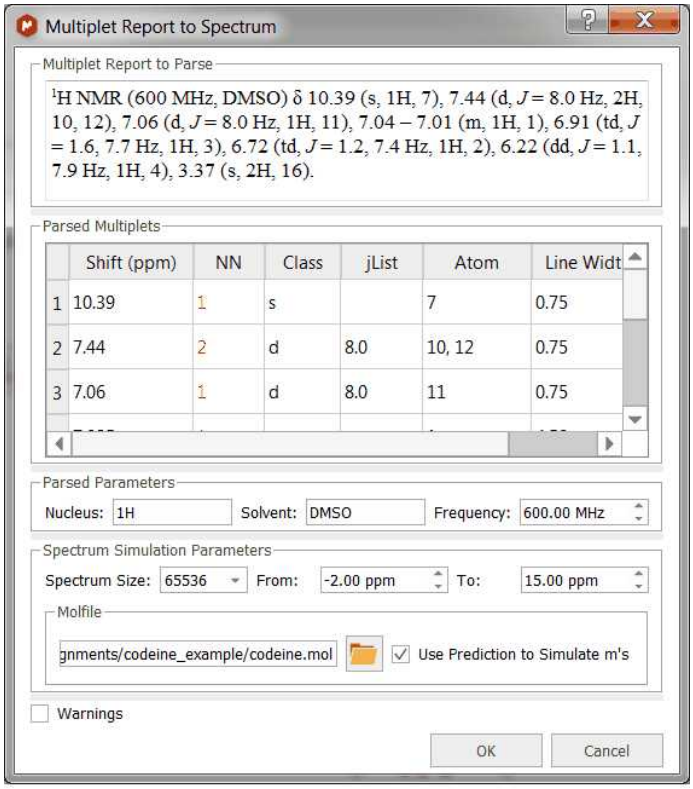
© 2024 MESTRELAB RESEARCH



After having clicked on the OK button, we will get the synthetic spectrum:

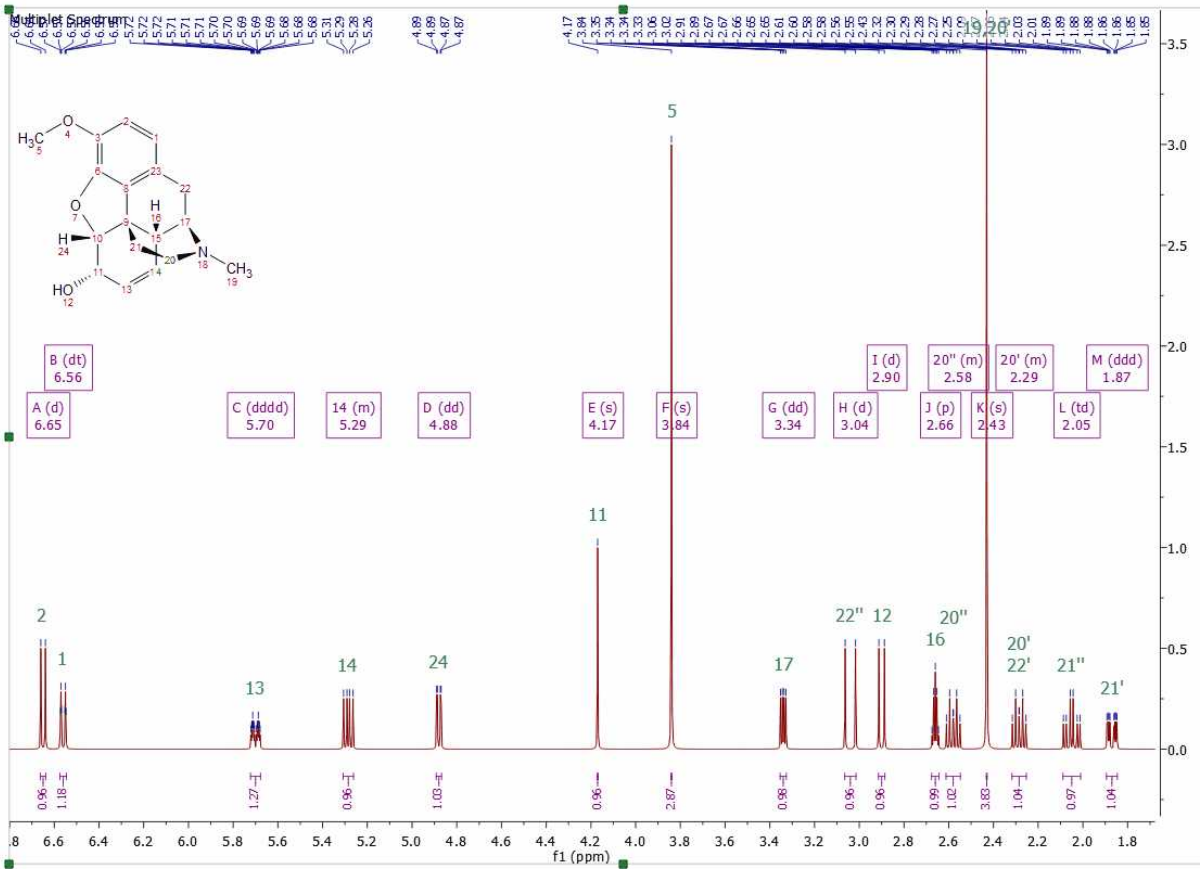


By the default the 'not defined multiplets' (labelled as 'm') will appear as 'broad singlets', however you can predict the multiplet shapes by loading a mol file and selecting the atom numbers for each multiplet (labelled as 'm') in the table:



For this example, we have assigned the multiplets, we loaded the mol file and checked the option to 'predict' the shape for those assigned multiplets. Here you can see the result:

© 2024 MESTRELAB RESEARCH



For further information, please see this video and take a look at this post

8.5 How to carry out assignments?

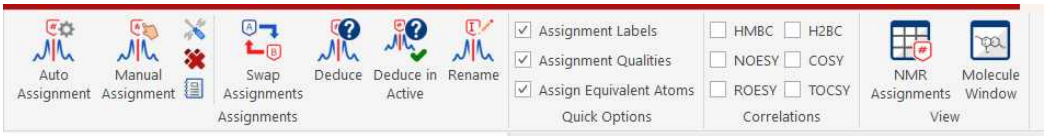
You will find the quinine datasets under the 'File/Help/Download Example datasets' menu)

You can carry out automatic assignments and atom to peak assignments both to 1H, 13C and HSQC spectra.

Automatic Assignment of NMR spectra has been a pipedream for many years, especially in the organic chemistry community. In spite of successful applications of computer algorithms for the automation of the processing and analysis of NMR spectra, fast and robust unsupervised assignment of NMR spectra of small molecules has so far been a discouraging endeavor.

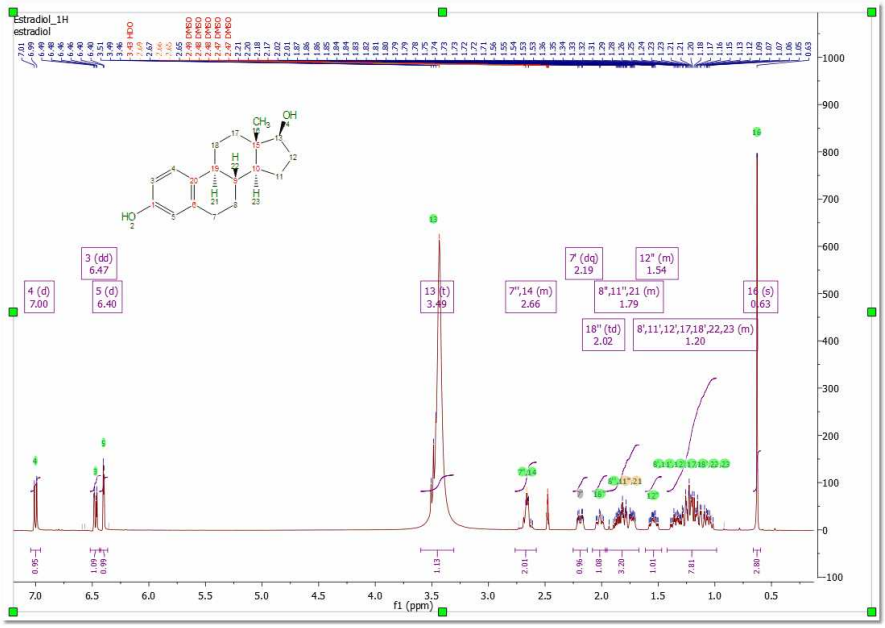
See this publication for further information.

Mnova provides a very simple interface to automatically assign your molecule. Just load your NMR spectrum (1H, 13C and HSQC; alone or combined) with the applicable molecular structure and follow the menu 'Assignments/Auto Assignment':

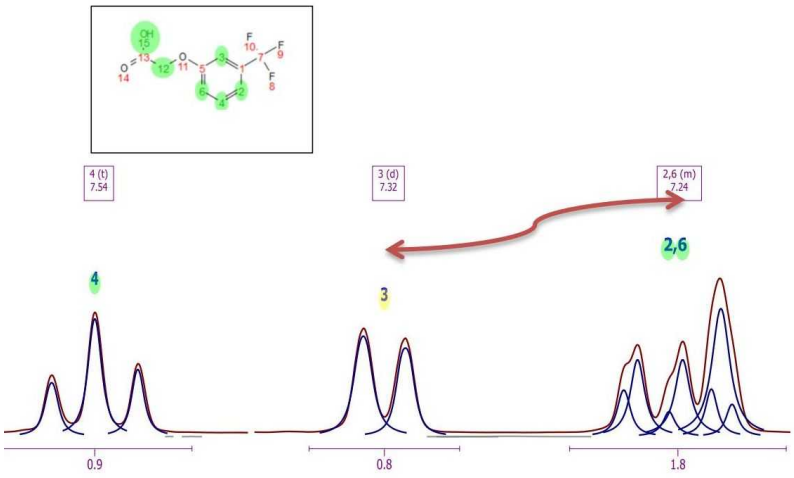


Mnova will automatically run a multiplet analysis and will fully assign your spectrum with the applicable molecular structure. If you have a license of the prediction plugin, the assignments will be more accurate.

The multiplet boxes will be renamed with the corresponding atom number of the molecule and you will get an assignment label with the atom number over the applicable peak (including a colour quality coding). In addition, hovering the mouse over the atom will highlight the applicable signal in the spectrum:



This algorithm for automatic assignment of NMR spectra has to be understood as a quick method to generate an initial 'rough' assignment that in some cases will require manual intervention from the user to easily amend any potential incorrect auto-assignment.
The auto-assignments could assign, for example, all the protons properly except two which are swapped (as illustrated in the figure below) where assignments (3) and (6) must be swapped.



You can **swap assignments** by following the menu 'Analysis/Assignments/Swap Assignments' (shortcut: S). Then you will only need to click on one assignment label (or multiplet box) and drag and drop it to the target assignment label (or multiplet box).

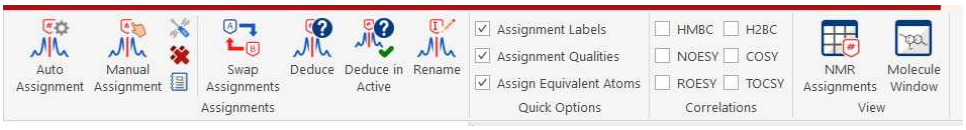
© 2024 MESTRELAB RESEARCH

If the target multiplet has more than one assignment, you will be asked for which assignment must be used for swapping.

The assigned chemical shift can be also modified by moving the assignment labels. In 2D spectra, it is possible to move vertical and horizontal assignments. When moving assignments to a position inside a multiplet range in the 1H spectrum the new assignment will be linked to the multiplet. The new assignment will be the multiplet shift or the selected position depending on the option "Use always 1H multiplet shift when adding 2D assignments".

MANUAL ASSIGNMENTS

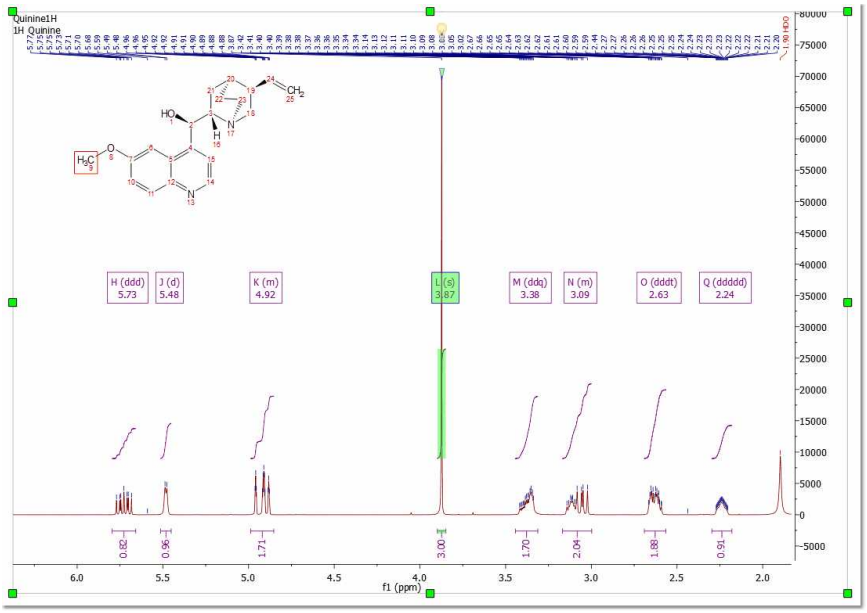
Mnova provides a very simple interface to manually assign your molecule. Open your NMR spectra in the same document and load/draw the molecule structure. Next, follow the menu 'Assignments/Manual Assignments' (or use the shortcut 'A').



Finally, click on an atom in the molecular structure (or a spectrum region) and then release the mouse and drag it to your desired peak, peak range, multiplet box or integral curve. We recommend you to assign your atoms to your multiplet boxes in order to transfer assignments through datasets.

The assigned chemical shift is added (automatically) to the assignments table when assigning to a multiplet, integral, peak or position, either in 1D and 2D spectra.

With the 'Assisted Assignment mode' (which works for 1H and 13C spectra having a multiplet analysis), a 1H and 13C predictions are made in the background. When hovering the mouse over the atoms of the molecule, the suggested multiplets in the spectrum will be highlighted (following a color code: green, yellow, red, gray- depending on the quality: grey: -1 to -0.5; red: -0.5 to -0.25; yellow: -0.25 to 0.25; green: 0.25 to 1) and viceversa if you hover the mouse over the multiplet boxes, the suggested atoms in molecule and compounds table will be also highlighted. This feature can be disabled from the 'Assignments options'.

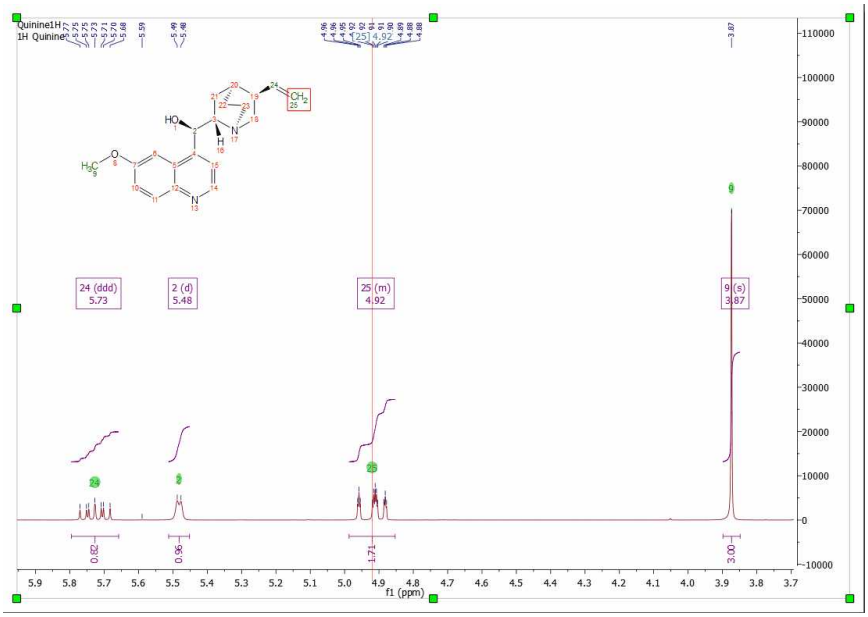


NOTE

Hold down the 'Ctrl' key and click, to select several atoms at a time.

Hold down the 'Alt' key while you are assigning to display the Assign dialog with Options to 'Add/Replace/Keep Original'

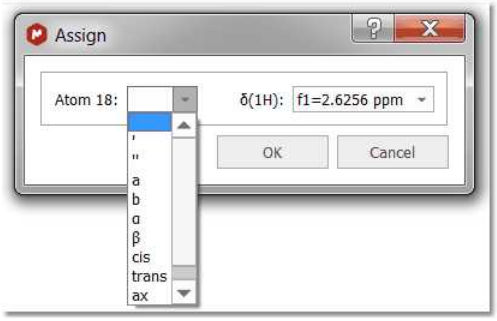
This multiplet will now be assigned to the atom (which will turn to green). Once the assignment has been made, you will get an atom number label on the chemical shift (with quality colour coding). Hovering the mouse over the atom will highlight the applicable peak in the spectrum and hovering the mouse over the peak will highlight the corresponding atom on the molecular structure. To customize the assignments labels, click here.



If you do not want to show the assignments labels or the quality colour coding; just uncheck the applicable option from the 'Assignments Properties' dialog box. From the Properties, you will also be able to customize format of the correlations labels.

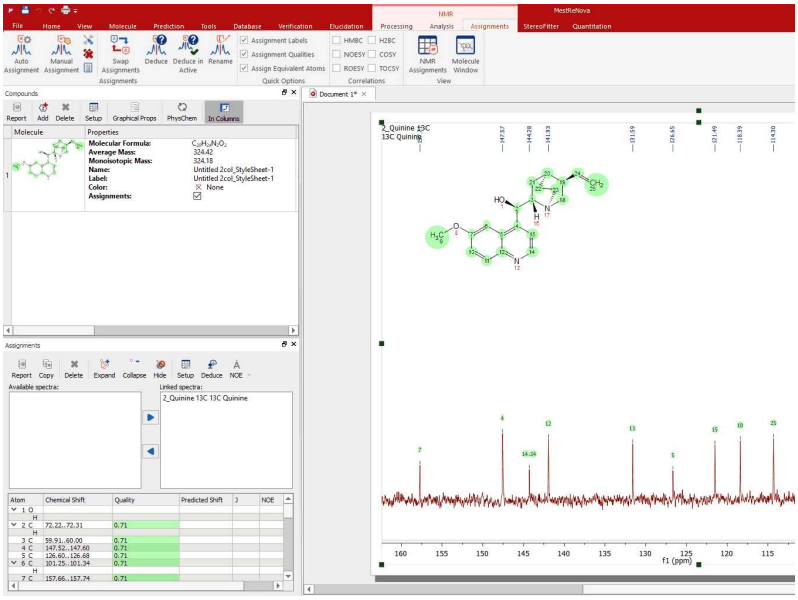
The user can also assign a region of the spectrum just by clicking, dragging and releasing the mouse over the desired region. In the example below, we have assigned a -CH_2 group. If you want to assign the diastereotopic protons, you would need to hold down the 'Alt' key when assigning, so a new window will be displayed to allow us to select which atom we want to assign, 18, 18', both (in blank) or even we can select any other annotation: 18a, 18b, cis/trans, ax/eq, α/β :

© 2024 MESTRELAB RESEARCH

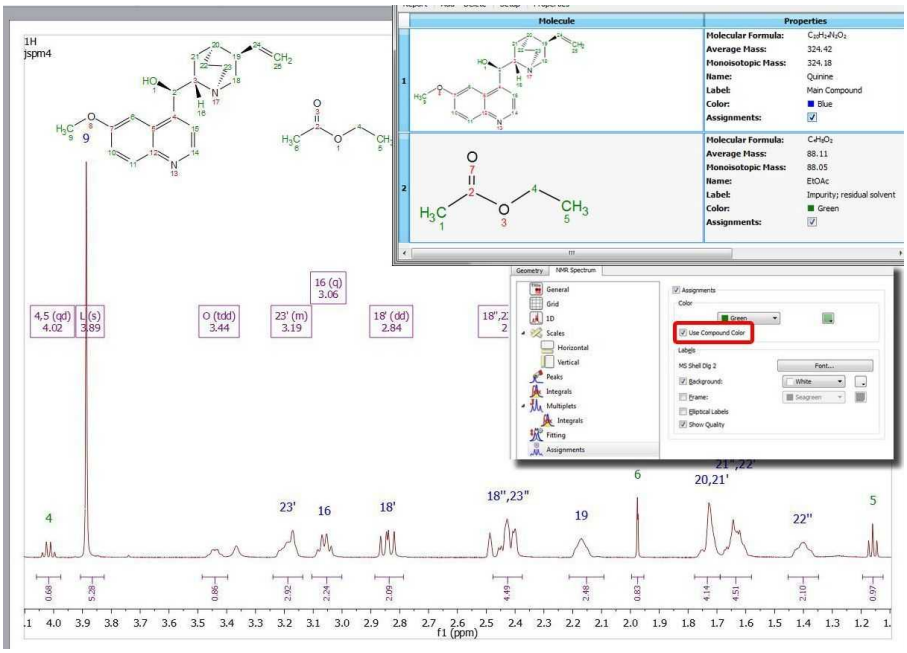


You can assign a 13C-NMR spectrum of the same molecule in the same document. To do that, select the atoms from the 'Molecule Window' panel by following the menu 'View/Panels/Molecule Window' (or from the Compounds Table; from 'View/Tables')

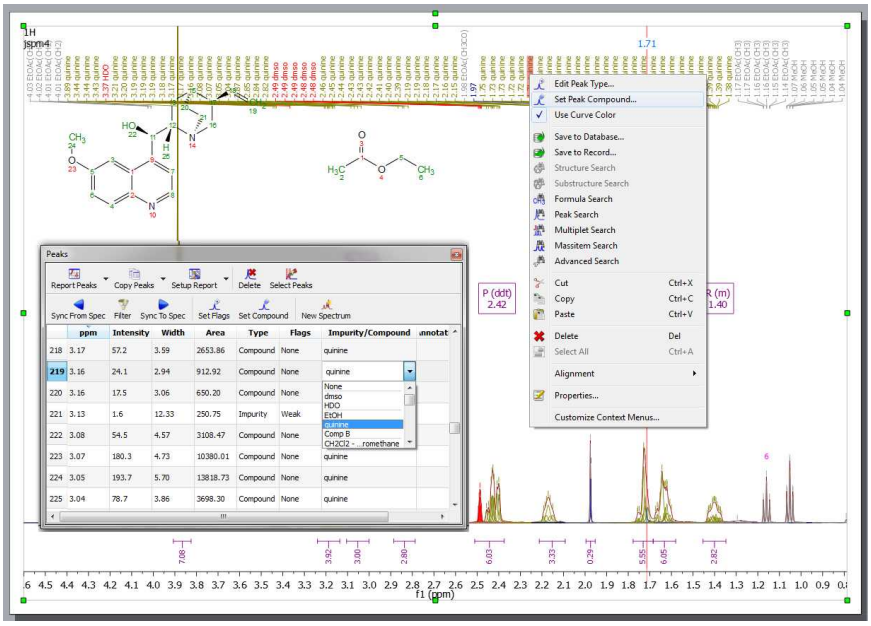
To keep the assignments propagation, follow the menu 'View/Tables/Assignments' and make sure that the desired are linked (in the example below, we have selected the 13C).



If your document has more than one molecule (see also this tutorial), use the compounds table to select a different colour for the assignments of each compound. In the example below the assignments labels of the quinine appears in blue and the assignments labels of the EtOAc are green (make sure that 'Use Compound color' is checked in the Assignment Properties):



If your document contains more than one compound, you can select the compound for each peak from the Peaks Table (by clicking on the 'Set Compound' button or by double clicking on the 'Impurity/Compound' cell). The same effect will be obtained by right clicking on the Peak label and selecting ‘Set Peak Compound’ (please make sure that the 'Use Curve Color' box is checked in the 'Peaks Properties'):



Rules for 1D assignments:

<u>For 1H Assignments:</u>

-CH2 atoms with diastereotopic protons: assign the not assigned proton first.

-Show the Assign dialog (automatically) when both -CH2 protons are assigned and the user wants to assign again the same atom. (Options Add/Replace/Keep Original)

© 2024 MESTRELAB RESEARCH

-Other atoms: assign all protons to the same chemical shift. Replace the chemical shift when the user wants to assign again the same atom.

-Other nucleus:

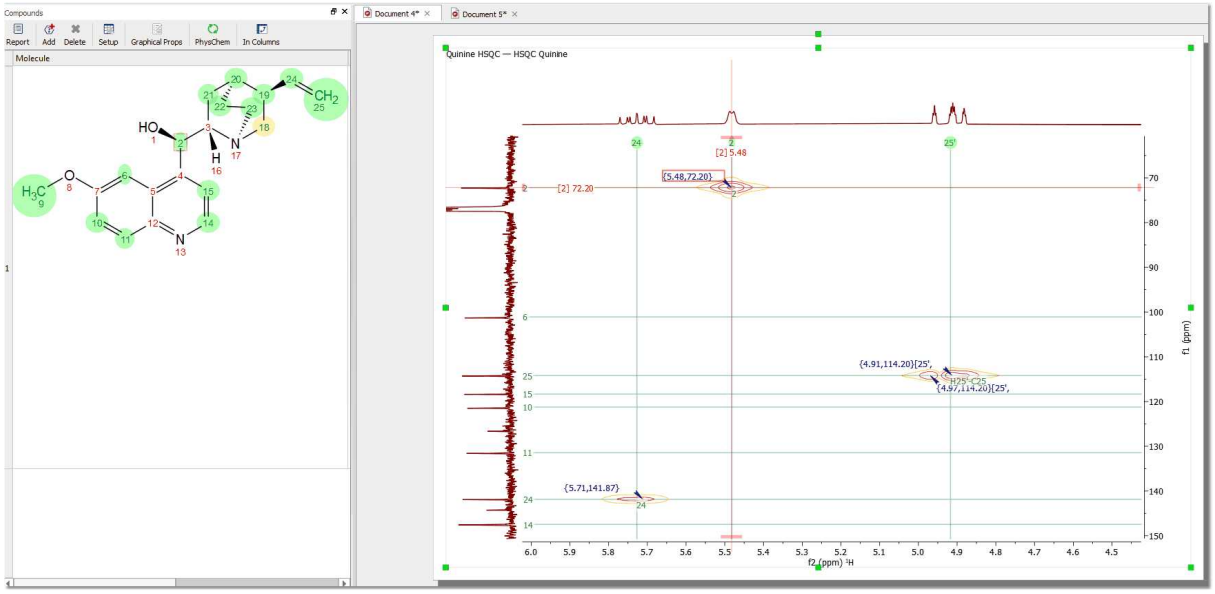
Assign to the selected chemical shift. Replace the shift value when the user wants to assign again the same atom.

Note: when the user assigns a diastereotopic proton, the notation is ', " by default. It can be changed in the Assignments Table, if the user doesn't want to use the dialog.

The user can force the visualization of the Assign Dialog by pressing the Alt key together with the second click of the mouse (when assigning).

Assignments in 2D

Once you have assigned the 1H and the 13C spectra, if you jump to a 2D-NMR spectrum (in the same Mnova document) you will see the assignments graphically on the screen.



From the Assignments options, you can use the unified chemical shifts when you assign a 2D spectrum. With the option "Do not calculate unified chemical shifts" is off, the unified shifts are calculated taking into account all the shifts provided by the linked spectrum objects (multiplets/peaks/integrals), either when the assignments are made manually, by moving an assignment and getting a new correlation deduced or from other deduction routines. When you assign to a position (in a 1D or 2D spectrum), all multiplets in the document corresponding to this/these chemical shift/s will be assigned (the assignment for the selected atom will be the shift and range of the multiplet with the narrowest range).

Note: the highlighting would show all the linked objects because all of them are considered for the unified shift calculation.

When the option "Do not calculate unified shifts" is on, each assignment to a spectrum object adds a link, so several links are still allowed. But no unified assignment is calculated. The assignment is taken from the last mutliplet/peak/integral assigned.

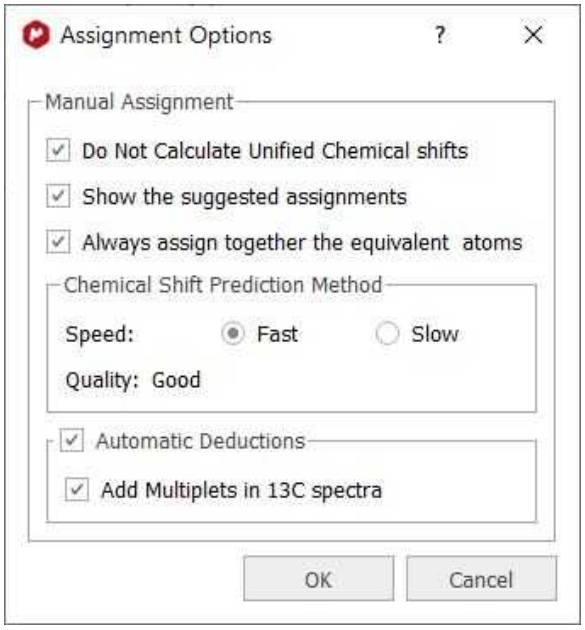
Note: only one linked object would be highlighted, which is the one used for the assignment value of each atom.

Basically when the:

a) Do not Calculate Unified Chemical Shifts is checked: it doesn't matter if you assign first to the 1H or HSQC, both assignments will be taken into account, and the resultant chemical shift will be the one corresponding to the narrowest assignment range of the two assignments.

b) Unified Shifts is unchecked: the assignment can change when you assign to 1H or HSQC, it will be always the last assignment done.

© 2024 MESTRELAB RESEARCH



From the dialog above, you can also select the prediction algorithm used for the 'assisted assignment mode' and you will have an option to always assign together the symmetrical atoms (this option will also appear as a check box in the Assignments ribbon)

When the 'Automatic Deductions' option is not checked:

- o assignments are not deducted in 2D/1D spectra when the user assigns manually a 1D/2D spectrum
- o assignments are not deducted when an assignment label is moved.

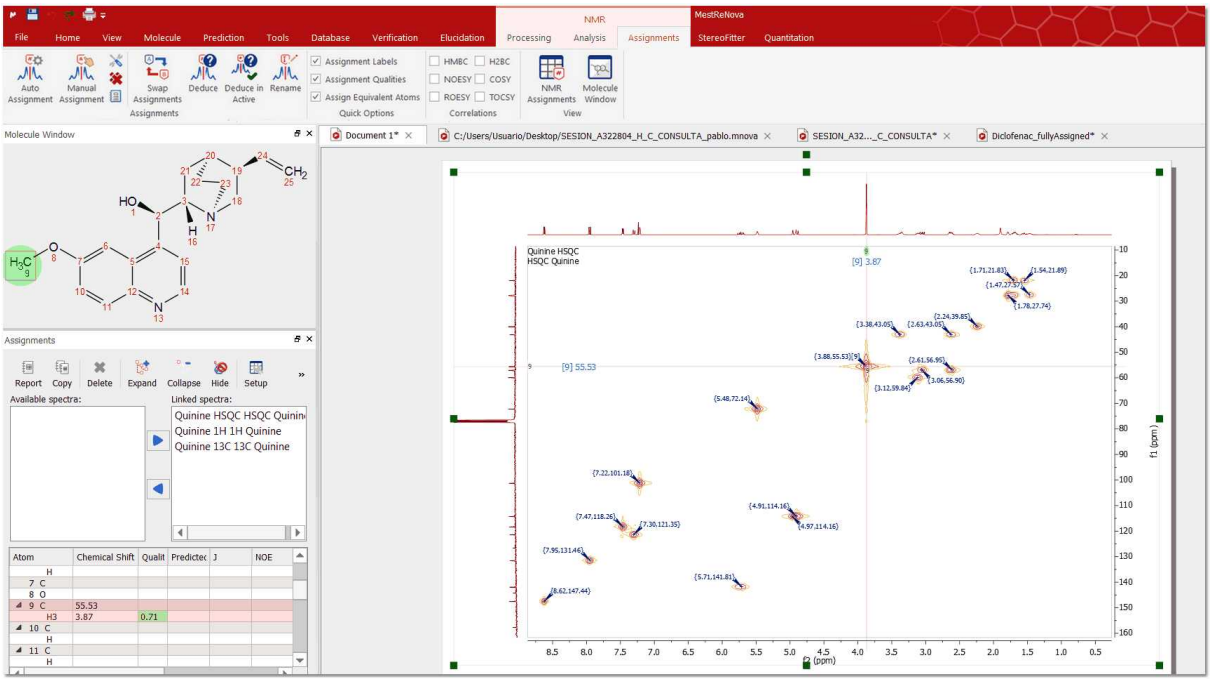
When the Deduce button of Assignments Table is pressed, the automatic deduction routines are executed, independently on this option is checked or not.

The "**Add Multiplets in 13C spectra**" tool needs the "Background Analysis" enabled (in Preferences/NMR/Processing) to work. This is a general feature, valid for all 2D spectra, not only for HSQC spectra, when the user assigns to a peak or multiplet.The assignments made in the 2D peaks will be transferred to the existing multiplets in the 13C spectrum.
If there are no multiplets in the 13C spectrum, they will be deducted, created and assigned automatically using the background information. (this behavior is produced only when the option Background Analysis is checked, and the new option "Add multiplets in 13C spectra" is also checked).

The searching of the 13C-multiplets compatible with the 2D assignment will be done in a different way according with the Use Global Assignment option:
- Global Assignment ON: the 13C-multiplet is auto-assigned (or created and auto-assigned in case of background multiplets) only if it's the unique 13C multiplet with a shift inside the range 2D chemical shift ± 0.25 ppm.
-Global Assignment OFF: only the 13C-multiplets whose range contain the 2D chemical shift are automatically assigned (or created and autoassigned if background multiplets are needed).

To carry out assignments to 2D spectra, type the applicable number of the atom in the 'Assignments Table' or **graphically** select the atom in the molecule structure and the applicable signal (peak label, chemical shift or multiplet box) in the 2D:

© 2024 MESTRELAB RESEARCH





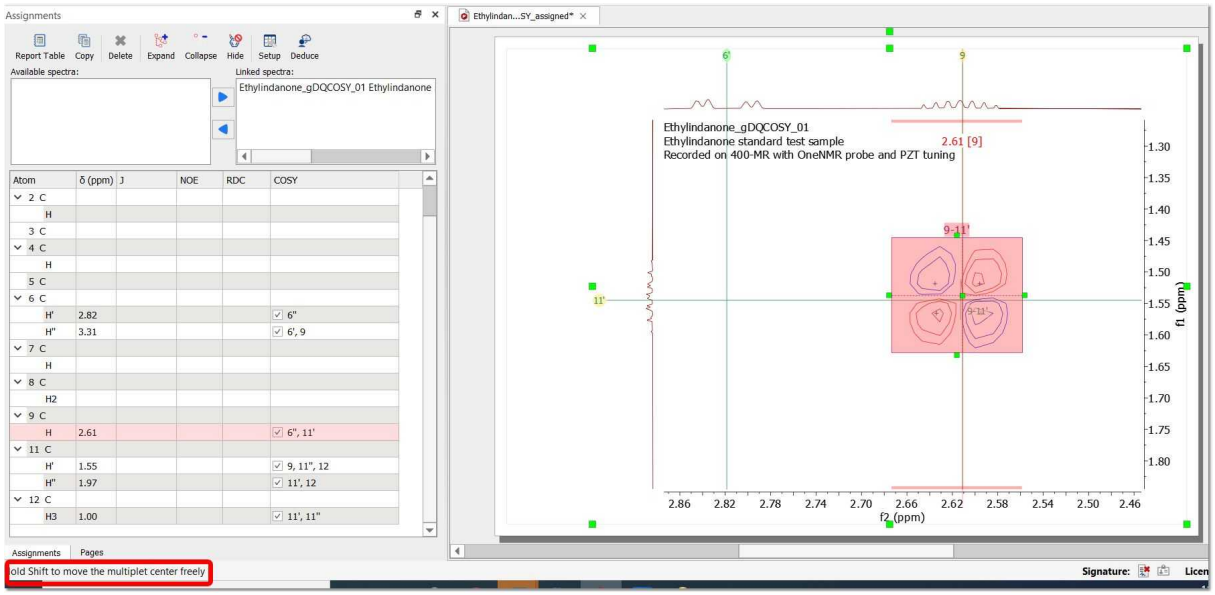
ATTENTION

By pressing and holding the Shift key while you are in the manual multiplet analysis mode for a 2D spectrum, it forces the creation of the multiplet (even if no peaks were found using the current peak picking settings); a single peak is searched from the center of the region and if none is found, a new peak will be created (at the center of the region with a fixed width of 3 points).The status bar will show a message informing about this behavior.

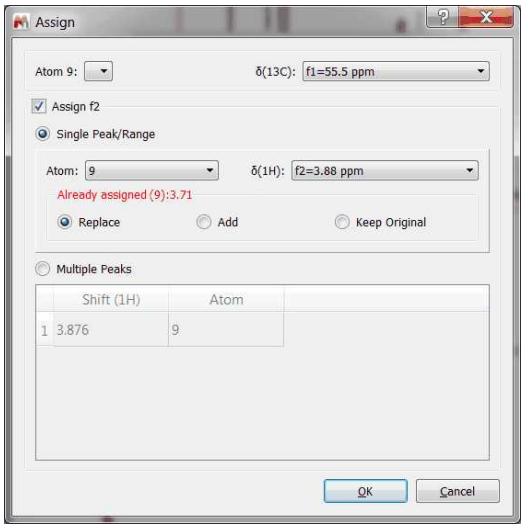
Once an assignment is made in a 2D spectrum, this assignment will be **transferred** to the applicable **multiplet** box in the 1H spectrum.

When hovering the mouse over the 2D multiplet area, a handler at the "center" of the multiplet box will be displayed to allow you to modify graphically the f2 and f1 multiplet shifts of the multiplet. When moving it towards a gridline, it snaps to the gridline; by holding down the Shift key, it will allow you to to freely move the position (a message is displayed in the status bar):

© 2024 MESTRELAB RESEARCH

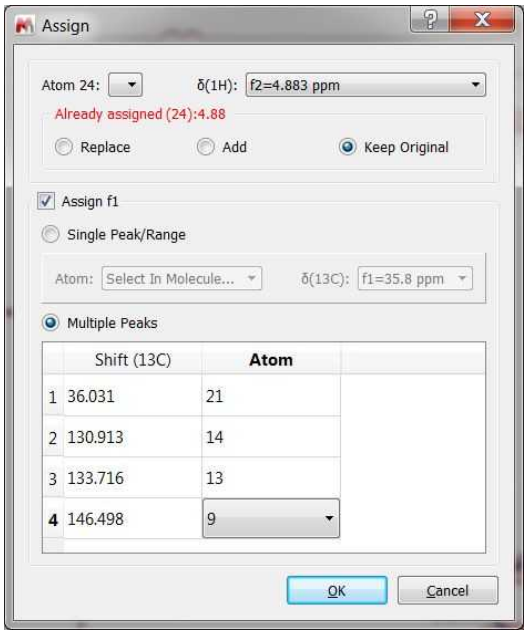


If you had already assigned the atoms in the 1D datasets and you assign in the 2D, the assignment will be replaced. You can force the visualization of the Assign Dialog by pressing the Alt key together with the second click of the mouse (when assigning); so you will be allowed to keep the assignments from the 1D, replace or add new assignments:



If you are working with a HMBC, you could assign one of the protons and then all the 13C in the same step (by selecting 'Multiple Peaks' from the panel):

© 2024 MESTRELAB RESEARCH



Alternatively, you could assign only one of the carbons and the next times that you need to assign carbons to the same proton, you will not need to select again the applicable proton (as it is already assigned). If you have overlapping in the proton dimension, you could hold down the Alt key and click to get the 'Assign' dialog displayed which will allow you to select any other proton.

When you manual assign to a 2D Multiplet:

- Assignment shift and range are set to the multiplet shift and range.
  - When the option 'Always assign together the equivalent atoms' is checked, the assignment shift is made to the 1H multiplet shift (when it exists).
- The 2D multiplet name is updated in order to contain the assigned atoms.
  - This name is not updated if only one dimension is assigned.
- A link between the assignment and multiplet is created:
  - When the assignment is deleted (one dimension), the atom name is removed from the multiplet name.
  - The multiplet name is updated when the corresponding correlation is removed, graphically from the spectrum or from Assignments Table.
  - When an assignment label is moved (horizontal and vertical dimension) and the final position corresponds to a 2D multiplet, the assignment get linked to that multiplet.
    - When the option 'Always assign together the equivalent atoms' is checked, the assignment shift will be the corresponding 1H multiplet shift (when it exists). Assignment Range will be the 2D multiplet range in all cases.
  - When 2D assignments are swapped, involved multiplets are updated. The user has the option of swap also (or not) the HSQC correlations.
    - Swapping can be done using the assignment labels (horizontal and vertical) or using the 2D multiplet boxes.
  - When the assignment shift is modified from the Assignments Table:
    - if the new shift belongs to the multiplet range: The assignment range is not modified. The user will be asked if these changes should be applied also to the multiplet:
      - YES: the multiplet shift is changed. The link is maintained.
      - NO: The multiplet is not modified. The link is broken, so correlations, constants, etc. are removed. The assignment is made to the new shift.

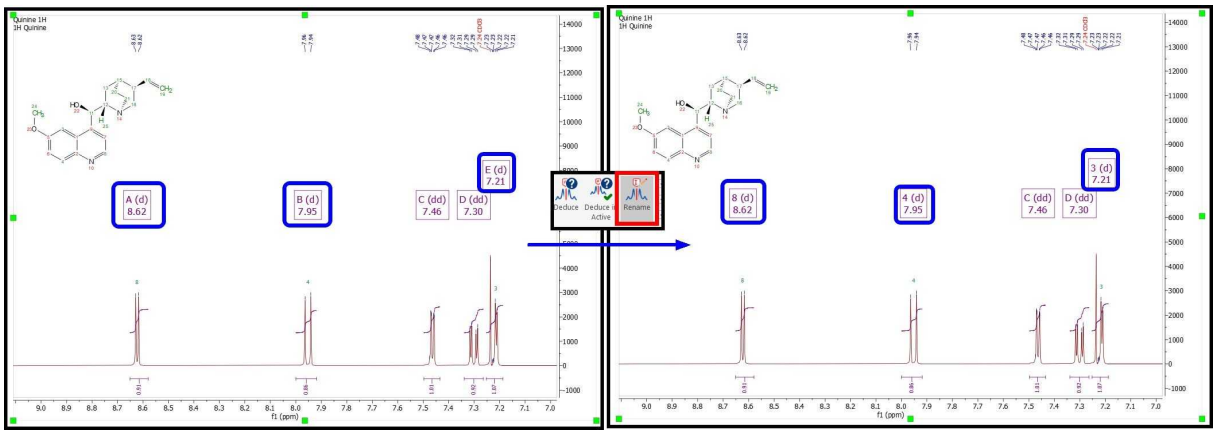
© 2024 MESTRELAB RESEARCH

- if the new shift is outside the multiplet: the link is broken, the assignment range is calculated using the default margin value (0.01). (The multiplet shift and range are not modified. The multiplet name is updated.)

o When the multiplet is removed, the assignment is deleted (both dimensions).

RENAME

When this button is pressed, the active spectrum multiplets are renamed with the assigned atoms information (a new link will be added between each multiplet and the corresponding nucleus).
That button will be enabled only when the active molecule is assigned and there are multiplets in the active NMR spectrum (and both, molecule and spectrum are linked).



It can be used for 1D and 2D:
In 1D: the multiplet will be renamed only if the assignment chemical shift is inside the multiplet range.
In 2D: the multiplet will be renamed only when each shift of the two dimensions are inside the corresponding multiplet range, and the correlation is already defined.

Existing assigned multiplets remain unaltered. Chemical shifts, ranges and qualities could change, as a new link is being added to the link list.

If "do not calculate unified chemical shift' option is OFF: the chemical shift, range and quality will recalculated taking into account the new link (the assignment will correspond to the narrowest assigned linked object). Background prediction will be executed if there is no prediction data (needed for the assignment quality calculation).

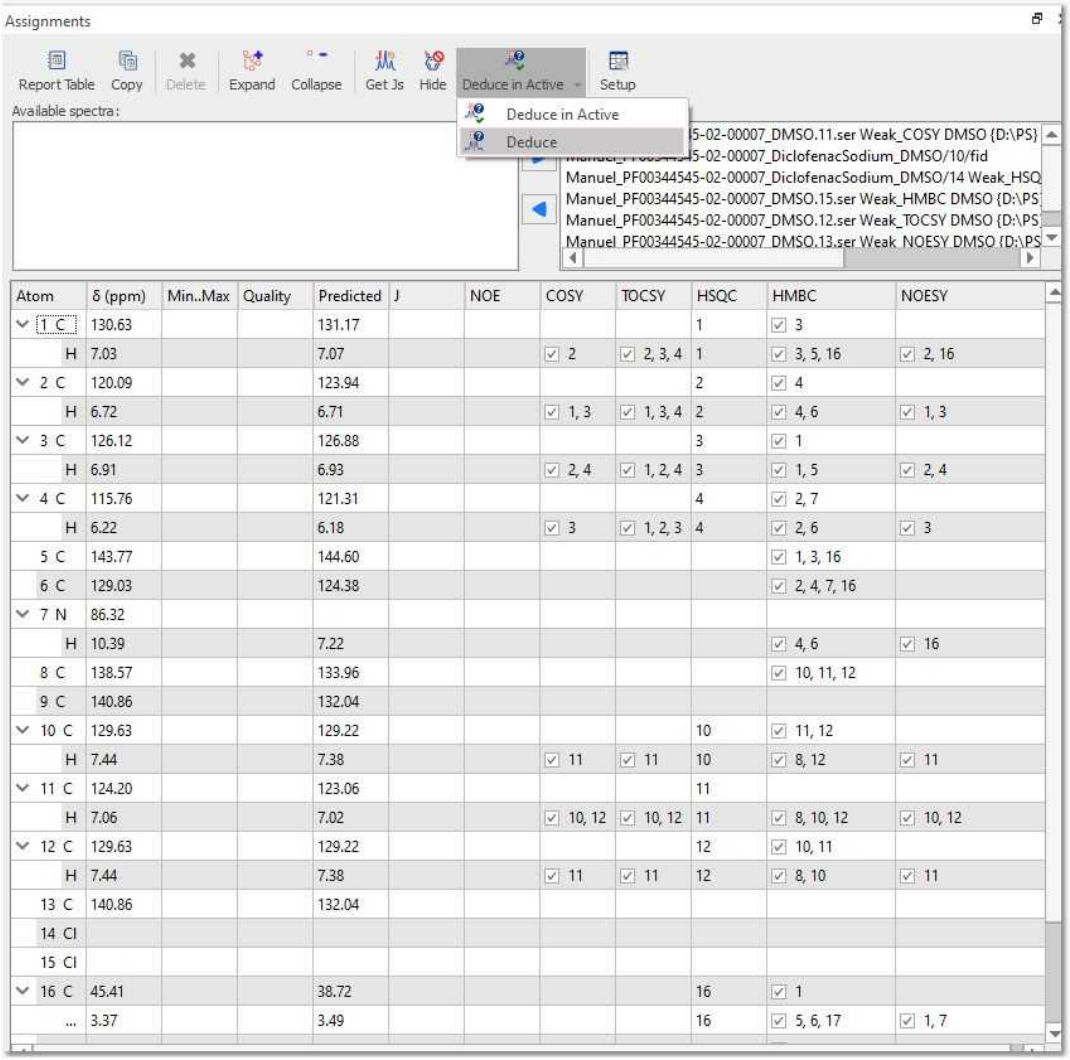
If 'do not calculate unified chemical shift' option is ON: the assignment would have to be taken from the last multiplet/peak/integral assigned.

As it's assumed that the user wants to maintain the current assignments as they are, the chemical shift, range and quality will not be modified.

DEDUCE TOOL

Mnova provides this tool to deduce 2D correlations by following the 'Assignments/Deduce' Menu.
You will need to firstly assign the 1H and the 13C NMR spectra (with multiplet analysis in the 1H) and apply a Peak Picking in your 2D plots. Finally, click on the 'Deduce' button to get the correlations in the 'Assignment table' for each 2D experiment (COSY, HSQC, HMBC, NOESY, etc..):

© 2024 MESTRELAB RESEARCH



Atom	δ (ppm)	Min..Max	Quality	Predicted	J	NOE	COSY	TOCSY	HSQC	HMBC	NOESY
1 C	130.63			131.17					1	3	
H	7.03			7.07			2	2, 3, 4	1	3, 5, 16	2, 16
2 C	120.09			123.94					2	4	
H	6.72			6.71			1, 3	1, 3, 4	2	4, 6	1, 3
3 C	126.12			126.88					3	1	
H	6.91			6.93			2, 4	1, 2, 4	3	1, 5	2, 4
4 C	115.76			121.31					4	2, 7	
H	6.22			6.18			3	1, 2, 3	4	2, 6	3
5 C	143.77			144.60						1, 3, 16	
6 C	129.03			124.38						2, 4, 7, 16	
7 N	86.32										
H	10.39			7.22						4, 6	16
8 C	138.57			133.96						10, 11, 12	
9 C	140.86			132.04							
10 C	129.63			129.22					10	11, 12	
H	7.44			7.38			11	11	10	8, 12	11
11 C	124.20			123.06					11		
H	7.06			7.02			10, 12	10, 12	11	8, 10, 12	10, 12
12 C	129.63			129.22					12	10, 11	
H	7.44			7.38			11	11	12	8, 10	11
13 C	140.86			132.04							
14 Cl											
15 Cl											
16 C	45.41			38.72					16	1	
...	3.37			3.49					16	5, 6, 17	1, 7

'Deduce in active' is only available when there are assignments in the active molecule.

Rules for 2D assignments:

HSQC C-H (HSQC, HSQC_EDITED, HMQC, HETCOR): Assign the dimension/s not assigned to the 2D position. When the Carbon/Protons/both of the selected atom are already assigned, the current chemical shift is replaced by the new value. (correlations, Js, NOEs, RDCs change according with the new shifts).

The Assign Dialog is only shown (automatically) when the user wants to assign a CH2 atom with diastereotopic protons and the carbon and the two protons are already assigned.

HMBC C-H (HMBC, H2BC): Two ways of assigning:
-Selecting only one atom in the molecule
-Selecting two atoms in the molecule, the ones of the correlation. It works selecting first the spectrum, second a first atom in the molecule, and then, a second atom. And also selecting first one atom, then the spectrum, and then the molecule again.

© 2024 MESTRELAB RESEARCH

When the user selects a 2D position in the spectrum already assigned to the f2 dimension, the program expects only one click on an atom. The f1-element of the selected atom is assigned to the f1 value. The correlation is added.

Same behavior when the selected 2D position is already assigned in the f1 dimension.

When the 2D position is not assigned in f2 nor in f1, the program expects two clicks in the molecule. The assignment (and the correlation) are made to the f2-element of the first atom and the f1-element of the second atom.

The same rules are applied when assigning NH or OH groups.

Assign Dialog is shown (automatically) in this cases:
-The 2D position is assigned in both dimensions.
-The 2D position is not assigned in any dimension and the first atom doesn't contain f2-elements.
-The 2D position is assigned only in f1 (not assigned in f2) and the selected atom doesn't contain f2-elements.
-The 2D position is assigned only in f2 (not assigned in f1) and the selected atom doesn't contain f1-elements.

COSY (COSY, DQF_COSY, NOESY, ROESY, TOCSY):
Same identical rules described in the first 4 points of the HMBC case
Assign Dialog is shown (automatically) when:
-The 2D position is assigned in both dimensions.

When assigning a NH2 to a proton, COSY, TOCSY or HMBC spectrum, both proton are assigned to the selected position.
-If you want to assign only one of the two protons of the NH2, you need to press Alt+the second click when assigning. The Assign Dialog is shown and you can choose '-'', a-b, cis-trans, etc.
- Once you have one of the NH2 protons assigned, the corresponding partner is chosen by default when selecting the same atom to assign the other proton.

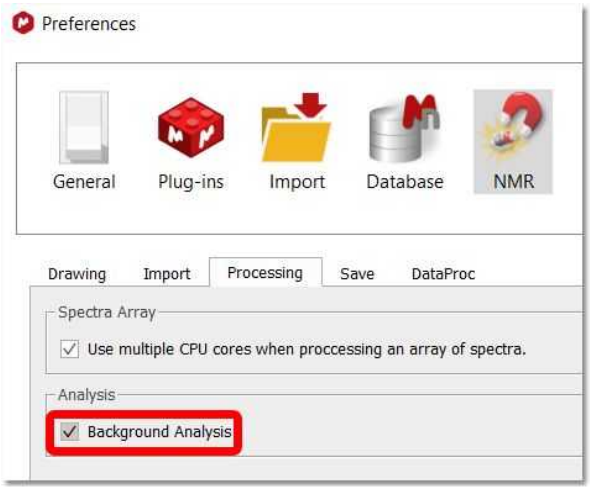
In the three cases (HSQC, HMBC, COSY) when the user selects a CH2 atom with diastereotopic protons, the assignment is produced always to the first not assigned proton.

General behavior for all 2D spectra: in the case the Assign Dialog is shown and the user chooses the option "Select in molecule", if the user selects a CH2 atom with diastereotopic protons, only the first not assigned proton is assigned.

So the assignments behavior is different depending on the assignments that you have in F1 and F2:
- If you do not have any assignment; the first assignment that you will make will go to F2 and the next for F1.
- If the peak is already assigned in one of the dimensions, when you make the assignment, Mnova waits only for the selection of one atom (in the dimension which is not assigned) in order to complete the information. If you do not want to do it, you would need to do ‘ALT+click’ to launch the ‘Assign dialog’.
- If the peak is already assigned in both dimensions and you try to assign it to another atom; the ‘Assign dialog’ will appear (as Mnova does not know what you need to do).

There is a 'Background Analysis' check box under the 'File/Preferences/NMR/processing' menu which could be useful for your assignments.

© 2024 MESTRELAB RESEARCH



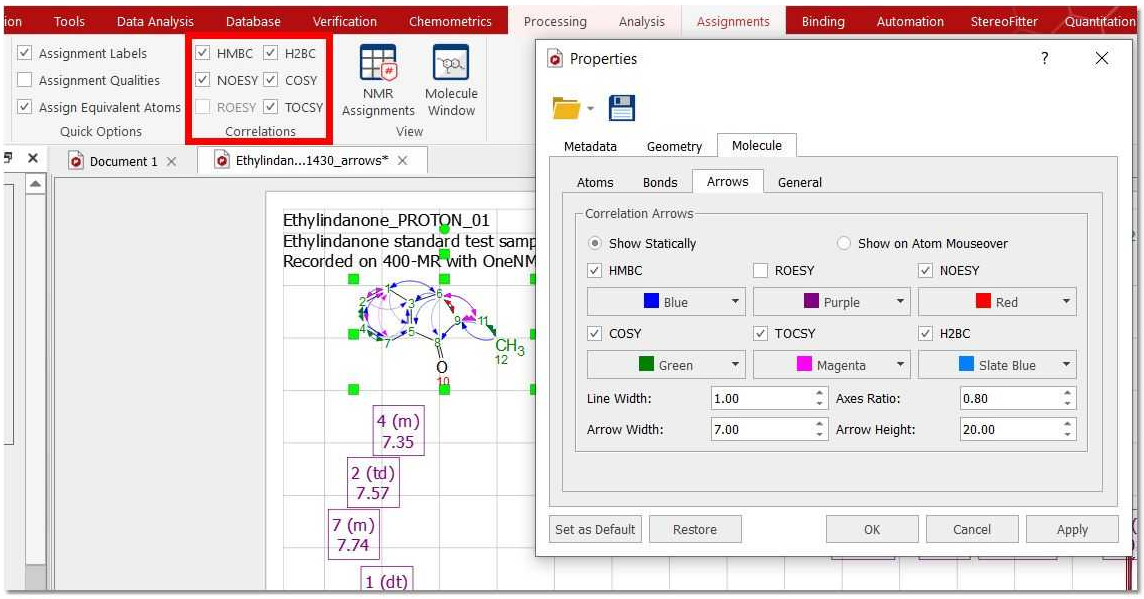
If it is checked; during the Manual Assignment mode, the Autodeduction routines create (and assign) on the fly the background multiplets when the 'real' multiplets are not defined by the user:

- When assigning to any 2D spectra (to multiplet/peak/range), the corresponding 1H multiplets are automatically created and assigned using the ranges of the background multiplets.
- 2D multiplets of any spectra are automatically generated and assigned when assigning to a 1H multiplet.
- If a Carbon was autoassigned in the previous point, this carbon shift is used to generate and assign the 2D corresponding multiplets in the other spectra.
- These routines analyze first the multiplets defined by the user, then, the background multiplets, and then, if these last are not found, the peaks defined by the user.

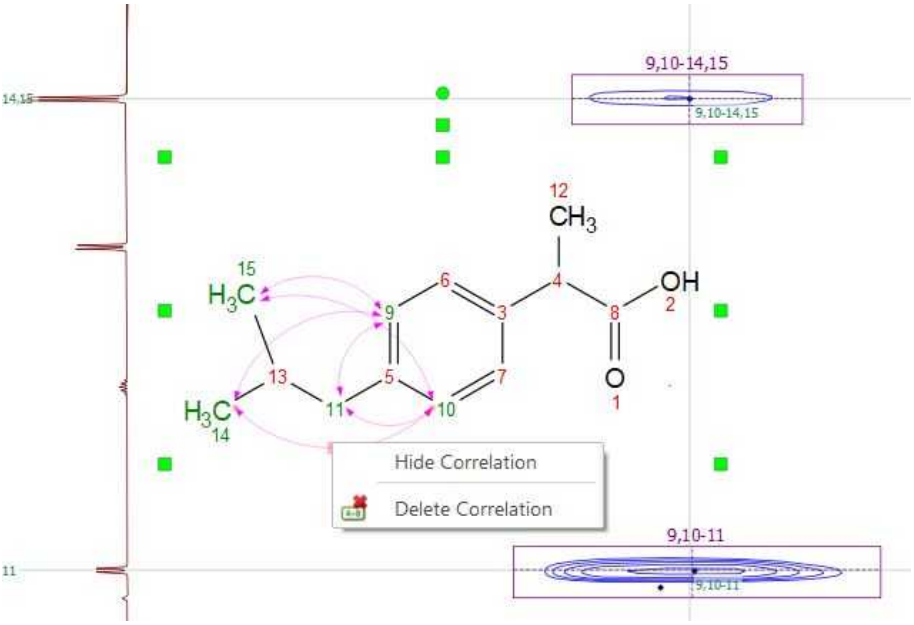
- Background multiplets are also analyzed in all 2D spectra when pressing the action Deduce Assignments.
  In this case, as the background analysis just starts when pressing the button, the program waits always until this analysis has finished in all spectra. (Or until the waiting time limit of 5 seconds has been reached).
- The background multiplets are also analyzed in all 2D spectra when the user moves an 1H assignment label to a 1H multiplet. The program waits until the analysis has finished (in all spectra) or the 5 seconds has been reached.

You can graphically visualize (by arrows between the coupling groups) the HMBC, ROESY, NOESY, COSY, TOCSY, H2BC and connectivity in the structure by checking the applicable boxes from the 'Assignments ribbon' or by double clicking on the molecular structure to display the 'Properties' and selecting the 'Arrows' tab to check the desired correlation arrows.

© 2024 MESTRELAB RESEARCH



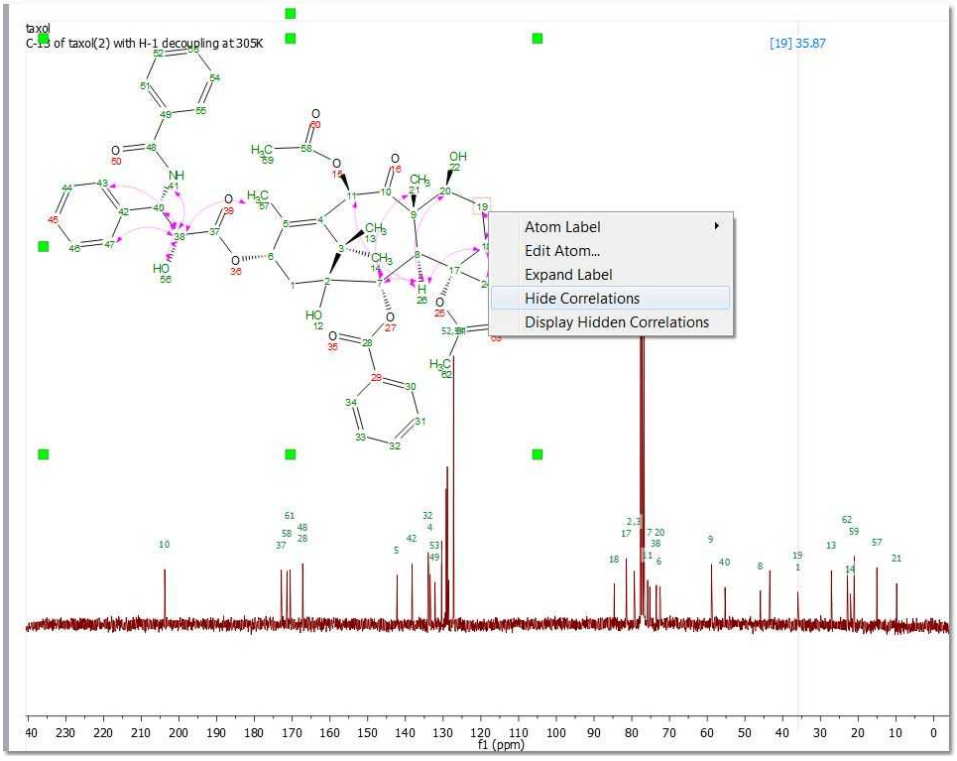
Hovering the mouse over the arrow and right clicking, will allow you to hide or delete the assignment:



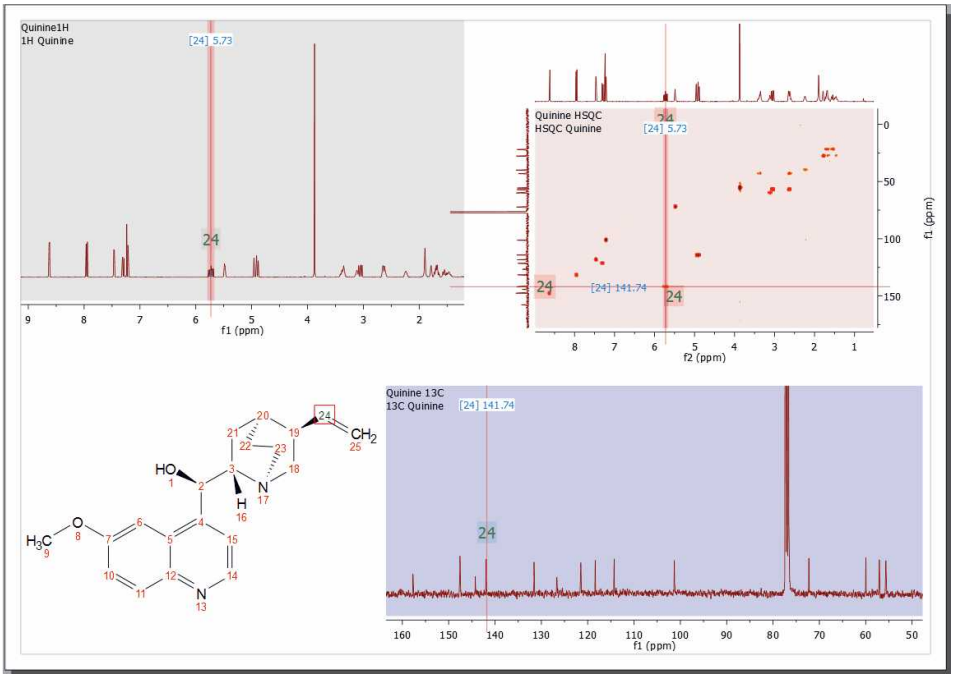
The Assignments table shows checkboxes for each row in the correlations that can be displayed as arrows in the molecule. Checking/unchecking these checkboxes will change the visibility of the correlations for each atom.

Right clicking on the atom will allow you also to 'Hide Correlations'; you can show them again by using the command 'Display Hidden Correlations'.

© 2024 MESTRELAB RESEARCH

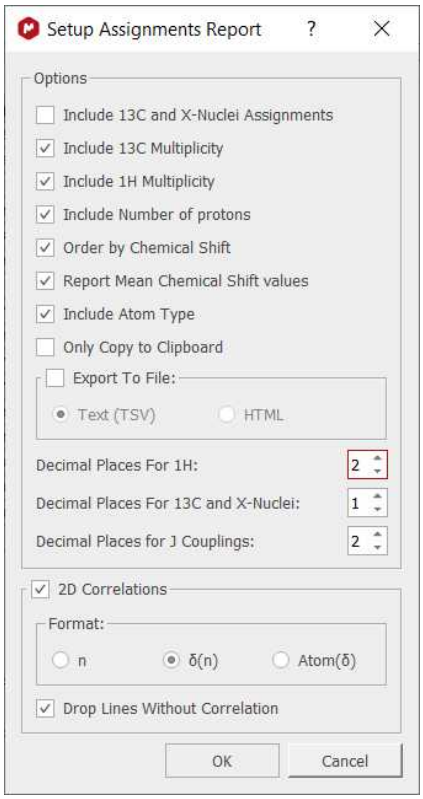


You can also copy several datasets in the same page (and after having resized them) to make the assignments. Here you can see a page which contains a 1H, 13C and HSQC datasets with an assignment in the molecular structure:



Click on the 'Report Assignments' button of follow the menu 'Tools/Report/Assignments' to **export your assignments** in journal format. Then, a new dialog will appear, to allow you to report the 2D correlations and the chemical shifts or to copy only the report to the clipboard (to paste it to another document) or

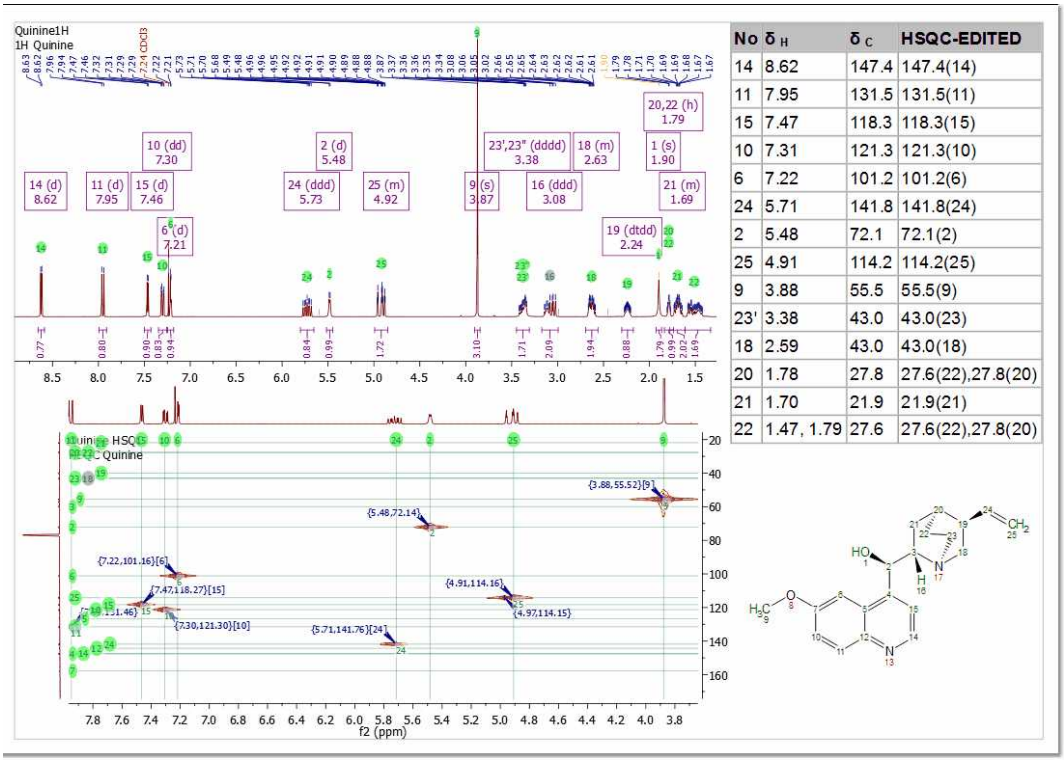
export it as CSV or HTML file. From here you can also order by chemical shift, include 13C and X-nuclei assignments or multiplicity and report ranges or mean chemical shift values. When no chemical shift is included, the correlations get ordered according to the atom number. When chemical shifts are included, the correlations get ordered according to the chemical shifts values.



It is required at least a 1H spectrum. The table only will include columns for the spectra present in the document containing assignments. It generates a table in which each row corresponds to each non-equivalent proton.

Finally, you will get the report of the assignments pasted on the spectral window:

© 2024 MESTRELAB RESEARCH



No	δ_H	δ_C	HSQC-EDITED
14	8.62	147.4	147.4(14)
11	7.95	131.5	131.5(11)
15	7.47	118.3	118.3(15)
10	7.31	121.3	121.3(10)
6	7.22	101.2	101.2(6)
24	5.71	141.8	141.8(24)
2	5.48	72.1	72.1(2)
25	4.91	114.2	114.2(25)
9	3.88	55.5	55.5(9)
23'	3.38	43.0	43.0(23)
18	2.59	43.0	43.0(18)
20	1.78	27.8	27.6(22),27.8(20)
21	1.70	21.9	21.9(21)
22	1.47, 1.79	27.6	27.6(22),27.8(20)

You can export the assignments as an ASCII file by following the menu 'File/Save As/Script:Assignments' or copy the assignments from one molecule to another (with the same numbering of the common parts).

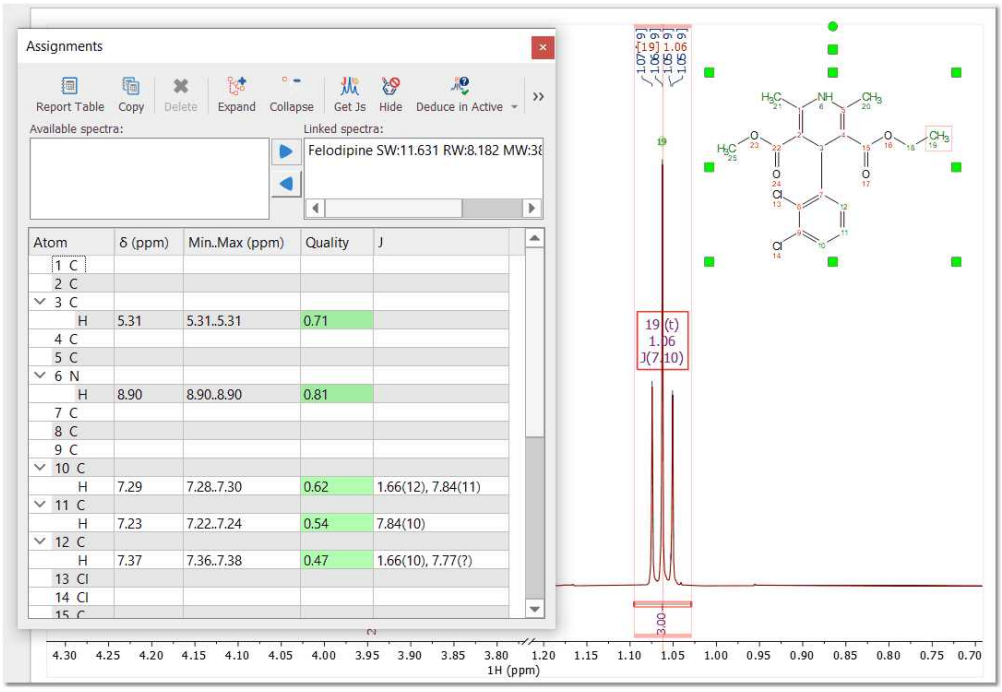
You can also export your assignments as NMReData format by following the menu 'File/Save As/Script: NMReData (*.zip)'. The zip file will contain the raw dataset, the processing templates and a SDF file with the molecular structure and the assignments. Loading that zip file into Mnova will display your processed NMR datasets with the assigned molecular structure.

8.5.1 Assignments Table

Once you have finished with the assignment, you can visualize the assignment list by following the menu 'View/Tables/Assignments'. From here, you can edit any entry by double clicking on it and overwriting the new value. Hovering the mouse over any cell of the table, will highlight the signals on the spectrum and the atom on the molecular structure.

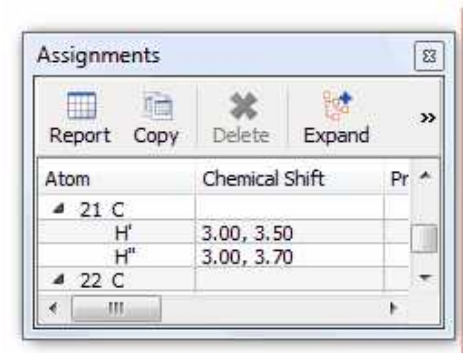
Bear in mind that you can carry out the peak assignment directly from this table by typing the applicable chemical shift in the edit box.

© 2024 MESTRELAB RESEARCH

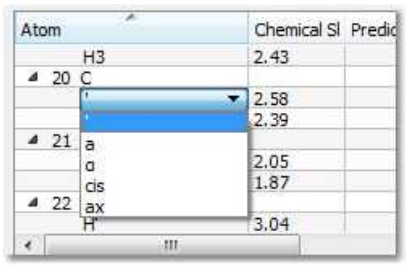


If you use the Assignment table to type directly the assignment of a CH_2 you would need add the prime marks if you want to assign only any of the two protons. If you do not type the prime marks, the assignment will be applied to both protons.

For example; typing this in a CH2 row: ***3.0, 3.5',3.7"*** means that 3.0 ppm is the chemical shift of both protons and in addition the H' has a signal at 3.5 ppm, whilst H" appears also at 3.7 ppm. The assignments table will automatically refresh showing something like this (after having typed: ***3.0, 3.5',3.7"***):

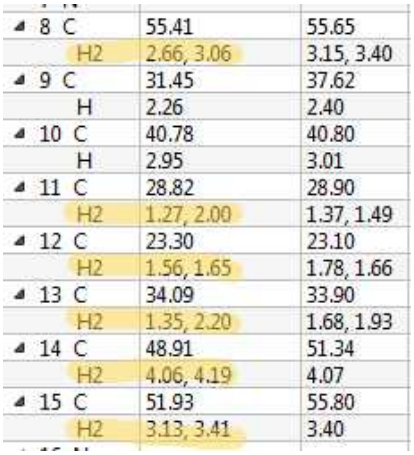


Double click on the applicable 'atom cell' to change the label of a -CH2 group. You can also change the atom number by double clicking on the applicable atom cell:



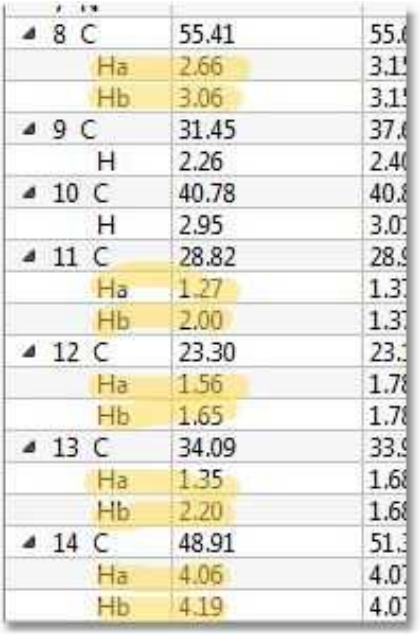
© 2024 MESTRELAB RESEARCH

You can do **ambiguous assignments** in a -CH2, just by typing both values in the chemical shift cell (both chemical shifts are assigned to both protons)



8	C	55.41	55.65
	H2	2.66, 3.06	3.15, 3.40
9	C	31.45	37.62
	H	2.26	2.40
10	C	40.78	40.80
	H	2.95	3.01
11	C	28.82	28.90
	H2	1.27, 2.00	1.37, 1.49
12	C	23.30	23.10
	H2	1.56, 1.65	1.78, 1.66
13	C	34.09	33.90
	H2	1.35, 2.20	1.68, 1.93
14	C	48.91	51.34
	H2	4.06, 4.19	4.07
15	C	51.93	55.80
	H2	3.13, 3.41	3.40

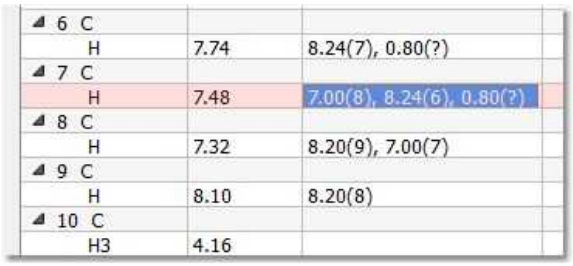
If you want to do **unambiguous assignments** (one chemical shift belongs to proton a and the other to proton b), you will need to assign each proton:



8	C	55.41	55.6
	Ha	2.66	3.1
	Hb	3.06	3.1
9	C	31.45	37.6
	H	2.26	2.40
10	C	40.78	40.8
	H	2.95	3.01
11	C	28.82	28.9
	Ha	1.27	1.37
	Hb	2.00	1.37
12	C	23.30	23.1
	Ha	1.56	1.78
	Hb	1.65	1.78
13	C	34.09	33.9
	Ha	1.35	1.68
	Hb	2.20	1.68
14	C	48.91	51.3
	Ha	4.06	4.07
	Hb	4.19	4.07

Type the value of the coupling followed by the atom number involved, to include coupling constants in the assignment table. If you want to add an *ambiguous assignment*, type the coupling constant value and press the 'enter key' (a question mark will be added between brackets):

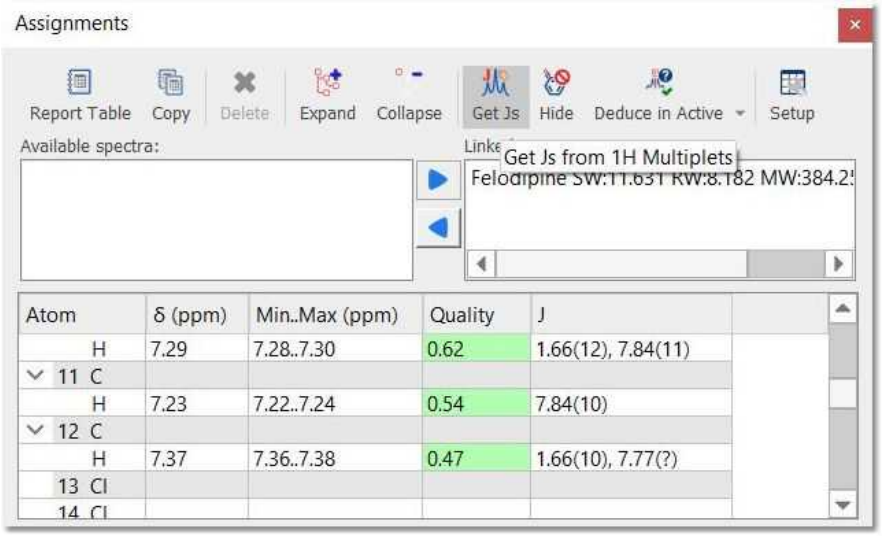
© 2024 MESTRELAB RESEARCH



6	C		
	H	7.74	8.24(7), 0.80(?)
7	C		
	H	7.48	7.00(8), 8.24(6), 0.80(?)
8	C		
	H	7.32	8.20(9), 7.00(7)
9	C		
	H	8.10	8.20(8)
10	C		
	H3	4.16	

The couplings will be added by pairs, in the same way as in the correlations columns, i.e., when the J(2-3) is created, the coupling J(3-2) is also automatically added, with the same value.

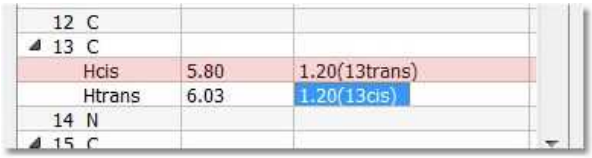
Click on the "Get Js" button to read the *J* couplings of the multiplets and to try to create pairs of similar *J* values. If this difference is small (less than the spectrum resolution), the pair between the corresponding assigned atoms will be added to the *J* column of the Assignments Table. The *J* value will be the average of the two values. Only atom pairs located at 5-bond distance or least will be added. If not, Js will be added as ambiguous values.



This button will only be enabled when the active molecule is assigned, there are assigned multiplets in the 1H spectrum, and both, molecule and spectrum are linked.

The table will give priority to the homonuclear couplings. Thus, when the user writes in a H row the atom number (without specify a proton), the coupling will be done between the protons. To add a coupling H-C, the user will have to write the *atomsymbol*, for example, "C". The same for the cases in which the user writes in a C row, the coupling will be done between the carbons, by default.

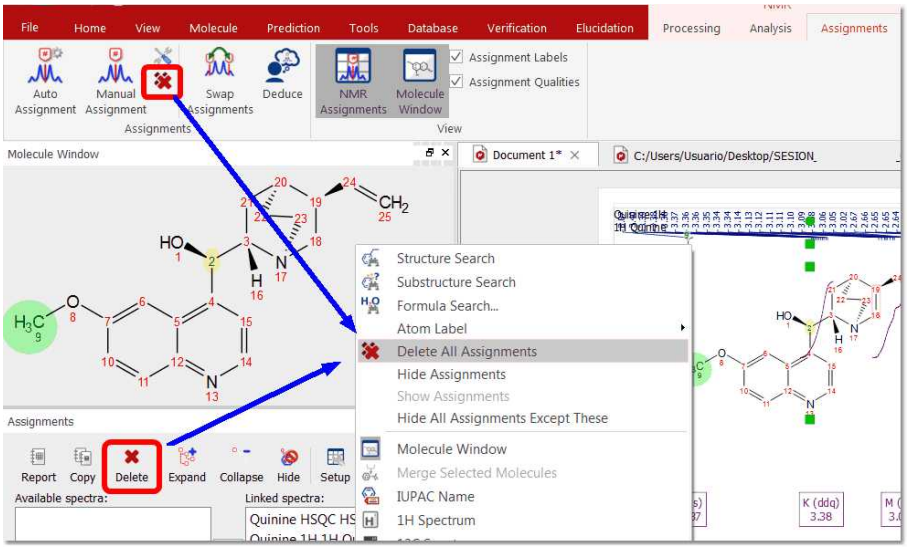
In the case of CH2 with no equivalent protons: the user should type the correct proton notation in the coupling. If we want to add a coupling with the first proton of $13CH_2$, and the notation is cis/trans, the text should be "1.20(13cis)". If we type "1.20(13')", we will get a message saying "the atom 13' doesn't exist in the molecule".



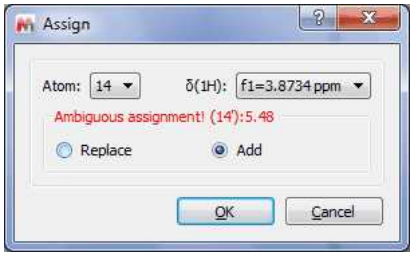
12	C		
13	C		
	Hcis	5.80	1.20(13trans)
	Htrans	6.03	1.20(13cis)
14	N		
15	C		

© 2024 MESTRELAB RESEARCH

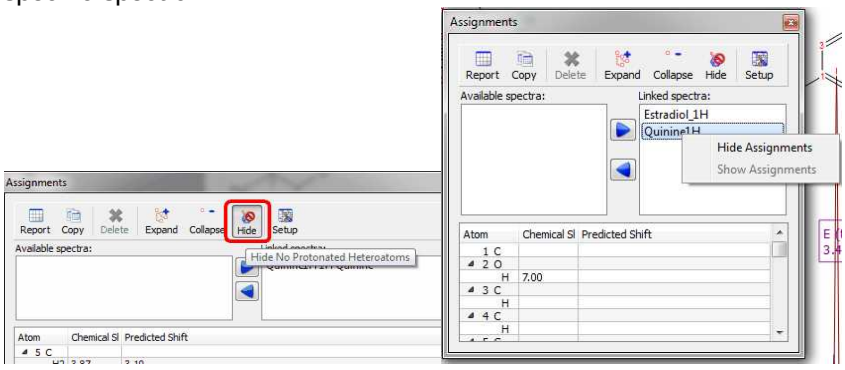
Click on the 'Delete' button to delete any assignment selected on the table. To delete 'All the assignments', do 'Ctrl/Cmd+A' to select all the assignments and click on the Delete button. The same effect will happen if you follow the menu 'Assignments/Delete All Assignments' or if you right click on the molecule to display the context menu:



You can add (or replace) a second chemical shift assignment to a single nucleus. This feature will be very useful if you need to assign rotamers or for ambiguous assignments.

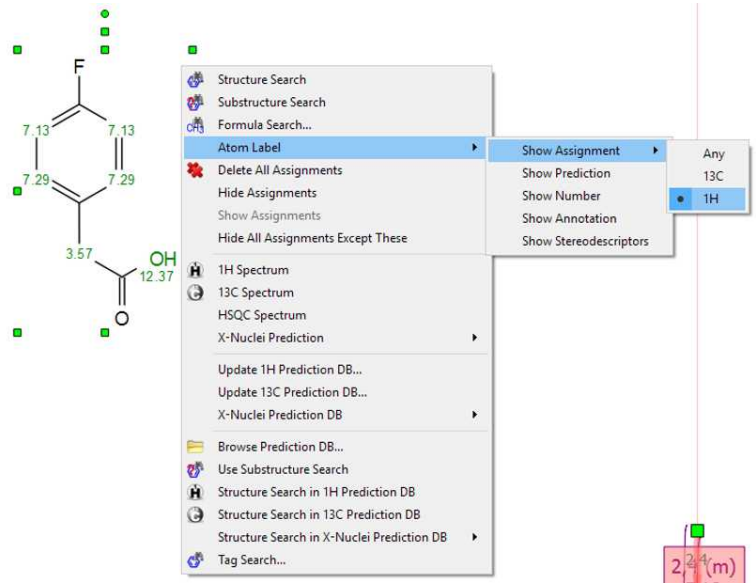


From the assignments table, you can hide "no protonated heteroatoms" or the the assignments of any specific spectrum:

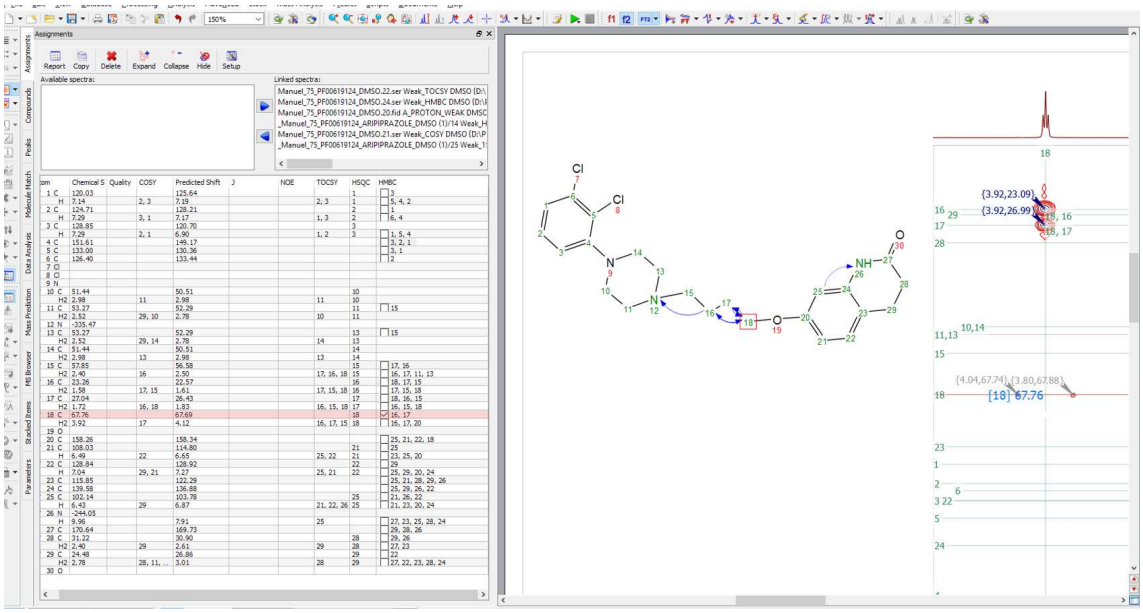


It is possible to display the 1H and 13C assignments in the molecule by right clicking on it and selecting 'Atom Label/Show Assignment'

© 2024 MESTRELAB RESEARCH

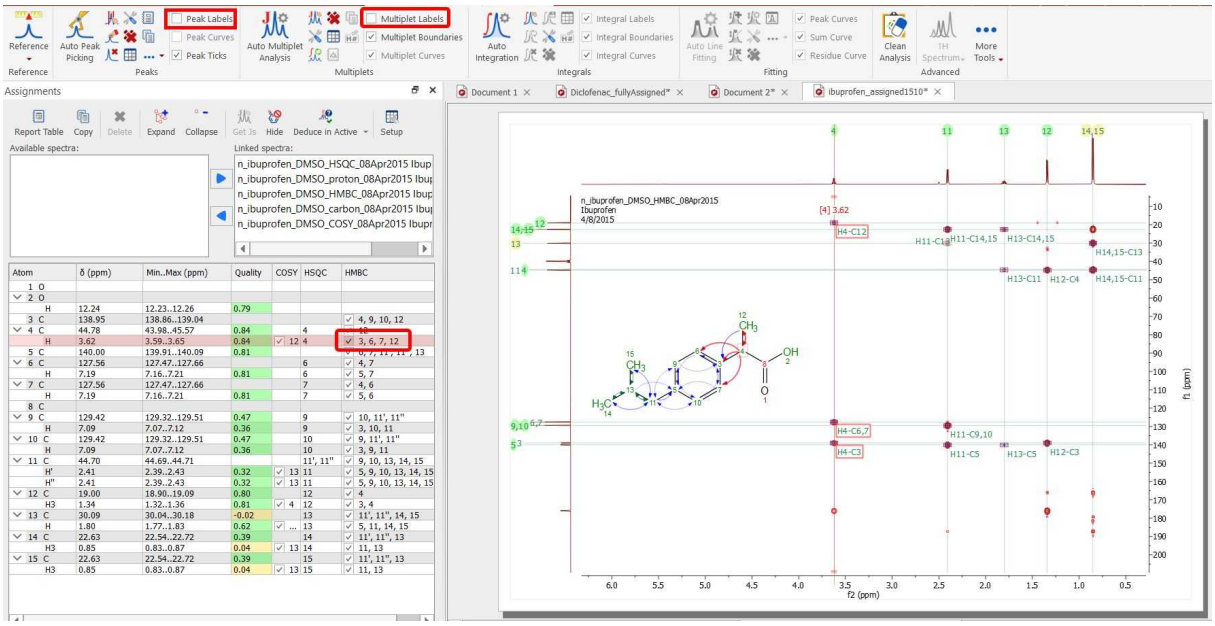


The Assignments table shows checkboxes for each row in the correlations that can be displayed as arrows in the molecule. Checking/unchecking these checkboxes will change the visibility of the correlations for each atom:

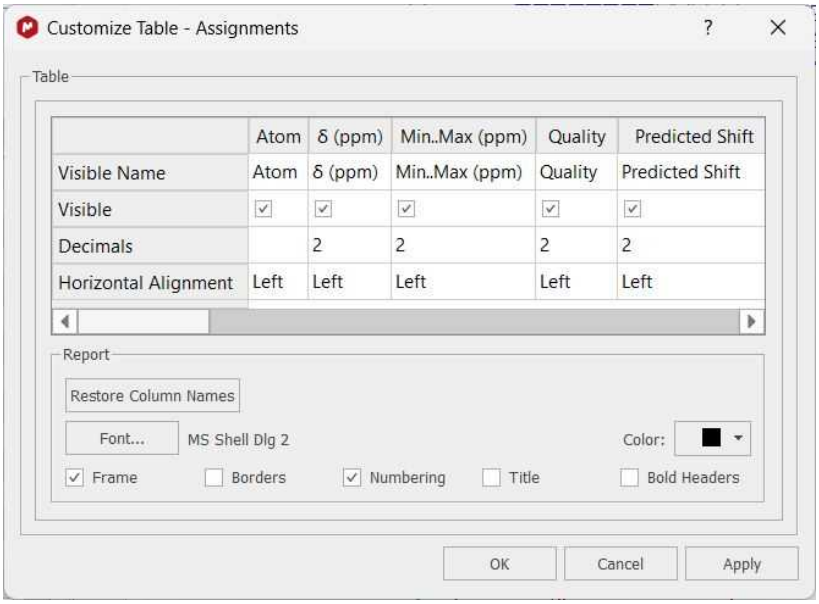


Hovering the mouse over the assignments table row, will highlight the assignments labels in the spectrum (if the multiplets and peaks labels are hidden), the atom in the molecular structure and the applicable correlation arrows:

© 2024 MESTRELAB RESEARCH



You can customize the assignments table by clicking on the Setup button:



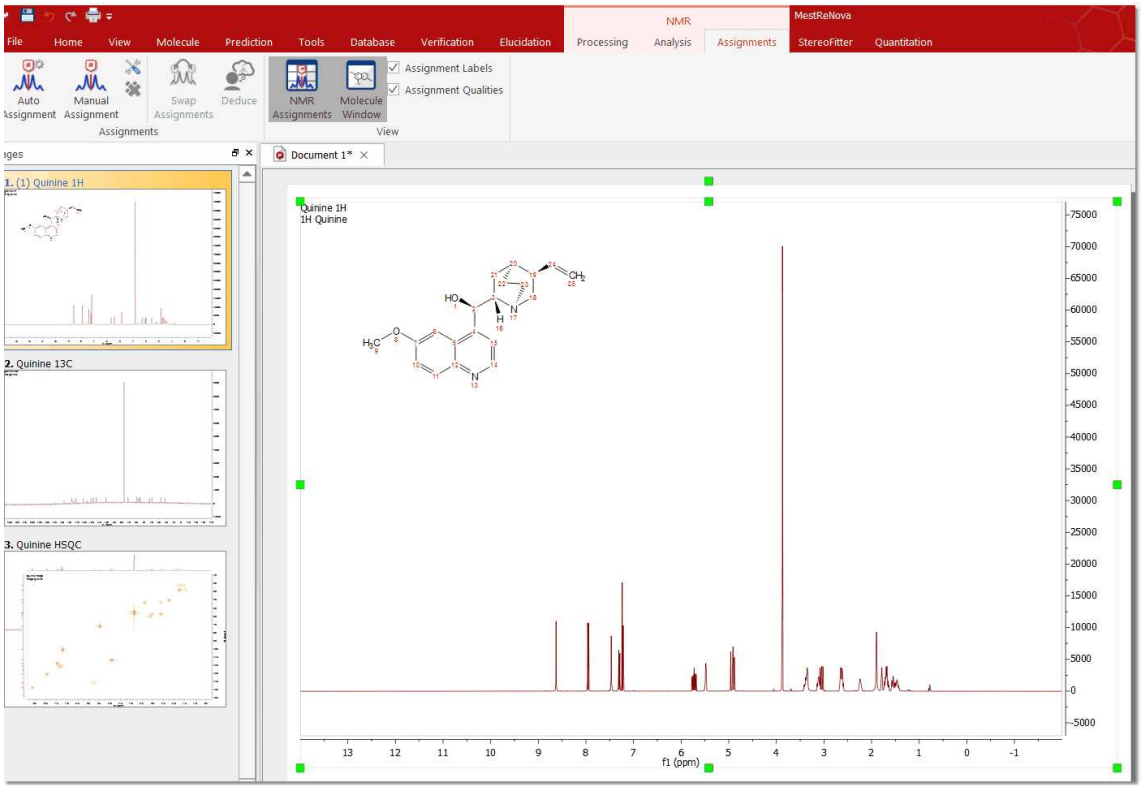
8.5.2 Transfering assignments through datasets

The **automatic assignment deduction** from 2D spectra are applied only when the user makes a manual assignment to a multiplet box in the 1H spectrum and 2D peaks have been picked in 2D spectra. The automatic deductions are made only when there are no other peaks very close, so that correlations are clear. In those cases where there is more than one peak peak label per signal, you would need to delete the extra peak labels or use the 'Merge Peaks' feature (under the 'Peak Picking' scroll down menu) and re-do the assignment to the multiplet box in the 1H-NMR spectrum (to get the deduction).

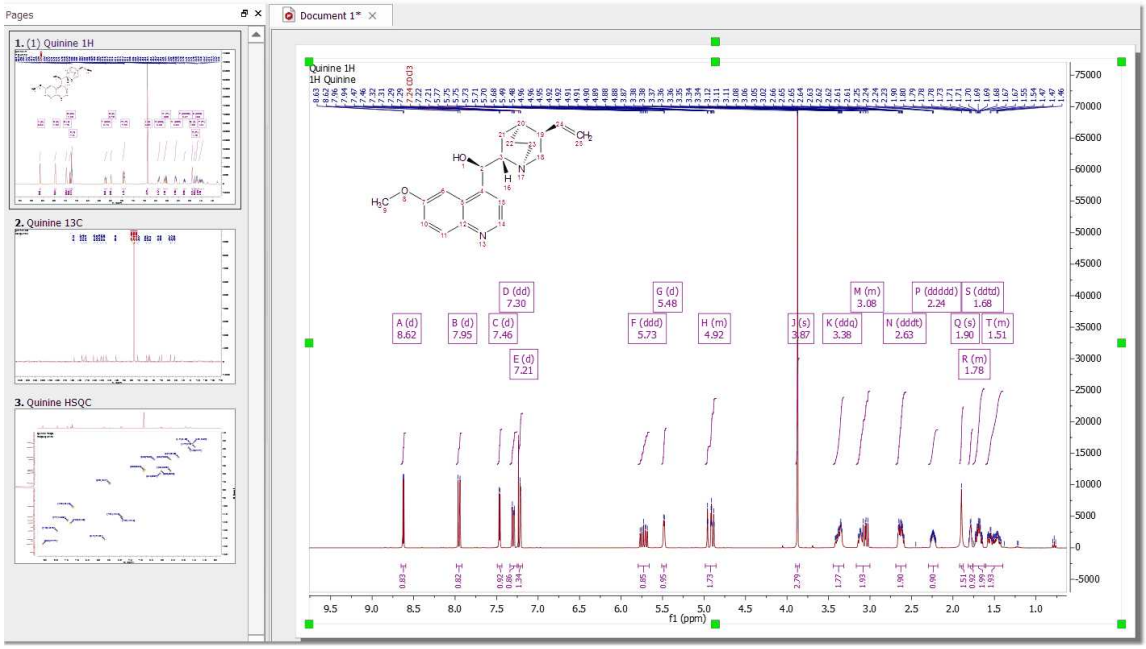
Let´s see how it works:

© 2024 MESTRELAB RESEARCH

1st: Open for example a 1H, 13C and HSQC datasets and the applicable molecular structure in the same document.

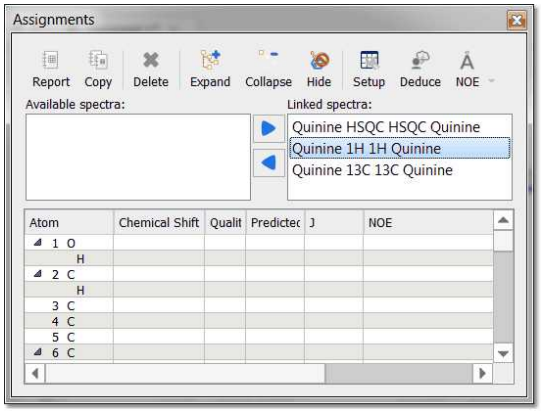


2nd: Apply a Peak Picking in all the datasets and a Multiplet Analysis in the 1H-NMR spectrum:



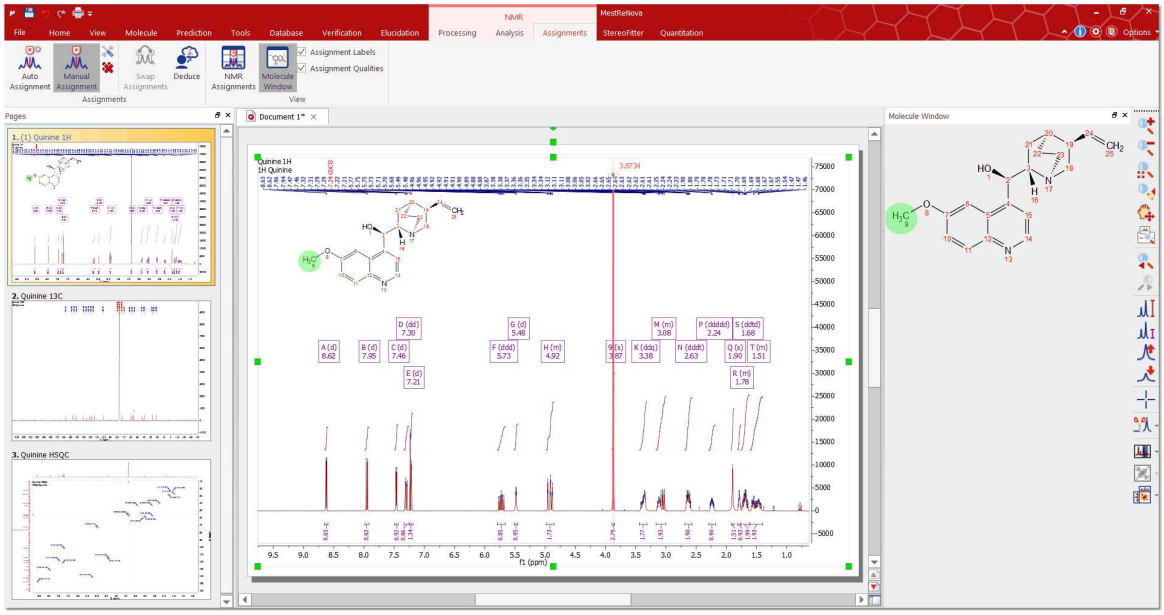
© 2024 MESTRELAB RESEARCH

3rd: Make sure that you link all the spectra of the document from the Assignments table:

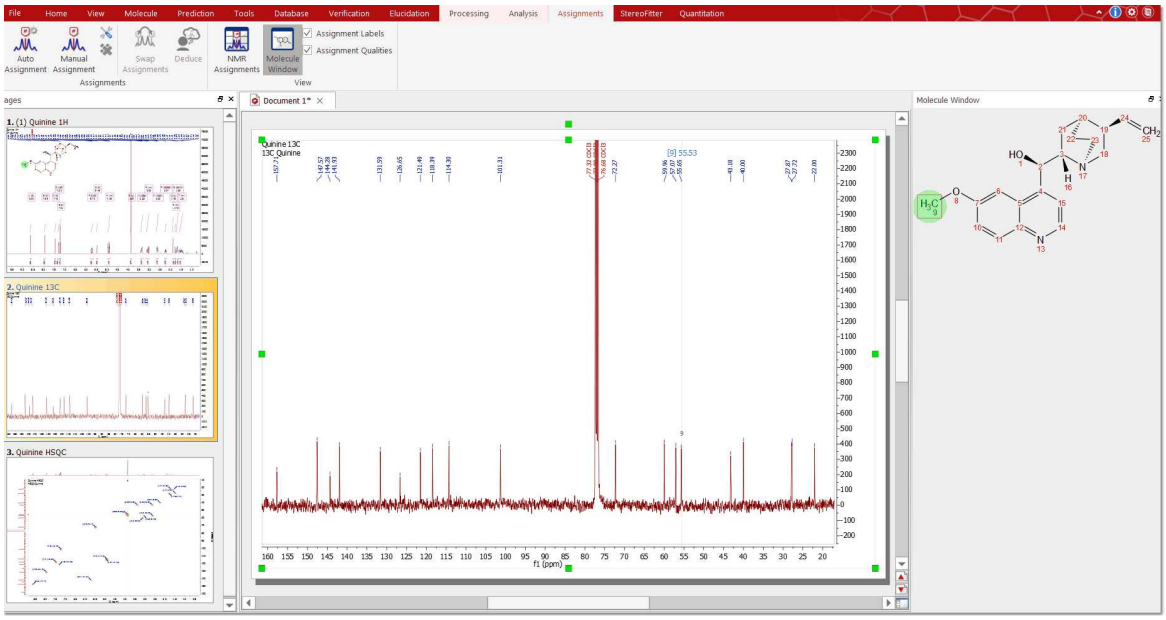


When adding or deleting spectra to the assignments linked spectra; the columns of the assignments table will be updated.

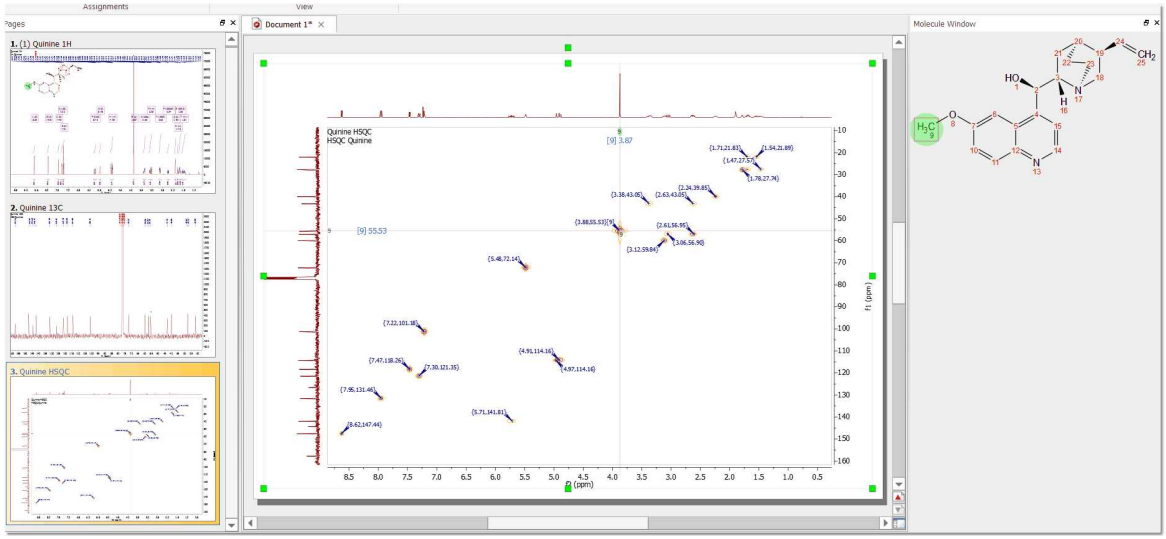
4th: Make an assignment to a multiplet box in the 1H-NMR spectrum. In that case we have assigned the atom 9 to the multiplet box at 3.87 ppm:



5th: And that´s all! Jump to the 13C and automatically you will find the applicable assignment:



....and the same with the HSQC as you can see below:



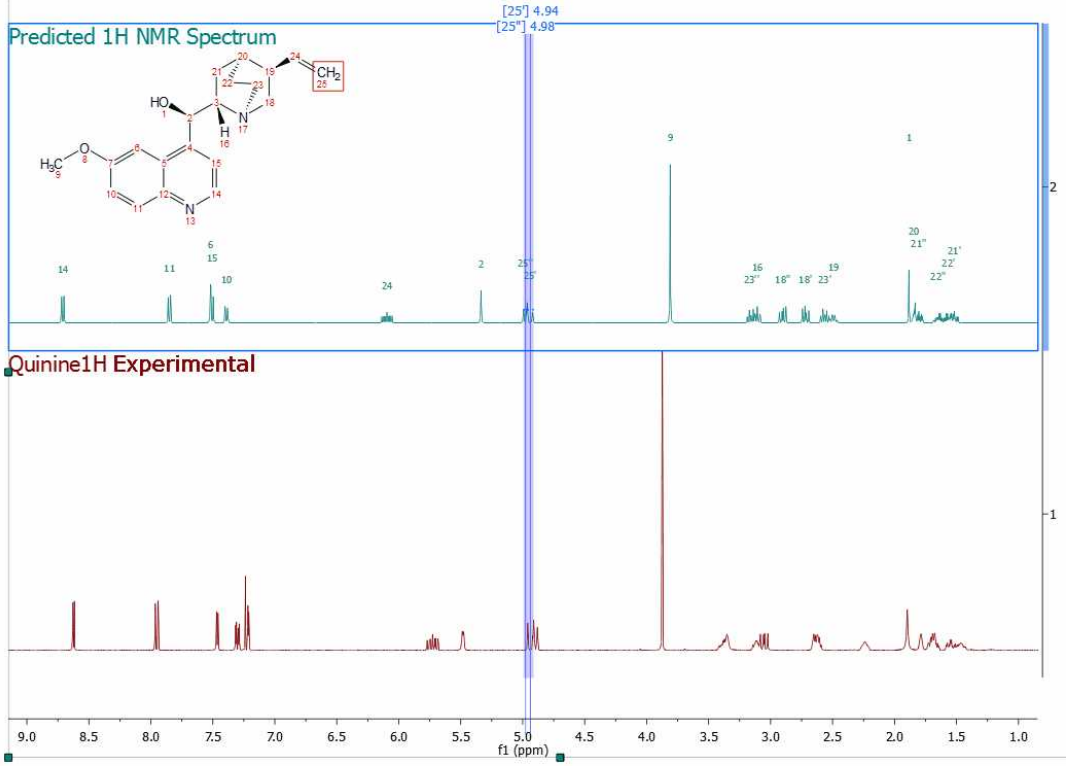
8.5.3 Assisted assignment by using the predictions

You can use Predict&Compare (or Predict&Verify) to make your assignments.

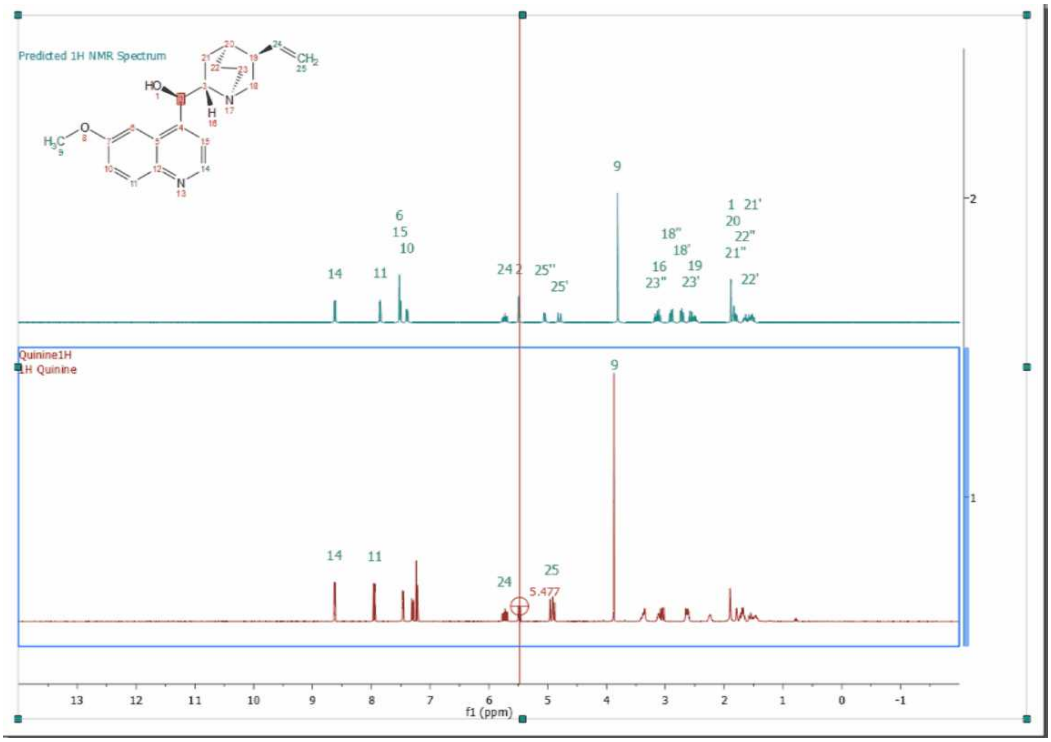
Just open a 1D-NMR dataset with a molecular structure and follow the menu 'Prediction/Predict&Compare'. After the prediction is run, Mnova synthesizes the corresponding 1H-NMR spectrum using a rigorous full quantum mechanics method taking into account any second order effect present in the predicted spin(s) systems(s).

For example, in the figure below, you can see the results of running a prediction and comparing the resulting 1H spectrum with its experimental counterpart and the applicable assignments for the predicted spectrum:

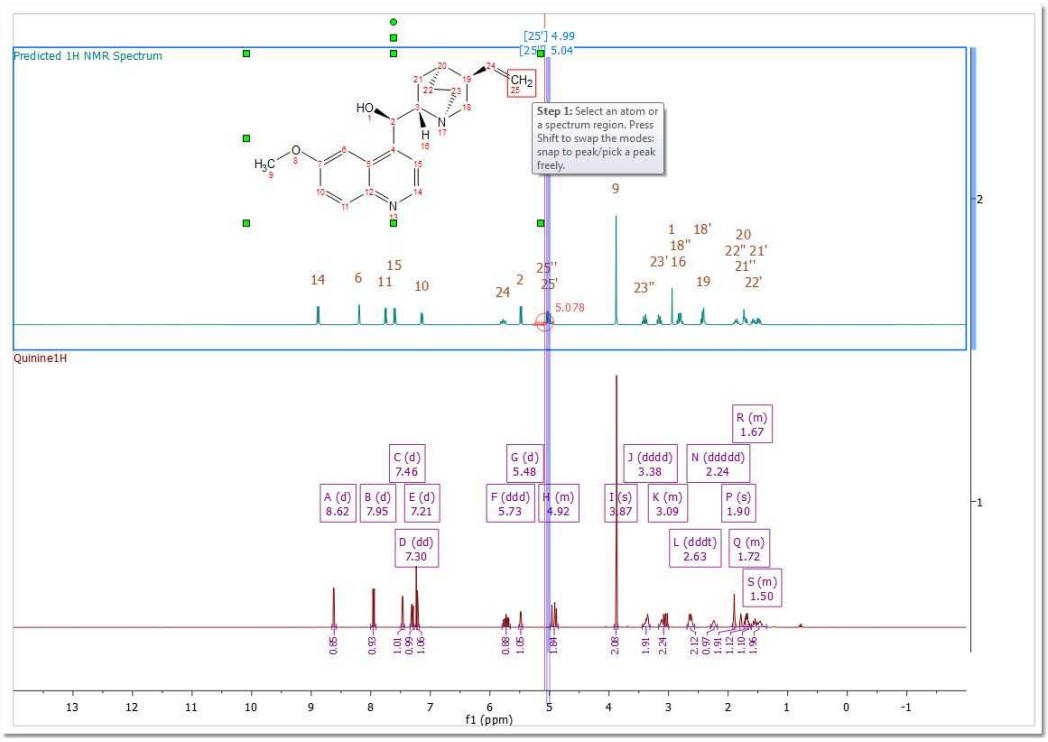
© 2024 MESTRELAB RESEARCH



Next, you will only need to press the 'A' key to activate the assignment mode and select the atom and the applicable region of the experimental spectrum to be assigned (following the assignments of the predicted spectrum):



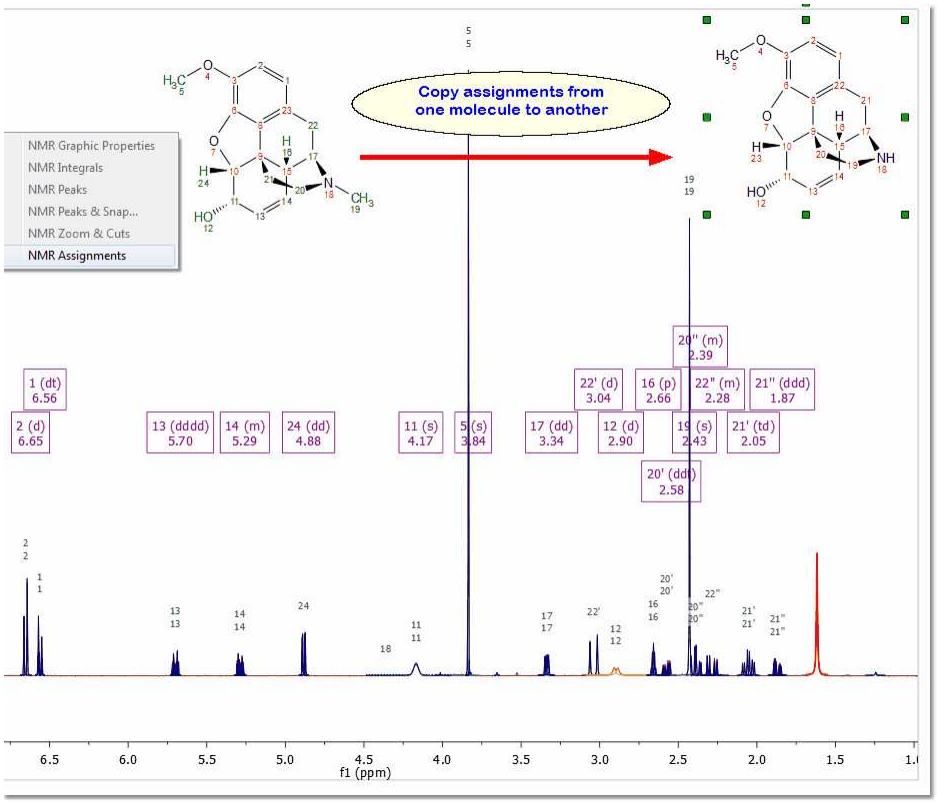
If you load a 1H-NMR spectrum with a multiplet analysis and run Predict and Compare; you will get a stack plot with the multiplet boxes displayed in the experimental spectrum, so you can easily assign those multiplets to the molecular structure and finally update the 'Prediction DB' if you wish:



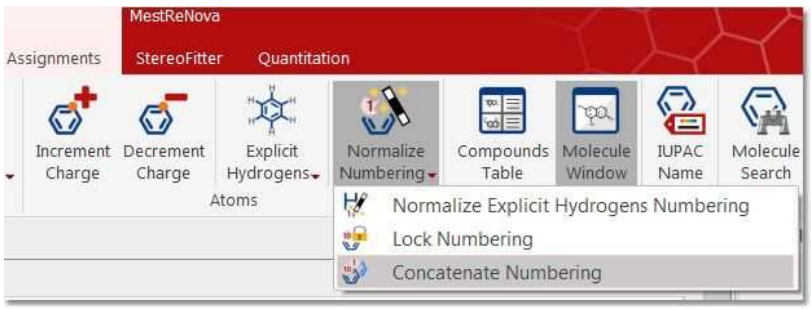
8.5.4 Copy assignments from one molecule to another

You can **copy assignments** from one molecule to another (the numbering of the common atoms must be the same) by selecting the first molecular structure with the assignments and doing 'Ctrl/Cmd+C'. Finally, select the second molecular structure and follow the menu 'Home/Paste/NMR assignments'. Please bear in mind that the numbering of the common parts of both molecules must be the same.

© 2024 MESTRELAB RESEARCH

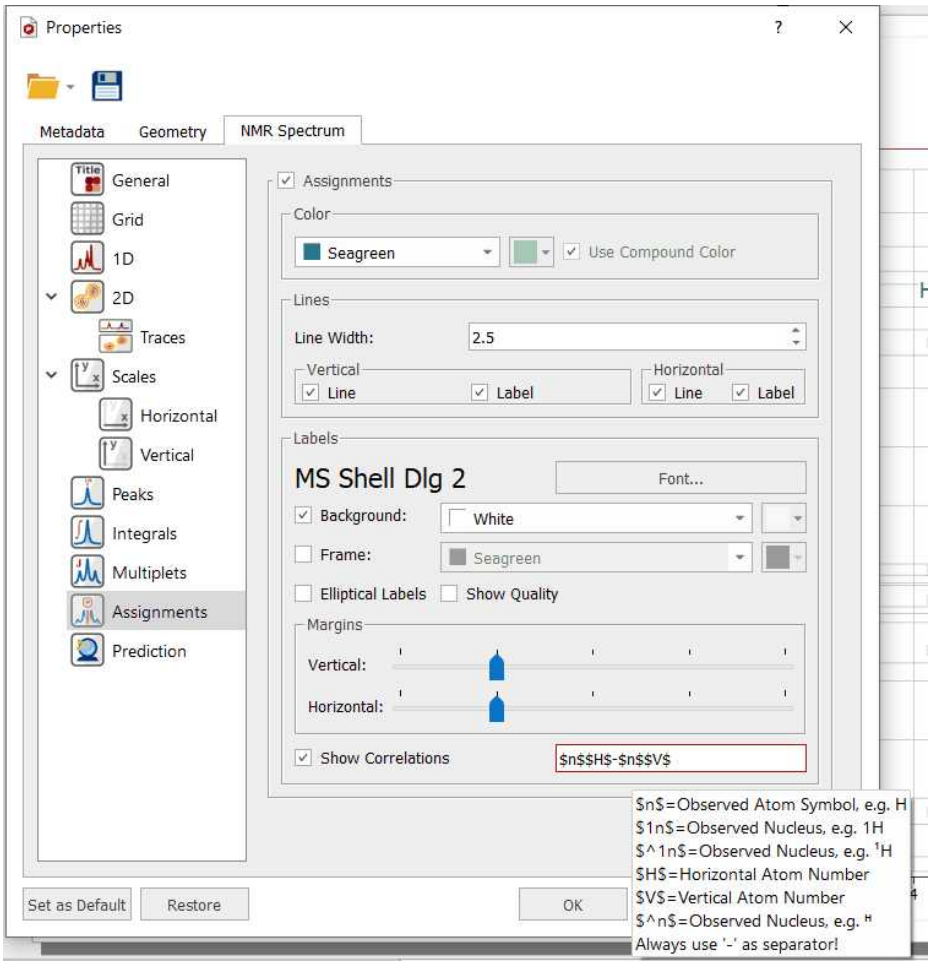


Another way would be to copy&paste the molecular structure with the assignments and make the structural changes in one of the molecules. You could renumber the resulting molecules by selecting 'Concatenate Numbering' from the 'Molecular Drawing Toolbar':



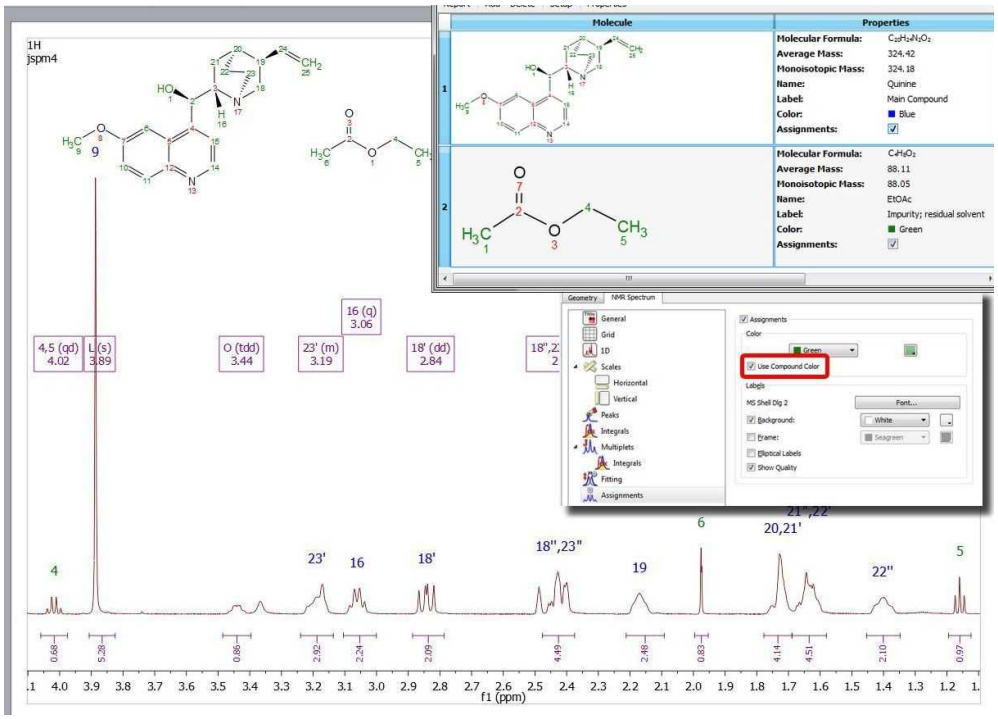
8.5.5 Assignments Properties

To change the properties of the Assignments labels, double click on the spectral window and select 'Assignments' in the left tabbed menu. Once there you will be able to select the color, line width, font, background, frame, form and margin of the labels (with the capability to display the correlations):



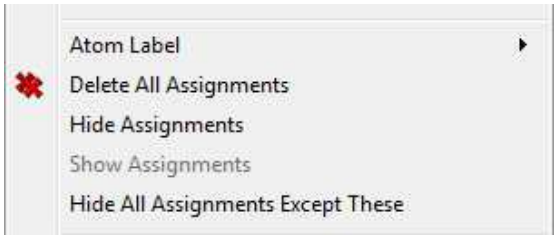
If you have several compounds in your document, you can use the same color compound for the assignments labels (green and blue in the example below) by checking the applicable box:

© 2024 MESTRELAB RESEARCH

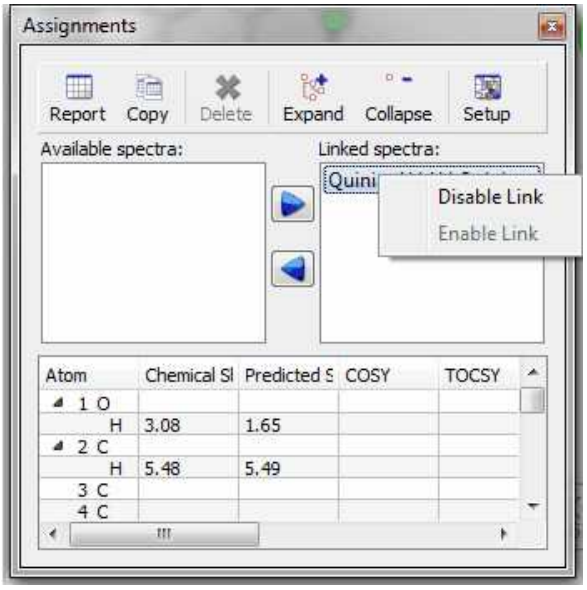


You can find a tutorial about handling mixtures, **here**.

You can hide the assignments in a spectrum by unchecking the 'Assignments' box (or lines boxes for 2D) in the Assignments Properties. You can also do the same by right clicking on the molecule and selecting the applicable option:

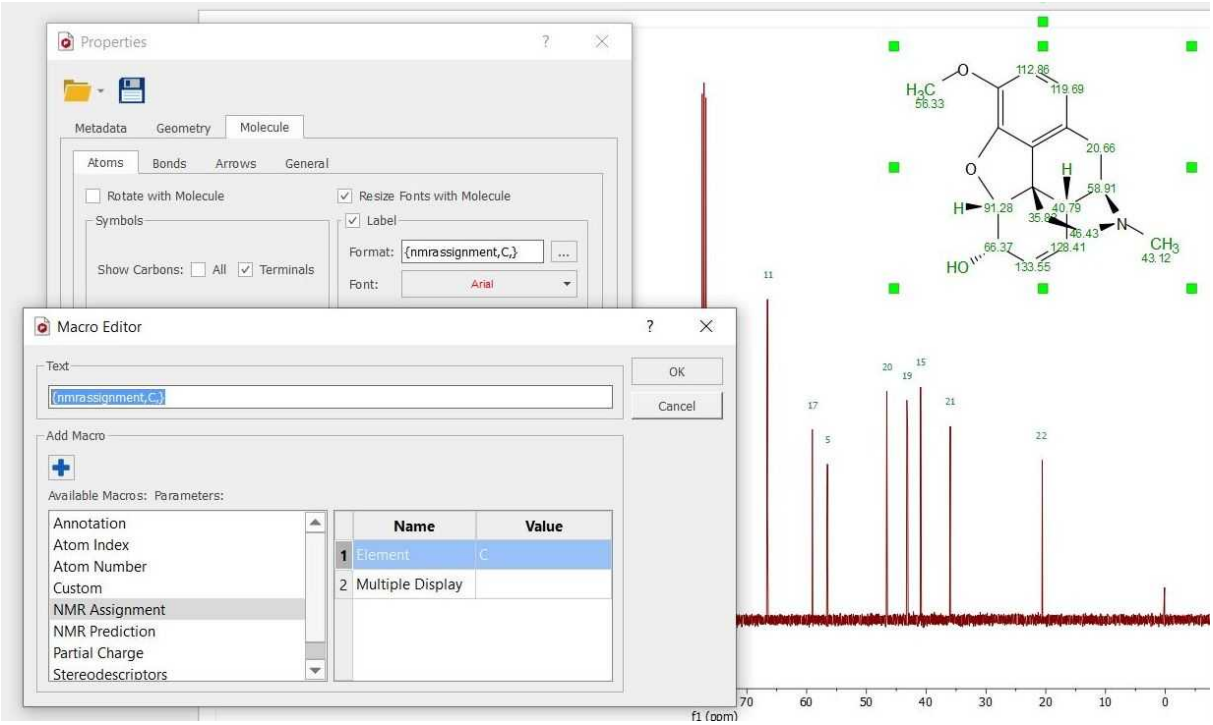


The same effect will be obtained by right clicking on the 'Linked Spectrum' from the Assignments table and selecting 'Disable Link':



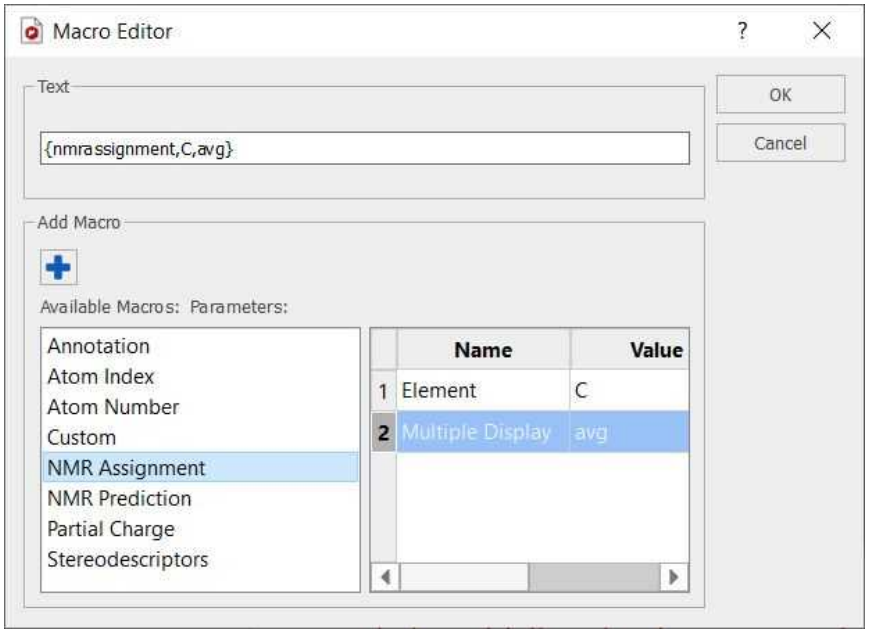
From the Properties, you could also customize the format of the assignments labels. The default configuration is "$n$$H$-$n$$V$", showing only the nucleus symbol and atom number from both dimensions. The info about the configuration format is shown in the tooltip.

You can display the assignments chemical shifts values in the molecular structure, just by adding the applicable macro from the Molecule Properties dialog box. In the example below, you can see some 13C assigcments added to the molecular structure.



© 2024 MESTRELAB RESEARCH

When you have more than one assignment to the same atom, you can use the 'Multiple Display' parameter to show the assignments as a list (when no value is added), as a range (when setting rng) or as an average value (when setting avg).



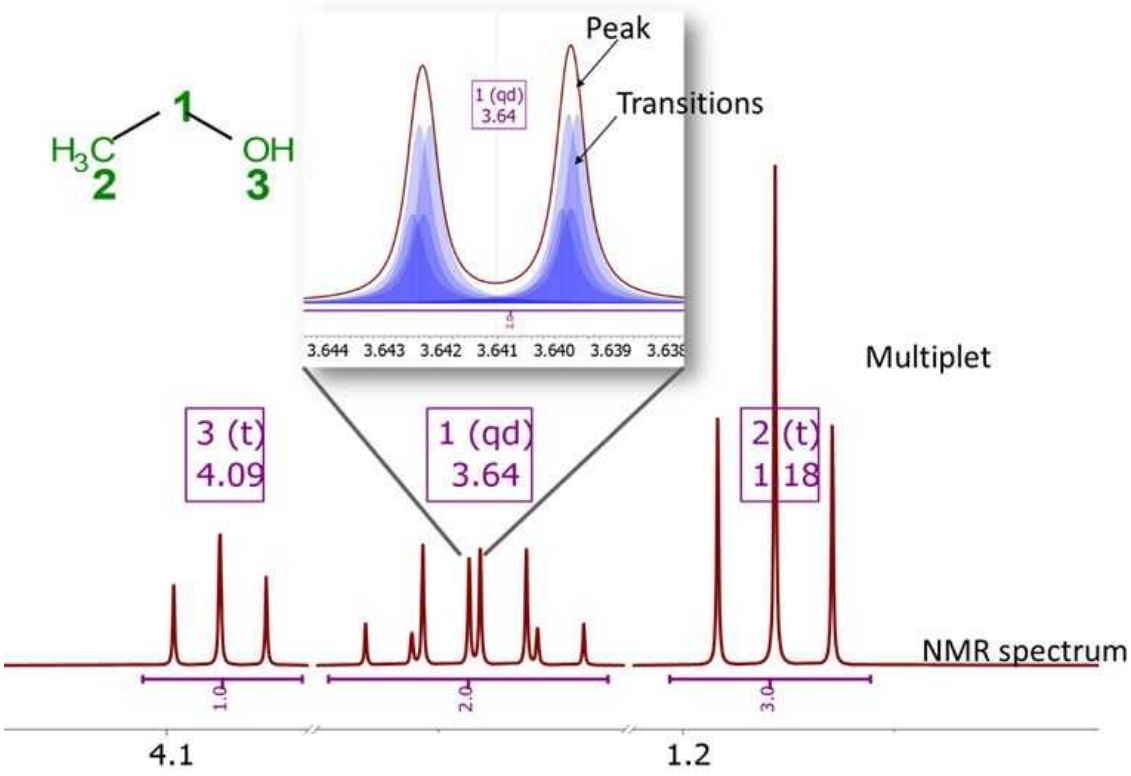
8.5.6 Assigning Estradiol - Practical Case

Introduction

NMR spectra are built up from **peaks**, which are considered as the basic unit of information. A peak in turn is made from **transitions.** A transition is the observable associated with a jump from one energy level to another in a quantum-mechanical system. A single NMR peak can be constituted by one or many transitions and very often many of them can collapse in one peak and cannot be resolved.

NMR peaks can be grouped into what are known as **multiplets**: Broadly speaking, a multiplet can be defined as the set of peaks that can be assigned to a particular spin (note: in the case of strongly coupled spin systems, there are the so-called *combination lines*, which no longer can be assigned to any one nucleus appear). Hence, a multiplet can be just a single transition, a single peak (composed by one or more transitions), or a set of peaks due to the interaction of the assigned spin (or nucleus) with other particles (e.g. due to scalar or dipolar couplings).

© 2024 MESTRELAB RESEARCH



From practical reasons, in the context of small molecules, **NMR assignments** are made from the **molecule atoms** (e.g. 1H or 13C atoms) to **multiplets** rather than to individual peaks or even transitions (note: assignments to transitions were popular in the past in NMR spin fitting software packages, such as those following the LAOCOON approach).

In former versions of Mnova, the concept of multiplets existed only for 1D spectra and assignments to 2D spectra had to be made to peaks, integrals or specific spectral regions. Version 14 and higher fill this gap and multiplets can be used for both 1D and 2D spectra. Whilst the other NMR elements (e.g. peaks, integrals) can still be used, it is highly recommended to use always multiplets when assigning 1D and 2D spectra.

The features described below have been described using the this **Mnova document**

1D assignments

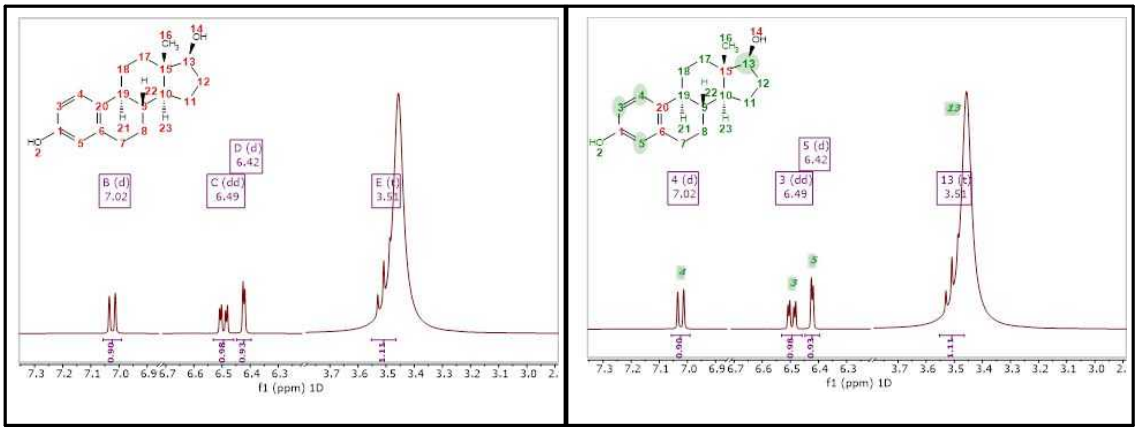
There are several workflows that can be used to assign molecules to 1H (and 13C) NMR spectra, namely:

1) **AutoAssignment**: Whilst this method usually achieves a >85% of correct assignments, it is usually necessary to correct some potential misassignments. In any case, as a result, protons in the molecule will be assigned to 1D Multiplets in the spectrum

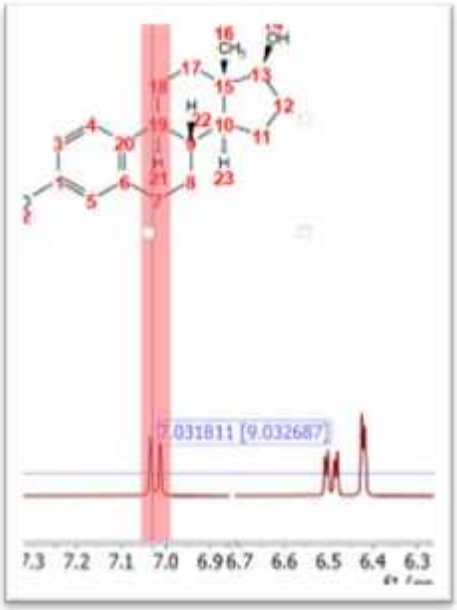
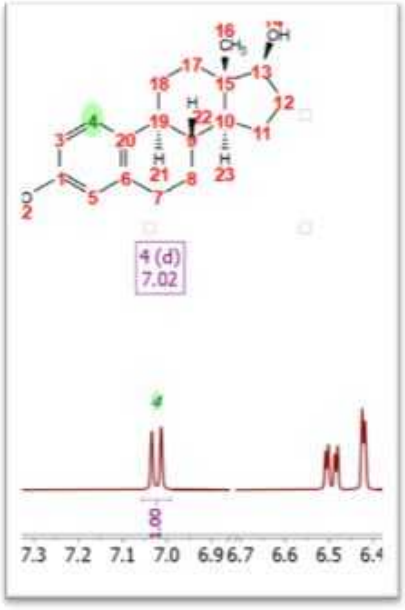
2) Run first **Automatic Multiplet Analysis** so that the multiplet will be first automatically generated. Next, edit (e.g. delete or add) any multiplet that is not considered necessary or it is missing. Finally, enter into the **manual assignments** mode (e.g. *shortcut: press A or a in the keyboard*) and add the assignment.

1) Automatic Multiplet Analysis	*2) Manual Assignments*

© 2024 MESTRELAB RESEARCH



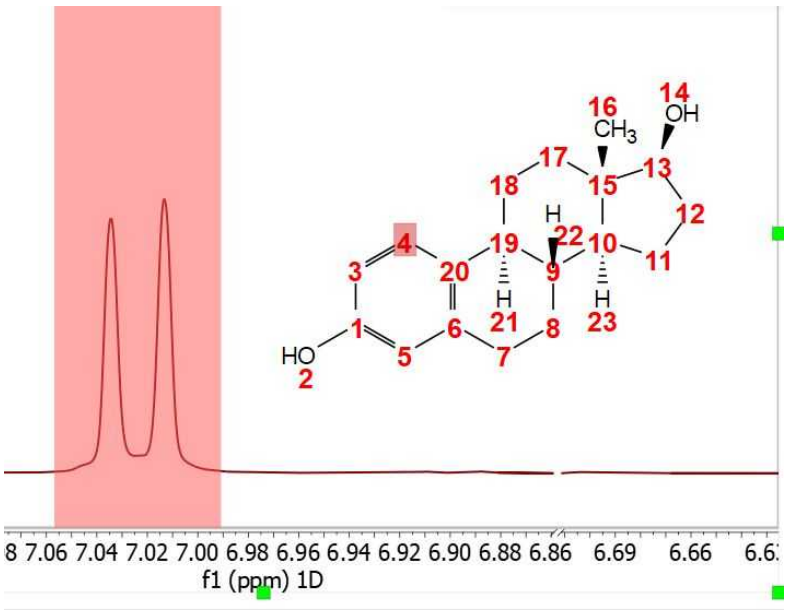
3) You can also create **the multiplets manually**, one by one. Mnova 14 introduces a quick method: once the program enters the manual multiplet mode (e.g. after pressing the J key), as you hover the mouse over the spectrum, the program will highlight the corresponding multiplet. If satisfied with that suggestion, just click and the program will create the multiplet. Of course, you can also manually define the multiplet region following the same procedure used in former versions of Mnova.

1) In the manual multiplet analysis, left click if the suggested multiplet is fine.	*2) Enter into the assignment mode and add corresponding assignment*
	

4) The two steps in point 3) can be executed **directly within the manual assignment mode**. Once in this mode, as you hover the mouse over the spectrum, the program will highlight a tentative multiplet. Just left click to create it and then make the assignment to the molecule. Alternatively, you can start the assignment directly from the molecule and then select the multiplet that is highlighted in the spectrum.

© 2024 MESTRELAB RESEARCH

1) In this example, while in the assignment mode, atom 4 was first selected in the molecule and then the mouse moved close to the spectrum so that a multiplet is highlighted. Just left click and the program will create corresponding assignment

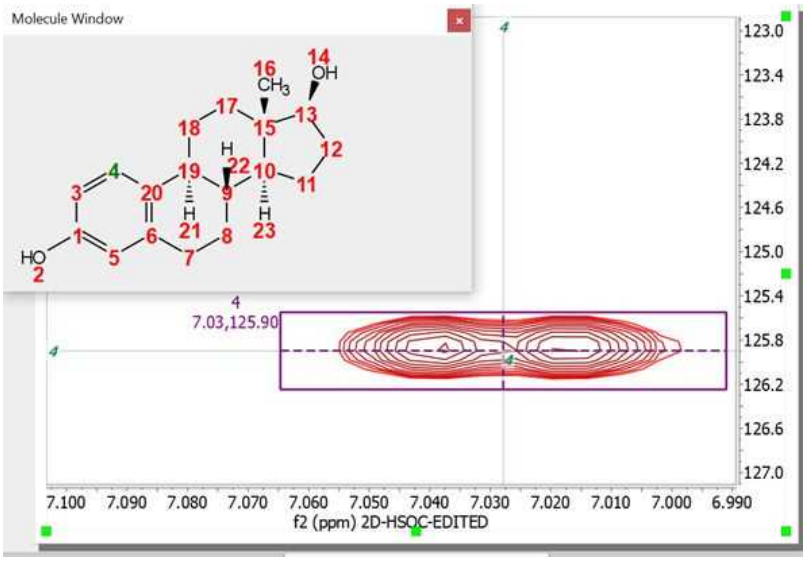


Note: In the case of points 3 and 4, the program creates a list of multiplets in the background. Whilst this process is computationally quite efficient, it can take a few seconds when the spectrum has many peaks. As a result, it is possible that when entering into the manual multiplet analysis of assignments, a wait cursor indicating that the program is doing some calculations may show up. Those calculations are carried out in a separate thread so that the Mnova User Interface should still be responsive.

2D assignments

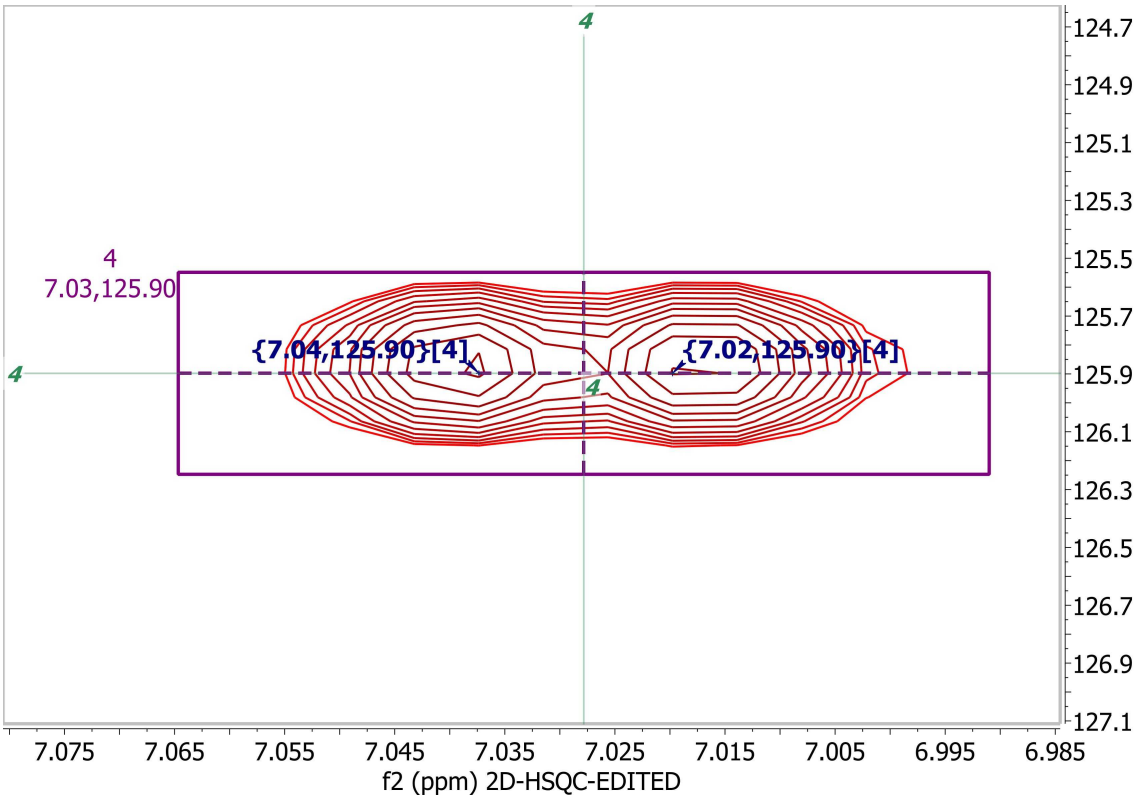
This is probably one of the major novelties introduced in Mnova 14, the concept of 2D Multiplets. This is essentially a direct extension of the idea of 1D multiplet to 2D spectra: all peaks corresponding to the same assignment atom(s) are grouped into one single entity. For instance, using the same estradiol example as before, this is how the assignment to H4 in a HSQC would look like:

© 2024 MESTRELAB RESEARCH

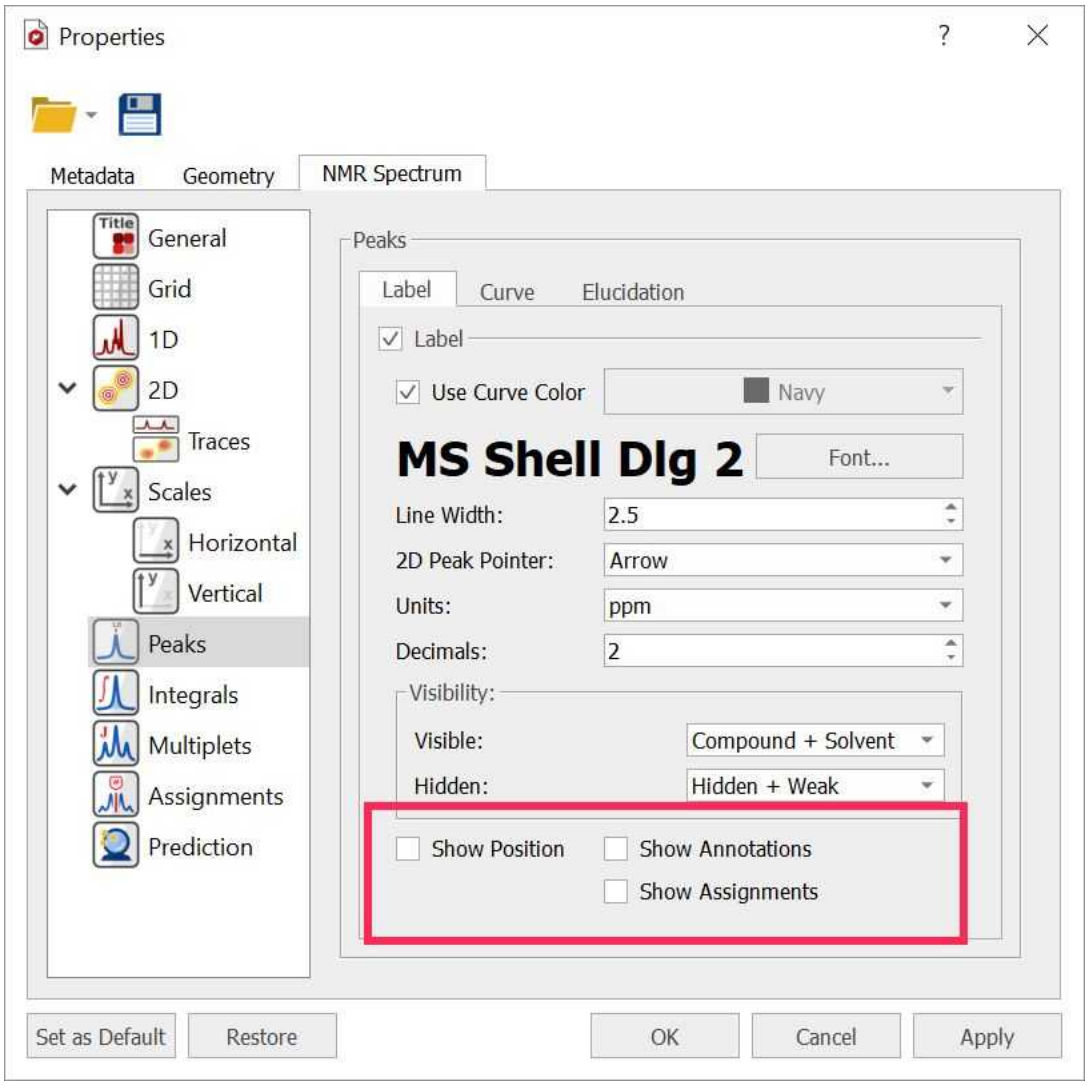


Note that this multiplet is comprised of two peaks which are merged together into one single multiplet. The central coordinates of the multiplet is showed as a pair of dotted cross hairs. Such coordinates are computed by using a weighted average of all peaks within the multiplet.

The information displayed together with the 2D multiplet box can be customized according to User preferences. For example, the peak position (coordinates), labels, etc, can be turned on/off from the preferences. For example, in the figure below all information about a 2D multiplet is displayed:

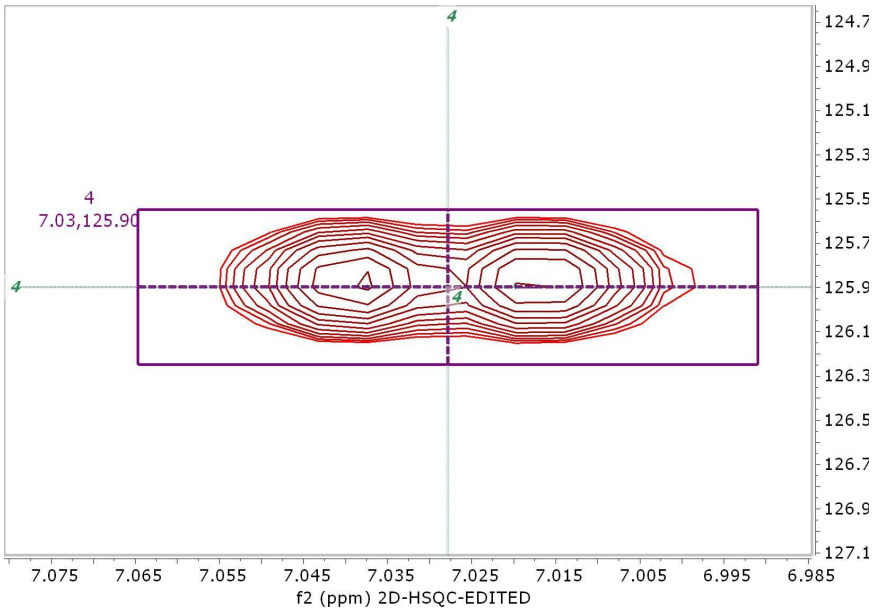


If the peaks values and assignments are not needed, they can be changed from the preferences:

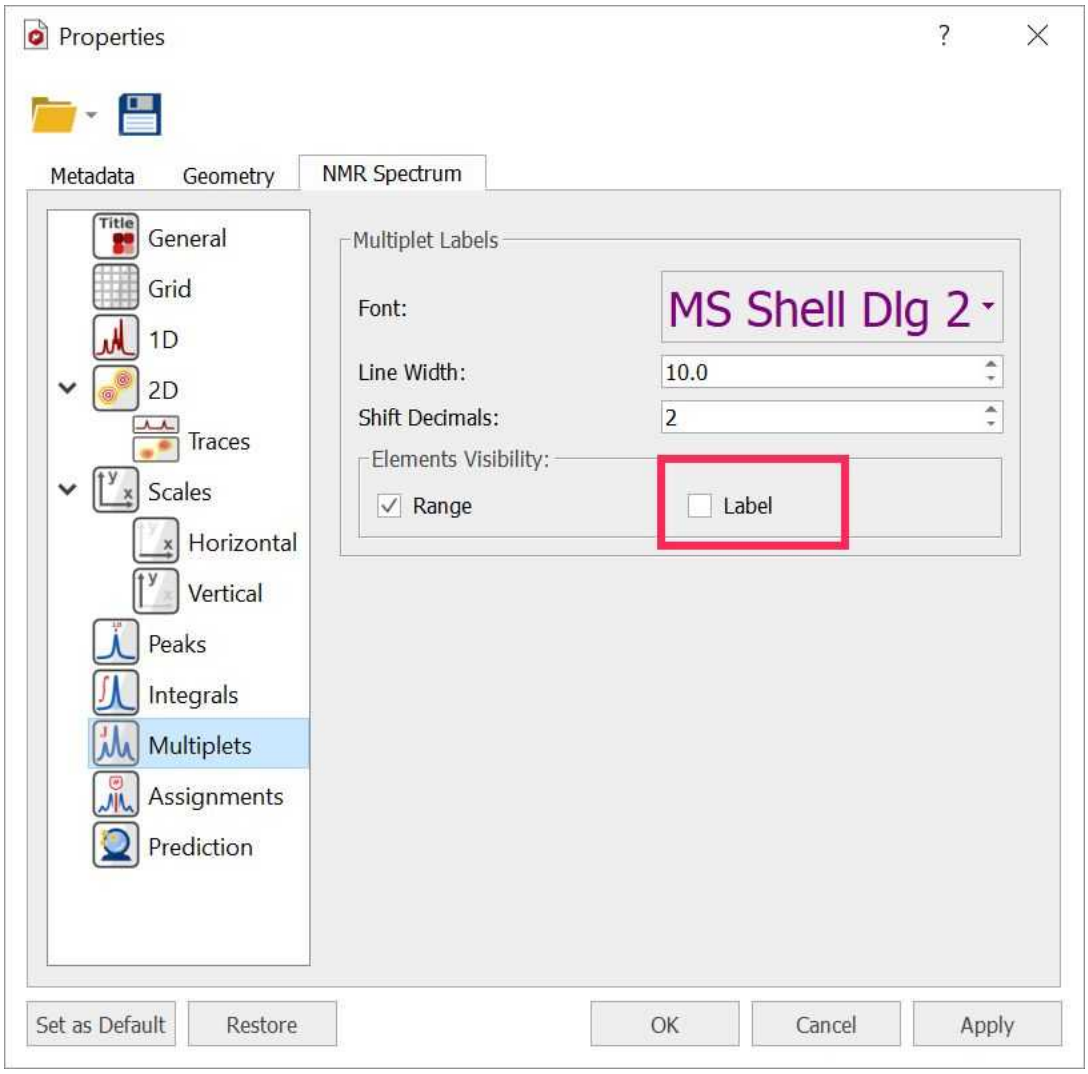


With all those options off, the multiplet will look like this:

© 2024 MESTRELAB RESEARCH

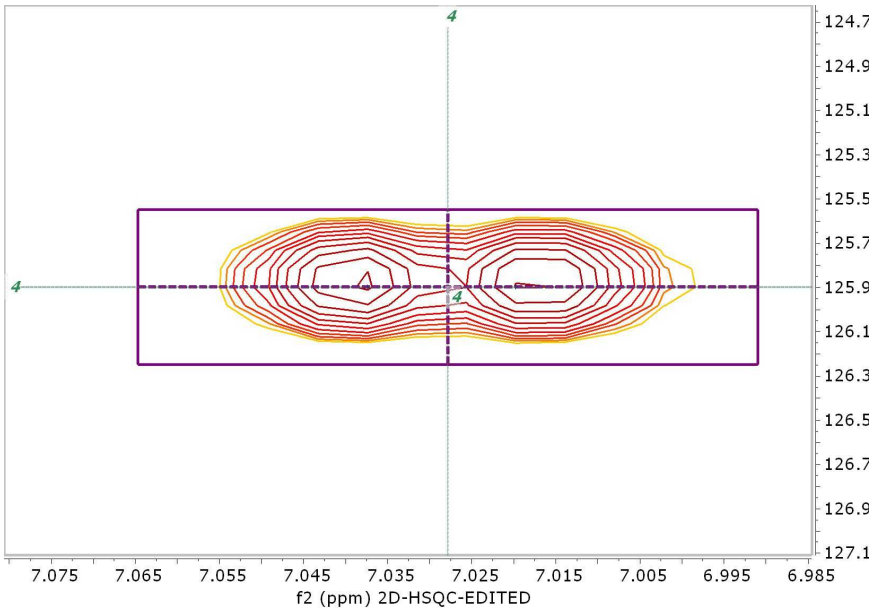


Finally, the multiplet legend can also be turned off from the preferences:

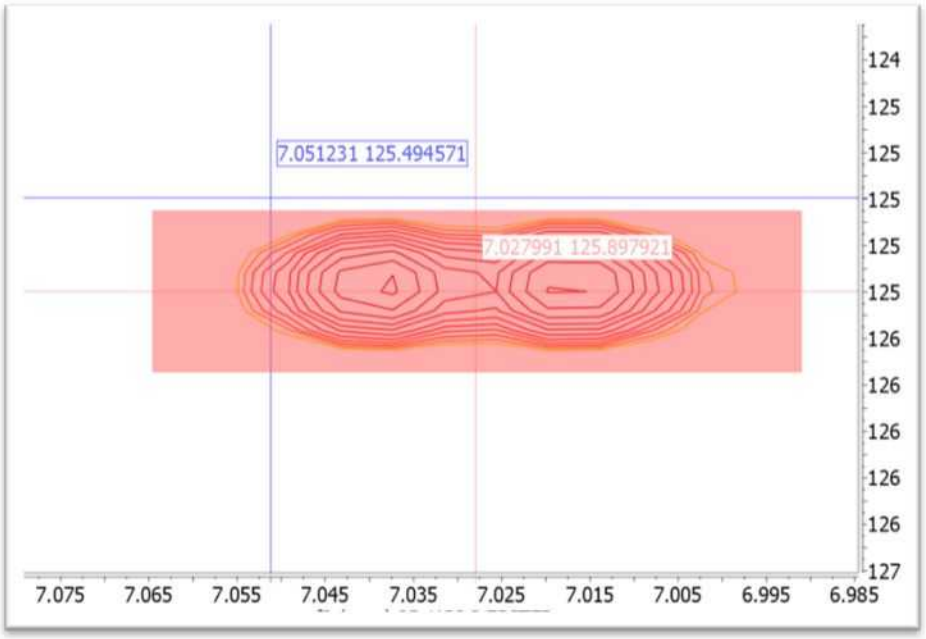


Resulting in this:

© 2024 MESTRELAB RESEARCH



As in the case of 1D multiplets/assignments, the program will automatically highlight a 2D multiplet as the mouse cursor is hovered over the 2D spectrum. Again, at that point, just left click to create the multiplet/assignment.



1D and 2D assignments for the same molecule

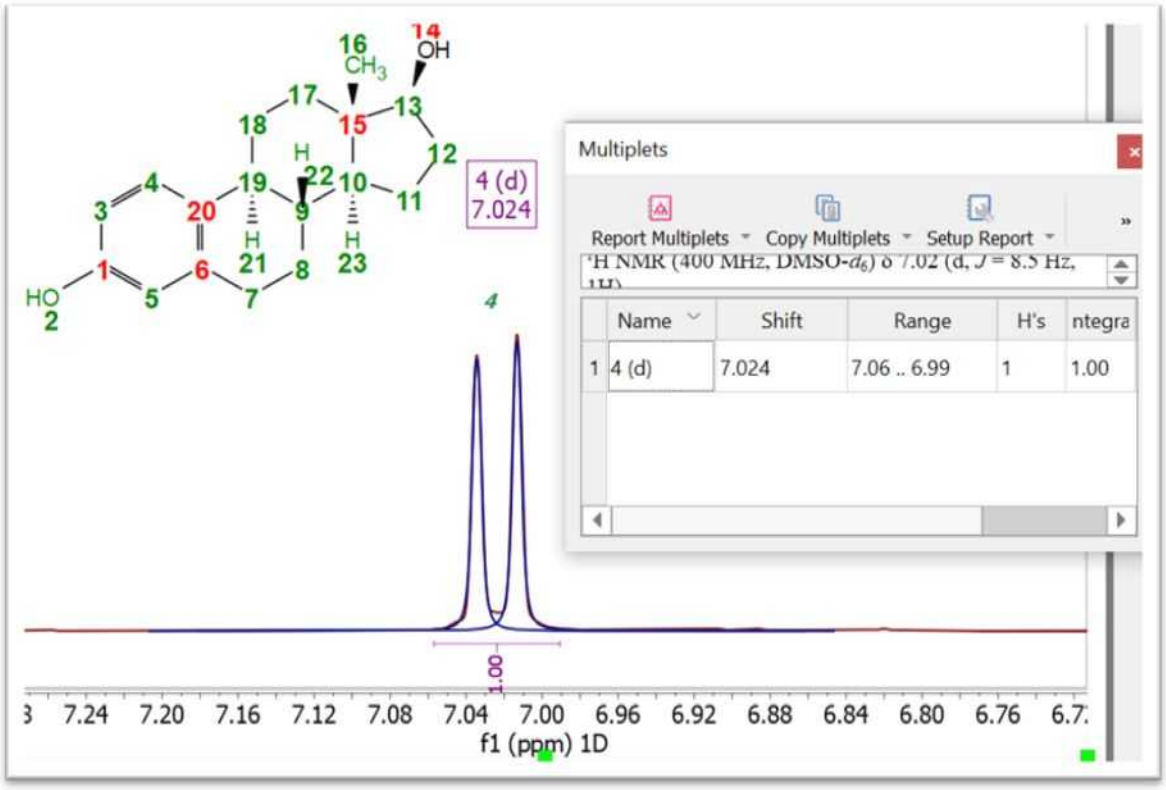
Note: It is very important that the 1H and HSQC spectra (in general, ALL spectra) are properly aligned (e.g. referenced) before assigning the spectra.

An important question arises when several spectra are used in the assignment process. For example, let's consider the simple case of 1H and HSQC. We will have the situation in which, for example, one 1H multiplet is assigned to a particular proton in the molecule and, correspondingly, another multiplet in the HSQC is also assigned to the same 1H spectrum (in the proton dimension). In an ideal situation, the

© 2024 MESTRELAB RESEARCH

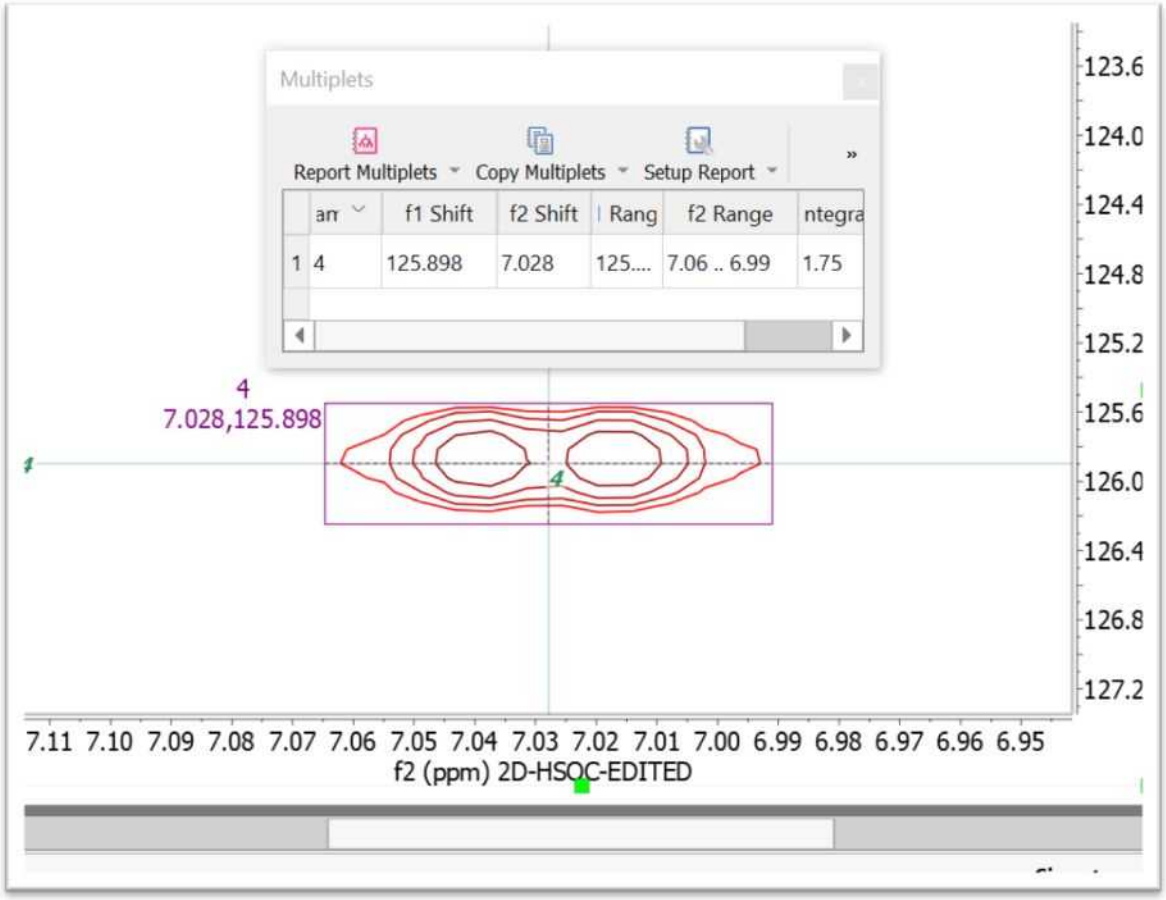
assigned chemical shift for both multiplets will be the same, or very close within experimental error (e.g. digital resolution).

For instance, let's consider the Estradiol example. If we assign atom 4 in the 1H spectrum, the chemical shift of corresponding 1H multiplet would be **7.024**



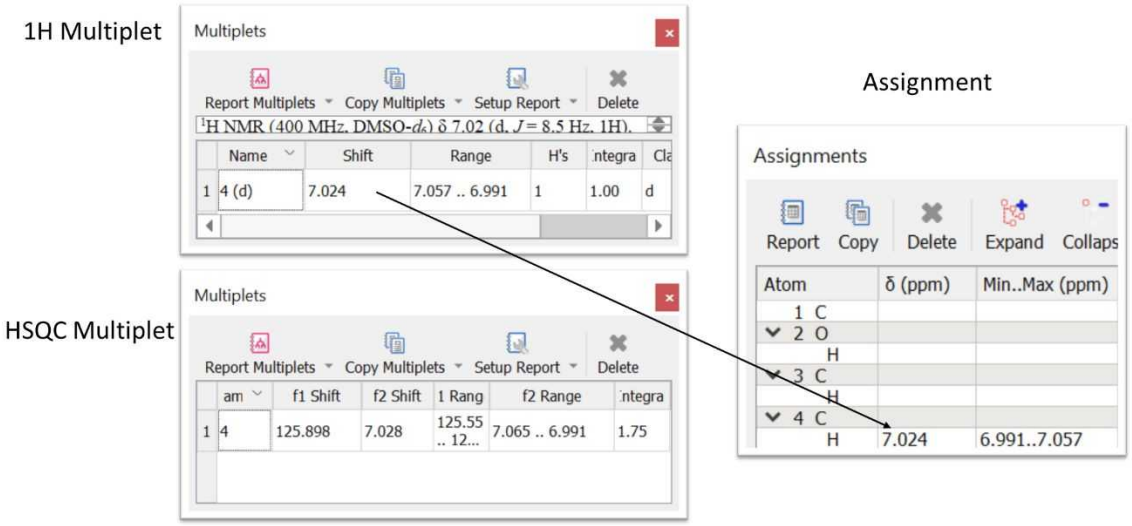
Next, if we do the same in the HSQC spectrum, the 1H chemical shift of corresponding 2D multiplet will be 7.028 ppm:

© 2024 MESTRELAB RESEARCH

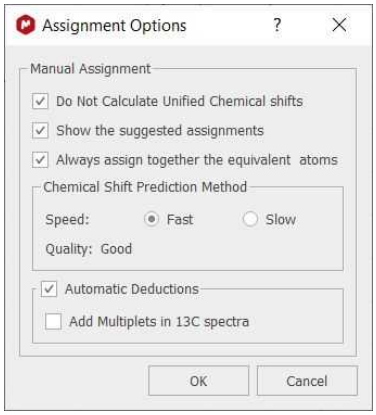


So the question is: given those two assigned multiplets, which would be the *global* assignment chemical shift? Obviously, there can be only one assigned chemical shift that must be computed from the two multiplets. There are a number of potential solutions to this question, but the approach taken in Mnova 14 is the following: ***always take the chemical shift of the multiplet with the narrowest interval***. In the particular case of H4, the difference is really tiny, both multiplets have a very similar interval (HSQC = 0.074 ppm; 1H = 0.066 ppm), but as the 1H multiplet is narrower, the final assigned chemical shift will be taken from the 1H multiplet, that is, 7.024 (from the 1H multiplet) and not 7.028 (from the HSQC multiplet). This is depicted in the figure below:

© 2024 MESTRELAB RESEARCH

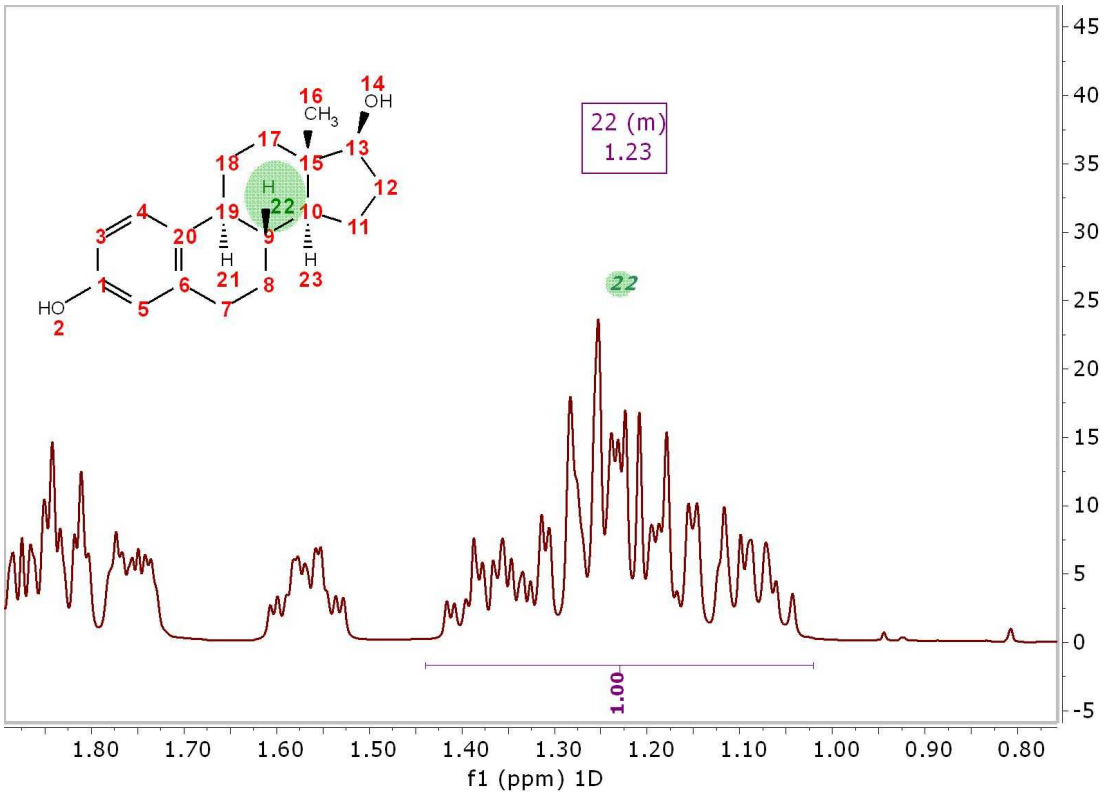


Note: This behavior can be turned off in the assignments properties so that the assigned value will correspond to the last assigned multiplet. Of course, the table of assigned can be manually edited so that any value can be entered.

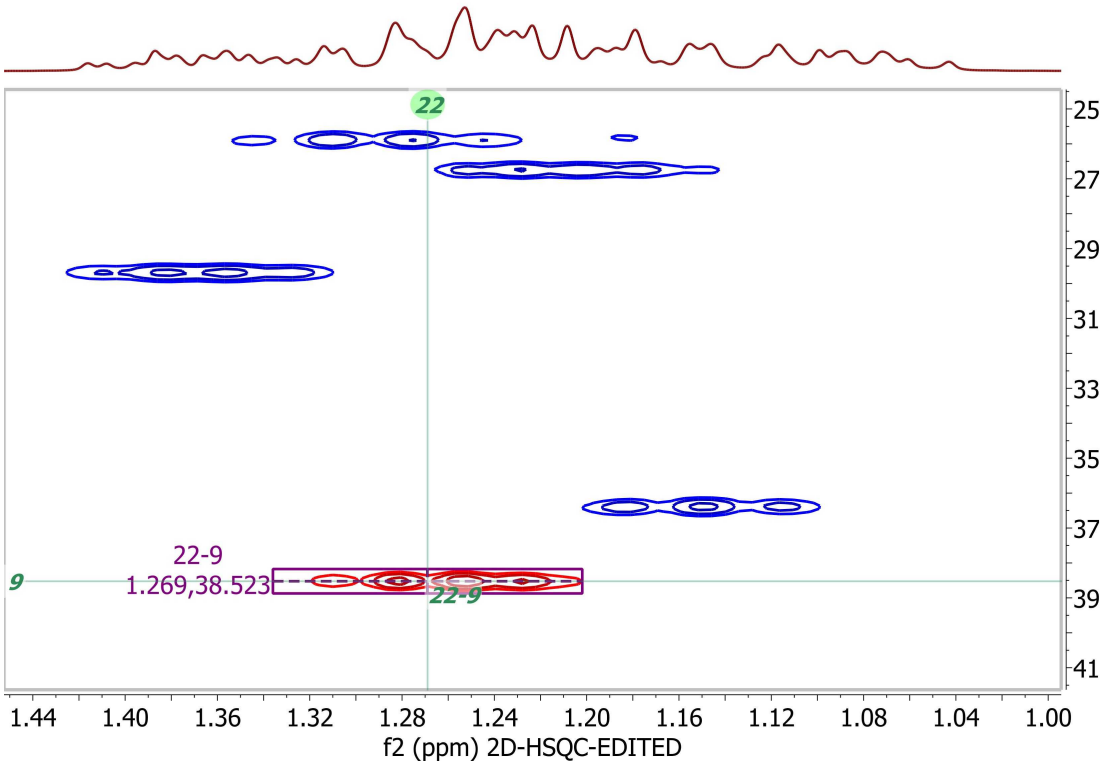


The reason why this calculation of the *unified* chemical shift when several multiplets are available makes sense can be better understood with another couple of multiplets from Estradiol. For example, looking at the aliphatic part of the 1H spectrum, we see one highly crowdy multiplet that can be assigned to several distinct protons, one of them being H9. We can make such assignment:

© 2024 MESTRELAB RESEARCH

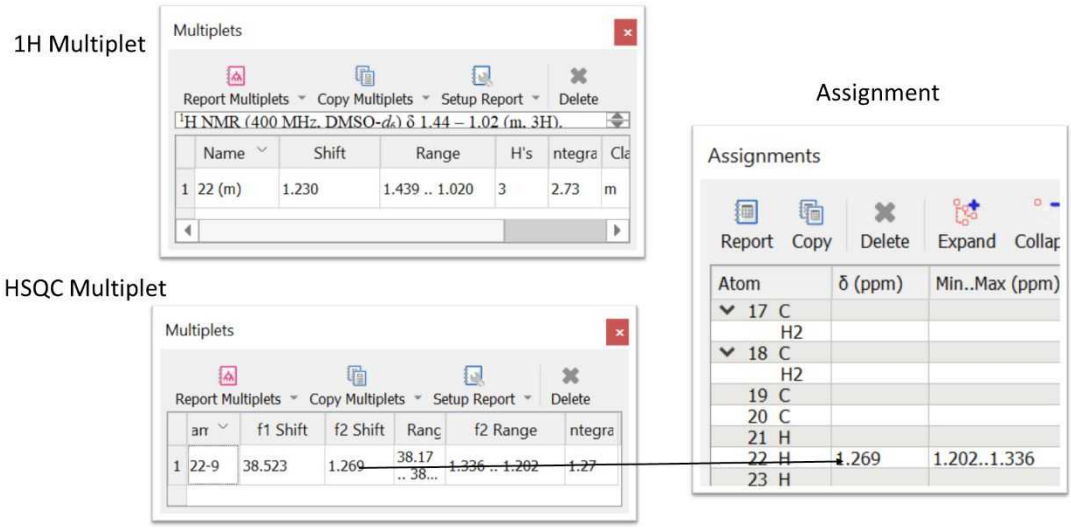


If we move to the HSQC spectrum, that assignment can be easily located:



© 2024 MESTRELAB RESEARCH

Since HSQC multiplet is better resolved than the 1H multiplet and hence, its interval (along the 1H dimension) is shorter, Mnova will take the chemical shift from this multiplet as the final assignment value, as illustrated in the figure below.

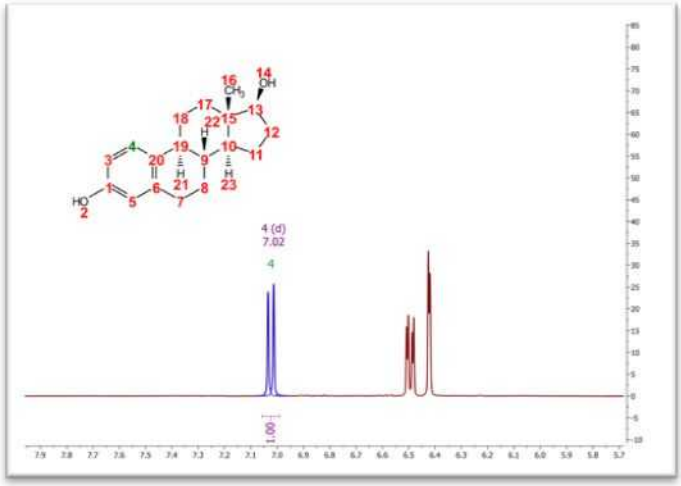


Additional new assignment features in Mnova 14

In addition to the features just described, Mnova 14.1 comes with more tools aimed at facilitating the manual assignment of NMR spectra. One of them is that when the Mnova contains a 1H and a HSQC spectra loaded, when one assignment is made (for example, in the 1H spectrum), the program will attempt to create another assignment (and with corresponding multiplet) in the other spectrum (e.g. HSQC) even if no multiplets have been created.

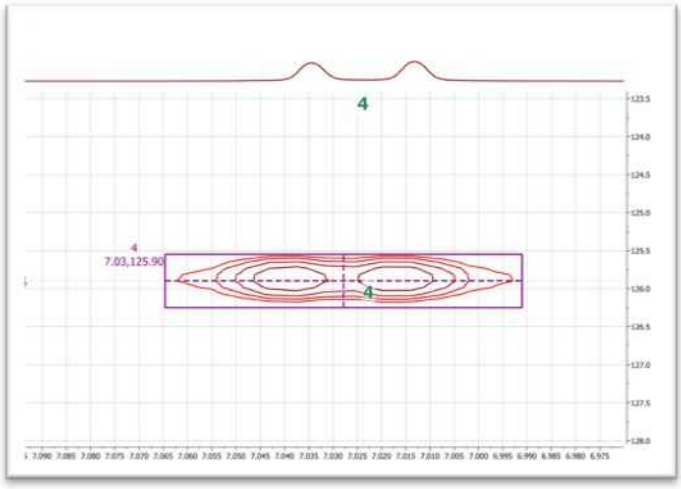
Under the hood, what the program does is calculate a list of multiplets for all spectra in the background so that when a multiplet is needed, the program takes it from there. For instance, assuming that we have a 1H and an HSQC loaded in the program. No multiplets exist in any of the two spectra. We can assign H4 in the 1H spectrum. As soon as this is done, a new 2D multiplet will be created in the HSQC and assigned to the same H4 in the molecule. That is:

1) H4 is assigned in the 1H spectrum:



© 2024 MESTRELAB RESEARCH

2) A new assigned 2D multiplet will appear in the HSQC spectrum:



The same workflow can be followed in the other direction: when a 2D multiplet is assigned in the HSQC spectrum, another multiplet will be created in the 1H spectrum and the assignment deduced.

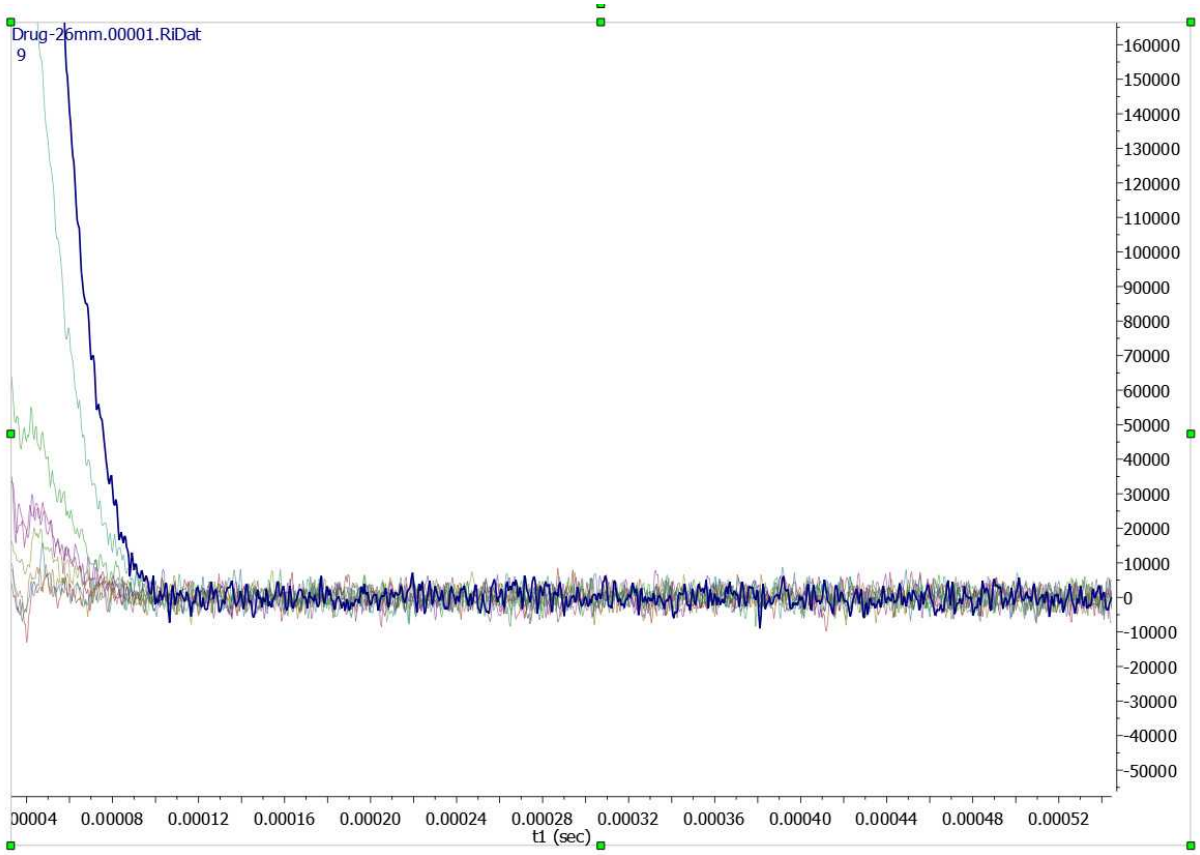
8.6 TD Quantitation

This feature will be used for the processing and analysis of low-field, time domain data acquired in Oxford Instruments.

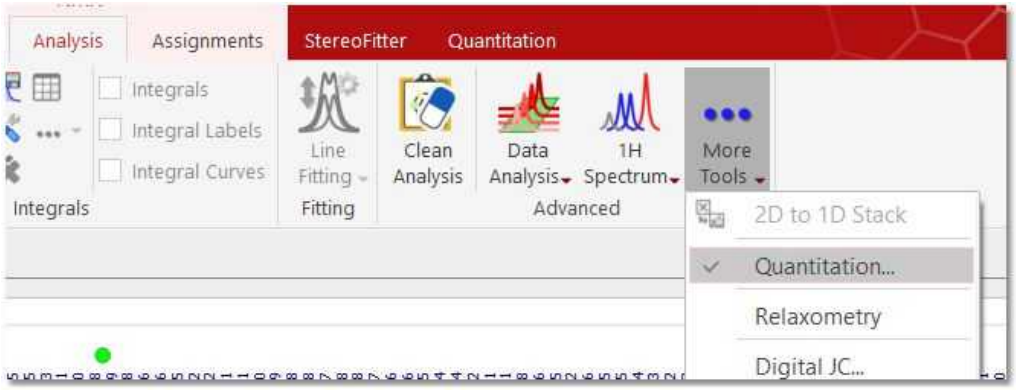
See this paper for further information.

First of all, you will need to load the NMR spectra which will be automatically displayed as an overlaid plot of FIDs. It is recommended to start with the datasets which belong to samples of known concentration, in order to build a calibration curve:

© 2024 MESTRELAB RESEARCH

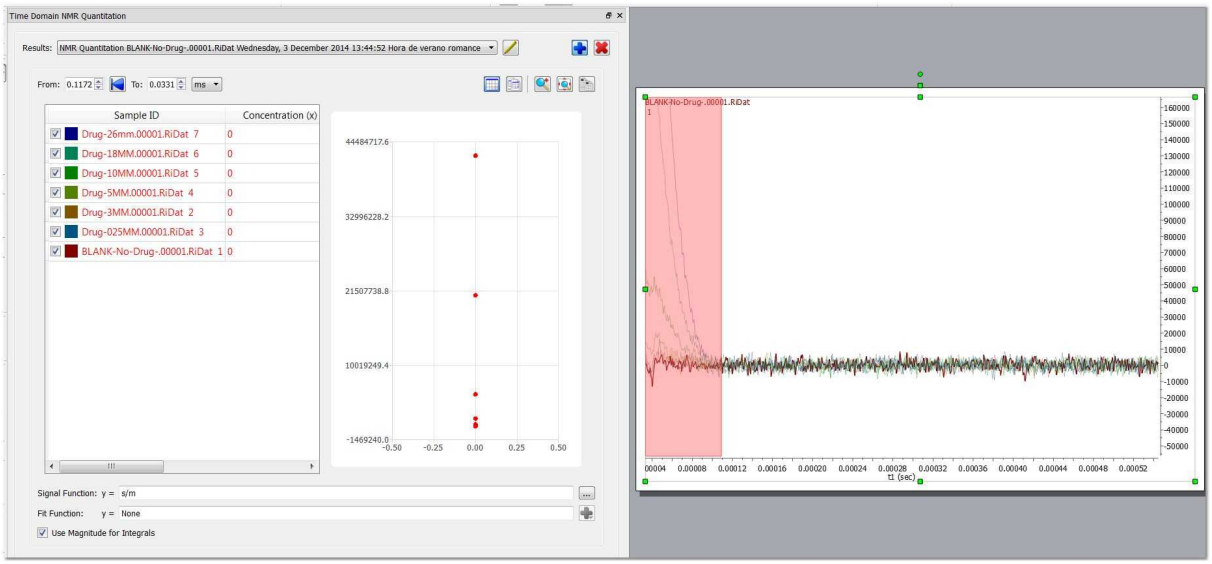


Next follow the menu 'Analysis/More Tools/Quantitation":

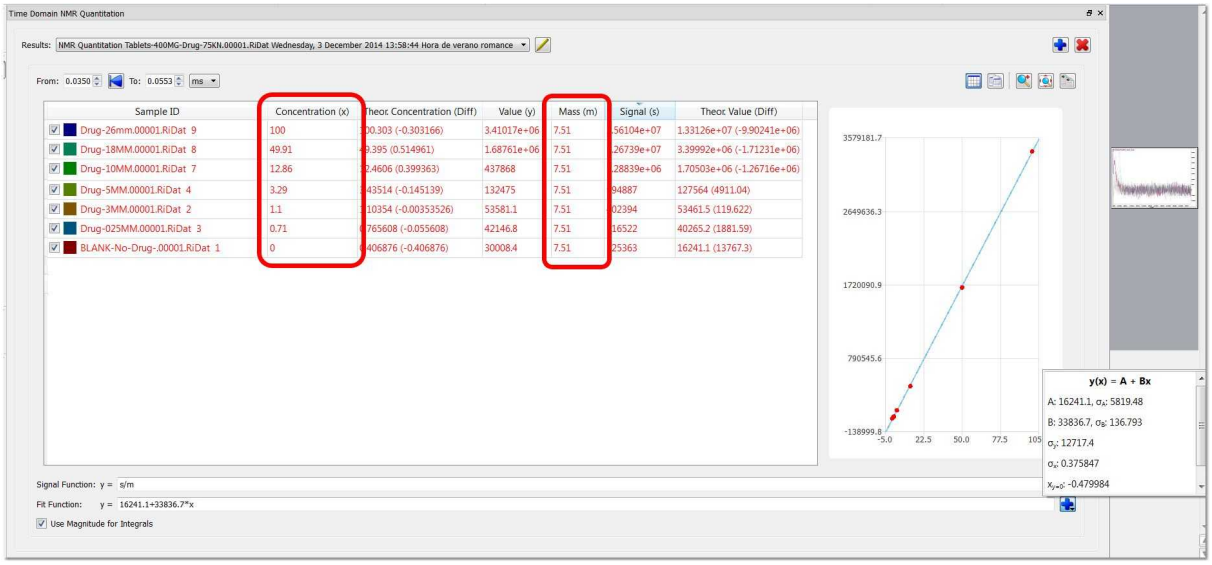


That will display the TD Quantitation window. Clicking on the 'blue plus button' will allow you to select the integration region (by clicking&dragging):

© 2024 MESTRELAB RESEARCH

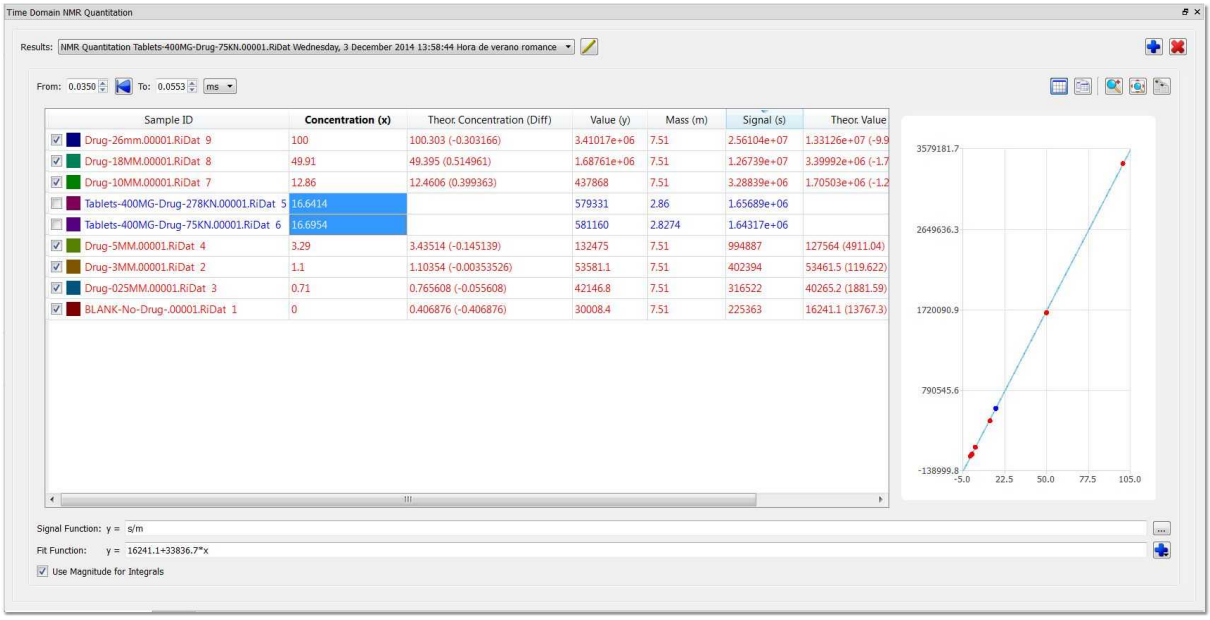


The next step will be fill the concentration and mass columns with the known values (for each of the samples used as reference):



Automatically, we will get a calibration curve with a fit function which will be used to calculate the concentration of unknown samples. Statistic information can be displayed by clicking on the plus button besides the Fit Function.

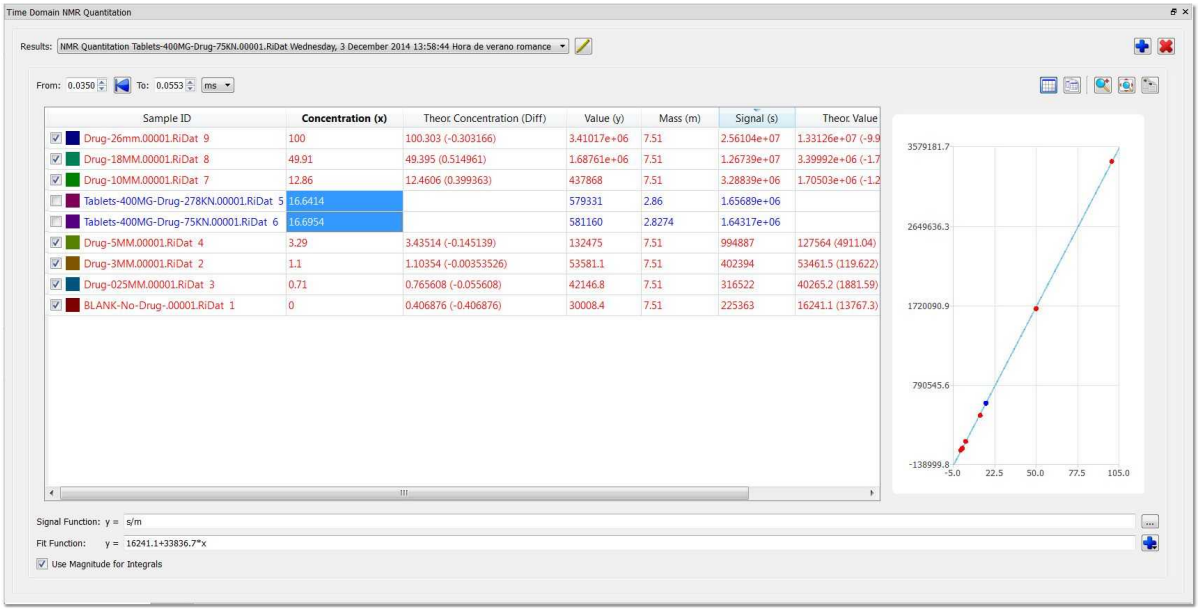
The next step will be to load the datasets of the unknown samples and enter the mass values for each of the samples. Automatically, we will get the applicable concentration values (according to the calibration curve):



As you can see in the screenshot above, the known references used for the calibration curve appear in red and with the check box selected, whilst the samples to analyze appear in blue and unchecked.

8.6.1 TD Quantitation panel

Let´s describe some of the features that appear in the TD quantitation panel:



 This feature will allow you to rename the experiment.

Enter the desired values in the boxes below to change the ranges. Additionally, you can select the first point in the spectrum as start point of the region. From here you can also change the units (seconds, milliseconds or μs)

© 2024 MESTRELAB RESEARCH



From the panel we can also 'show/hide' the table, copy the table to the clipboard, zoom in the graph, restore the zoom and copy the graph to the clipboard:

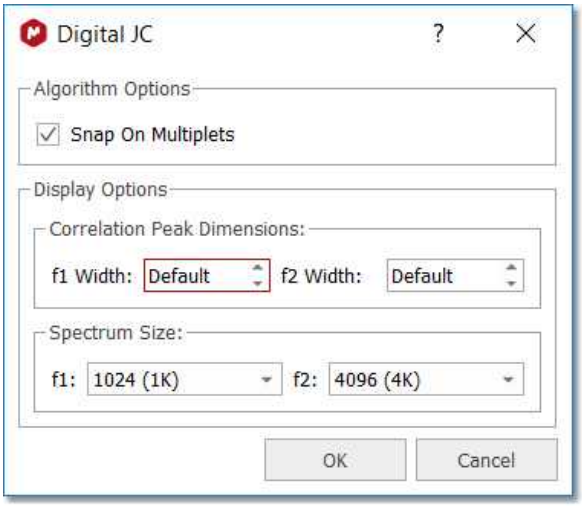


8.7 Digital JC

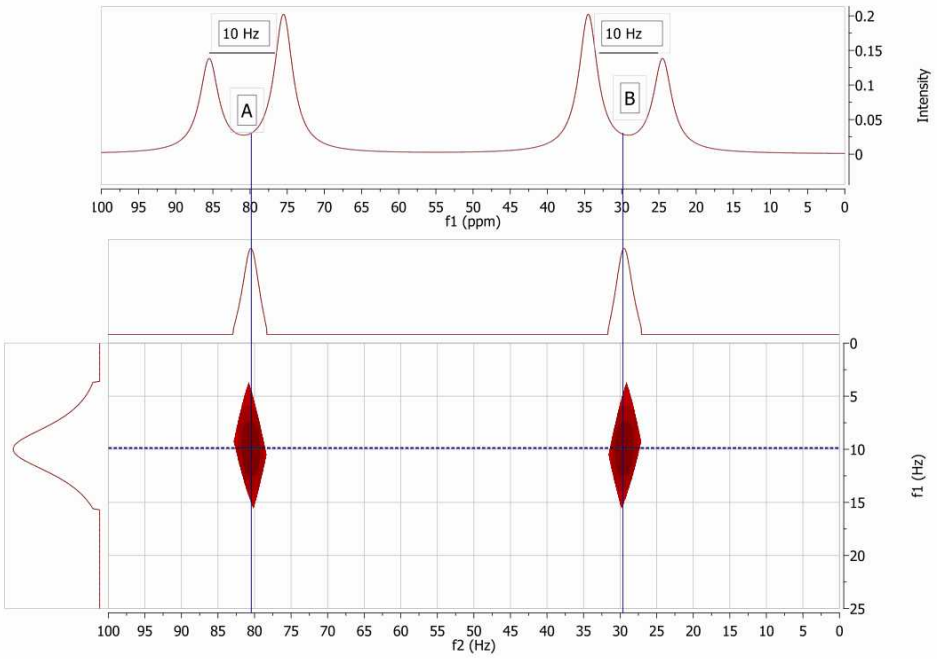
Digital JC (Not available in Mnova Lite): is a new method for detecting spin connectivities in ^{1}H NMR spectra. The main goal of this method is to convert a 1D spectrum into a 2D-like plot in which it is easy to correlate multiplets containing the same **splitting**. Its main purpose is to rule out couplings between multiplets. It is suitable for human inspection.

The basic idea is to generate a 2D plot from a 1H NMR spectrum in such a way that the horizontal dimension conveys the chemical shifts whereas the vertical one represents the separation between peaks in the spectrum (***splittings*** from now on). Basically, it is a special kind of 2D 1H - D experiment (where D = splitting).

Just load your ^{1}H NMR spectrum and follow the menu 'Analysis/More Tools/Digital JC' to display the J Correlation dialog box to select the desired algorithm and display options:



As usual, a picture is worth a thousand words:



In this figure we have simulated 1D spectrum with two coupled spins, A = 80 Hz and B = 30 Hz. JAB = 10 Hz.

In the new 2D plot (JC from now on) we can see two cross peaks at the positions of A and B respectively and at the same d-trace (vertical dimension or splitting dimension).

It's very important to remember that what we see in the JC plot are just splittings, not Coupling Constants, even though in some cases these splittings can coincide with coupling constants.

8.8 Arithmetic

Arithmetic (Not available in Mnova Lite)**:**

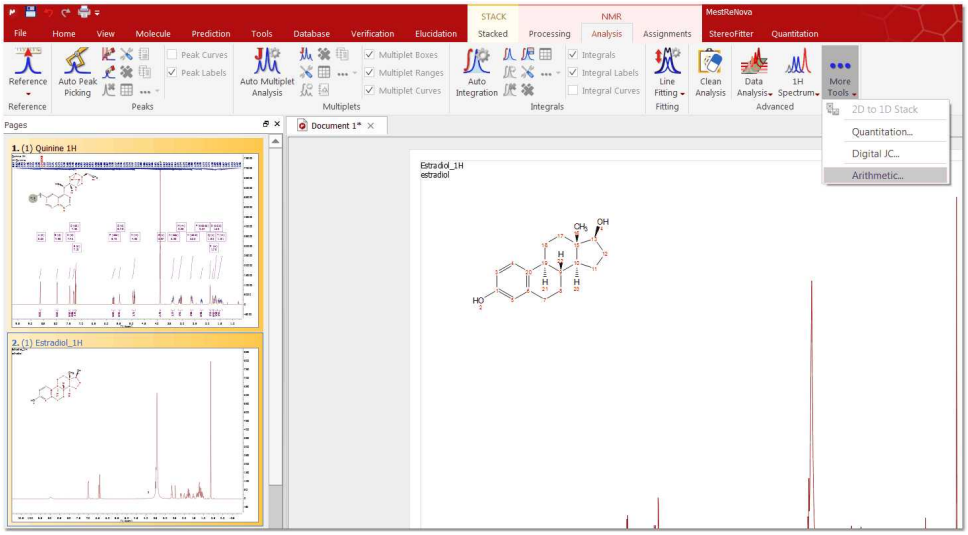
This feature is used to carry out arithmetic operations with 'fid"s or processed spectra. Typically, you will use the arithmetic module implemented in MestReNova to sum 'fid"s acquired under the same measurement conditions in order to improve the signal-to-noise ratio.

This tool is also very useful to **remove impurities** or solvent signals from a spectrum, to **analize DEPT experiments**. This feature will be enhanced in the oncoming versions of the software to be able to **subtract FIDs of Selective Experiments** (such as NOE or ROE).

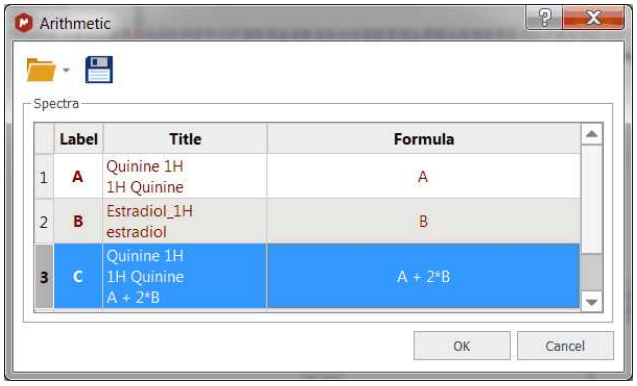
How to sum or subtract spectra?

Application of Linear Combinations (sums, subtraction and multiplication) to spectra in the MestReNova interface is extremely simple. Just select the desired spectra (make sure they are acquired under same conditions) on the Page Navigator (by holding down 'CTRL key' or 'Cmd key' while clicking on each spectrum) and follow the menu 'Analysis/More Tools/Arithmetic':

© 2024 MESTRELAB RESEARCH



This will display the 'Arithmetic' dialog box on screen. In this example, we want to add two times spectrum A to spectrum B, so we shall type the formula 'A+2B' in the applicable cell.

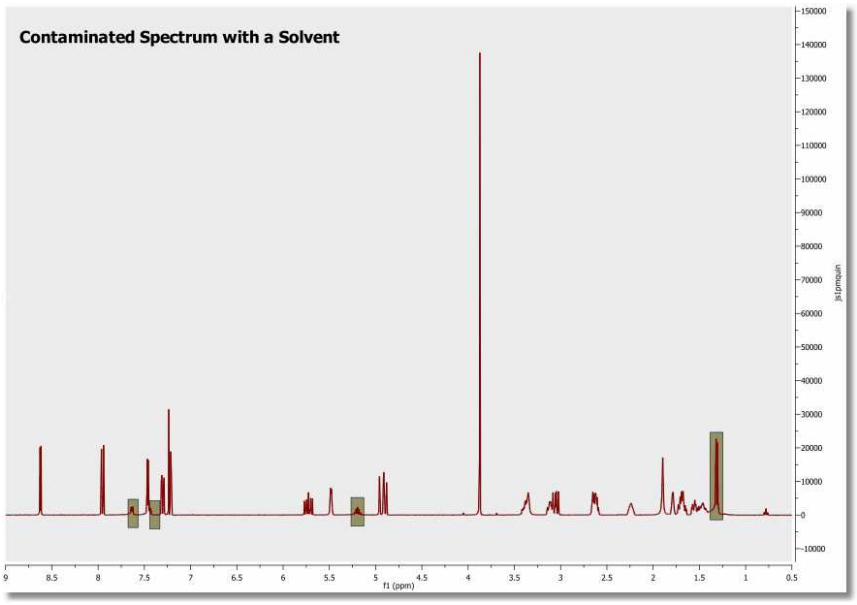


The '**Save**' icon will allow you to save your formulas, which can be loaded by using the '**Load**' button .

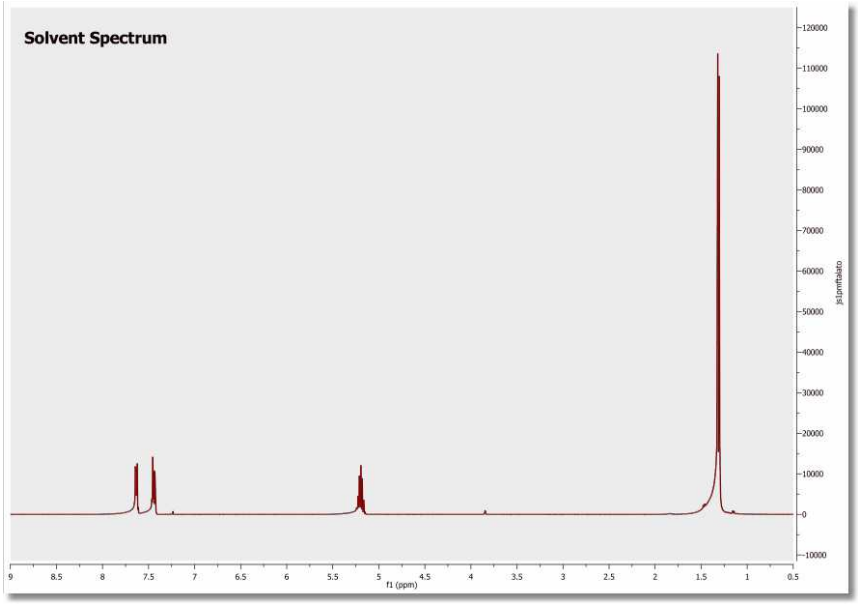
On pressing 'OK', the result is a new spectrum which will consist of the sum of spectrum B plus two times spectrum A.

You can also subtract spectra, but in this case it is important that the user calculates the intensity ratio of both spectra. For example, in this spectrum we have a sample contaminated with a solvent.

© 2024 MESTRELAB RESEARCH



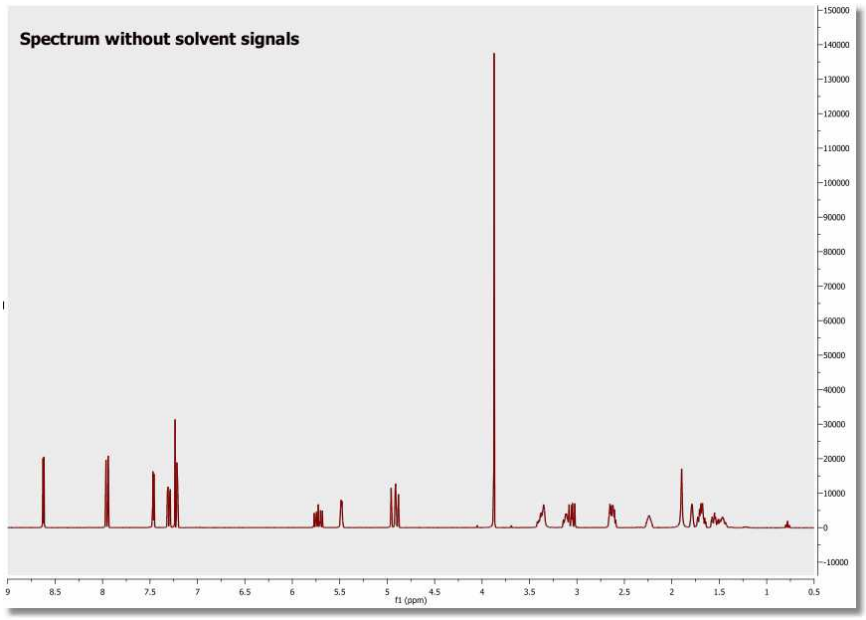
Whilst the spectrum below corresponds to the solvent which we need to eliminate from the sample.



To successfully carry out the subtraction, we need to calculate the intensity ratio between the contaminated spectrum and the solvent spectrum. To obtain this ratio we can measure the intensity of a signal present in both spectra (by applying the command ‘Peak by peak’ and getting the result on the applicable table). For example, we measure the intensity of the singlet which appears at 1.32 ppm. For the first spectrum, we obtain an intensity of 22617 and for the second, 115031, indicating the the intensity ratio to be 1/5, or 0.2.

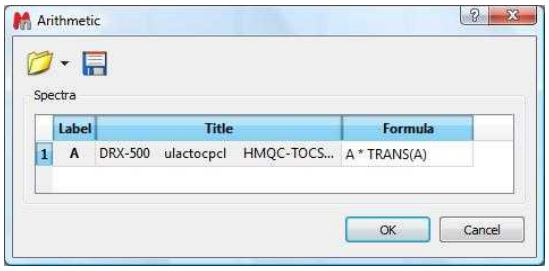
Typing the formula **'A-0.2B'** (or 'A-0.2*B') in the 'Arithmetic' dialog box, we shall obtain the spectrum below, where the solvent signals have been removed:

© 2024 MESTRELAB RESEARCH



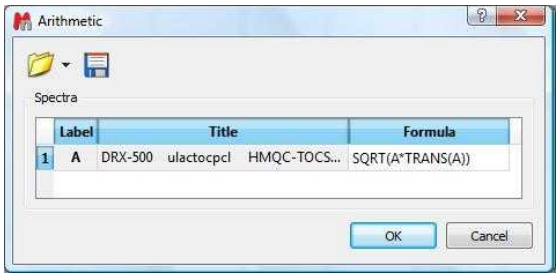
Arithmetic and Covariance NMR

2D NMR spectra are simply 2D matrices (Note: these matrices can be real, complex or hypercomplex, but for the sake of simplicity, we will consider real matrices only) which can be subject to standard matrix algebra operations. You can read the Covariance NMR chapter where it is shown how Indirect and Direct Covariance NMR can be applied by proper matrix algebra. These methods are incorporated in Mnova as a dedicated module which also makes the filtering of spurious resonances possible . In order to show you that Covariance NMR actually involves these matrix operations, you can use Mnova's powerful Arithmetic module which we have recently completed. This module has been designed in such a way that it operates as a simple spectral calculator. You enter the equation which the program parses and produces the expected result. For example, if we have a HSQC-TOCSY spectrum (**A**), we can enter the Indirect Covariance formula like this:



Where **A** corresponds to the real part of the original spectrum and **TRANS** indicates the transpose operation. This operation will produce the (unnormalized) Covariance NMR spectrum, in this case the 13C-13C correlation spectrum. Of course, in order to better approximate the covariance spectrum to the applicable standard 2D FT counterpart, it's necessary to calculate the square-root using, once more, matrix algebra. This is again very simple with our arithmetic module by just adding the square root operation (**SQRT**) into the equation:

© 2024 MESTRELAB RESEARCH



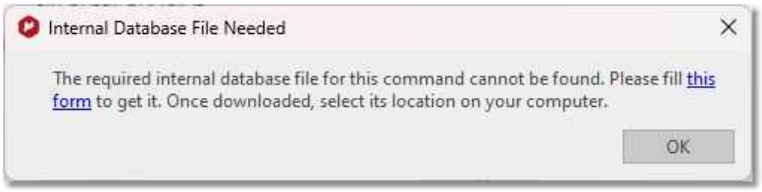
This arithmetic module is not restricted to matrix operations within a single spectrum. We can freely combine as many spectra as we want. For instance, if we have, in the one hand a COSY spectrum and in the other a HSQC spectrum, we can combine both in an analogous way so that the indirect covariance NMR spectrum yields a HSQC-COSY 2D spectrum.

8.9 13C/HSQC Molecular Search

Etymologically, dereplication means “removal of replicas” although today this term is used in many different processes with very broad applications. Dereplication aims at answering one fundamental question: given this experimental data set (e.g. an NMR spectrum), does it correspond to a known compound? Is it new?

Dereplication by NMR usually consists of a spectral search in a large database of assigned NMR data and this will clearly only be of value if the DB contain as complete a collection of compounds data as possible.

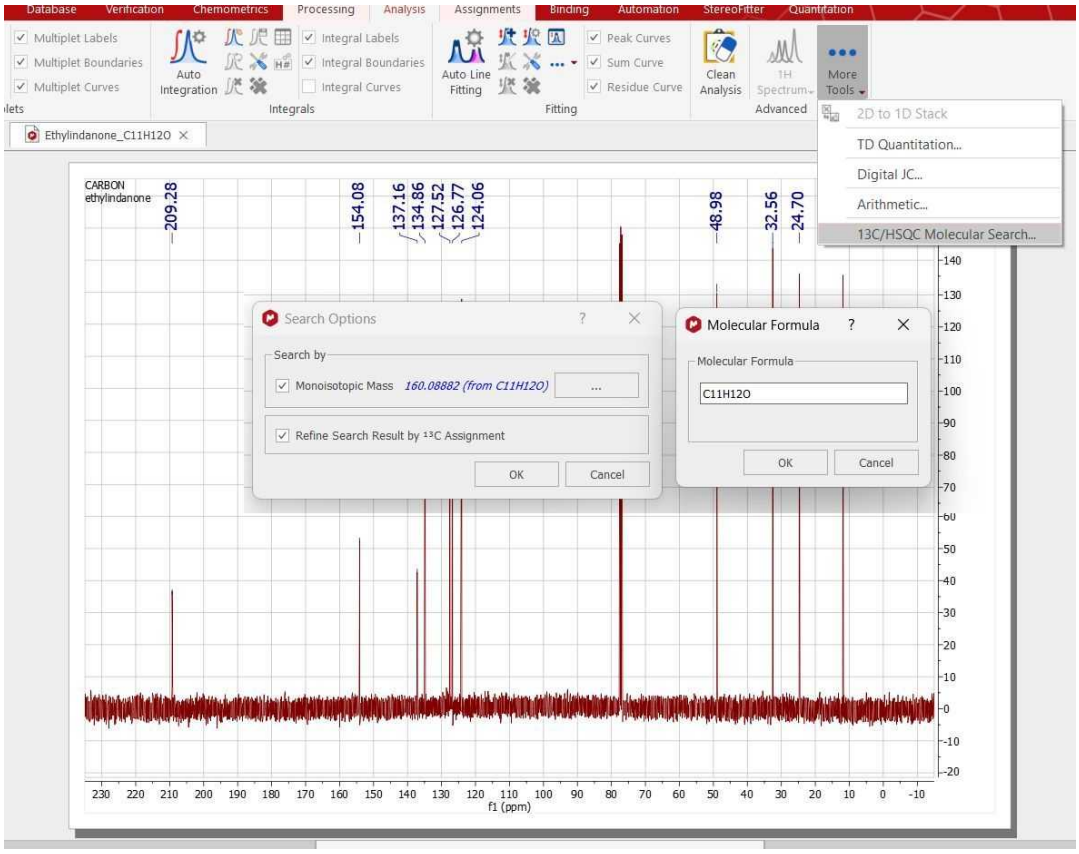
The first time that you use this tool, you will need to follow the menu 'Analysis/More Analysis/13C/HSQC Molecular Search" and select the path of the .madb file containing the 13C predicted spectra database (after having clicked on the OK button of the error message below):



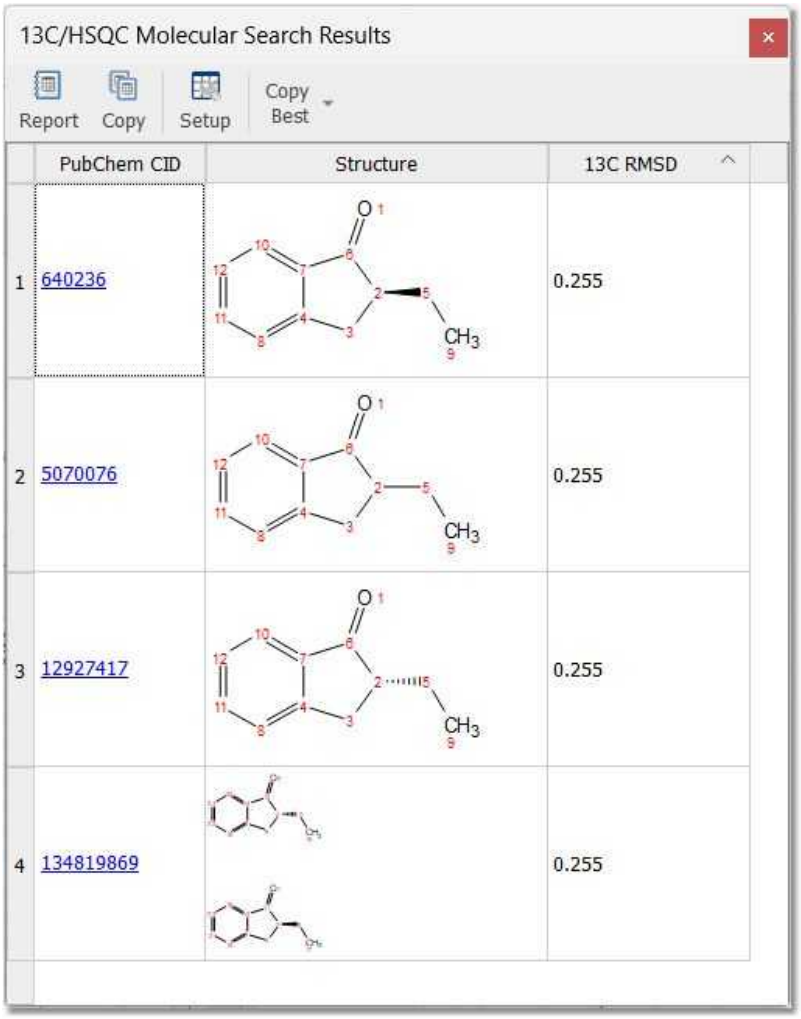
You can download the Database file by filling this form.

Next, apply a Peak Picking or Multiplet analysis in a 13C NMR spectrum (making sure that you do not select impurity signals), follow the menu 'Analysis/More Analysis/13C/HSQC Molecular Search and enter the molecular formula:

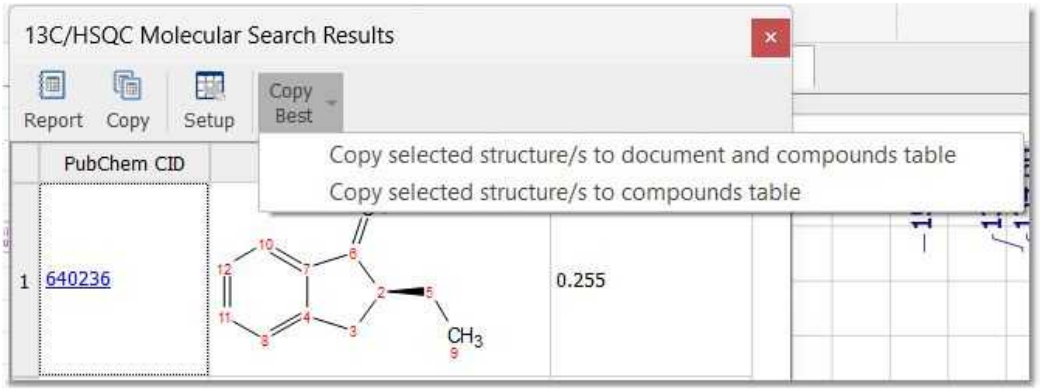
© 2024 MESTRELAB RESEARCH



Check the 'Refine Search Result by 13C assignment' box to autoassign structures (and reject imposible structures). After having clicked on the OK button, you will get the result with all the possible candidates sorted out by the 13C RMSD value:



From the table above, you can report the result, open the PubChem link for each compound and copy the molecules to the Compounds table and/or to the Mnova document:



Clicking on the 'Copy Best' button will paste the molecular structure of the compound with the lowest 13C RMSD value into your Mnova document.

© 2024 MESTRELAB RESEARCH

8.10 Line Fitting (Deconvolution)

When the spectrum is crowded with peaks, or affected by excessive noise, deconvolution may be the only way to measure the compounds ratio; but please bear in mid that this feature does not make miracles and it can not be a substitute for higher magnetic fields, more concentrated samples or longer acquisitions.

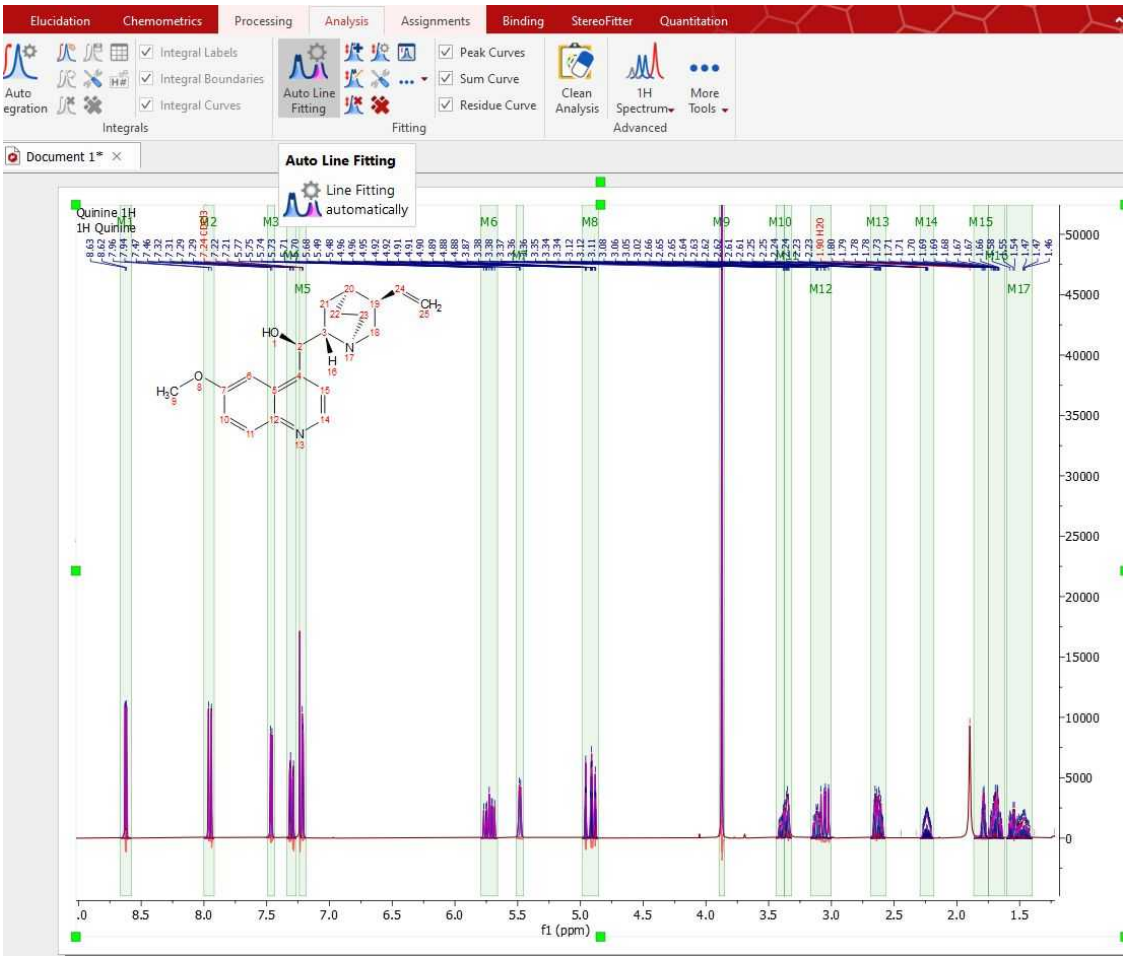
The name “deconvolution” means (more or less): “removing the shape”. The theoretical shape of an NMR peak is given by the Lorentzian equation. Field inhomogeneity and weighting functions can however yield partial Gaussian line-shapes. From a merely graphical (and practical) point of view, they are two complementary shapes, in the sense that most symmetrical peaks can be approximated with suitable combinations of Lorentzian and Gaussian components. For your convenience, Mnova defines a mixed function, for which you can vary at will the percentages of Lorentzian and Gaussian character.

MestReNova includes a fully interactive interface for the deconvolution of experimental spectra into individual lines.

This module includes options for locking any of the parameters during optimization and the ability to compare the resulting calculated spectrum, as a sum of the individual components, with the experimental spectrum.

It is really easy to run the Line Fitting feature. Just click on the 'Auto Line Fitting' button of the ribbon. If the spectrum has already regions (i.e. integral regions or multiplet regions), it uses them to create the fit regions. If not, it creates the fit regions using the current "autodetect" configuration (under the integration options) to calculate the integral regions.

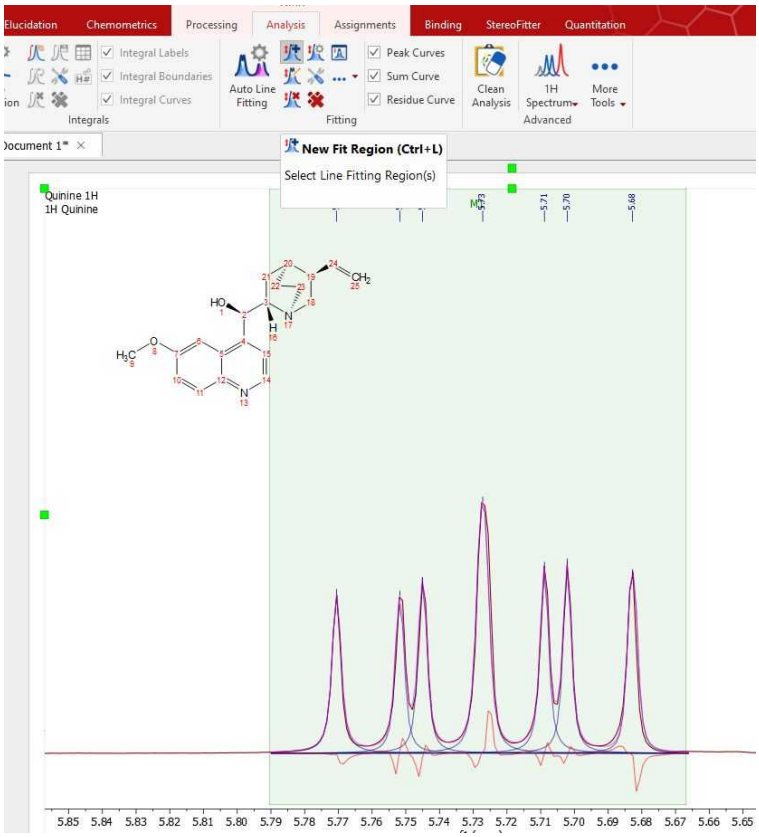
© 2024 MESTRELAB RESEARCH



You can also add manual regions by clicking on the 'New Fit Region' icon  (shortcut: Ctrl+L) and next clicking and dragging over the desired region of the spectrum. (Please make sure that you have previously corrected the baseline of the spectrum).

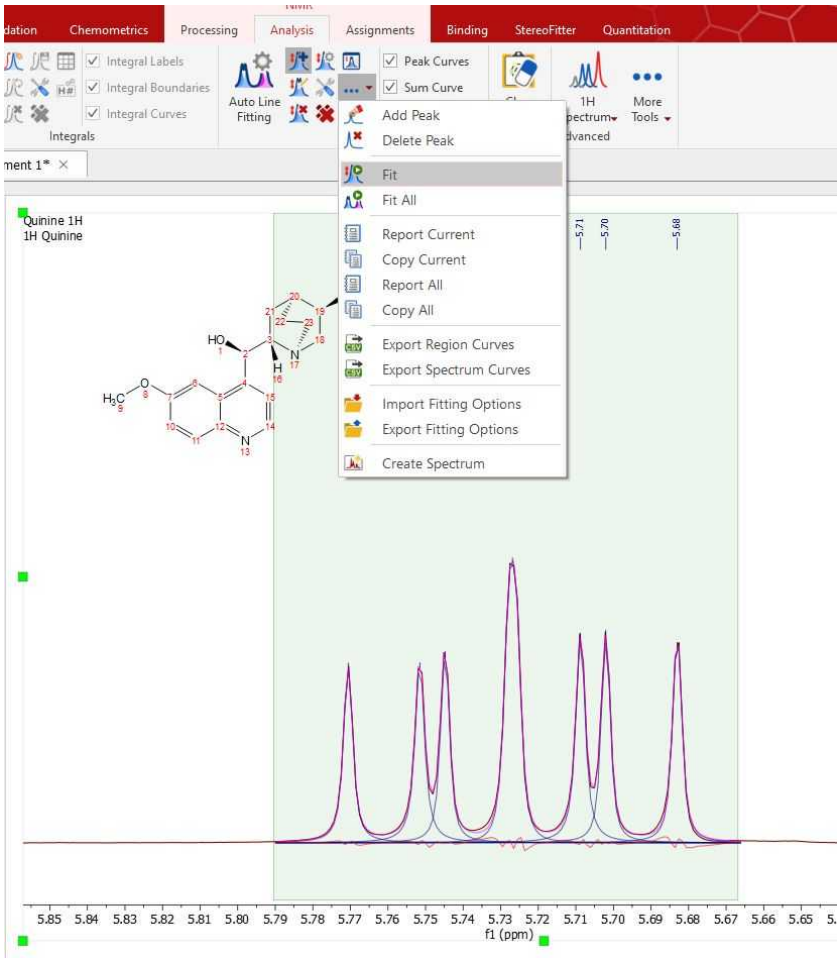
NOTE: It you prefer, you could apply a previous peak picking before selecting the Line Fitting region. To have a better control over the peak picking, you could use the ‘Peak by Peak’ mode or the ‘Manual Threshold’.

© 2024 MESTRELAB RESEARCH



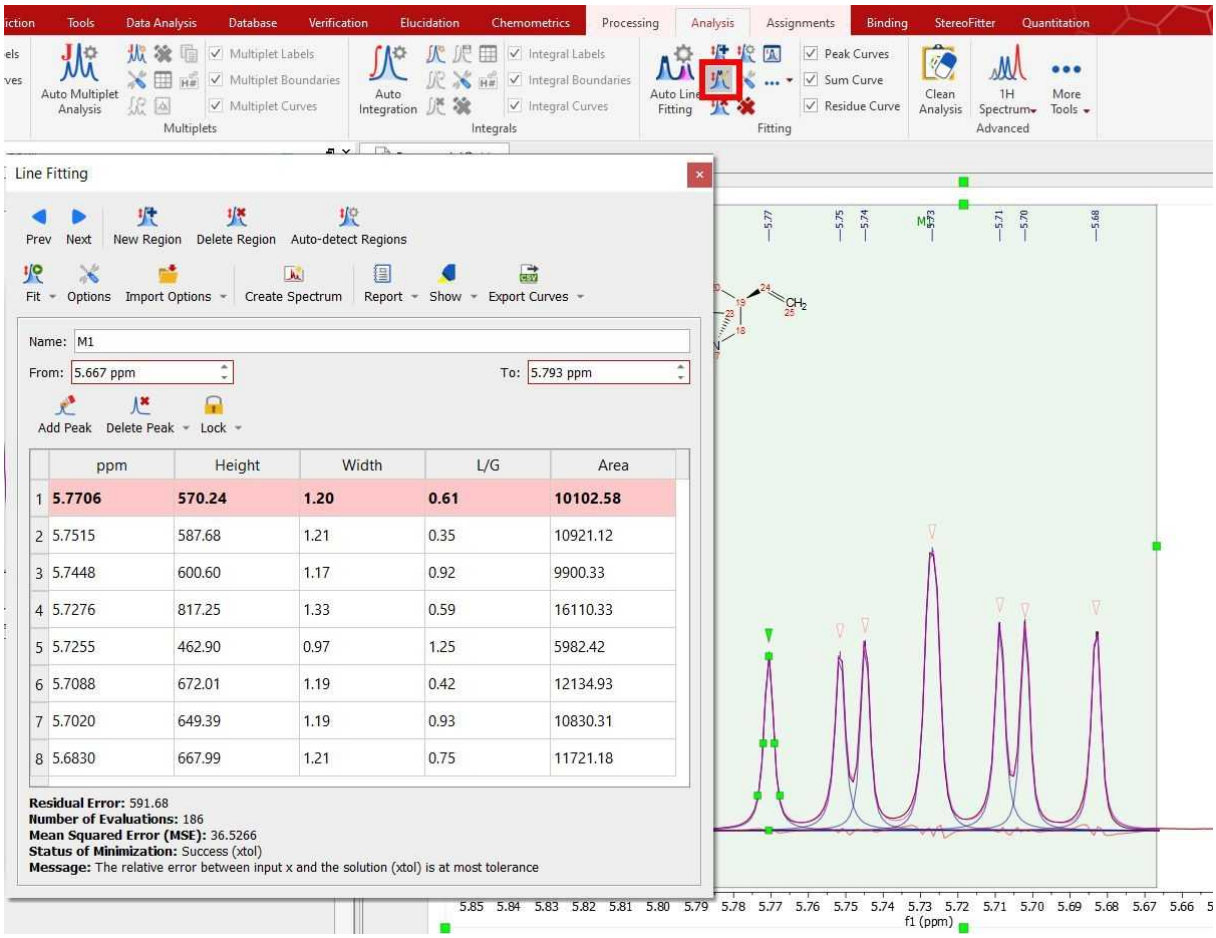
You can see in the picture above a blue line which corresponds with the fit peak, a red line which corresponds which the fitting residue and a magenta line which corresponds with the sum.

Next, select Fit under the scroll down menu to carry out the Fitting:



You will notice that the residue, the fit peak and the sum lines have changed. They constitute a starting point for the fitting (unless you are happy with the first fitting). If you are not happy with the initial fitting; you can add/remove components and change their parameters, just by selecting 'Edit Fit Region'. This will display the 'Line Fitting' panel, where you will find information about the peaks of interest (the chemical shift, in ppm; the Height, the Width, in Hz; the Lorentzian/Gaussian ratio and the Area).

© 2024 MESTRELAB RESEARCH

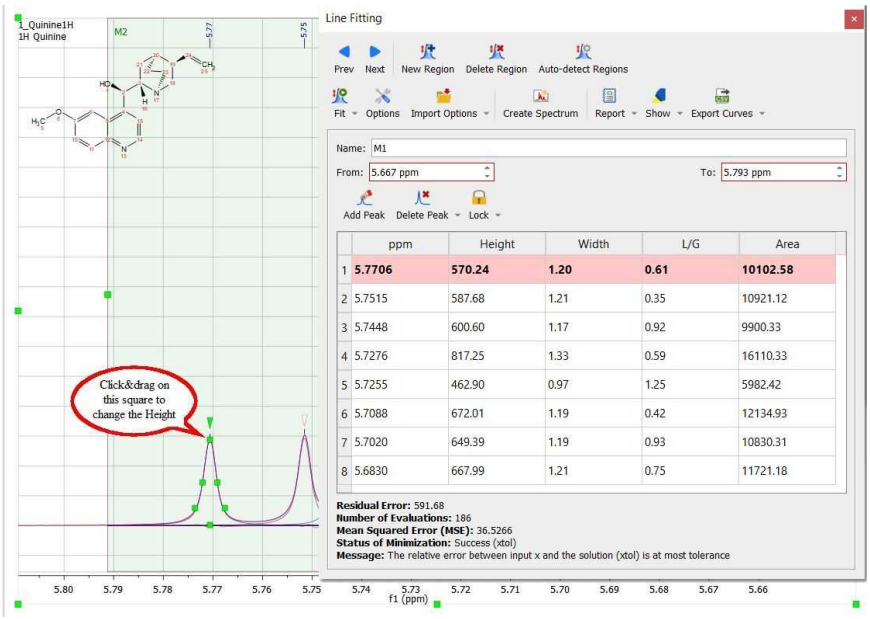


These parameters can be changed interactively over the spectrum (just by clicking and dragging over the green little squares) or by double clicking on each cell of the Line Fitting panel. Changes on any parameter will obviously affect to the 'Residual Error' (the smaller error, the better). For each point of the region, Mnova calculates the difference between the experimental and theoretical points, this value is powered by two and Mnova calculates the average of all the errors in the region of interest.

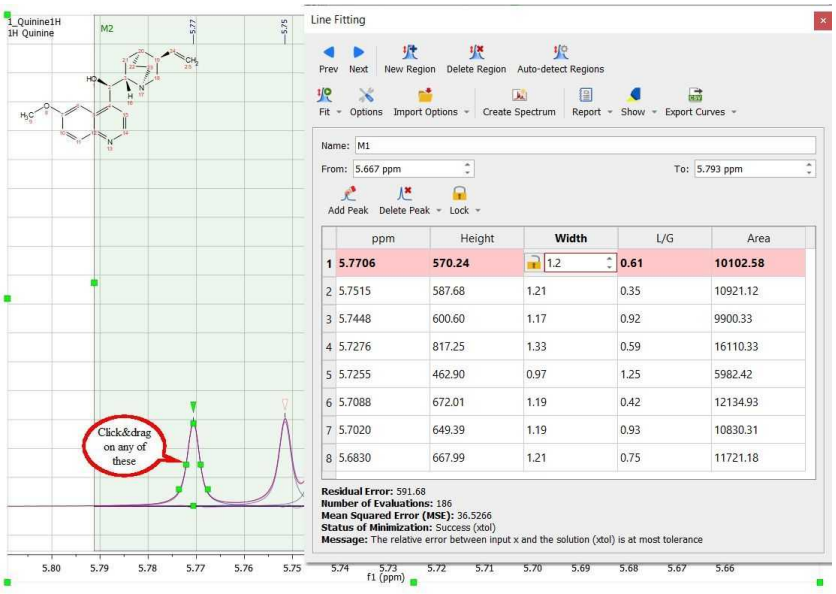
The MSE is a value obtained by the Levenberg-Marquardt algorithm after having applied the fitting. This algorithm returns the norm of the residual vector (resNorm), then MSE = (resNorm * resNorm) / numPoints

Clicking and dragging the green square at the top of the peak, will change the Height of the peak.

© 2024 MESTRELAB RESEARCH



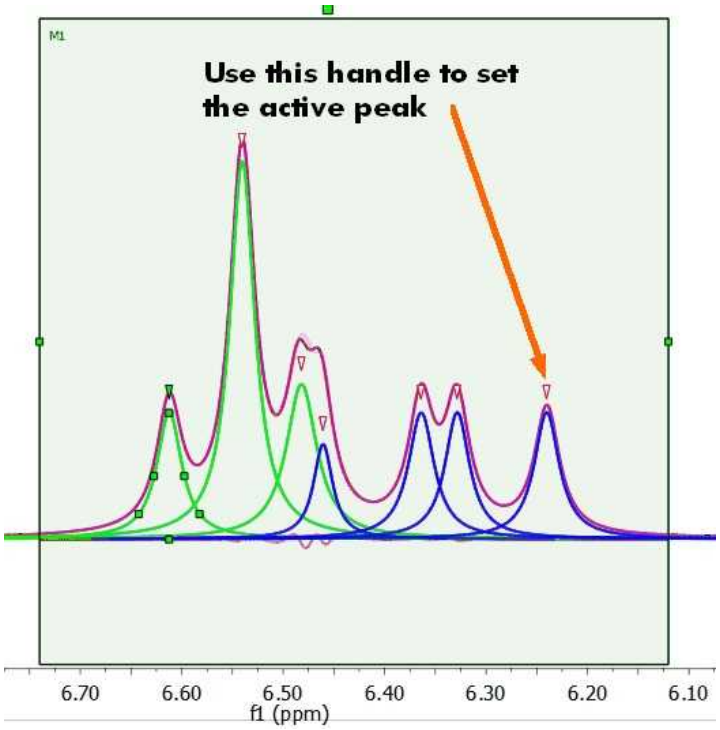
The squares at the mid-height of the peak can be used to modify the Width of the peak:



Clicking and dragging on the next two squares will modify the Lorentzian/Gaussian ratio of the signal and finally the last square will be used to modify the chemical shift of the signal.

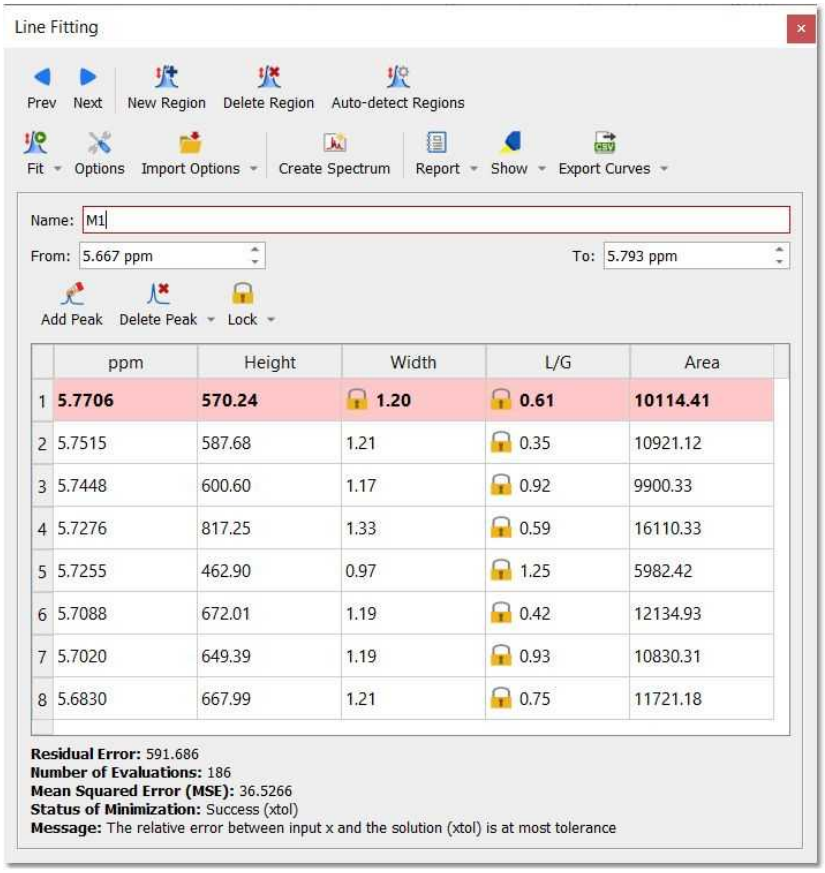
Clicking on the red triangles will set the active peak:

© 2024 MESTRELAB RESEARCH



Any of these parameters can be locked for the Fit, just by double clicking on the applicable cell of the panel and closing the lock which appears. In the picture below it is shown how we have locked the width parameter of the peak 1 and also the L/G ratio of all the peaks, so these parameters will not change when the fitting will be applied. So a lock in front of a value indicates that it will not be optimized in the fitting.

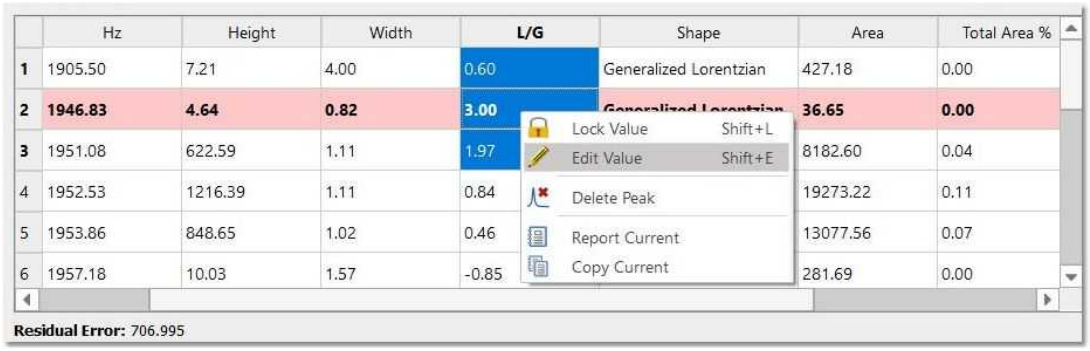
© 2024 MESTRELAB RESEARCH



You can also use the shortcuts below:
SHIFT+L: locks all the selected values
SHIFT+U: unlocks all the selected values
SHIFT+E: opens a dialog to edit the selected values

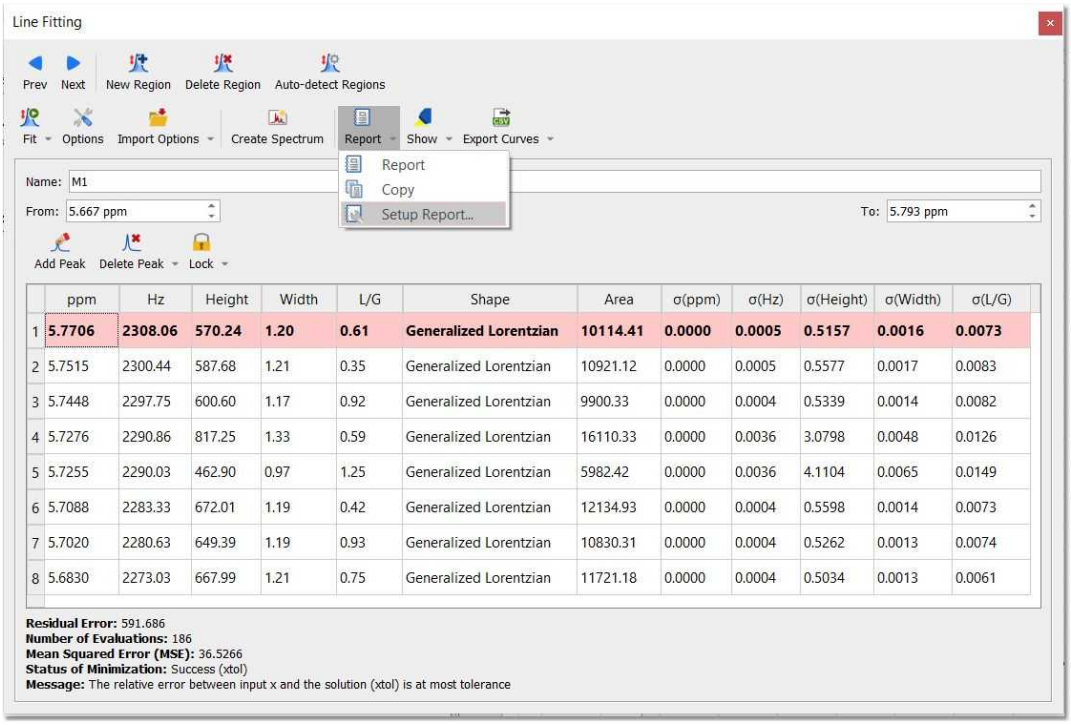
You can go through the table editing each cell by using the Enter key; which will display the editor dialog. Pressing the Enter key a second time, will change the value and close the editor.

You can edit several cells together just by highlighting them, right clicking to select 'Edit Value' and typing:



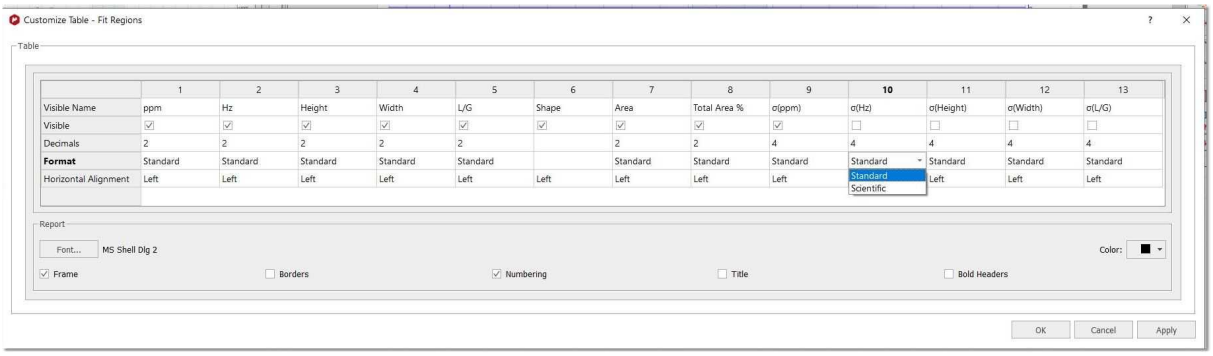
From the setup report dialog, you can display the standard deviation of ppm, Hz, Height, width, L/G and the peak shape:

© 2024 MESTRELAB RESEARCH



	ppm	Hz	Height	Width	L/G	Shape	Area	σ(ppm)	σ(Hz)	σ(Height)	σ(Width)	σ(L/G)
1	**5.7706**	**2308.06**	**570.24**	**1.20**	**0.61**	**Generalized Lorentzian**	**10114.41**	**0.0000**	**0.0005**	**0.5157**	**0.0016**	**0.0073**
2	5.7515	2300.44	587.68	1.21	0.35	Generalized Lorentzian	10921.12	0.0000	0.0005	0.5577	0.0017	0.0083
3	5.7448	2297.75	600.60	1.17	0.92	Generalized Lorentzian	9900.33	0.0000	0.0004	0.5339	0.0014	0.0082
4	5.7276	2290.86	817.25	1.33	0.59	Generalized Lorentzian	16110.33	0.0000	0.0036	3.0798	0.0048	0.0126
5	5.7255	2290.03	462.90	0.97	1.25	Generalized Lorentzian	5982.42	0.0000	0.0036	4.1104	0.0065	0.0149
6	5.7088	2283.33	672.01	1.19	0.42	Generalized Lorentzian	12134.93	0.0000	0.0004	0.5598	0.0014	0.0073
7	5.7020	2280.63	649.39	1.19	0.93	Generalized Lorentzian	10830.31	0.0000	0.0004	0.5262	0.0013	0.0074
8	5.6830	2273.03	667.99	1.21	0.75	Generalized Lorentzian	11721.18	0.0000	0.0004	0.5034	0.0013	0.0061

From the setup report dialog, you can also select the option to display the values in scientific notation:

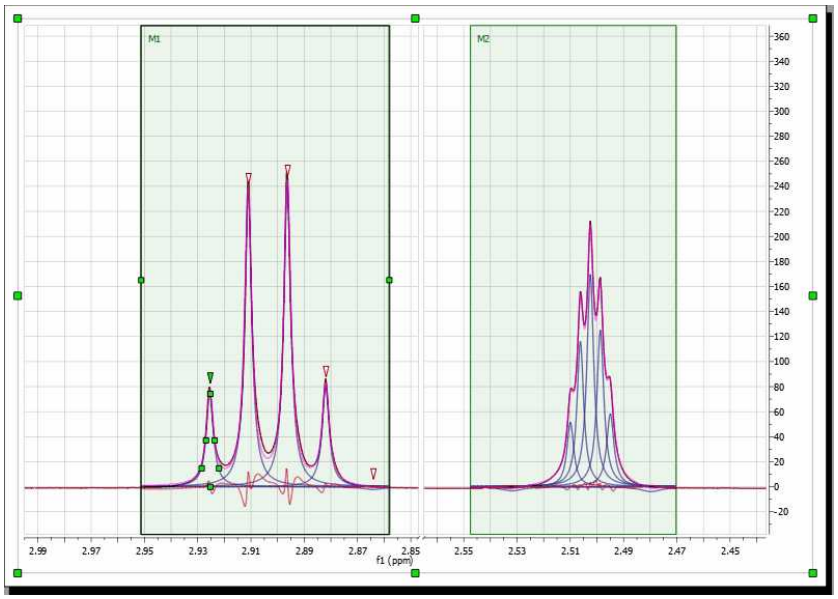


Once you have run the fitting of several regions, you will find information about the residual error, number of evaluations, MSE (Mean Squared Error), status of minimization (the result of the fitting) and a message (explaining the meaning of the status of minimization).

L/G ratio: it does not pretend to be a mathematic representation. It is like a ratio between L and G. The value of the function to be fit would be:
LG(x) = LGFactor*L(x) + (1-LGFactor)*G(x)

Of course you can fit several regions within a single spectrum, just by selecting a 'New Fit Region':

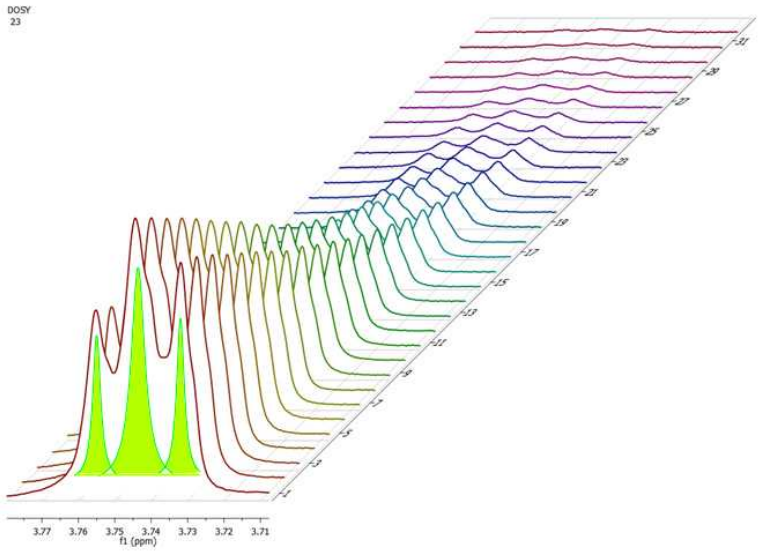
© 2024 MESTRELAB RESEARCH



Once you are happy with all the parameters, you will only need to apply a new Fit, just by clicking on this icon 

. If you have several regions, you will need to select 'Fit All' in the Fir scroll down menu. Once the fitting has been made, if you want to get the Fitted spectrum just click on the 'Create spectrum' icon 

You can also use the Line Fitting feature for the simultaneous fitting across arrayed spectra. Just select the region and the fitting will will take place along all the arrayed spectra:



You can apply line fitting options and parameters for all stacked spectra.

Follow the menu 'Tools/Export/Fit Regions' to export the results in a .txt file.

You can copy the Line Fitting regions from one spectra to another by selecting the first spectrum and doing 'Ctrl+C'; next jump to the second spectrum and follow the menu 'Home/Paste/NMR Fit Regions'. A new dialog box will be displayed with the options below:

© 2024 MESTRELAB RESEARCH



- The ***Regions*** option pastes only the regions and fulfill them with an automatic peak picking. The regions are bounded to the current spectrum's range.

- The ***Regions & Peaks*** option pastes the regions and their peaks, exactly as they are in the copied spectrum (same Shift, Height, Width, and L/G).

- The ***All*** option pastes the regions, peaks and locks, exactly as they are in the copied spectrum.

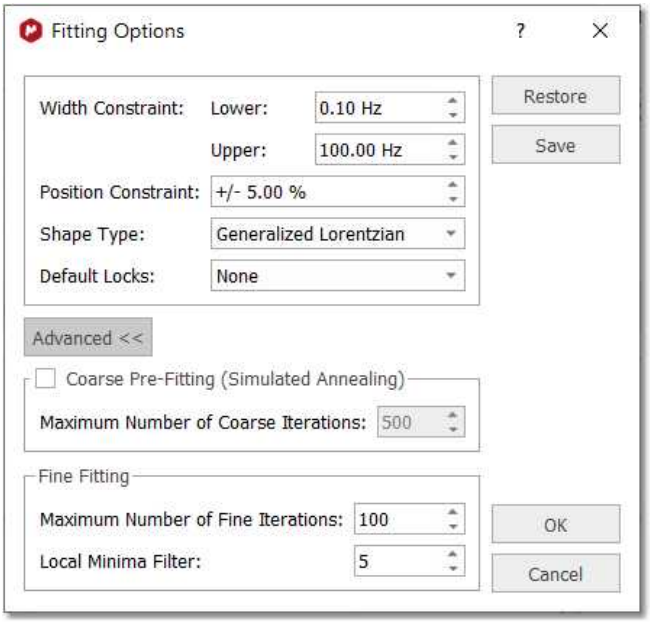
- The ***Apply Fitting*** option executes the fitting of the regions after pasting them.

Other Options of the Line Fitting panel

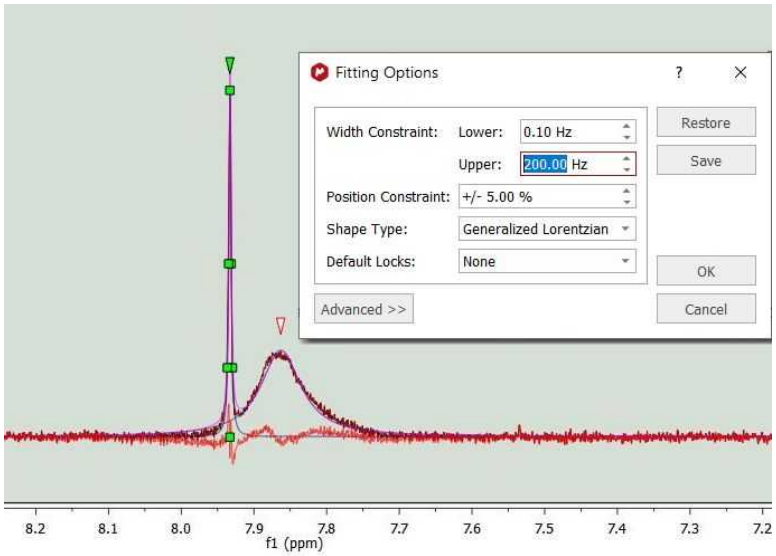
The Line Fitting panel includes other tools, let´s see the meaning of them:

New Fit region: use this icon to create another region to be fit.
Delete region: to delete the current region.
Detect Line fitting regions automatically: it will apply an automatic Line Fitting over the regions detected by the automatic integration tool.
Fitting Options: to display the Fitting Options window where the user will be able to establish some constraints for the fitting. Line Widths and Chemical Shift parameters can be constrained within user-defined values:

© 2024 MESTRELAB RESEARCH



If the spectrum contains broad signals, just increase the Width Constraint to get good results. In the example below the Upper width constraint has been increased to 200 Hz:



Position Constraint: it takes into account the values outside the range (p=shift) p*(1-pos_constraint*p). A big value of the algorithm will 'shift' the peak far. Normally, you will not need to change the default value, because the peak should be close to the original position.

Shape Type: you can select between 'Generalized Lorentzian' or 'Lorentzian-Gaussian'.

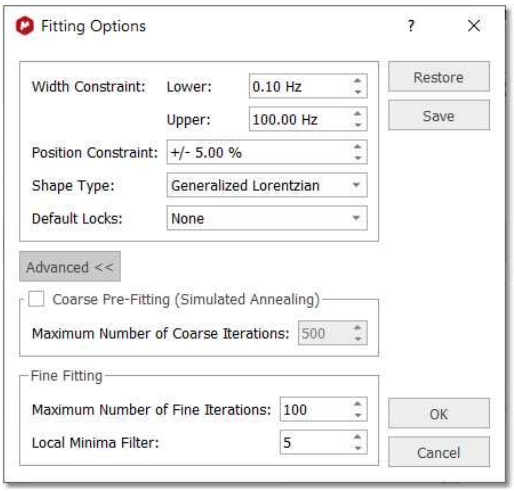
'Generalized Lorentzian': The new generalized NMR peak shape profile proposed (which is a lorentzian plus a factor by another function), generalizes the ubiquitous Lorentzian shape. The mathematical expression (to be published) for the profile function contains only one extra parameter in addition to the three parameters of the Lorentzian line (location, height, and width). The new parameter does not interfere with any of the three 'classical' parameters (orthogonality) but affects the peak's kurtosis (for this reason we call it the kurtosis parameter). It is a-dimensional and has a 'natural' physical range of -

© 2024 MESTRELAB RESEARCH

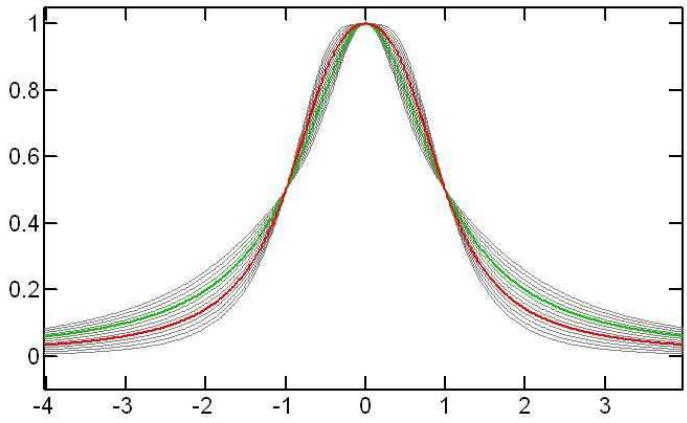
1.0 to 2.0 (beyond these limits the formula is still operative but the peak profile starts splitting into a doublet or triplet). There are strong physical and conceptual reasons to expect that the new family of shape profiles will be much more useful in NMR spectroscopy than any of the often-used mixtures of Lorentzian and Gaussian shapes. In particular, we expect it to improve the quality of quantitative assays through NMR spectra and help the process of automatic editing of NMR peaks prior to structure verification.

NOTE: When a GSD peak picking is done the assumed peak shape is the Generalized Lorentzian.

By default, Mnova uses the so-called “Generalized Lorentzian” Lineshape which is similar but not equivalent to the linear combination of a Loretzian-Gaussian lineshapes. The type of lineshape can be selected in the Line Fitting options, as shown in the figure below:



From a more theoretical point of view, to partially mitigate the unavoidable line shape imperfections found in NMR spectra, we have introduced the idea of a Generalized Lorentzian peak shape (GL). A single extra parameter describes the peaks shape deviation from the Lorentzian, which we call somewhat loosely the ‘kurtosis parameter’ (in the Line fitting dialog this corresponds to the L/G parameter which somewhat mimics the Lorentzian/Gaussian ratio, although this is not strictly the same, as discussed next). Briefly, the GL peak shape, though using just one ‘extra’ parameter, covers deviations from the Lorentzian about 3 times broader (in terms of kurtosis) and extending to both sides of the Lorentzian. This is illustrated in the figure below where the green line is pure Lorentzian, the red line is pure Gaussian (of the same half-height linewidth) and the gray lines are generalized Lorentzians:



© 2024 MESTRELAB RESEARCH

When a linear combination of the Lorentzian-Gaussian lineshapes is used, the result is essentially an interpolation between the green and red lines so that, as can be seen in the figure, the actual range covered by this composite lineshape is more limited compared to the proposed Generalized Lorentzian.

The GL thus provides more flexibility. At the same time because it is a rational function of the frequency offset, its imaginary part and its integral are easy to evaluate using exact, explicit formulas. This is not the case for a Gaussian line shape.

Mathematically, the Generalized Lorentzian (GL) function is defined by [1], where the amplitude has been assumed to be 1 and *x* corresponds to the frequency offset:

$$GL(x;k) = (1-k)L(x) + kG(x) \qquad [1]$$

where k is the “kurtosis parameter”, so called because it affects the peak’s kurtosis and

$$G(x) = \frac{1+x^2/2}{1+x^2+x^4} \qquad [2]$$

$$L(x) = \frac{1}{1+x^2} = \frac{1}{(x+i)(x-i)} = \mathrm{Re}\left(\frac{i}{x+i}\right) \qquad [3]$$

As opposed to a L/G ratio which is within the [-1,1] range, the kurtosis parameter can take broader values, but they should usually be restricted to the [-2,+2] interval.

Default Locks: you can select from here what parameters you want to lock by default.

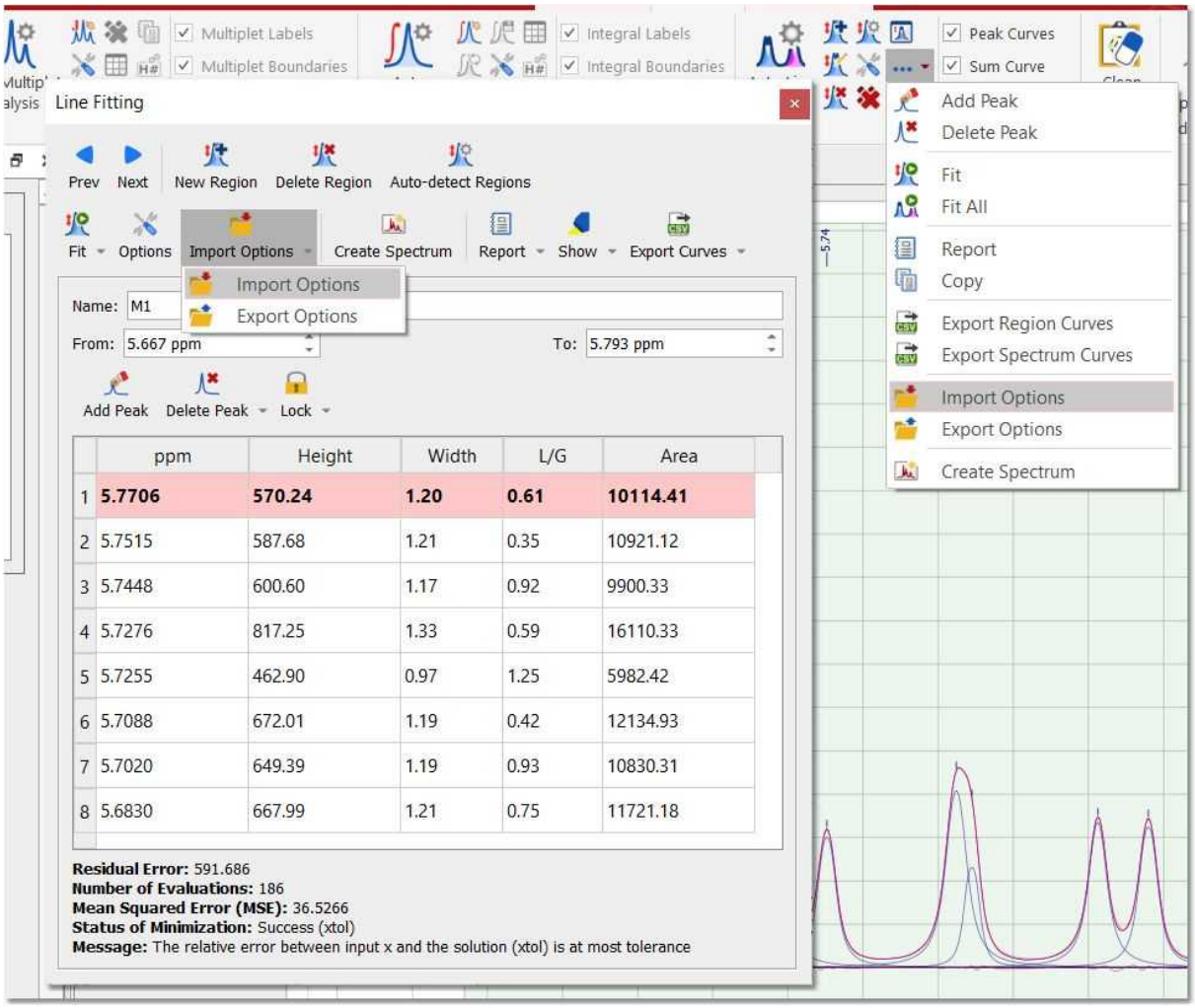
Coarse Pre-fitting (Simulated Annealing): coarse fitting algorithm which will look for random solutions, keeping the best one as starting point for the *Fine Line Fitting* in order to get a better solution. The user will need to select the Maximum Number or coarse iterations (to find the initial starting point). This algorithm will be faster but less precise than the Fine Line Fitting.

Fine Fitting: this algorithm uses a variation method to find the solution. With the initial parameters, it calculates the difference between the result and the real curve; after that, it modifies a little these parameters to achieve the real curve.

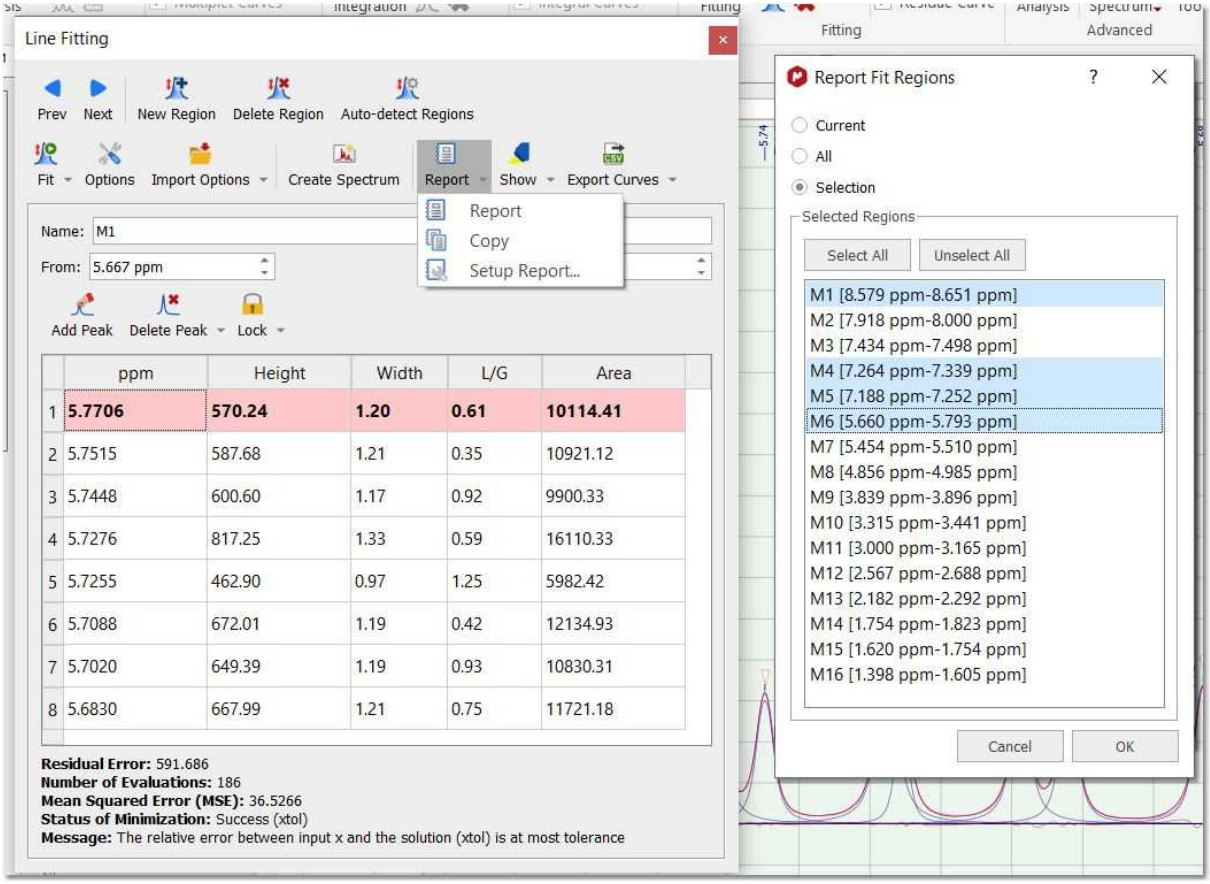
Local Minima Filter: The iterations stop when you reach the maximum number of iterations, when the difference with the real curve is too small, or when after several iterations, the variation of the parameters does not improve the fitting result. This parameter controls how much the parameters vary on each iteration. A high value means that the parameters change a lot on each iteration, which will be very useful in those cases where the initial curve is really different with the real curve. The user should not need to change this value.

You can export (and import) the Line Fitting options from the 'Line Fitting ribbon' and from the 'Edit Line Fitting' dialog box:

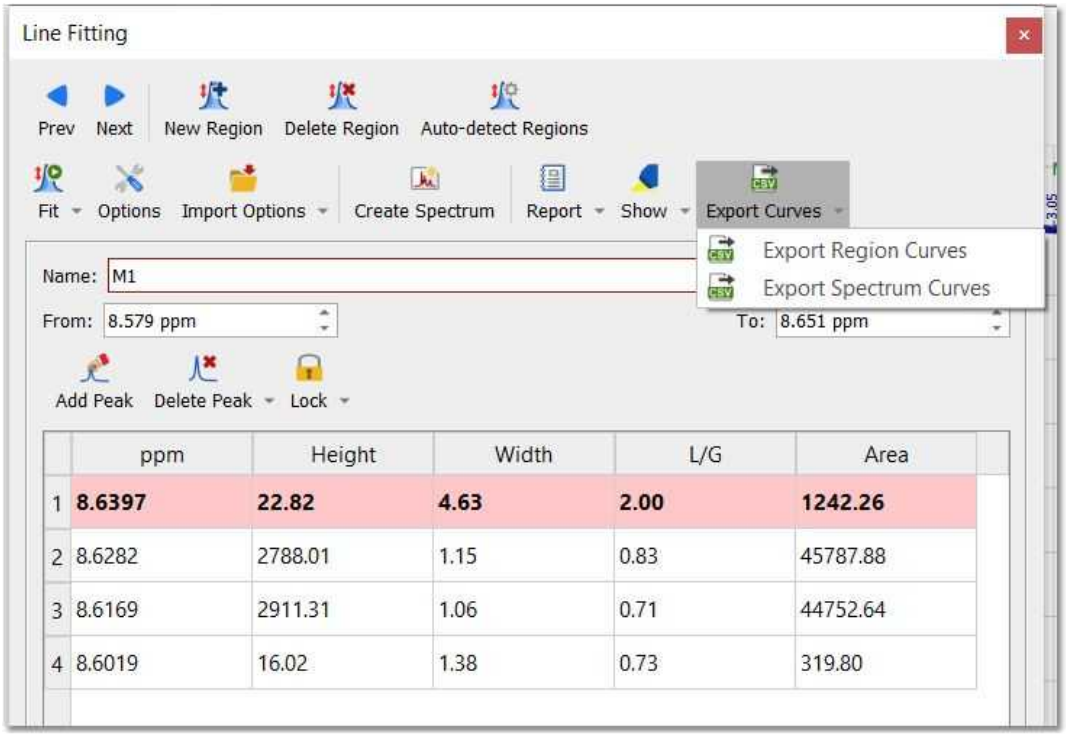
© 2024 MESTRELAB RESEARCH



Report: to report the Line Fitting panel. Selecting the 'Report' option will allow you to report the current region, all or a selection. From here you can also copy the report to the clipboard or customize the format of the report.



Export: to export the current fit regions or the entire spectrum curves as a CSV file.



© 2024 MESTRELAB RESEARCH

Once you have selected one of those options, you will have the capability to select if you want to include the intensities, sum, residue or peaks:



The resulting CSV file will have the column ppm under the "x" coordinates, and the respective columns with the "y" coordinates of those type of data selected (i.e. Intensities, Sum, Residue, Peaks). In the case of Peaks, there will be one column for each peak in the fit region / spectrum. The values are separated by commas.

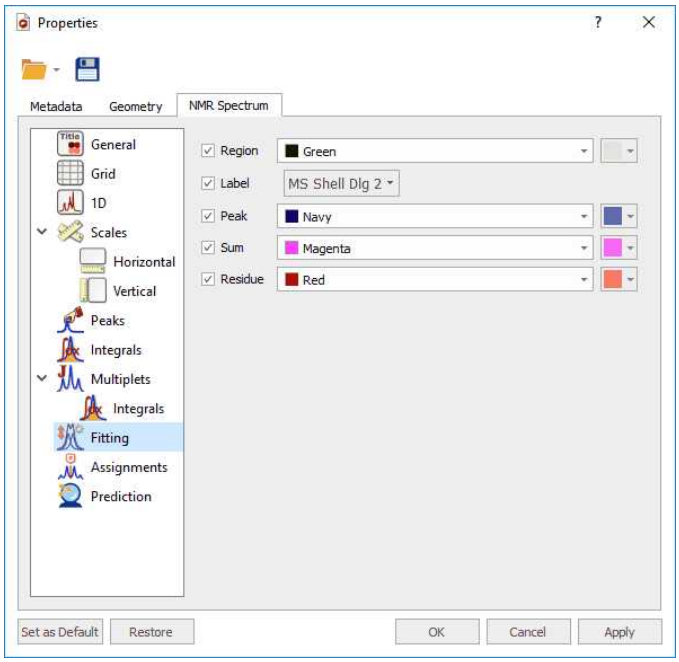
Follow the menu 'Tools/Export/Fit Regions' to export the results in a .txt file.

Select Lines to Show: toggle button to show or hide the fitting lines (peaks, sums or residues)
Previous/Next Region : to navigate through the fit regions.
Name: to change the name of the fit region.
From/To: to change the rang of the fit region.
Add Peak: to add a new peak to the region
Delete Peak: to delete the current peak of the region (or all peaks at the same time)
Create spectrum: To generate a new spectrum from the fit regions.

8.10.1 Line Fitting Properties

You can customize the colours (and transparency) of the Fit regions, label and lines (Peaks, Sum and residue) from the 'Properties' dialog box, which can be accessed by double clicking on the spectral window and selecting 'Fitting' in the left tabbed menu:

© 2024 MESTRELAB RESEARCH



© 2024 MESTRELAB RESEARCH

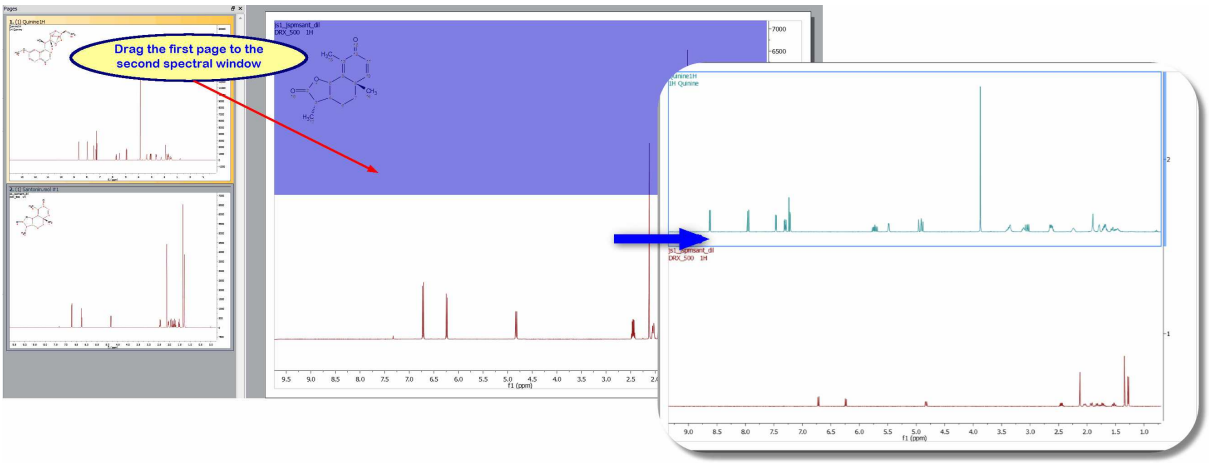
Part



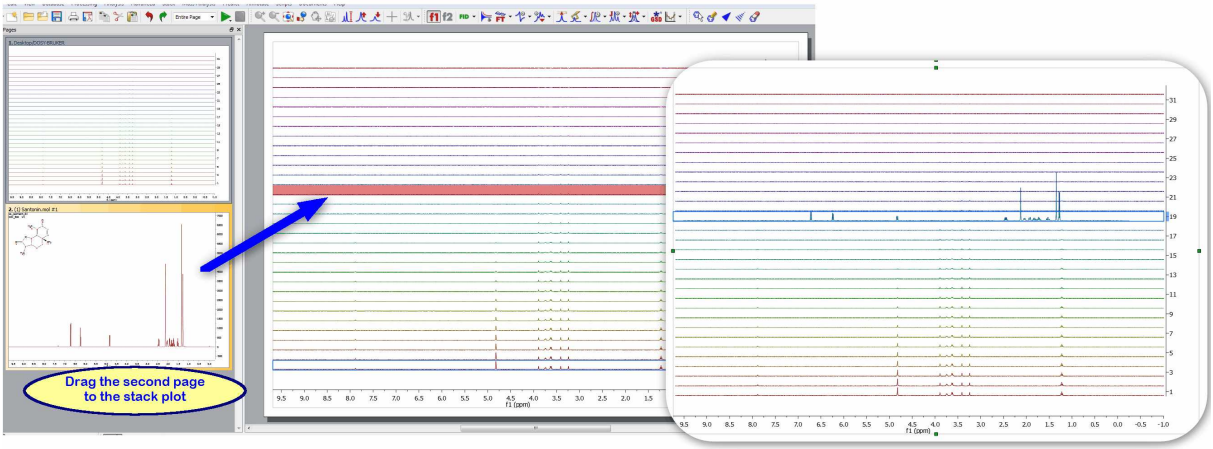
9 Stacked NMR Processing

Many types of experiments require obtaining a series of FIDs, related to each other through the variation of one or more parameters (temperature, concentration, solvent...). The user, for example, may need to run several spectra at different temperatures: 270K, 300K, 330K, 360K and 390K. Instead of acquiring five different sets of data, the user is able to create an array in which the temp parameter is given for successively different values.

Creating a stacked (or pseudo 2D) spectrum with Mnova is very easy; just by **dragging** one spectrum from the 'Page Navigator' to another spectral window. In the example below we are dragging the first page into the second spectrum to generate a stack plot:

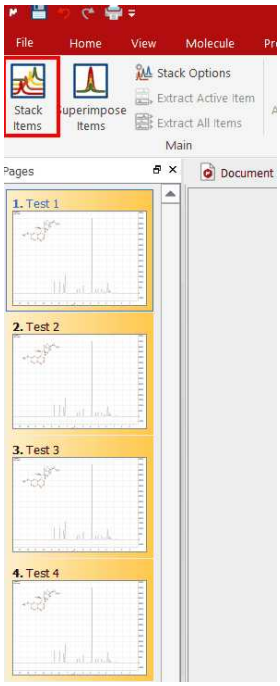


You can use this method to add a spectrum to an existing stack. In the example below we are adding the spectrum in the second page as the trace 19th of the stacked plot:

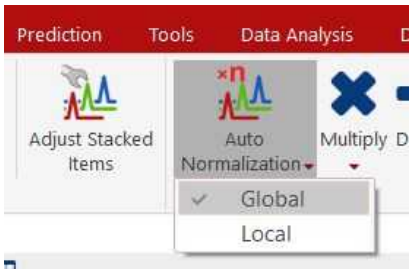


You can also create a stack plot by selecting the desired spectra in the page navigator (by holding down 'CTRL/Cmd key' while clicking on each spectrum) and then issue the command 'Stack/Stack Items':

© 2024 MESTRELAB RESEARCH



You can also normalize the traces of your stacked plot by clicking on the 'Auto Normalization button, which has a dropdown menu to select if the action applies to the full spectrum (Global) or to the zoomed region (Local).



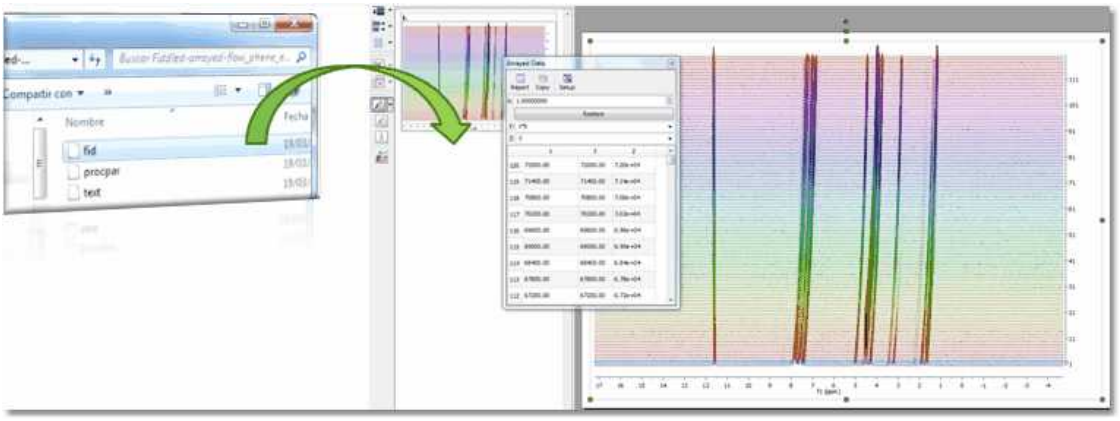


NOTE

By default Mnova will not delete the 1D spectra after having stacked them. If you want to modify this default, you will need to follow the menu 'Stack/Stack Options' and uncheck the applicable box.

Additionally, Mnova can detect spectra which have been acquired in the so-called arrayed-mode (or pseudo 2D), typically used in relaxation, kinetics or diffusion experiments. For all these cases, Mnova will only process the acquired dimension (t2). Basically, Mnova will process only the rows of the data matrix and, by default, will display the spectrum as a stacked plot (see below):

© 2024 MESTRELAB RESEARCH



You can also import all the spectra from the same folder and create a stacked plot by using the 'Directoy Spectra Stack' script from the 'Tools/Import' menu.

A few tips on Stacked Spectra

- Change the stacked angle to zero (15º by default) in the properties dialog box (by double clicking on the spectral window).
- Normalize the spectra (if they have different intensities) by using the command: 'Processing/Normalize'.
- Highlight the desired trace of the stack plot just by clicking on it.
- Create a stack spectrum just by dragging one spectrum to another.

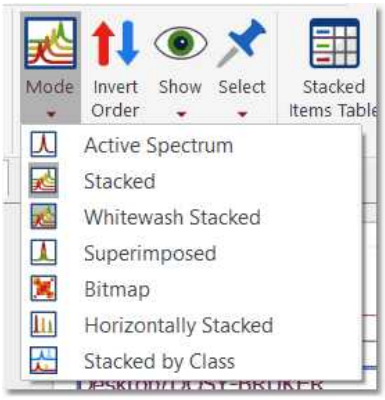
Display Modes:

The main features to handle arrayed spectra will be described below:

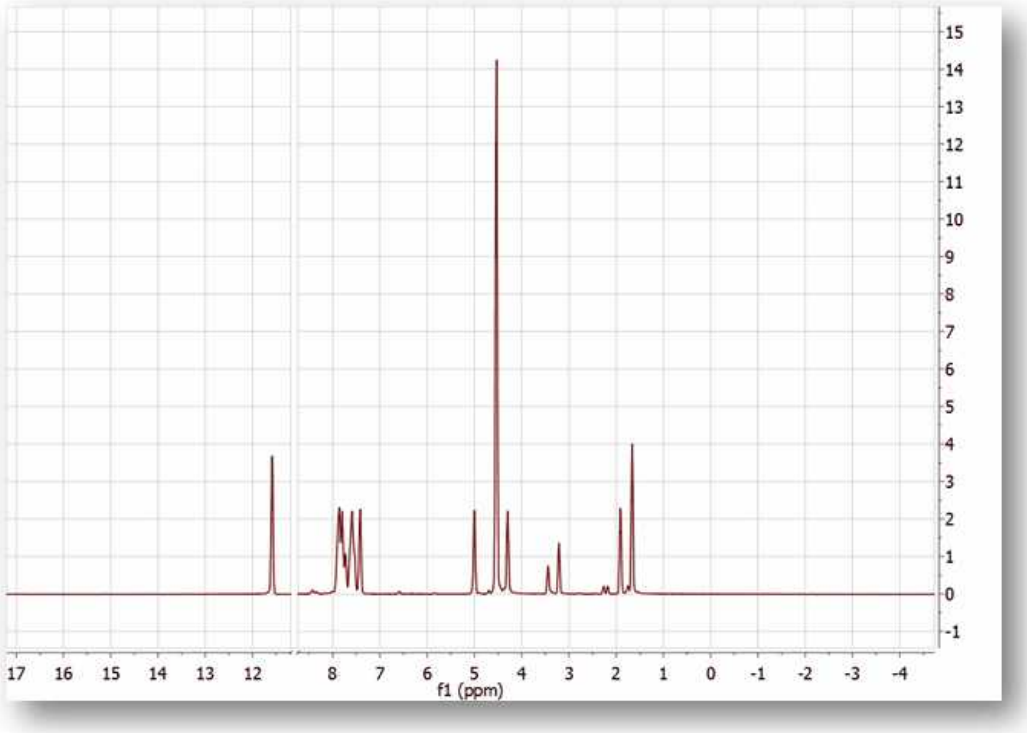
- Multiple display modes: Stacked, superimposed, active, bitmap, horizontal stacked.
- Ability to select multiple spectra and apply processing only to those selected spectra
- Ability to hide any number of spectra
- Multiple selection can be done either graphically in the stacked plot mode or by means of a table
- New powerful algorithm for the automatic alignment of arrayed spectra
- Ability to automatically import any number of spectra and automatically stack them
- New selection mode to extract NMR descriptors (e.g. integrals) with the ability to adapt its shape to any profile (useful when peaks are not aligned)

As you well know there are several display modes of arrayed datasets, as stacked, superimpose, active or bitmap. You can select the mode by using the applicable scroll down menu:

© 2024 MESTRELAB RESEARCH

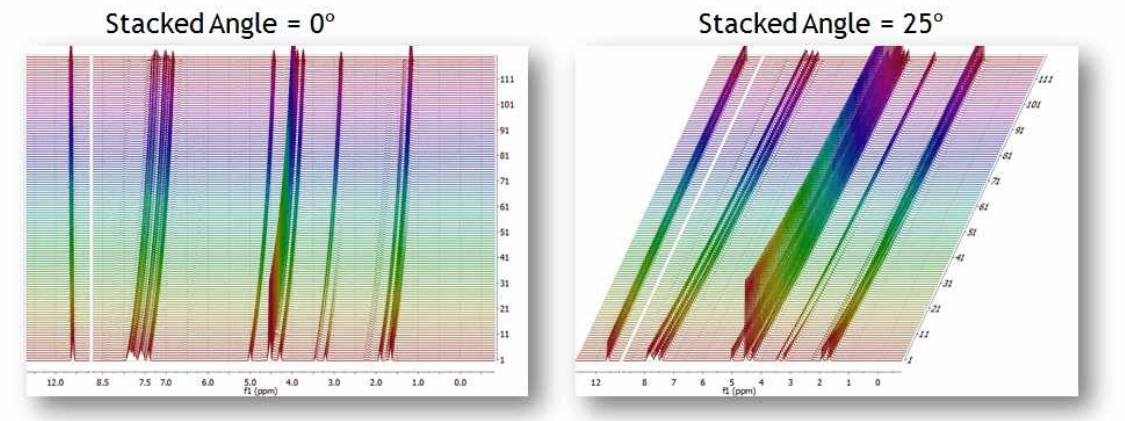


'Active Spectrum': to see only the highlighted spectrum. Within this mode, you can navigate along all spectra in this stack by using Shift+Mouse wheel (or arrow keys up&down).

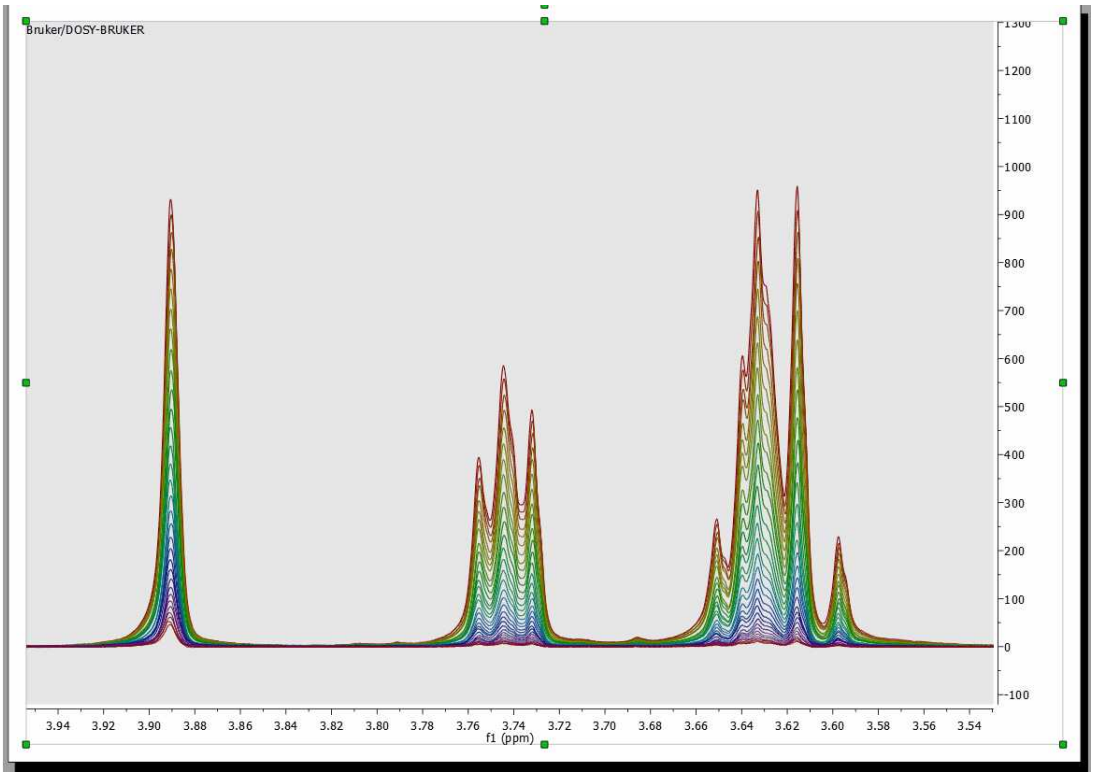


'Stacked': to see all the spectra in arrayed mode. You can change the angle from the Properties dialog box.

© 2024 MESTRELAB RESEARCH

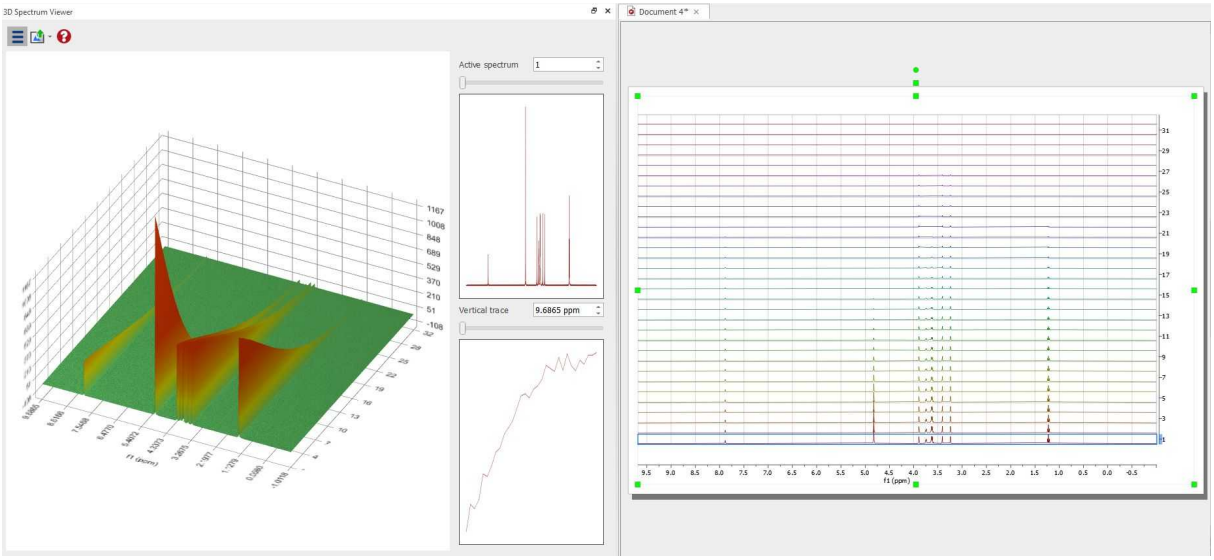


'Superimposed': to see all the spectra overlayed (one over another).

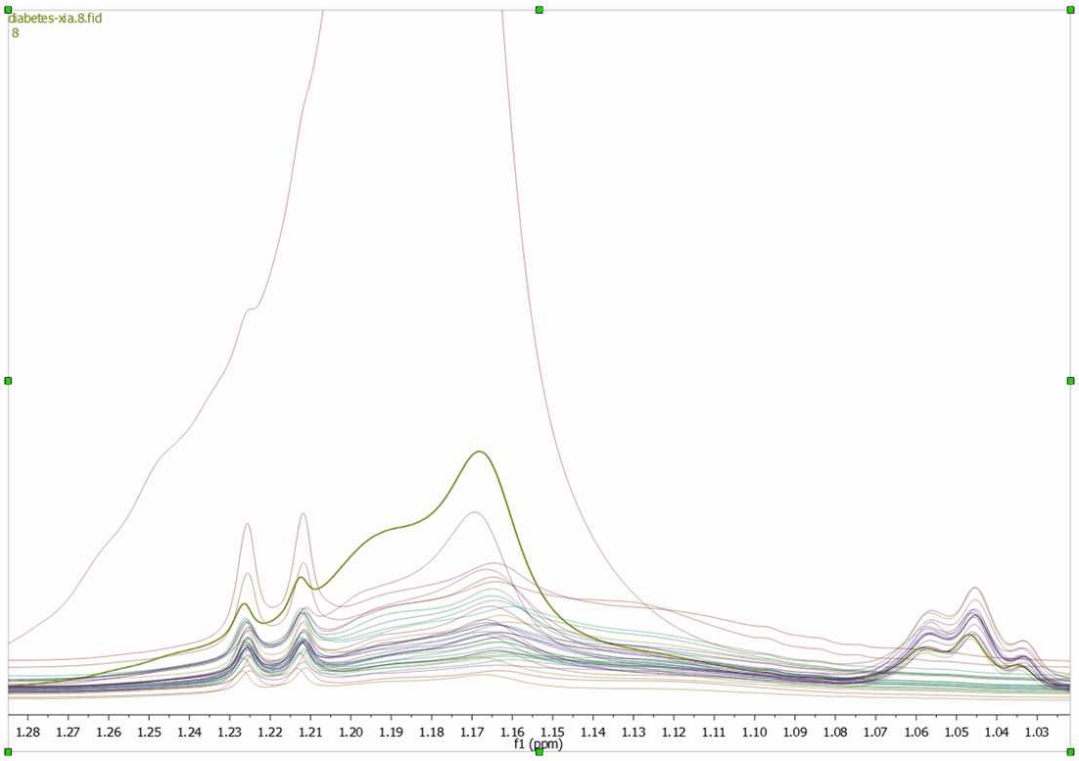


Loading a stacked plot of 1D NMR spectra will also display the result in the 3D viewer:

© 2024 MESTRELAB RESEARCH

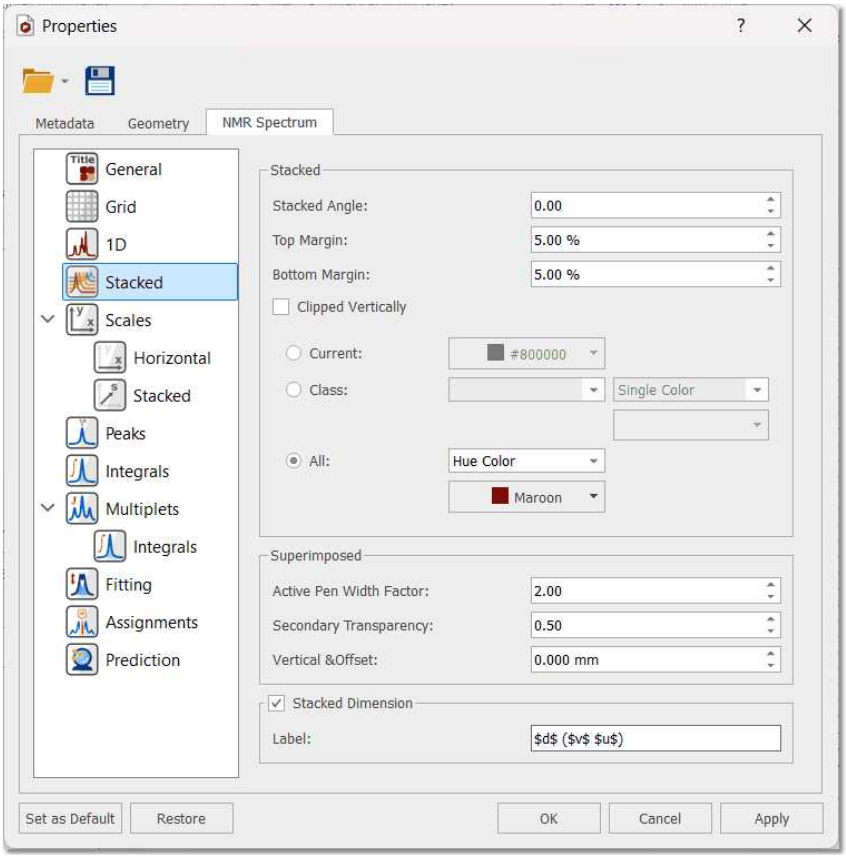


For metabolomics studies it is important to superimpose multiple spectra and compare their shape, e.g. to identify which ones are outliers. In such a mode, when the user sets a spectrum as the Active spectrum, that spectral curve will be displayed with a thicker curve:

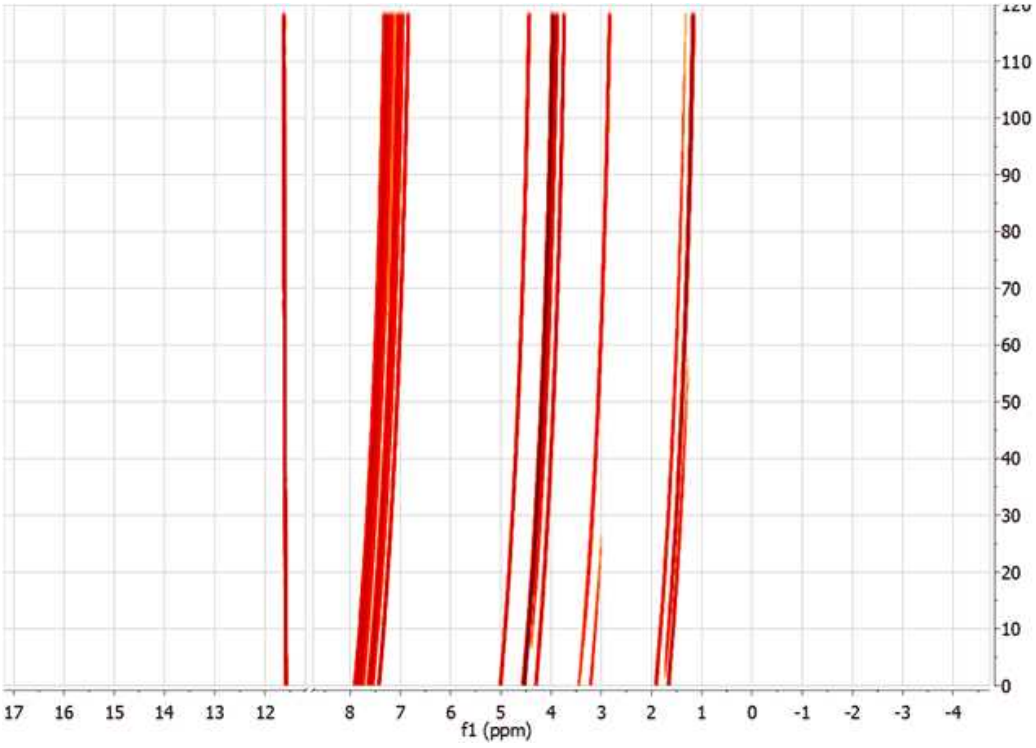


Follow the menu 'Home/Properties/Stack' to customize 'Active pen width factor' (2.00 by default) and 'Secondary Transparency:

© 2024 MESTRELAB RESEARCH

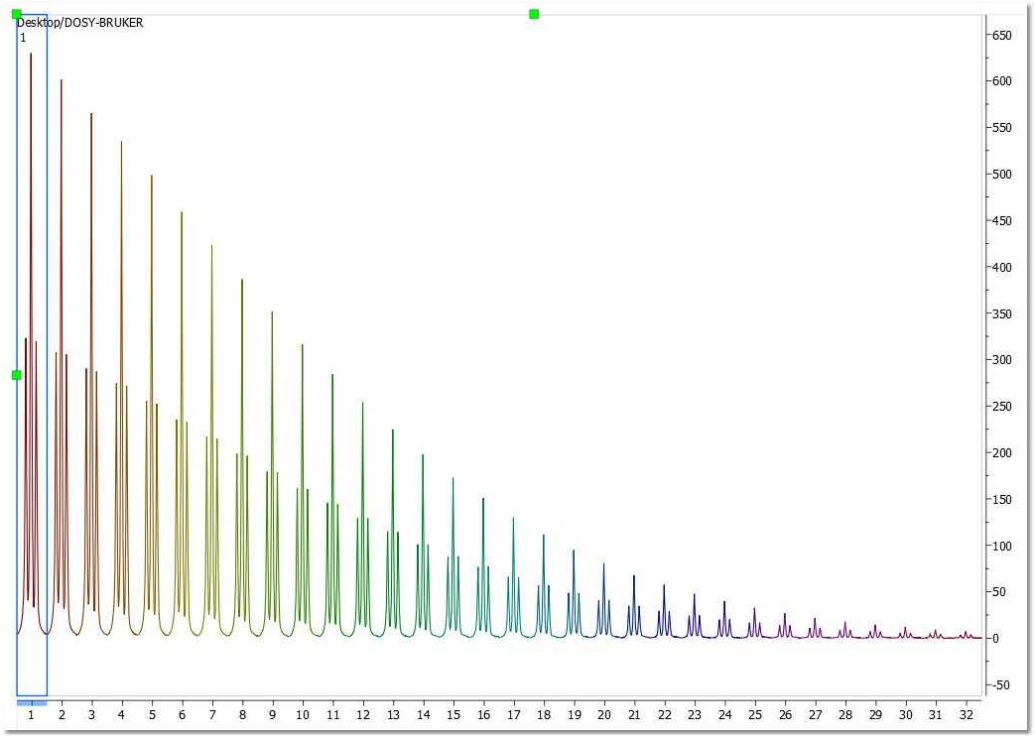


'Bitmap': too see all spectra in a bitmap mode.



© 2024 MESTRELAB RESEARCH

Horizontal Stacked: to display the spectra horizontally stacked.



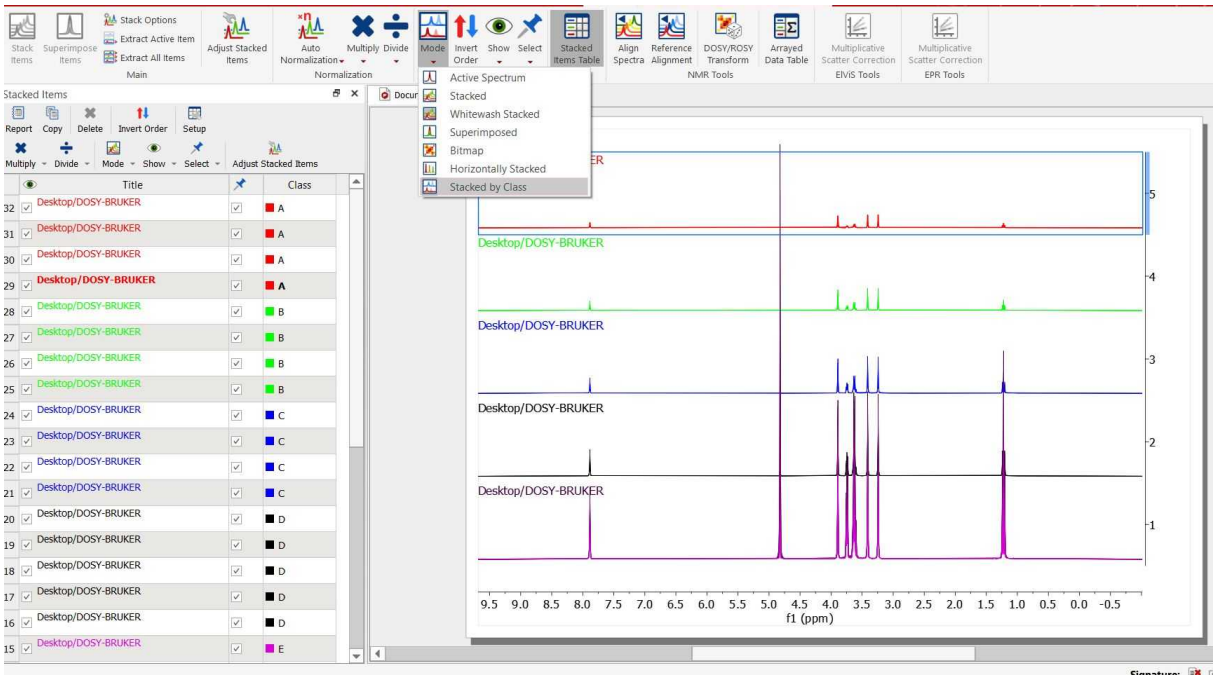
Stacked by Class

The Stacked by Class mode is similar to the Stacked mode, but in this case all items of same class are stacked as superimposed in the same row of the stack. Items without class are superimposed together in the same row.

When no sorting is selected, the original order of the items is kept as much as possible (considering that they are grouped by class)

When titles are visible, the title of the active item is shown in the corresponding row, the title of the first item of the class is shown if the active item is not one of that row

© 2024 MESTRELAB RESEARCH



Multiple Spectra Selection

Mnova includes a powerful, yet easy to use ***multiple spectral selection*** mode, which allows the user to optimize the processing of groups of spectra by selecting a bunch of spectra and applying any processing (e.g. manual phase correction to the selected group)

Multiple spectra selection can be done either graphically from the stack or directly from a table

In addition, Mnova offers the ability to hide any number of spectra in an arrayed item.

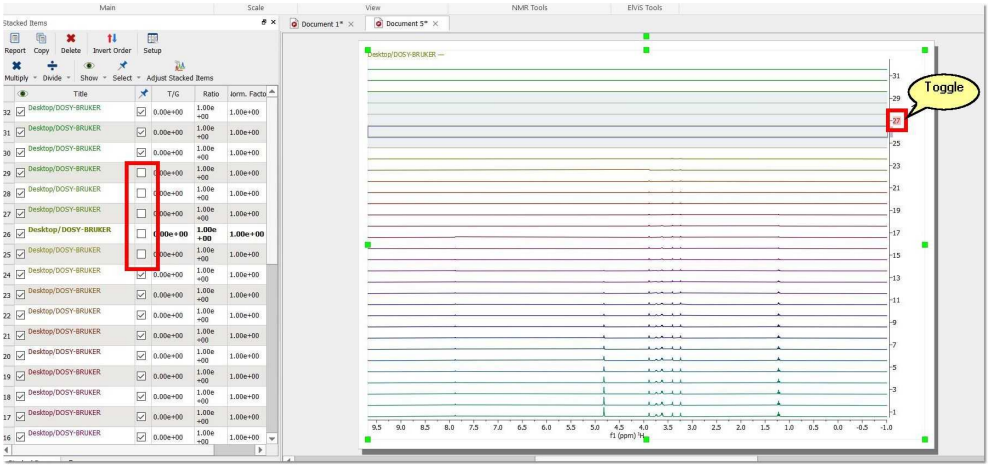
Graphical selection of spectra

In order to be able to select a bunch of spectra ***graphically*** with the mouse, it is necessary to display the spectra in the stacked plot mode.

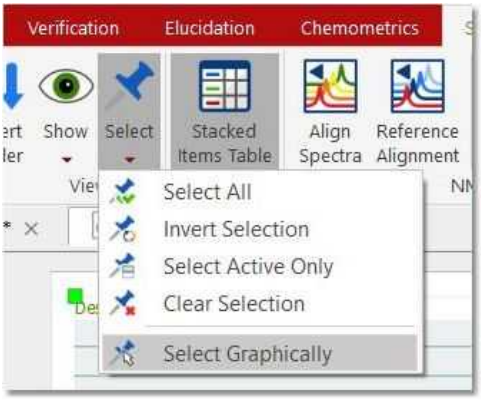
To select a single spectrum, **left click** on the spectrum trace while keeping pressed the **Alt key**
To deselect a spectrum: **left click + Ctrl + Alt**

By default all the stacked items are selected and a gray shade is drawn only when they are unselected. You can toggle the item selection by clicking in the *category* axis labels when the axis is visible.

© 2024 MESTRELAB RESEARCH



There is a mode to select the items graphically (it will only work on Stacked and Horizontally Stacked display modes)



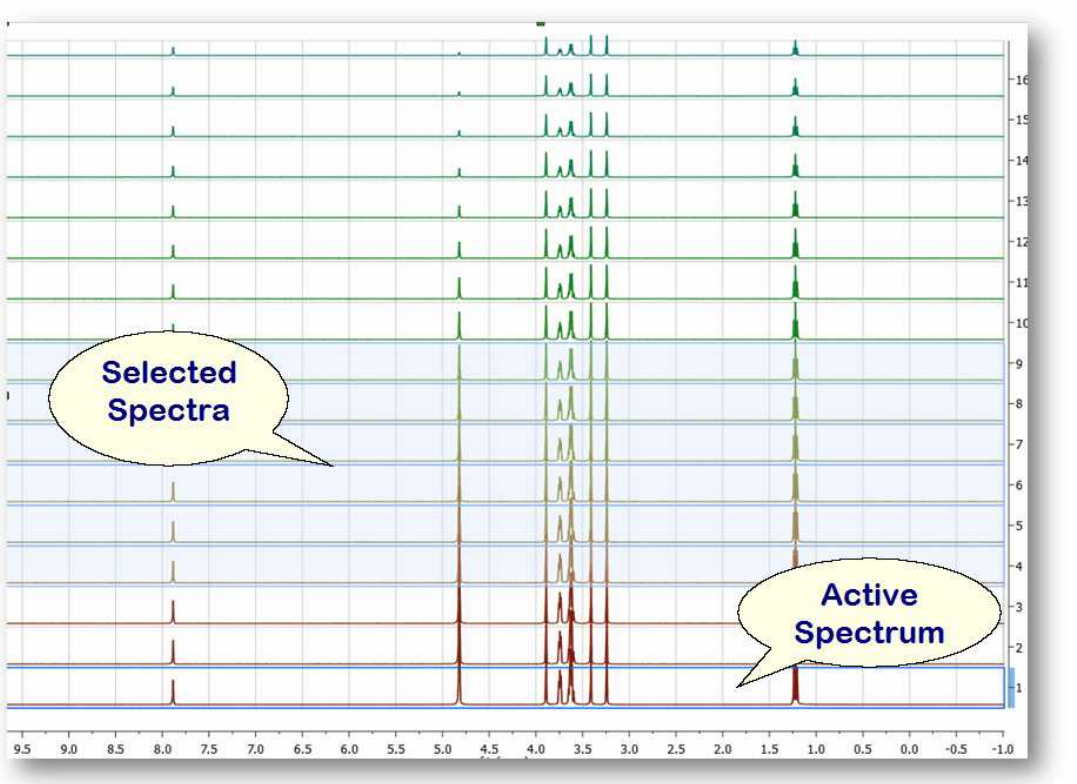
1. Pressing and holding Shift will append to the current selection.
2. Pressing and holding Ctrl will subtract from the current selection.
3. And without any of those keys pressed it will set the current selection.

In order to select multiple spectra, follow the same operation as above while keeping pressed **Alt and/or Shift keys**.
The following slide will show how to select several groups of spectra

Some important concepts: Active spectrum vs Selected spectrum/spectra

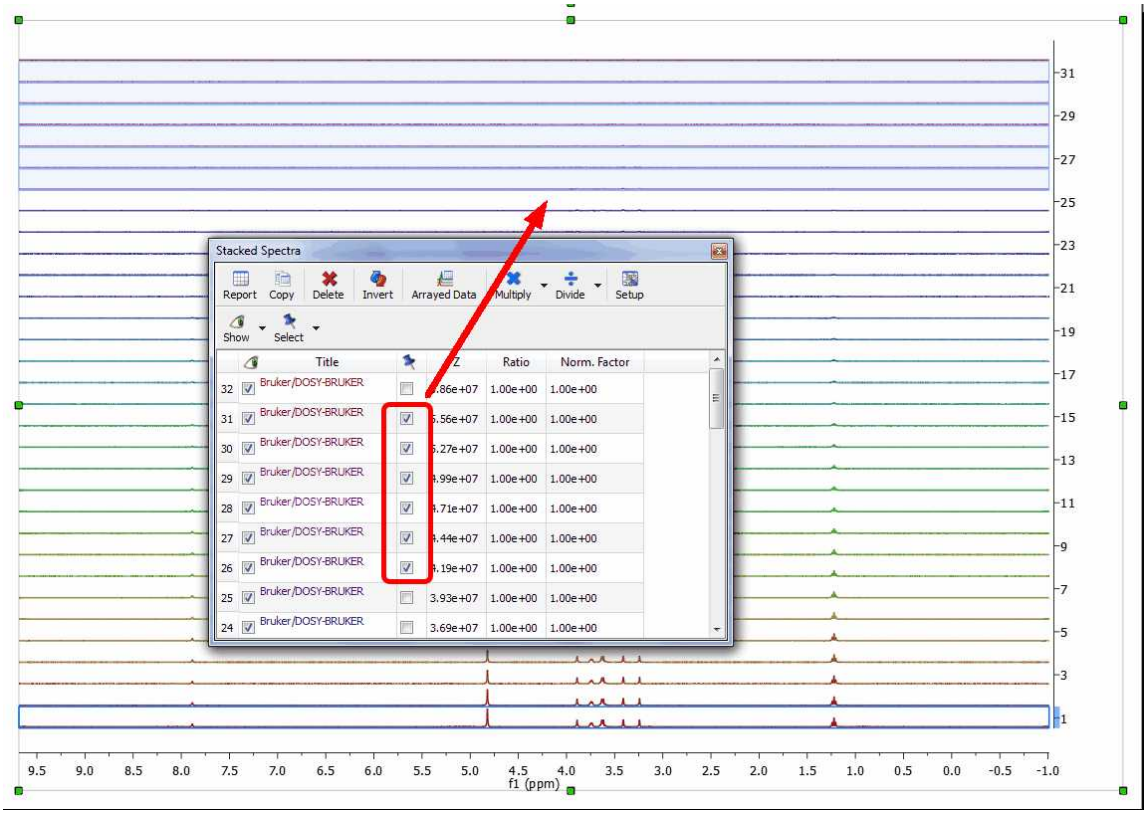
Selected spectra: when the spectra are selected, they are highlighted with a light blue background. Any processing operation will take place only for those selected spectra
Active Spectrum: active spectrum is marked with a blue box and it indicates the spectrum which will be displayed in the *active spectrum* display mode. It's also the reference spectrum used in some processing operations (e.g. alignment)

© 2024 MESTRELAB RESEARCH

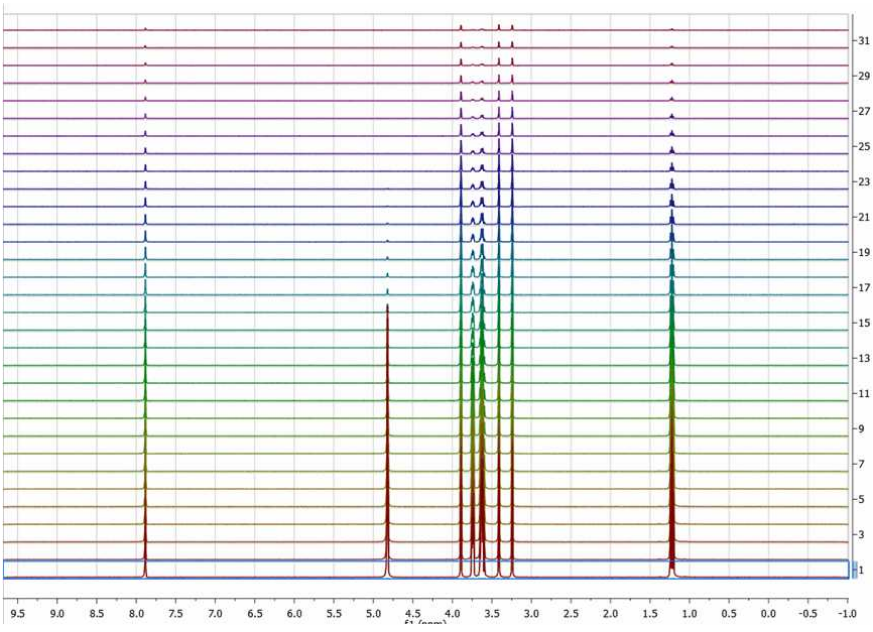


You can also **select** several slides by using the check boxes on the right of the 'Stacked Items Table'. When the spectra are selected, they are highlighted with a light blue background. Any processing operation will take place only for those selected spectra.

© 2024 MESTRELAB RESEARCH

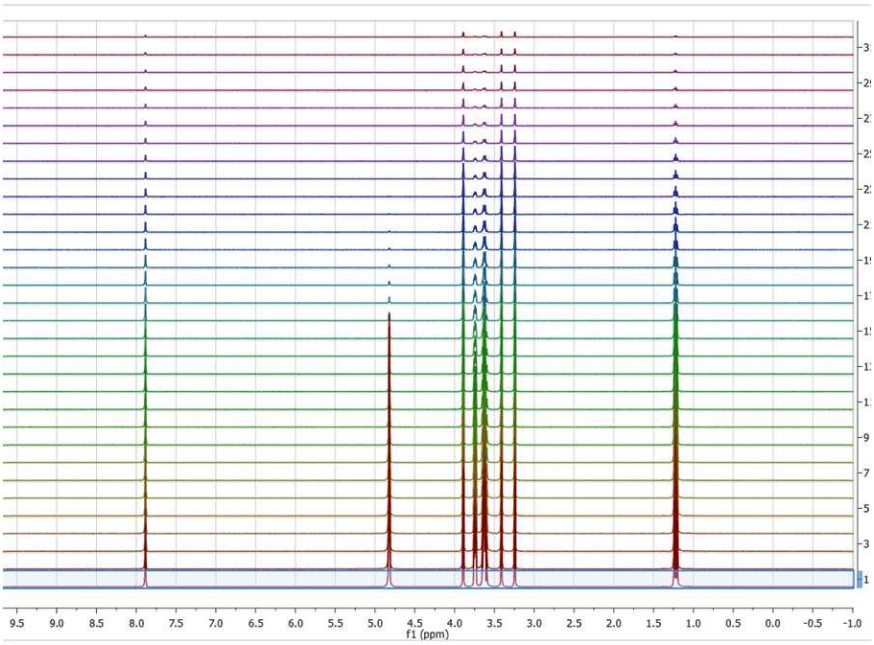


Example #1:
If there are no spectra selected and the active spectrum is not either selected (i.e. no spectra have a blue background) then ***any processing operations will be applied to ALL spectra (***Active spectrum is not selected)

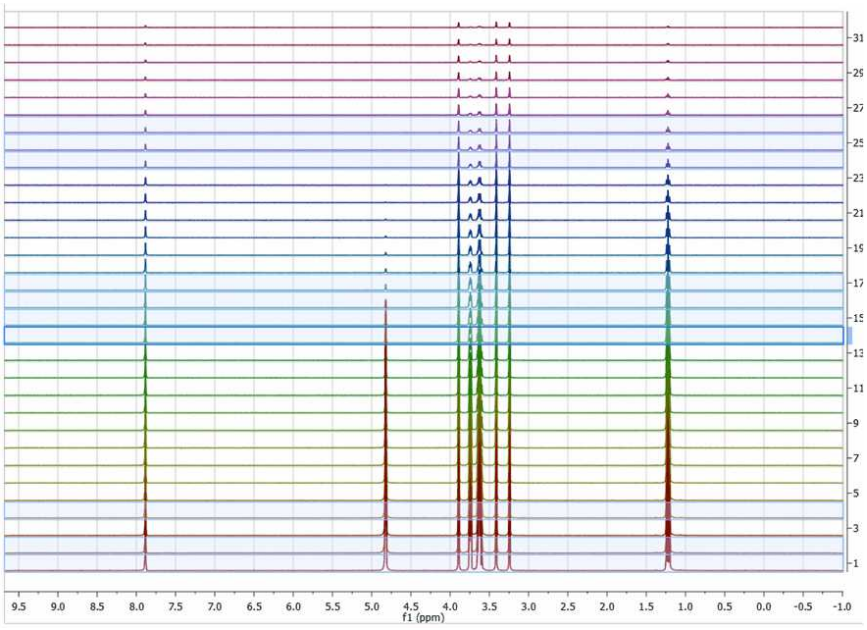


Example #2:

If only the active spectrum is selected, ***any processing operations will be applied ONLY to the active spectrum.*** (Remember, to select a spectrum: left click + Alt)



Example #3:
In general, ***any processing operation will be applied only to the selected spectra*** (and remember that if no spectra are selected, All spectra will be processed):



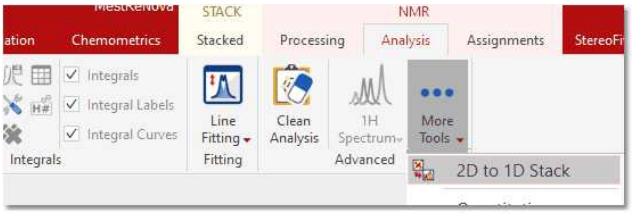
See also 'Table of stacked items'

To navigate through the traces in the stack plot, press and hold down **SHIFT** and use the up or down arrow key (or use the mouse scroll wheel). The **active trace** will be highlighted by default with a blue

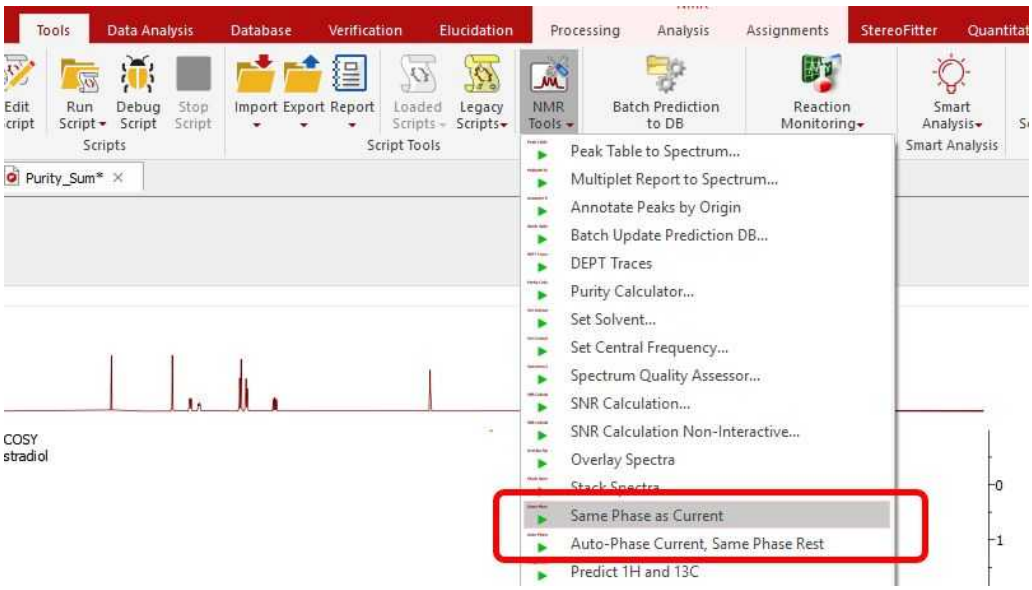
© 2024 MESTRELAB RESEARCH

rectangle, whilst **selected traces** will be highlighted with a light blue background (those marks will not be exported whit the stack plot). Any processing operation will take place only for those selected spectra.

Please note that in strange cases, Mnova shows some arrayed datasets as 2D plots. In those cases just follow the ribbon 'Analysis/More Tools/2D to 1D stack':



Scripts to autophase importing autophase stacked spectra when importing.
Following the menu 'Tools/NMR Tools', you will find a couple of scripts to apply automatic phase correction to a stacked plot:

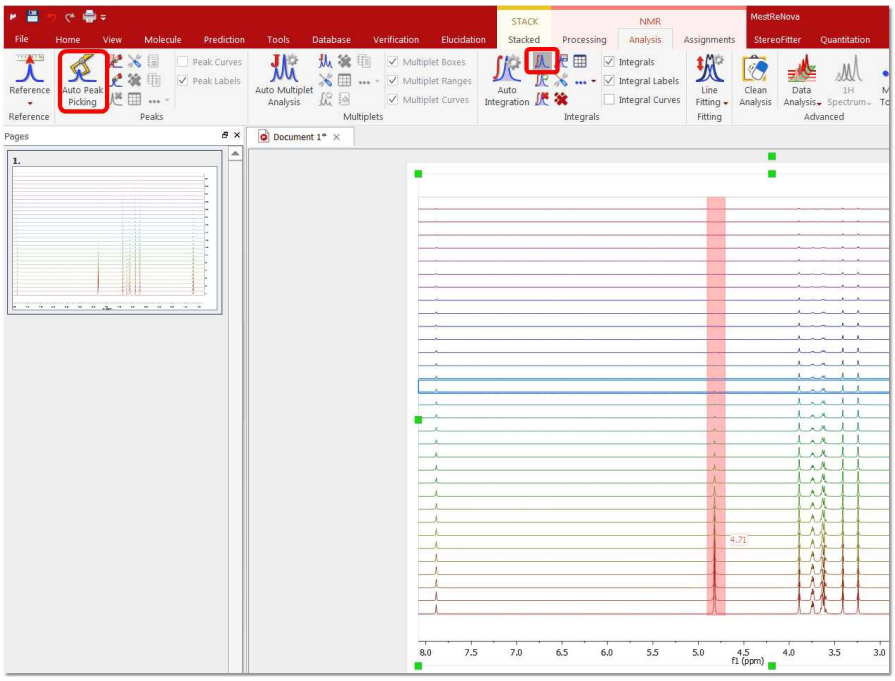


- **Auto-Phase Current, Same Phase Rest**: the active spectrum is automatically phase corrected, and the resulting values of PH0 and PH1 are applied to the other spectra in the stacked.
- **Same Phase as Current**: PH0 and PH1 values of the active spectrum are applied to the other spectra in the stacked.

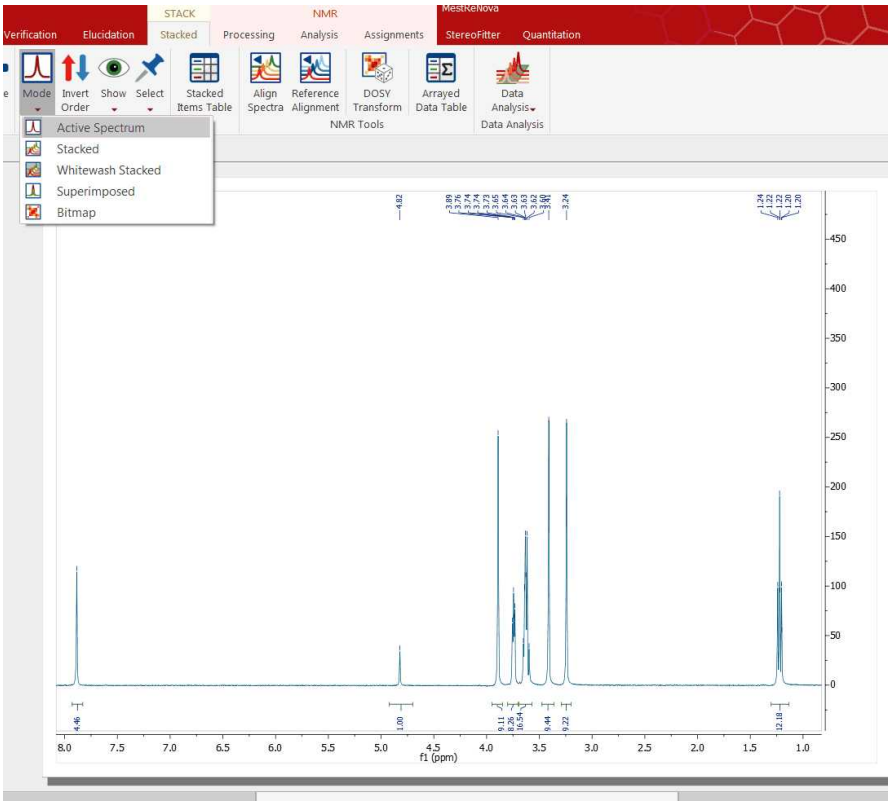
9.1 How to apply a peak picking or an integration analysis in stacked mode?

It is very easy to apply a peak picking or an integration analysis to a stacked spectra, just by clicking on the corresponding icon (or selecting the desired analysis function). In the picture below, we have applied an automatic peak picking and a manual integration of several areas of interest:

© 2024 MESTRELAB RESEARCH



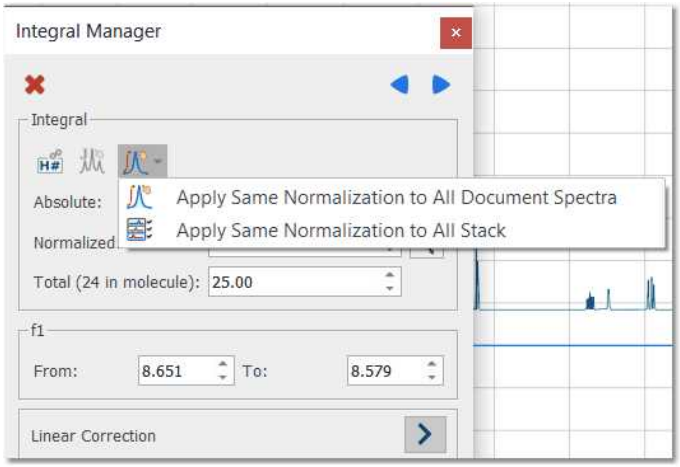
Then, just select 'Active Spectrum' under the 'Mode' scroll down menu to obtain only the highlighted spectrum and you will be able to see the integrals and the peak picking. To navigate through the traces, press and hold down **SHIFT** and use the up or down arrow keys (or the mouse scroll wheel):



© 2024 MESTRELAB RESEARCH

Finally you can export the peak picking or the integration analysis to a text file by following the menu File/Save as/Script:ASCII Matrix or 1D Integral Series (*.txt). This will generate a text file which can be loaded into Excel (for example). Saving as "Script: NMR CSV Matrix (.txt)" you will get the zoomed area of all spectra distributed in columns (blind region and cut areas will be filled with zeroes). If you save it as "Script: NMR CSV Matrix (Transposed) (.txt)" you will get them in rows.

The user can apply the same integral normalization to all the spectra (in the current document) or to all the traces of a stack plot by selecting the applicable option from the 'Integral Manager' (shortcut: SHIFT+I):



This is very useful if you need for example to determine relaxation times from a series of 1D spectra, to be able to compare the integral values between the different traces.

9.2 Setup Arrayed Spectra

This feature for the vertical & horizontal alignment of stacked spectra is available after having clicked on the 'Adjust Stacked Items' button:



The way in which this control works is explained directly in the panel, in the same manner as with the phase correction panel:

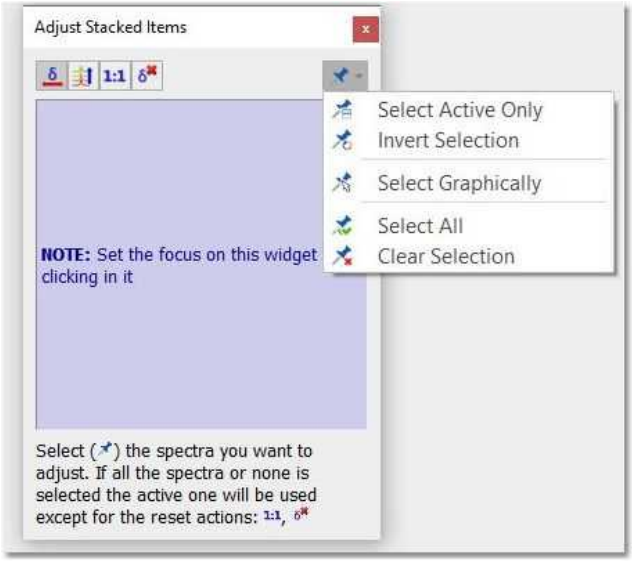
Horizontal shifting: To align the spectra just click&grag to right or left. When the spectrum is shifted to the left or to the right, its chemical shift reference is adjusted to reflect that horizontal move.

Vertical scaling: This consists in changing the internal data points in the spectrum array by multiplying each point by a scaling factor. Note that this will have implications in the quantitation results.

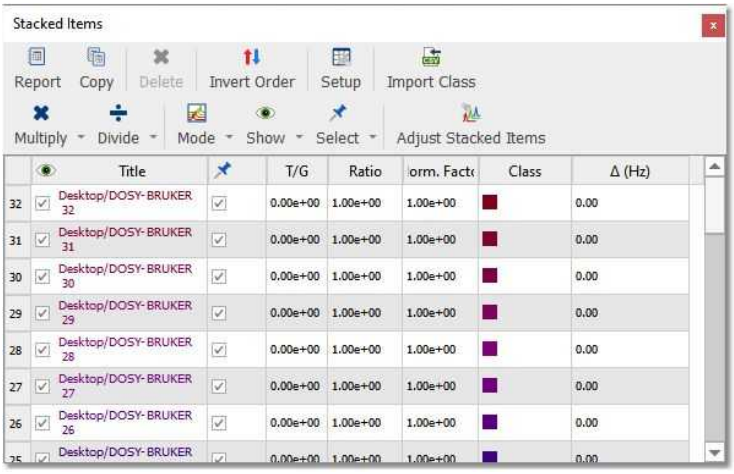
Reset Intensities: this button will restore the intensity changes

Reset Shifts: this button will restore the shift changes.

Clicking on the pin scroll down menu will allow you change the selection of your spectra:



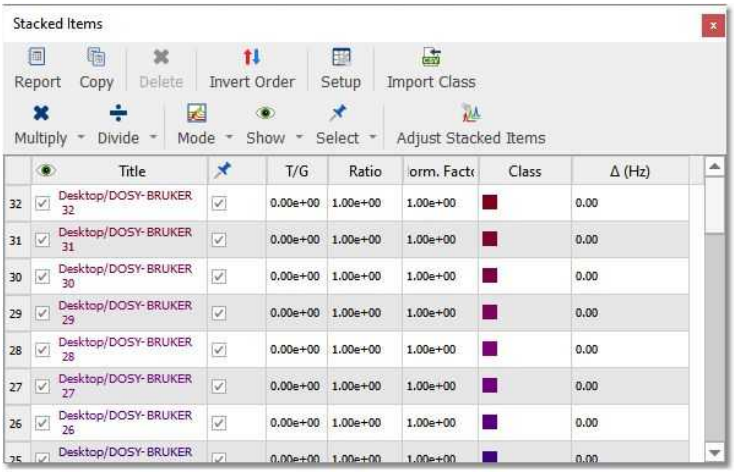
Every change carried out during this process will be reflected in the stacked table. For example, if you move one spectrum 15 Hz to the right, that result will be shown in that table, so that it will be possible to use that value to measure, for example, a coupling constant or the amount by which a spectrum was shifted so that it perfectly overlaps with another spectrum.



© 2024 MESTRELAB RESEARCH

9.3 Table of stacked items

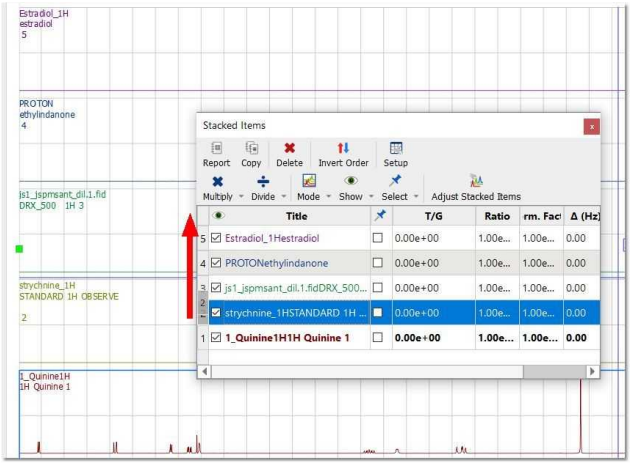
This table (View/Tables/Stacked Items) makes it possible to select spectra as an alternative way to the graphical method but it offers more features, including the ability to **delete**, **hide** or **change the relative intensities** of any spectra:



T/G column: 'T' stands for Time whereas 'G' stands for Gradient. The underlying idea is that the Data Analysis module is intended mostly to analyse (1) Reaction monitoring and relaxation experiments in which case the independent variable would be expressed in seconds and (2) Pulse field gradient (aca DOSY) experiments where the independent variable would correspond to field gradients strength.

Sorting traces:

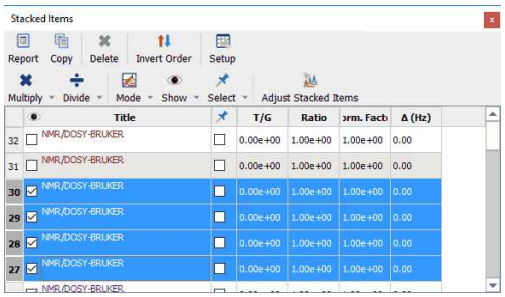
The user can easily **sort** the traces by **clicking on the number of the spectrum** for which you want to change the location and dragging to the desired location. Clicking on the title header will sort the spectra in descending/ascending order by title.



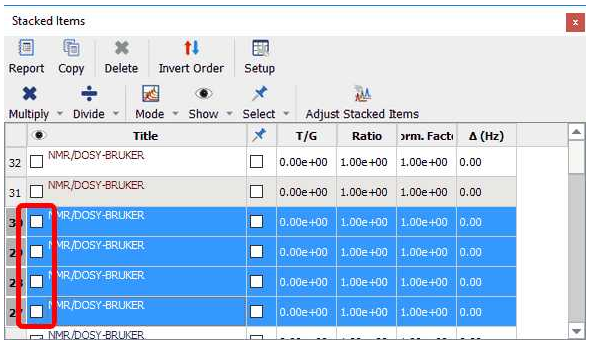
How to hide several spectra?

It is really easy to hide slides, just:

- Select in the table all those rows corresponding to the spectra you want to hide



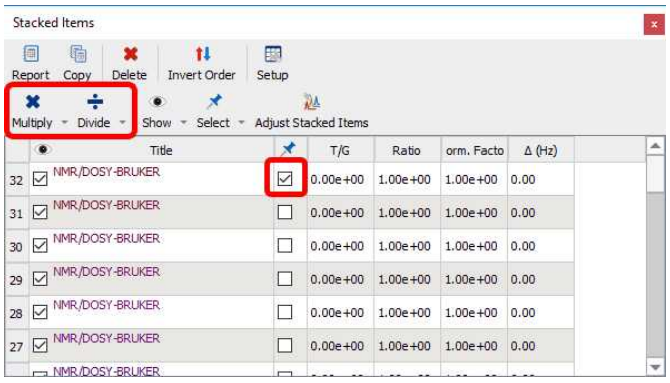
- and uncheck the boxes of selected rows:



You can follow the same operations to restore those spectra.

How to scale the traces of the stacked spectra?

The user can also change the intensity of the traces by checking the box of the applicable trace(s) and clicking on the 'Multiply' or Divide' buttons:

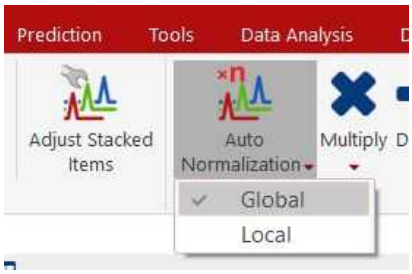


Clicking on the scroll down menu of the multiply or divide buttons will allow you to select the factor value.

Please bear in mind that you can also use the buttons of the Stacked ribbon to multiply/divide the intensity of your traces.

You can also normalize the traces of your spectrum by clicking on the 'Auto Normalization button, which has a dropdown menu to select if the action applies to the full spectrum (Global) or to the zoomed region (Local).

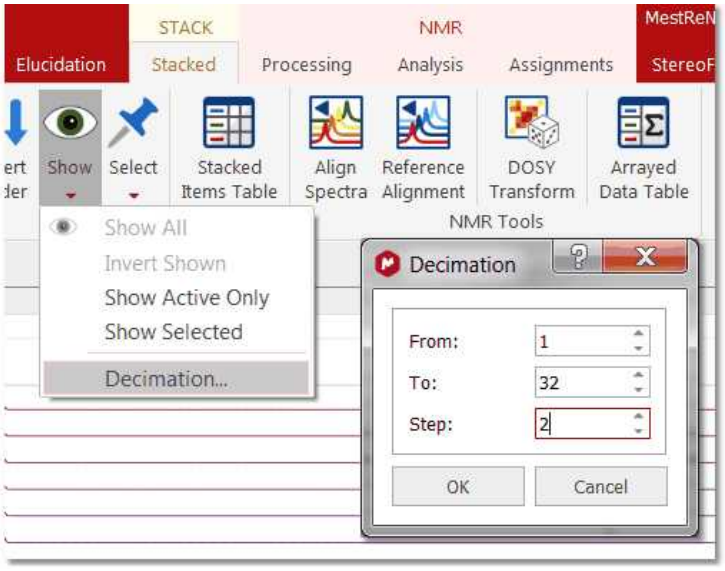
© 2024 MESTRELAB RESEARCH



You can extract the 'Active Item' or 'All Items together by selecting the applicable options from the ribbon above.

Decimation

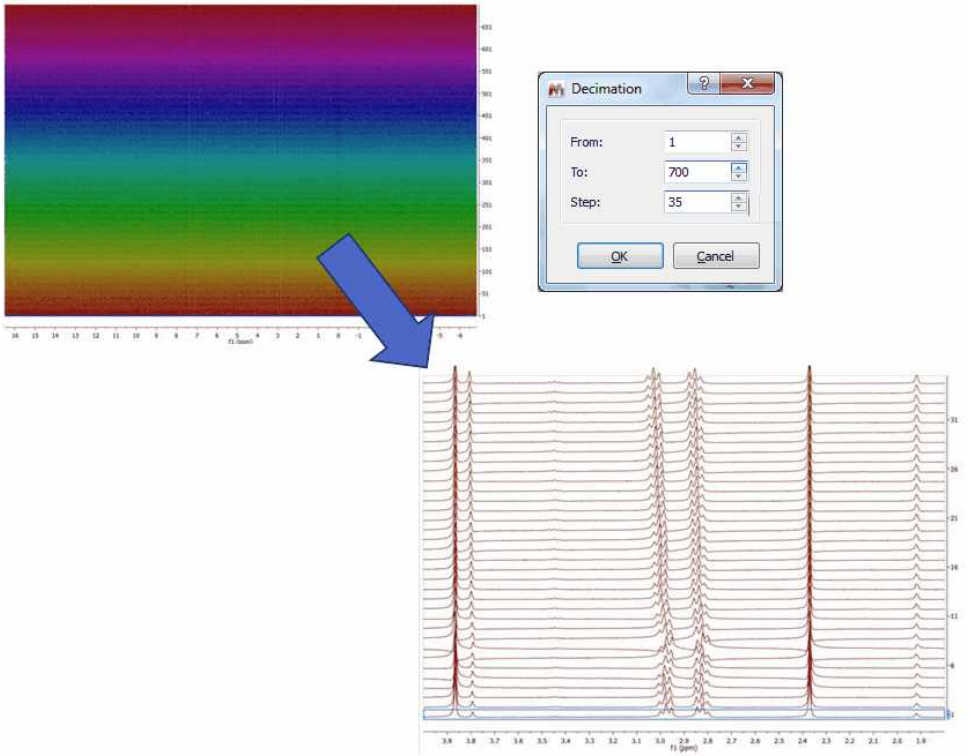
Worth mentioning is the '***Decimation***' option:



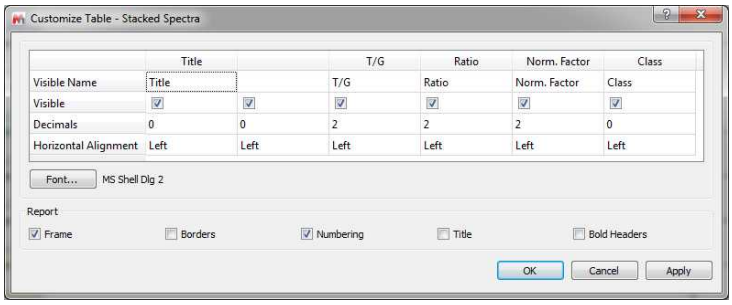
This option is very useful when the number of stacked spectra is very large, slowing down the plotting and not showing relevant information

This shows the result of a R-M experiment consisting of 700 spectra. After selecting **the decimation option**, a new dialog shows up where you can select the first and last spectrum to be displayed and the step or decimation factor. In the picture below you can see how the dataset will look like after decimation:

© 2024 MESTRELAB RESEARCH

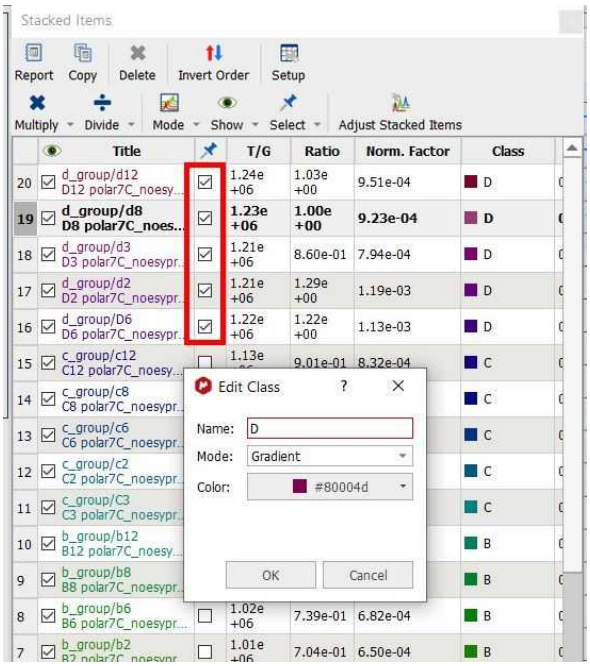


In the context of metabolomics, you would need to classify the stacked spectra by specifying a class. To do that, you can add an editable column to the Stacked Items table in order to input there a short text. You can show the 'Class' parameter by clicking on the 'Setup' button of the Stacked Items table (or on the Customize button of the 'Parameters Table'):

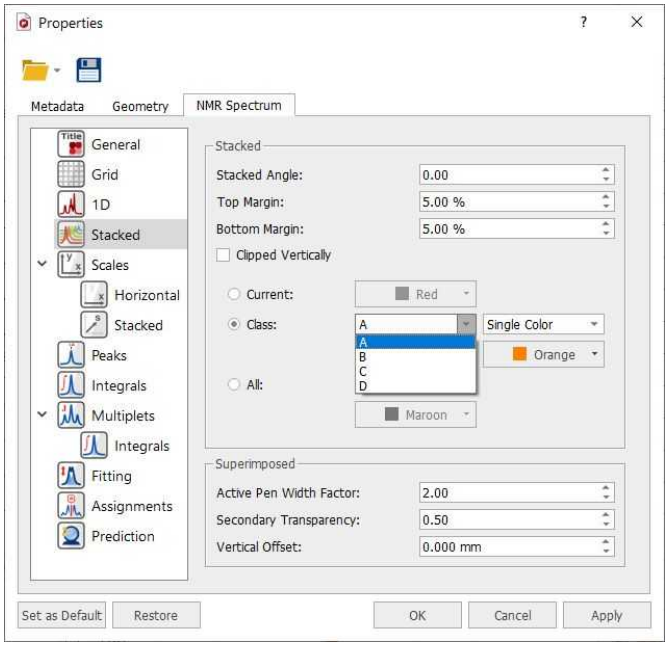


This parameter will be editable by double clicking on the 'class cell'. If you want to select a class for several traces at the same time, make sure that you check the applicable 'pin boxes':

© 2024 MESTRELAB RESEARCH

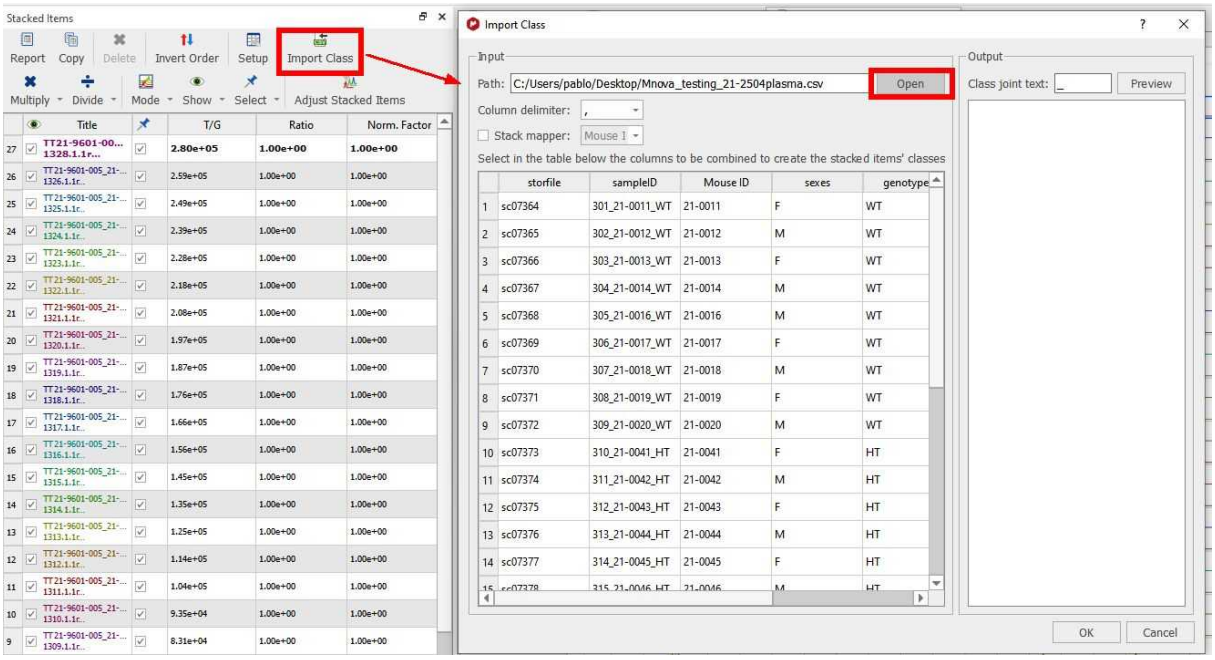


The Class properties can also be changed from the 'Properties' dialog box:



Click on the 'Import Class' button in the Stacked Items table to create the classes by combining columns of a CSV file. When this button is clicked the corresponding dialog "Import Class" is displayed. It contains 2 sections, Input and Output, and each of these sections have a set of settings and a table:

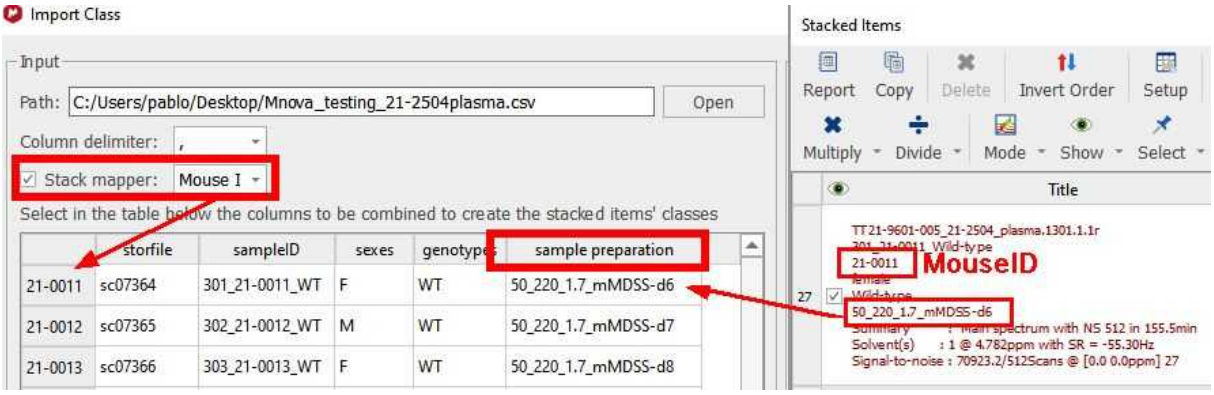
© 2024 MESTRELAB RESEARCH



In the Input section there is a button "Open" with which we can select the CSV file that will be used. If the number of rows in the CSV file uploaded matches with the number of spectra in the stack the table in the Input section will show the contents of the CSV file.

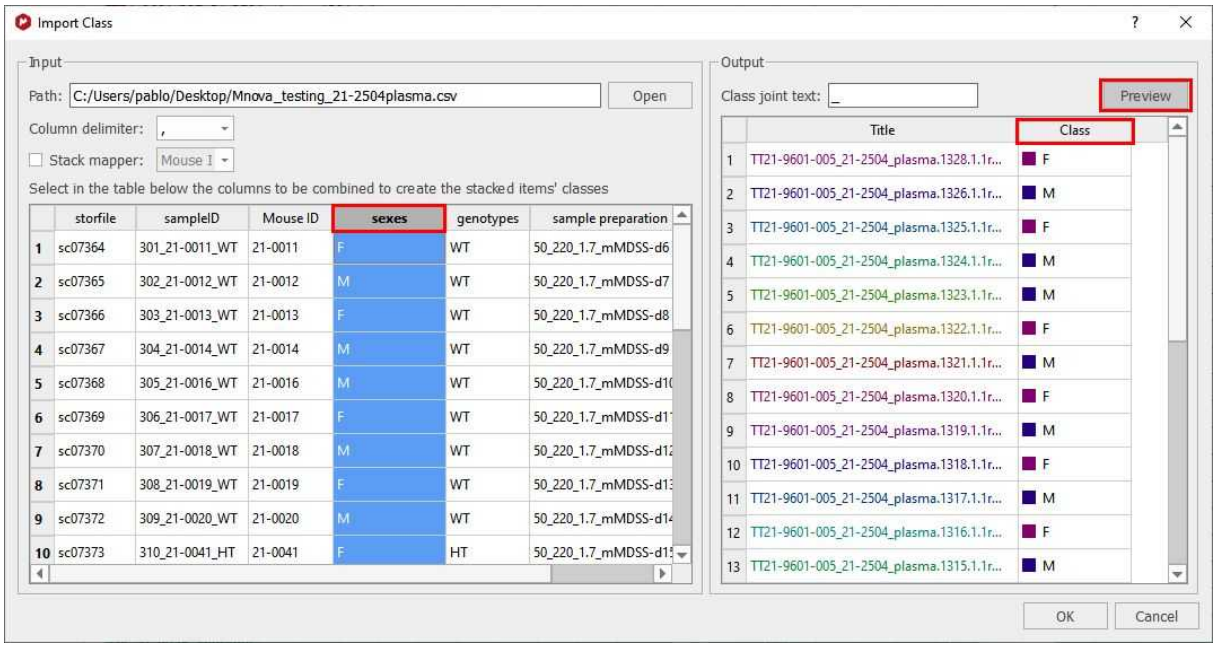
The Input section contains an option to select the column delimiter of the CSV file (comma, semicolon, single space, and tab):

When the CSV file has columns with values that matches unequivocally with the titles of stacked items, the Stack Mapper is enabled and its combobox shows these valid columns. See the example below, where columns "Mouse ID" and "sample preparation" appear in the combo box because its content (values) are defined in the spectra tittle:

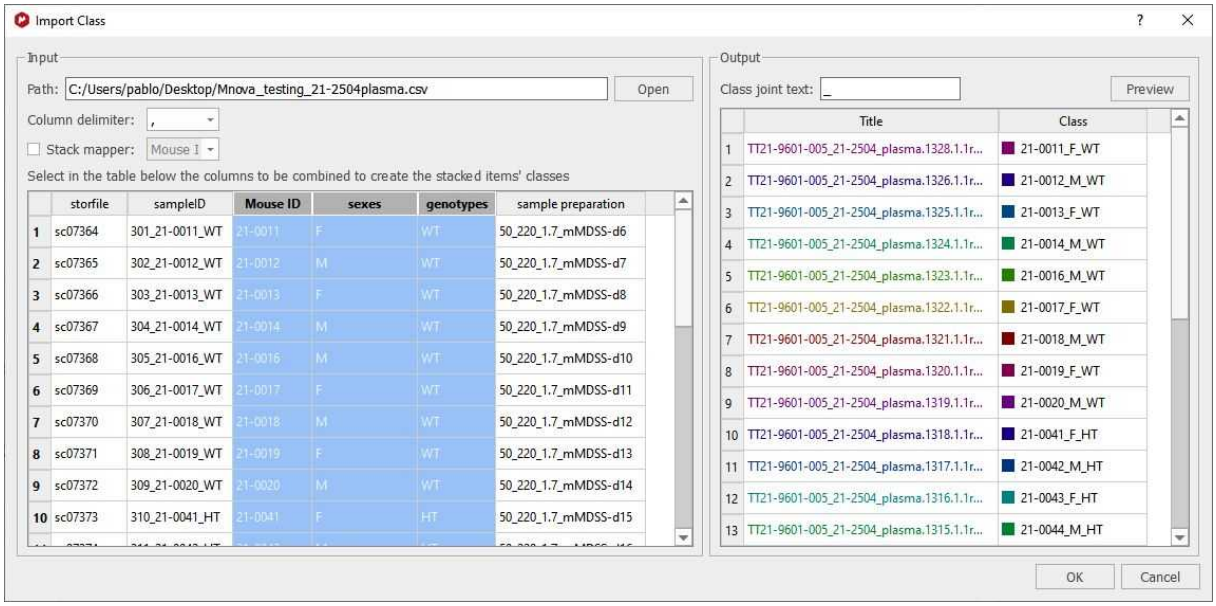


To create the classes; select one column of the table in section Input and click on "Preview": as expected, in the section Output the classes are created based on the info of the column selected:

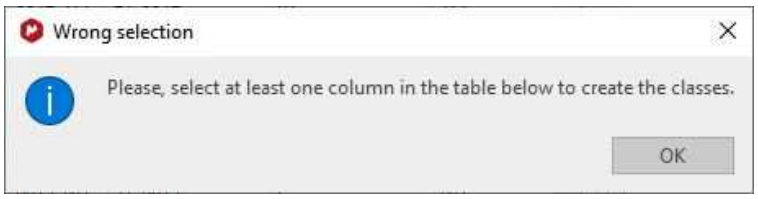
© 2024 MESTRELAB RESEARCH



You can also select two or more columns of the input table and the expected combined classes will be created:



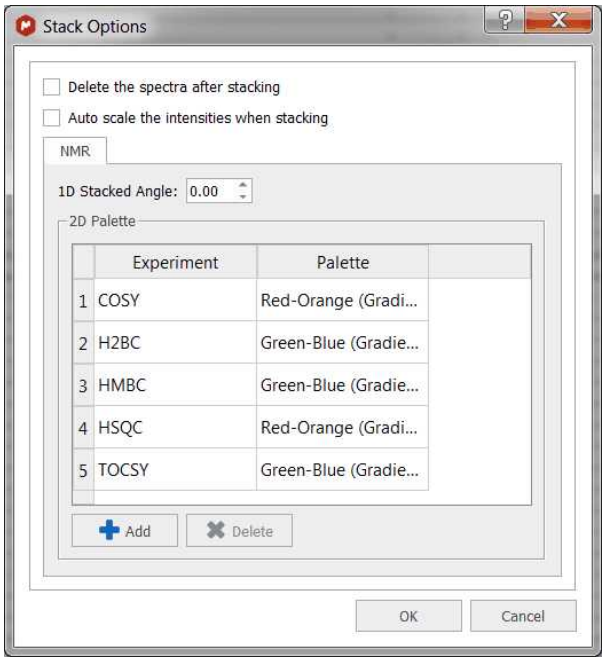
If no columns are selected and if we click "Preview", a warning message will be displayed:



9.4 Stacked Options and Properties

Options

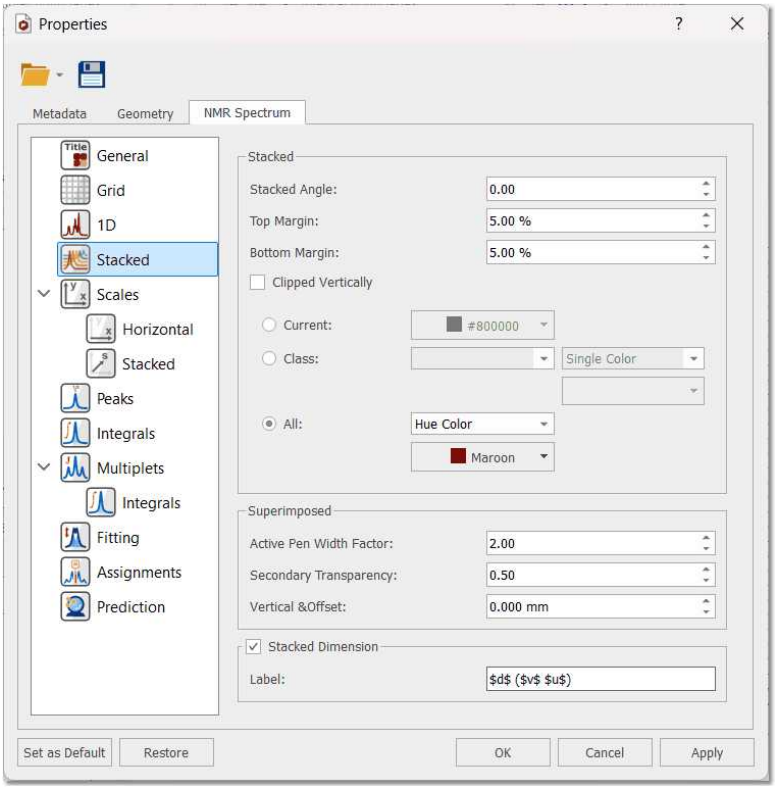
By default Mnova will delete the 1D spectra after having stacked them without autoscaling them. If you want to modify these defaults, you will need to follow the menu 'Stack/Stack Options' and uncheck the applicable box. From here, you will be able to change also the stacked angle and to select different palettes for your superimposed 2D experiments.



Properties

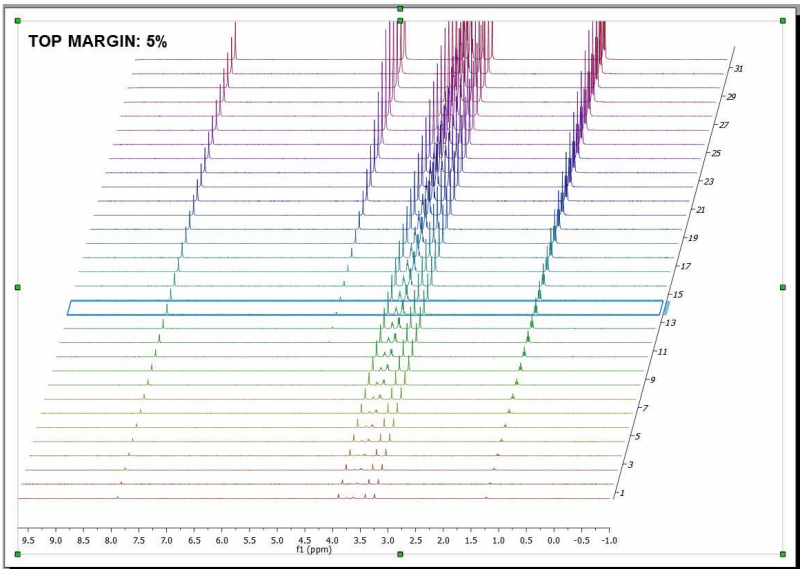
The user can change the Stacked Items properties by double clicking on the spectrum window; this will display the Properties dialog box, where the user can modify the Style (lines, crosses or circles), the Line Width, the Stacking Angle and the color of the current slice as well as that of the spectrum (single color, gradient or hue color). From here you can change the colour of the background or show the title of the spectrum and the title of each slide of the stacked plot, by checking the corresponding boxes:

© 2024 MESTRELAB RESEARCH

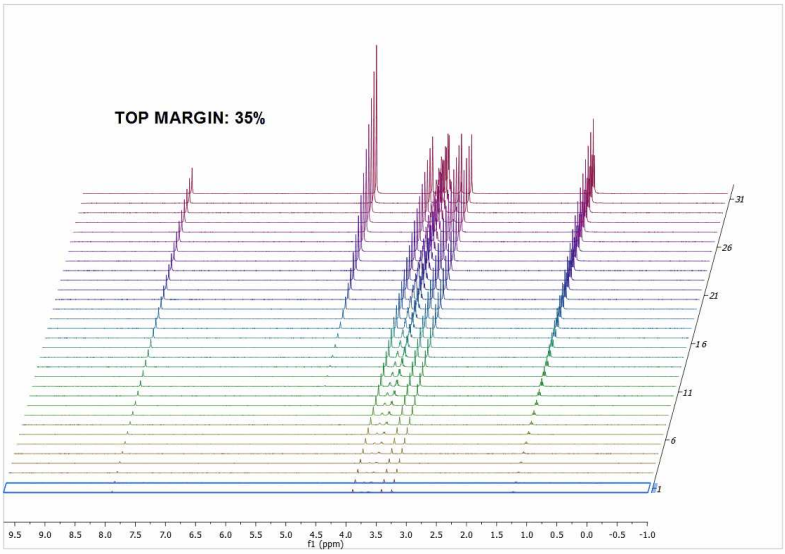


You can change the top and bottom margins of the stack plot, which is really useful in to avoid clipping a stacked plot at the spectrum border. With this property you will be able to adjust the distance between the top of the spectrum object and the baseline of the uppermost spectrum.

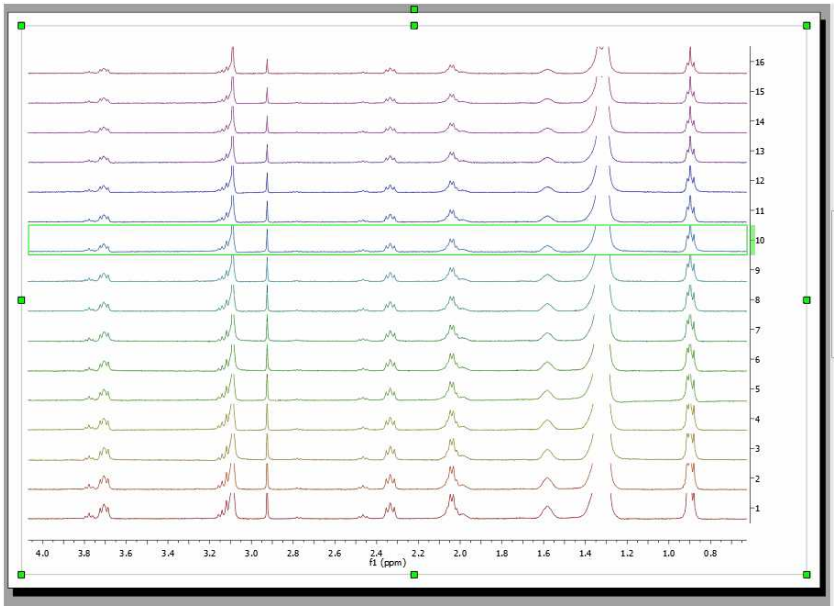
Here you can see an example with a top margin of 5%:



And the applicable result after having selected a top margin of 35%:

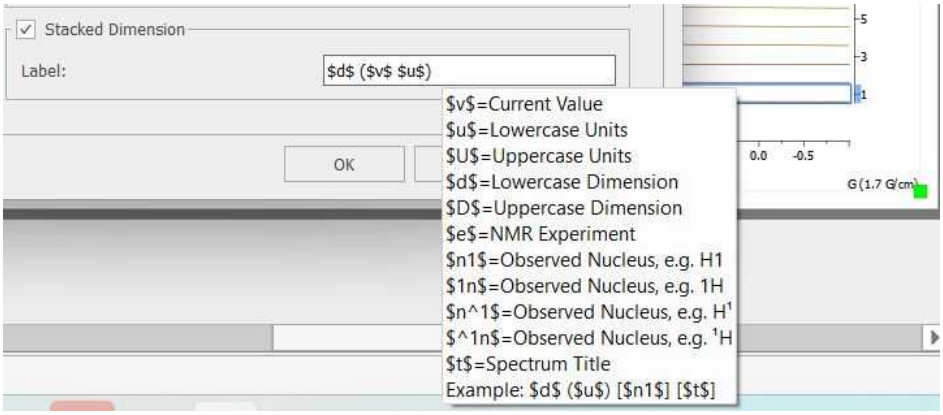


You can cut off the peaks to avoid overlapping just by checking the **'Clipped Vertically'** option:

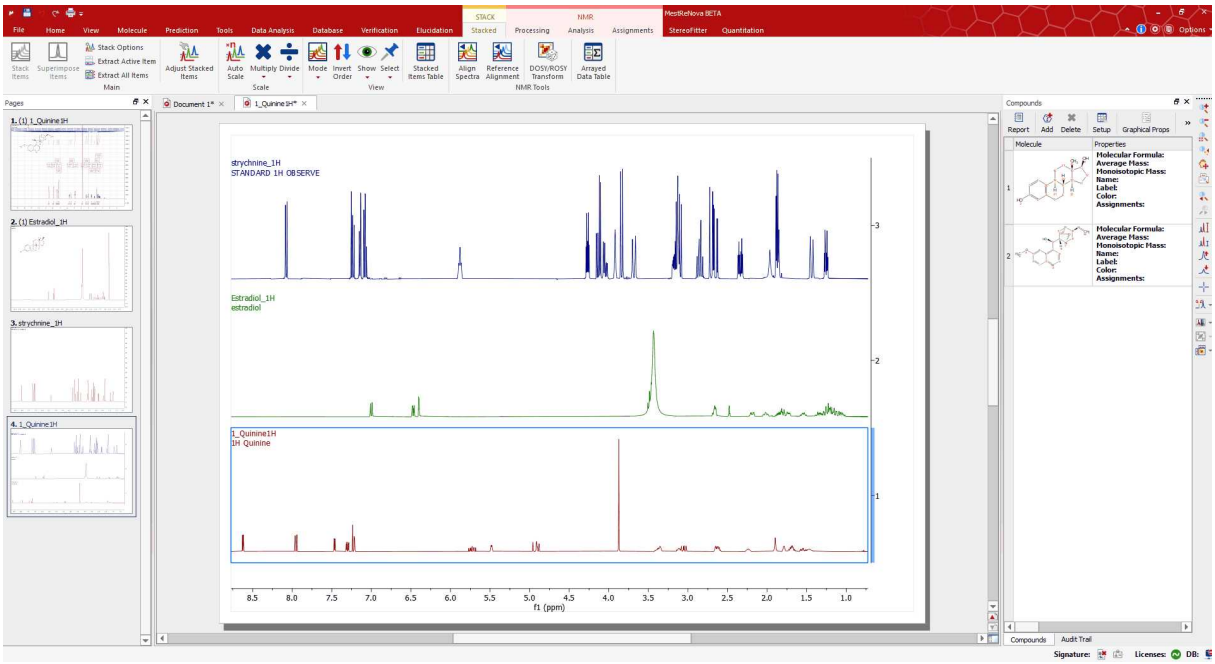


© 2024 MESTRELAB RESEARCH

You can customize the stacked label from here:



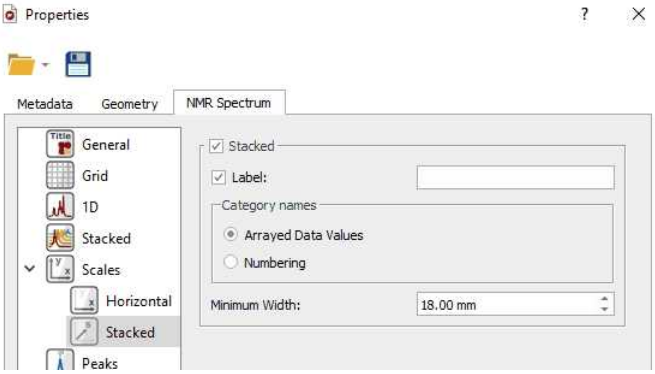
Here you can see an example of a stacked plot with the title of the different traces:



For metabolomics studies it is important to superimpose multiple spectra and compare their shape, e.g. to identify which ones are outliers. In such a mode, when the user sets a spectrum as the Active spectrum, that spectral curve will be displayed with a thicker curve. Follow the 'Home/Properties/Stack' menu to customize the 'Active pen width factor' (2.00 by default) and 'Secondary Transparency'.

Selecting 'Scales' in the left-vertical tabbed menu, will allow you to change the color of the scale, the font, add labels and also show/hide the horizontal and the stacked axes. From the Stacked menu, you can also change the label and the content (arrayed data or just numbers) of the stacked scale or the minimum width of the scale.

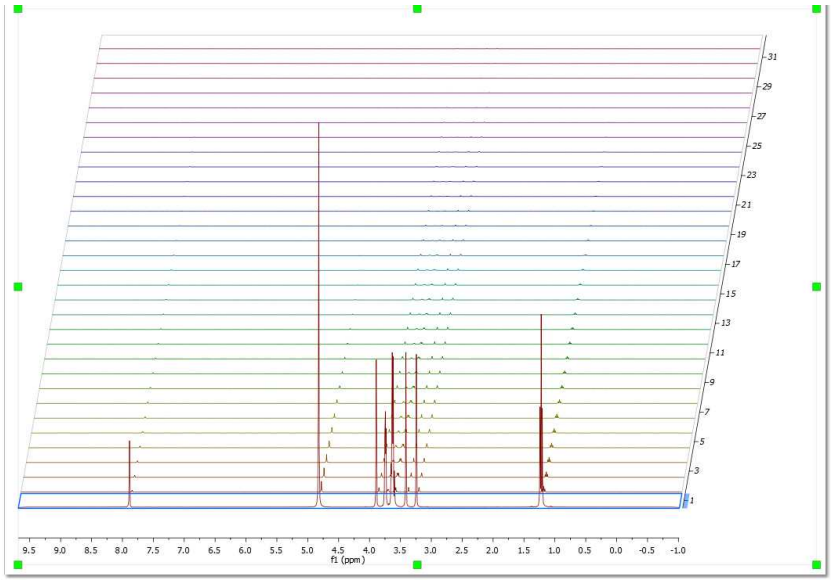
© 2024 MESTRELAB RESEARCH



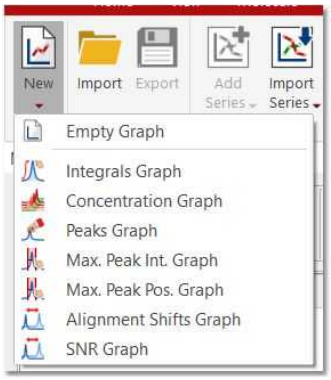
9.5 Data Analysis Quick Start Guide

First of all, please make sure that you are familiar with stacked plots. Take a look at this chapter for further information.

Let´s see how the Data Analysis feature works. Just load your experiment into Mnova:

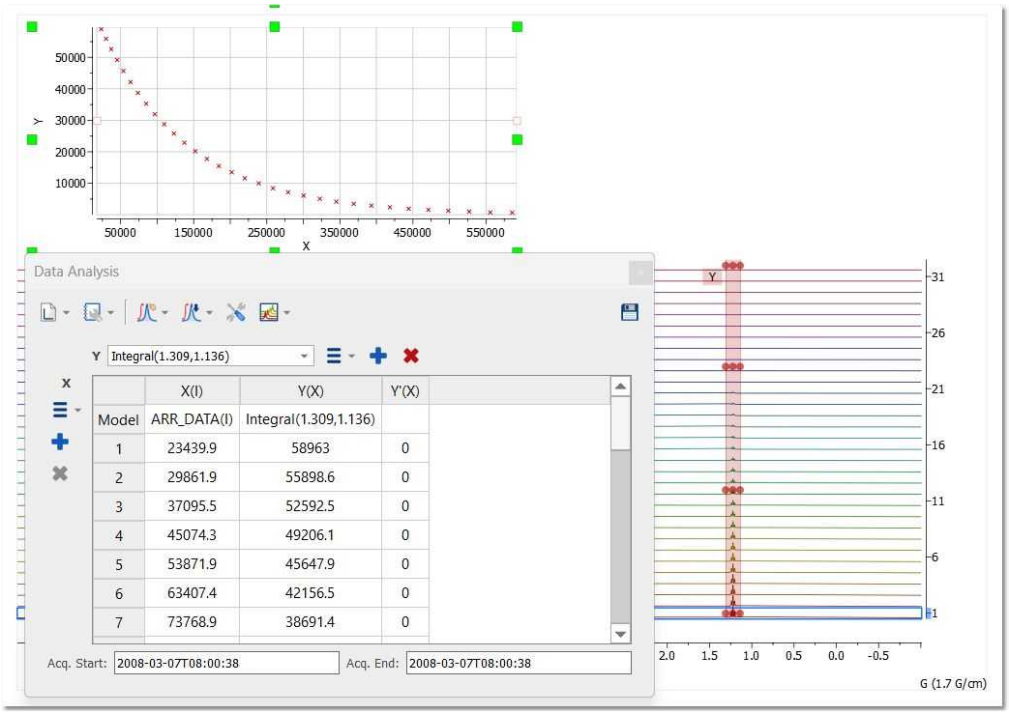


Follow the menu 'Data Analysis/New' and select the desired graphical representation. In that case 'Integrals Graph':



© 2024 MESTRELAB RESEARCH

You will notice how the cursor will change to a blue integral. Now you will only need to integrate the desired signal (in this case, we will select the signal at 1.20 ppm). Once you have integrated the desired region, you will notice how the graphical will change according with the decay of the signal. Please bear in mind that all values will appear in a tabulated form in the Data Analysis panel



NOTE: the integration calculation method used in the Data Analysis will be the one selected under the 'Integral Options' (Analysis/Integration/Options).

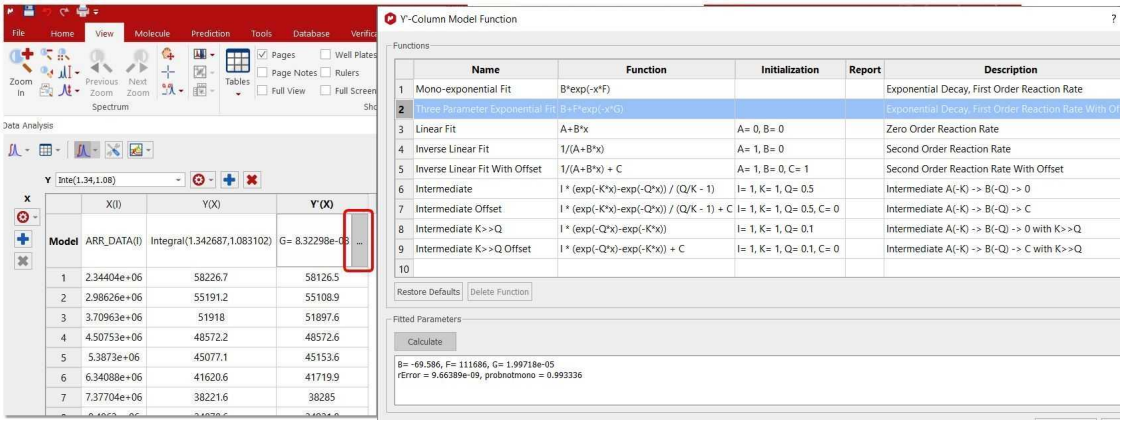
Finally, allow Mnova to try to guess the order of the reaction, fitting the data to standard equations and comparing the mean square error. To do it just follow the menu 'Data Analysis/Edit/Fit to Best'.



You can also set the 'Fit to Best' option by default by selecting 'Fit Function=Best Fit' on the 'Edit Model Options'.

You will find the result of the fitting in the 'Data Analysis' panel (Data Analysis/Show table). In that case, the best fit would be a 'Three Parameter Exponential'. If you are not happy with the fitting, just click on the first cell of the column Y'(X) to select any other function:

© 2024 MESTRELAB RESEARCH



NOTE: The Probnotmono value is important to assess whether or not the model for the fit we are assuming, in this case, a three parameter-exponential model satisfies. For instance, if you assume a mono-exponential model but the experimental data follows a bi or multi-exponential behavior, then the analysis will not be fully correct.
On the other hand, if we assume that the model is mono-exponential, the 'rError' value gives the [absolute] probable error in the value of 'r', where 'r' is:
y = a*exp(-**r***x)+b

This error is calculated based on the assumption that the final residues correspond to the actual noise in the data.

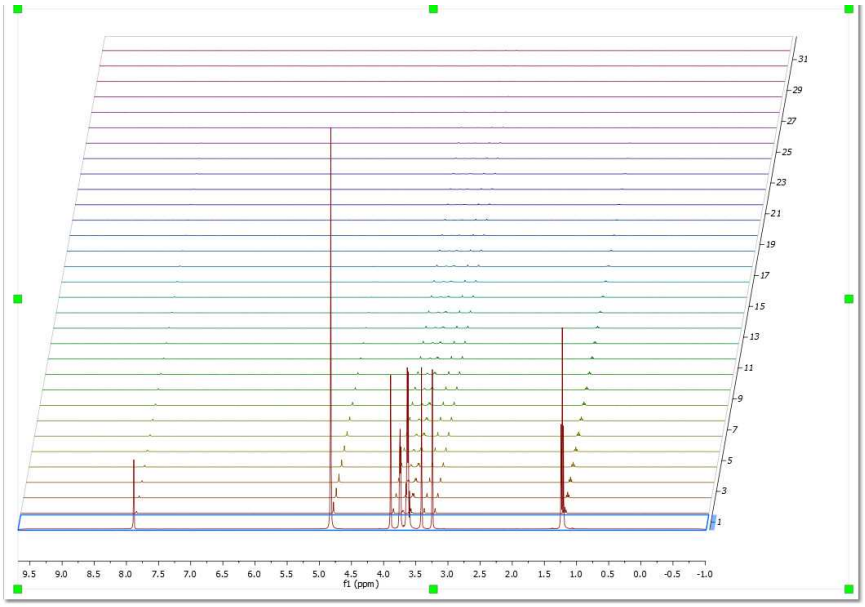
9.5.1 Data Analysis

Mnova incorporates a **'Data Analysis'** feature for the analysis of diffusion experiments. This feature covers also relaxation (T1/T2) experiments, kinetics, reactions monitoring, etc.

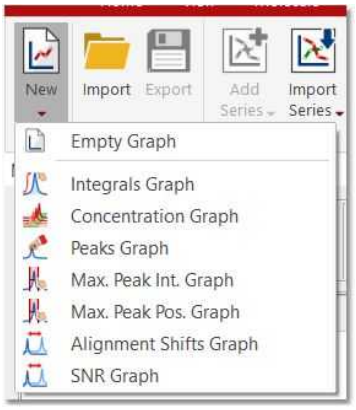
With this feature you will be able to extract the peak intensities and integrals in a tabular form from series of 1D NMR experiments and to draw graphical representations of the extracted values as well as any fit results.

Let´s see how this feature works in the case of a diffusion sample. Just load your experiment into Mnova:

© 2024 MESTRELAB RESEARCH



Follow the menu 'Data Analysis/New' and select the desired graphical representation (Peak Integrals, Concentrations, Intensities, or Maximum Peak Intensity, Alignment shifts and also SNR Graph).



Let´s select for example the 'Integrals Graph' option; you will notice how the cursor will change to a blue integral. Now you will only need to integrate the desired signal (in this case, we will select the signal at 1.20 ppm). Once you have integrate the desired region, you will notice how the graphical will change according with the decay of the signal. Please bear in mind that all values will appear in a tabulated form (in this case the integral values) and you will be able to export them to 3rd party apps for further post-analysis or reporting (just by clicking on the 'Copy to Clipboard' button of the table or by saving as XML files). Notice also that, if you integrate another region of the spectrum, the curve will be updated.

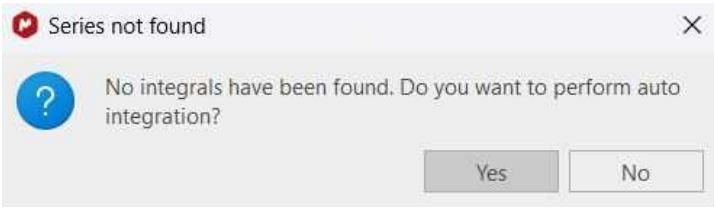
NOTE: the integration calculation method used in the Data Analysis will be the one selected under the 'Integral Options' (Analysis/Integration/Options).

If you have previously integrated (or picked) your stacked plot, you can use the 'Import Series' option from this dialog box and all the integral regions (or peaks) will be kept:

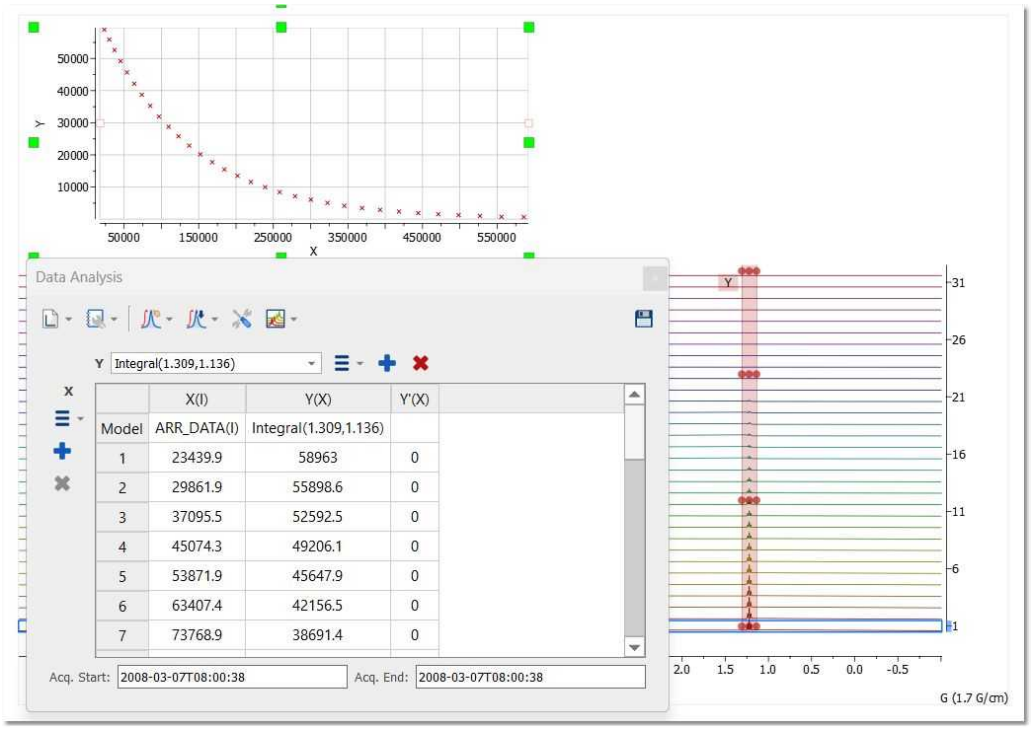
© 2024 MESTRELAB RESEARCH



If you use this option without having applied the integration, Mnova will ask you if you want to apply an automatic integration first:

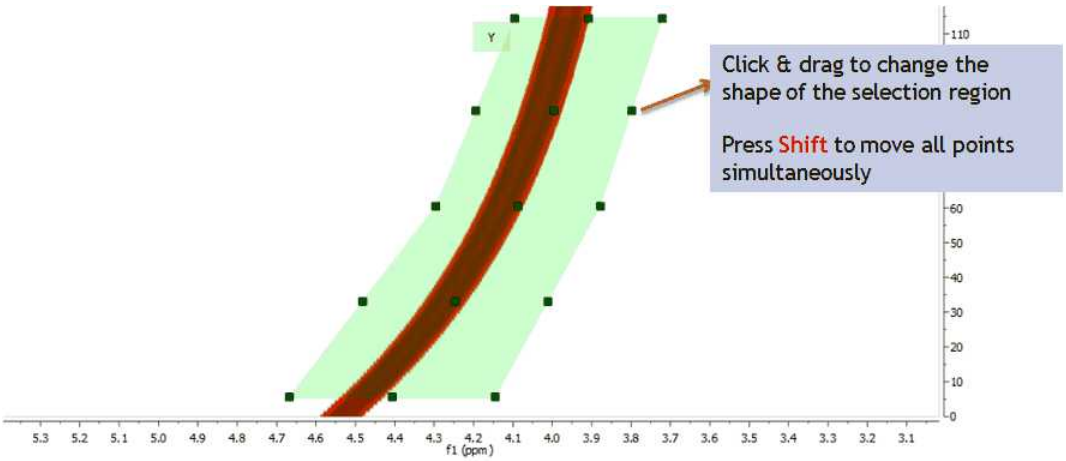


Go to this chapter to see a fully description of the Data Analysis panel

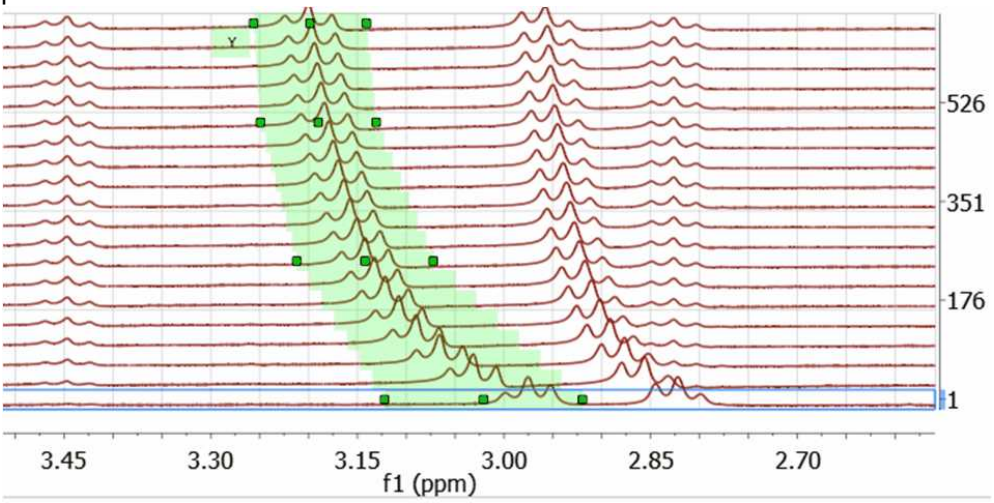


Please notice, that the integrated region will be highlighted in green and will contain several handles to modify the ranges of the integration just by clicking&dragging them (which will be really useful for 'reaction monitoring' in order to account for peaks movements). Holding down the 'shift' key, whilst you click and drag on any of the handles, will shift all the handles together of the same vertical.

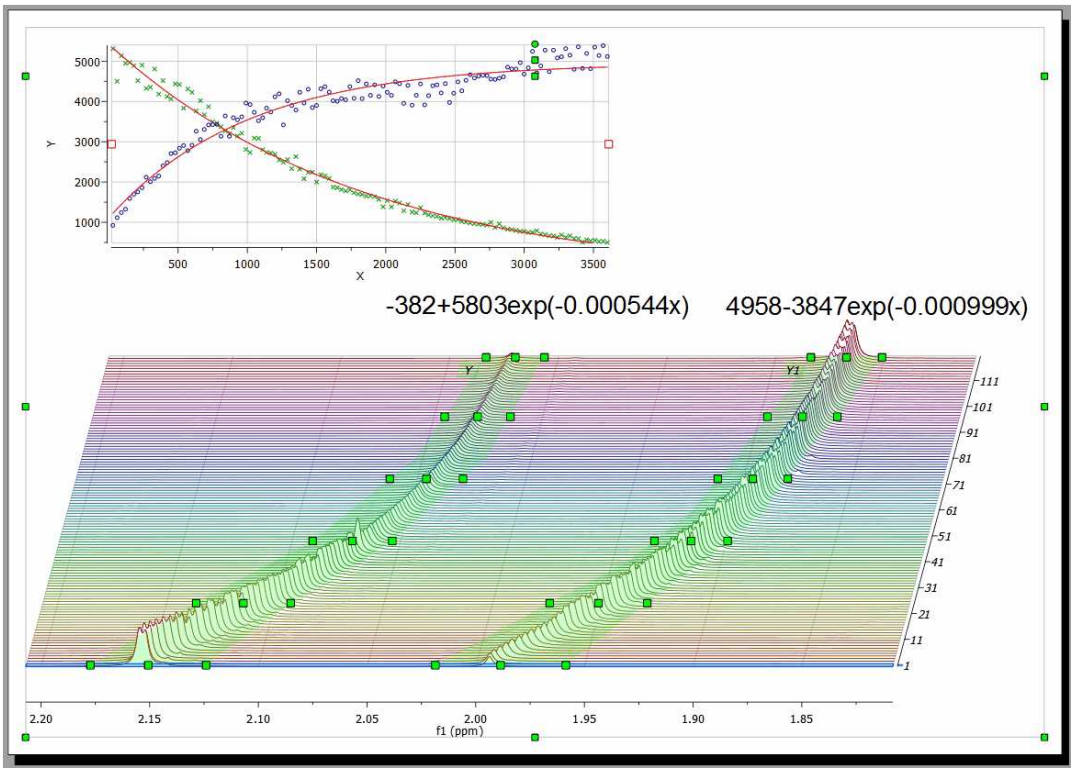
© 2024 MESTRELAB RESEARCH



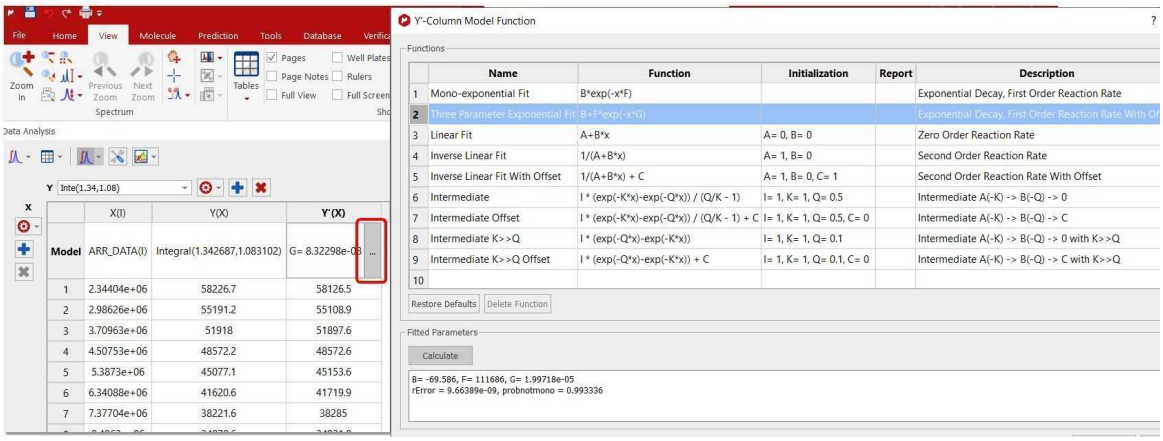
This new selection method works with any displaying mode: bitmap, stacked, active spectrum … The picture below shows this with the stack mode



Here you can see another example, where the graphical tool can be very useful to follow the evolutions of the signals:



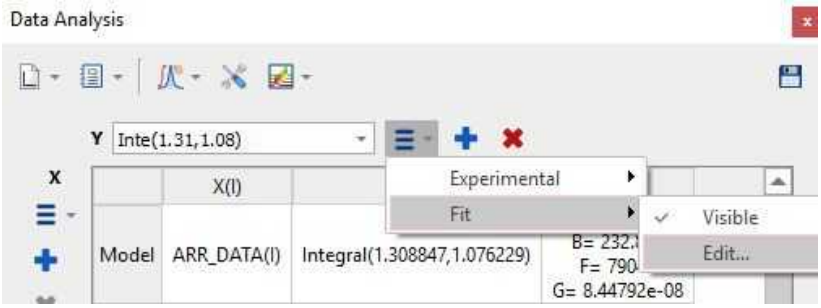
Clicking on the first cell of the column Y'(X) will allow you to fit the curve. In this case, we will fit it as a three parameter exponential curve.



Double click on the applicable cell to customize your NMR model by adding any other fitting formula. **Here** you can find a list of Mathematics javascript functions that you can use for your formulas (by using Math.sqrt(...))

You can also select the 'Edit Fit' option below:

© 2024 MESTRELAB RESEARCH



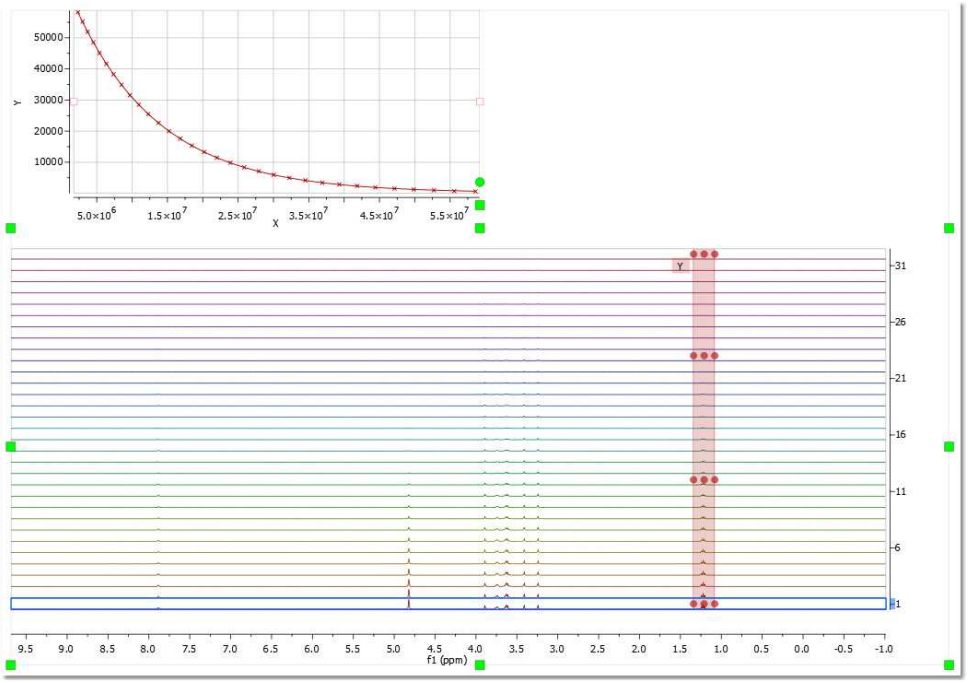
Next, click on the 'Calculate' button to generate the error and the parameters of the fitting and finally click on the OK button to draw the fitting curve.

NOTE

Probnotmono parameter gives the probability (0-1) that the decay is NOT monoexponential. For example, probnotmono>0.7 can be read as 'this could be non-monoexponential' while 'this is almost certainly non-monoexponential' when probnotmono>0.95

The rError is the quadratic error of the fit.

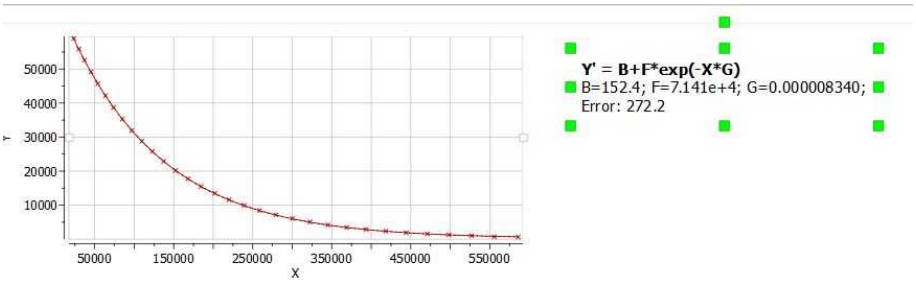
Ref: Gianni Ferrante and Stanislav Sykora, Technical Aspects of Fast Field Cycling, in Advances in Inorganic Chemistry, Vol. 57, Relaxometry of water-metal ion interactions, Elsevier 2004 (ISBN 0-120-23657-5), pp.405-470.



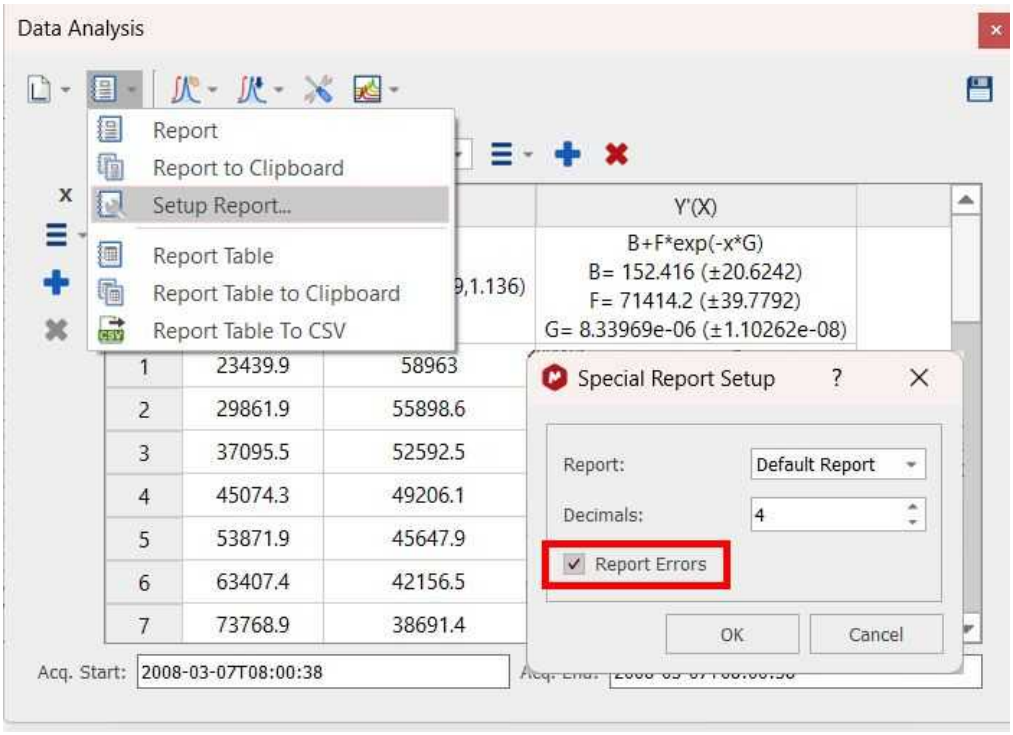
Follow the menu ' Data Analysis/Edit/Fit to Best' to allow Mnova to guess the order of the reaction (by fitting the data to standard equations and comparing the mean square error):



Click on the Report button to get the report of the fits:

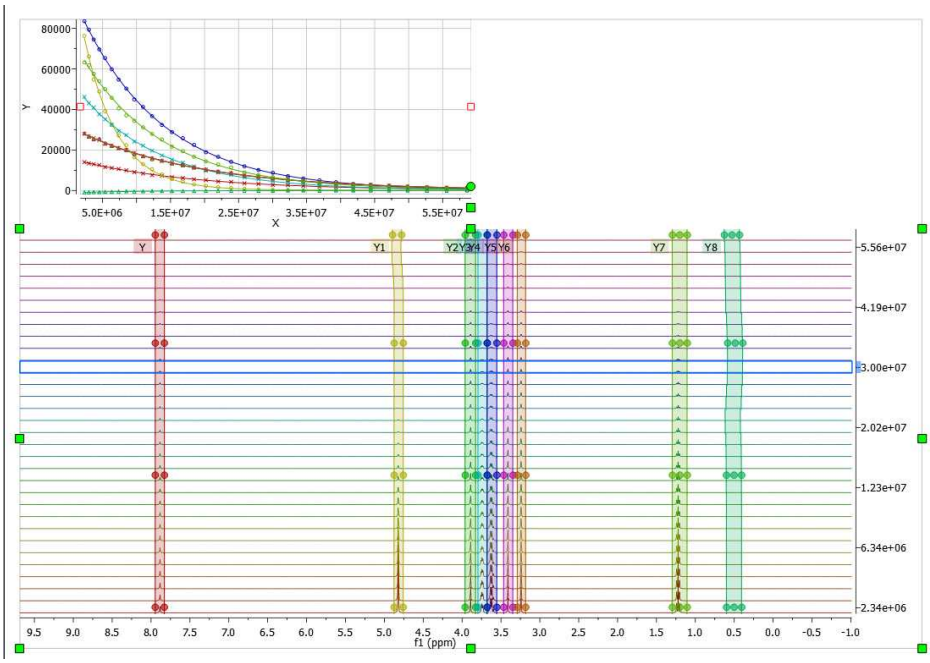


You can report the results with the error associated to each parameter by checking the 'report errors' box under the 'Setup Report' option:

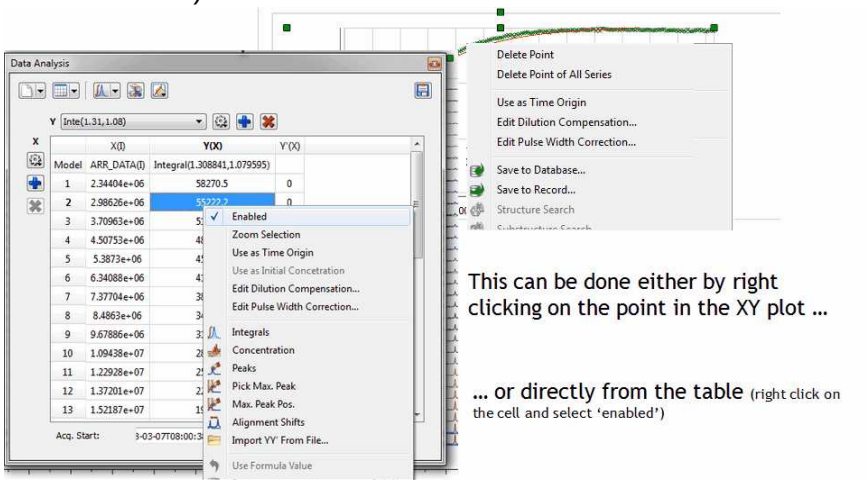


You can select other regions to be analyzed; each selected region will be colored differently (and the grab handle colors and graphical lines will have the same colors; which you can change by double clicking on the graph):

© 2024 MESTRELAB RESEARCH

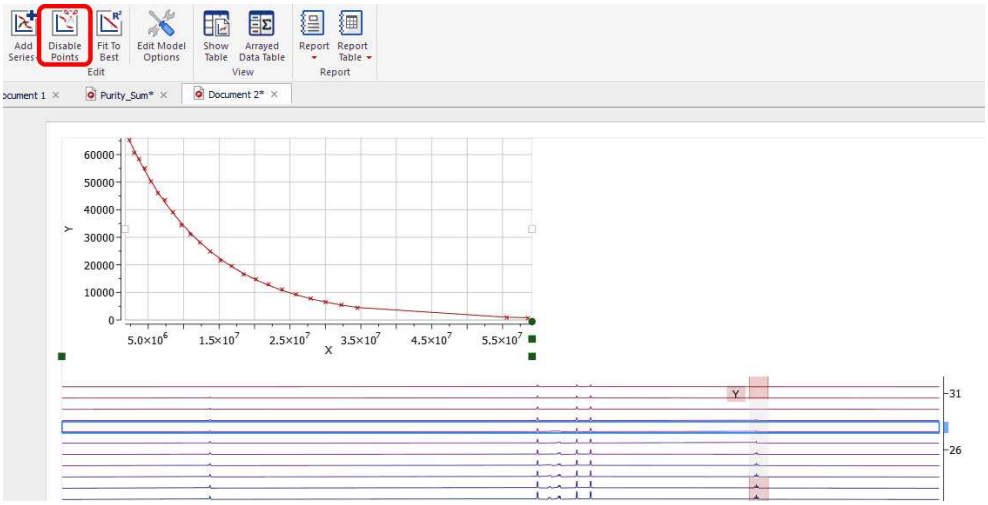


From the Data Analysis panel, you will also be able to disable points (either directly from the XY graph or from the table):

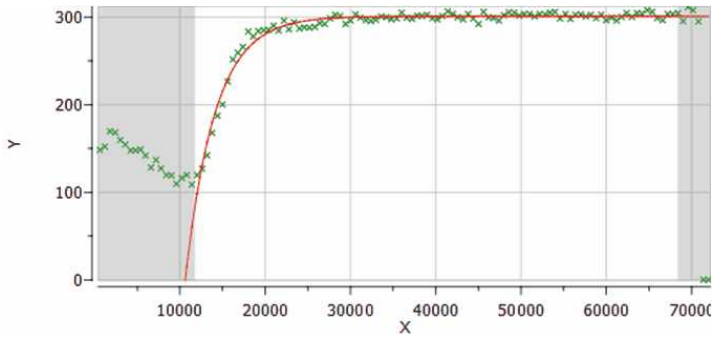


You can also disable points directly from your arrayed dataset, just by clicking on this button  and then selecting the undesired signals on the dataset. You will notice how the applicable points in the XY graph will disappear and the signals on the stack plot will be highlighted in grey.

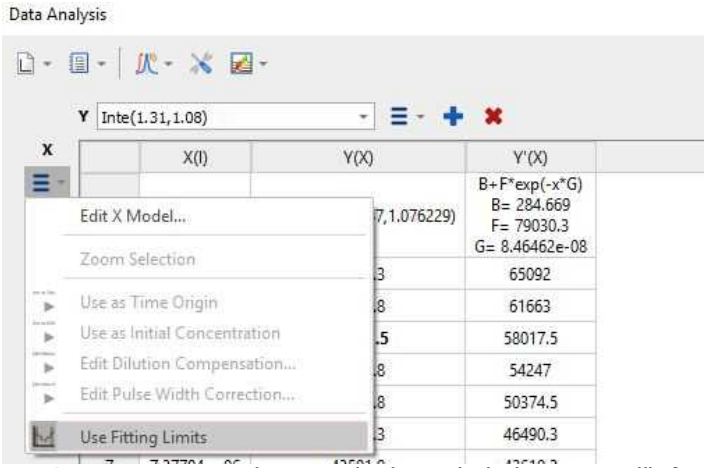
© 2024 MESTRELAB RESEARCH



For convenience, you can **exclude points from the left and right** edges of the graph (by selecting 'Use Fitting Limits' on the Data analysis panel and highlighting the graph):



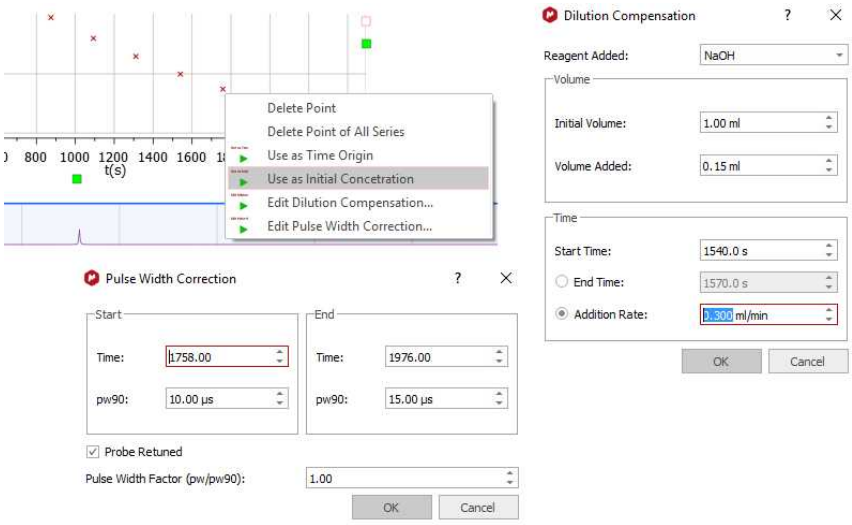
If you want to disable this option, just uncheck this button on the Data Analysis panel:



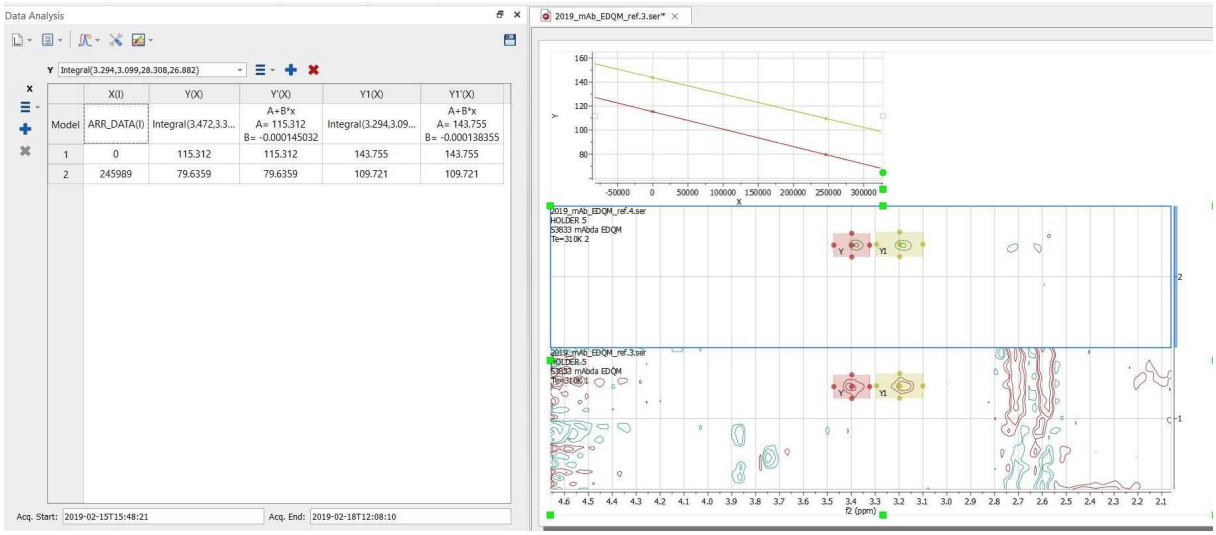
IMPORTANT: Every time a point is excluded, Mnova will ***dynamically*** recalculate the fitting function.

With the *Reaction Monitoring* plugin enabled, if you right click on any point of the Data Analysis Graph (or Table), you will be allowed to 'use that point as time origin', or to set the Dilution Compensation Parameters' and 'Pulse Width Correction':

© 2024 MESTRELAB RESEARCH



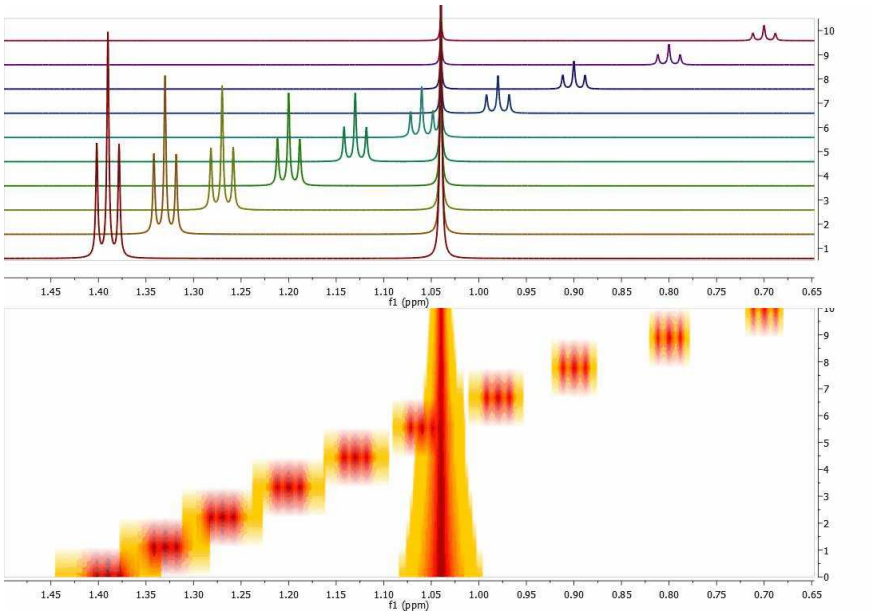
The Data Analysis feature can also be used with 2D stacked NMR plots:



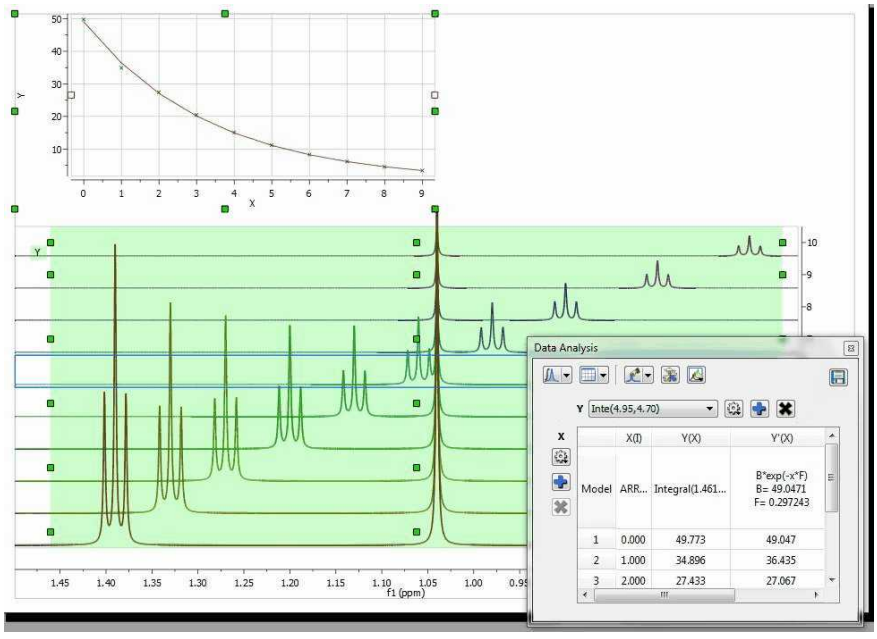
Crossing over of peaks

Crossing over of peaks is a very common event in Reaction Monitoring (RM) experiments. When this happens, the automatic alignment algorithm might not work properly. To illustrate this issue, we have simulated using Mnova a very simple data set comprised by a triplet and a singlet in such a way that the chemical shift of the triplet moves from 1.4 ppm to 0.7 ppm and having an exponential decay from spectrum to spectrum. This is depicted in the figure below, both as a stacked and a bitmap plot:

© 2024 MESTRELAB RESEARCH



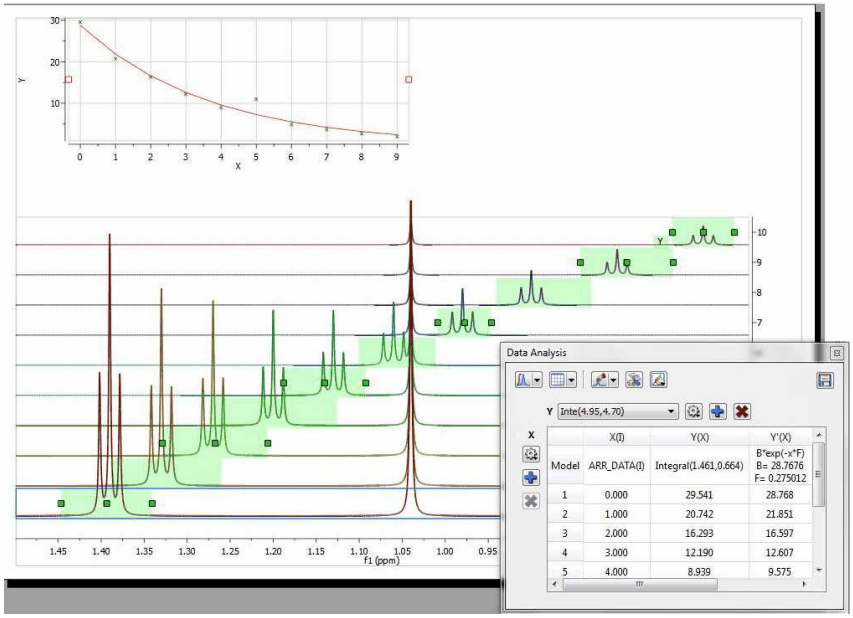
Now let's say you are interested in extracting the intensities of the triplet as the reaction progresses. There is actually no need to pre-align the spectra algorithmically; it is much simpler to have some kind of graphical tool to instruct the software which peaks (or multiplets) need to be used for the reaction monitoring analysis. Let me show you how this works in Mnova:
First of all, in the Data Analysis module you select the region to be analyzed. As a starting point, the region will have a rectangular shape (green rectangle in the figure below):



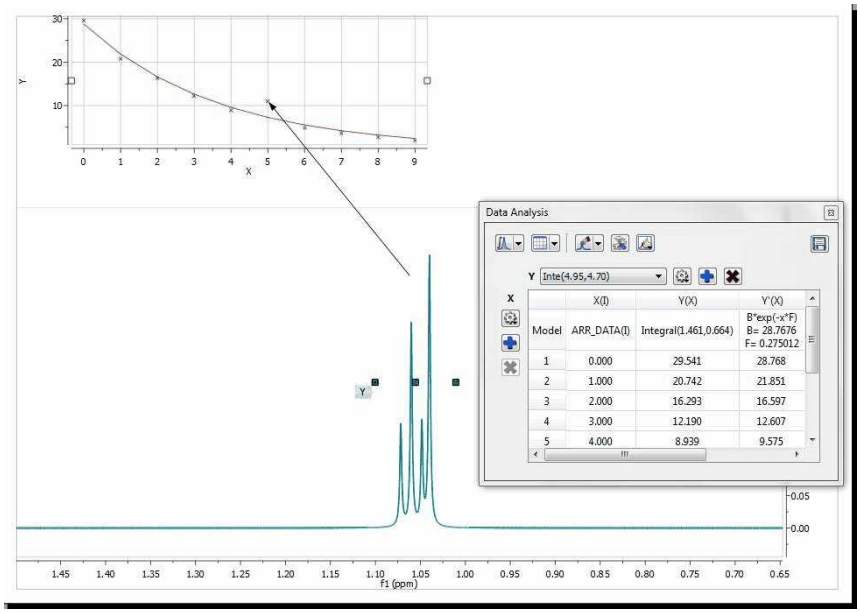
It can be noted that the graph shows an exponential decay, but the actual values must obviously be wrong as the values calculated, using the green rectangle as a boundary for the integration, include peaks from both the triplet and singlet, and we are interested in the analysis of the triplet resonances only.

© 2024 MESTRELAB RESEARCH

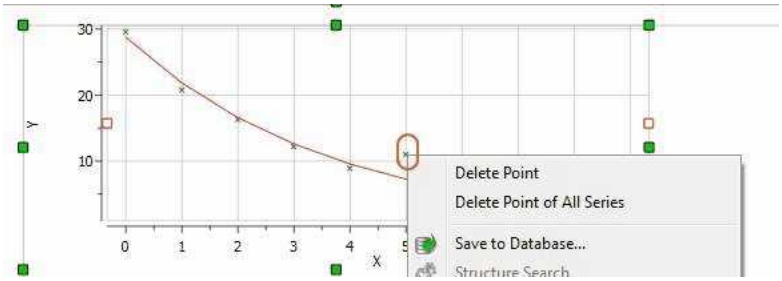
The selection rectangle has a number of handles (small green boxes). You can drag and move them freely so that you can adjust the selection feature to follow the triplet (the number of handles can be adjusted in the Data Analysis options. In the figure below, the result of adjusting the handles to follow the triplet is shown:



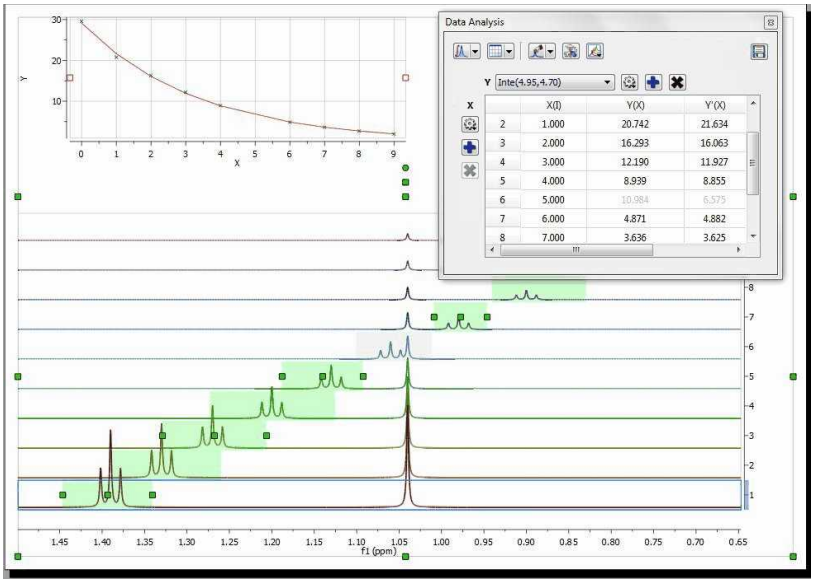
Now you can see that there is an outlier in the exponential curve which, obviously is caused by the singlet which overlaps with the triplet (spectrum number 6 which corresponds to data point #5, as in the graph the numbering starts from zero). Figure below shows that particular spectrum showing the singlet overlapping with the triplet:



At this stage, there are several approaches. The simplest one is to just discard that point for the analysis, for example, by right clicking on that point in the graph and disabling it:



As soon as that point is deleted / disabled, Mnova will update the graph automatically. This is the new result:



Another approach would involve using GSD to eliminate the singlet from the triplet so that it would not be necessary to discard the information from that particular spectrum.

Here you can watch a video about using Data Analysis for pKa calculation by NMR.

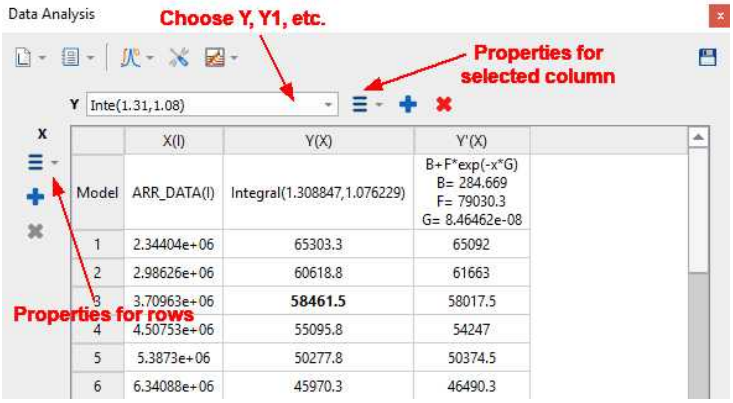
See this blog entry for the automatic peak tracking feature:
http://mestrelab.com/blog/automatic-peak-tracking-in-arrayed-nmr-data/

You can also use the Data Analysis for stacked chromatograms. See this chapter for further information.

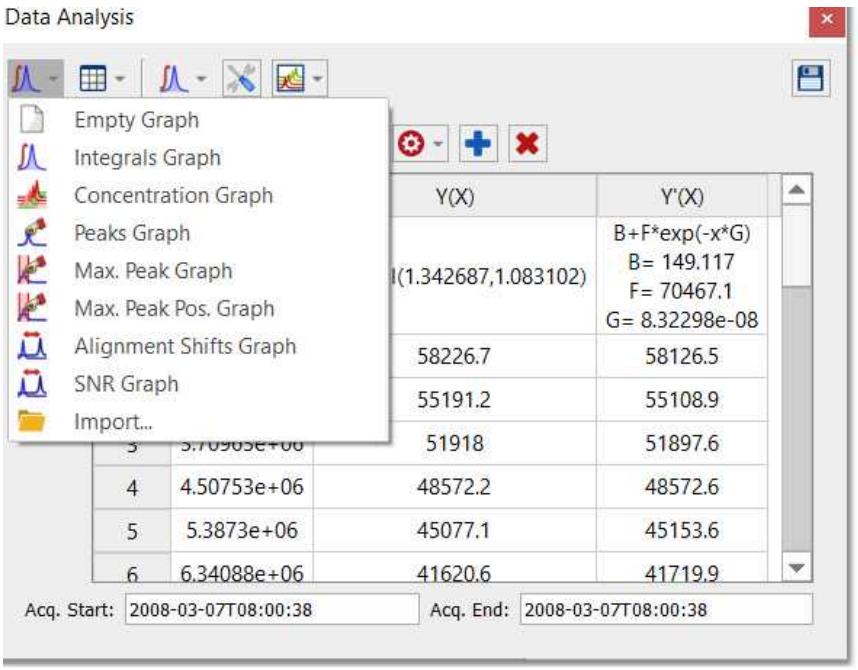
9.5.1.1 Data Analysis panel

The Data Analysis panel contains several options to create your graphs. You can import a .txt or .csv file (via File/Open dialog of Mnova) to fill the Data Analysis panel with X,Y values and generate the applicable graphical representation.

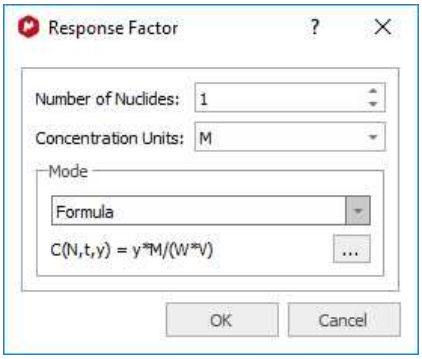
© 2024 MESTRELAB RESEARCH



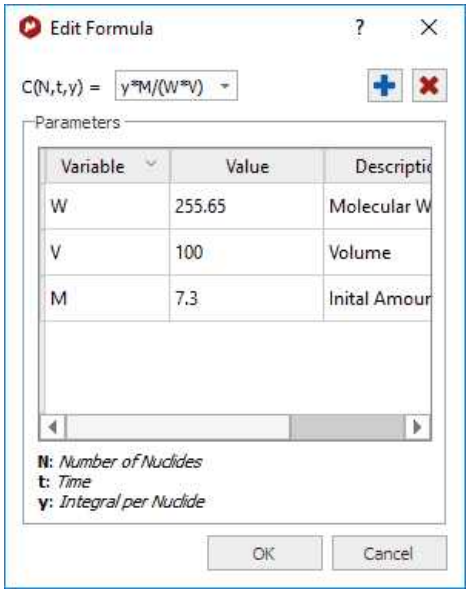
You can select any graph from the scroll down menu below (and then a region of interest in the arrayed spectrum):



If you select the Concentration Graph, a new dialog box will appear to ask you for the number of nuclides of the selected region



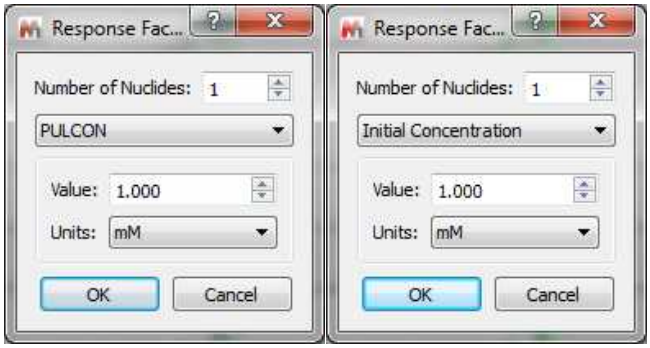
Click on the 3 dots button to customize the formula:



The terms in the equation and table are as follows:
N=Number of nuclides
W = molecular weight of chemical species (g/mole)
M = initial amount, or mass
V = volume

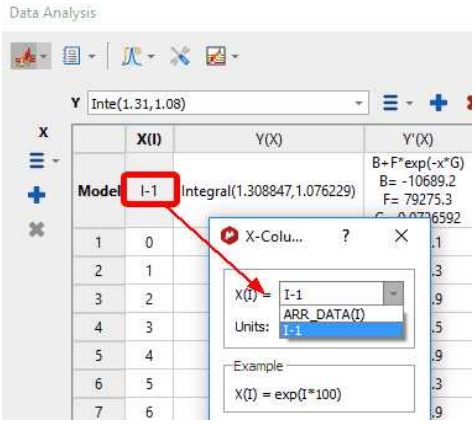
So if M is in grams, and V is in litres, then the equation calculates concentration in Molar. (Obviously the user can introduce factors to convert this to mM or whatever is convenient).

With the *'Reaction Monitoring'* plugin enabled you will be able to select 'PULCON' or 'Initial Concentration' modes:



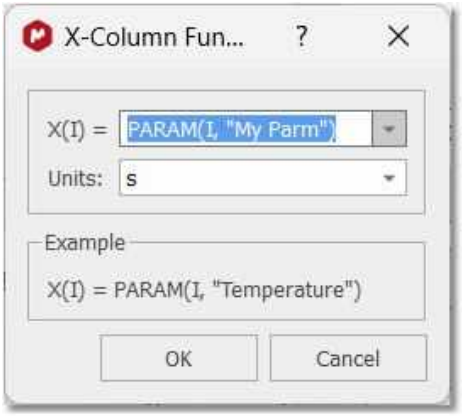
NOTE: If the X column in Data Analysis is filled with zeros or you need to change the function, just double click on the model cell to derive t' not only from t but also from i-spectrum index:

© 2024 MESTRELAB RESEARCH

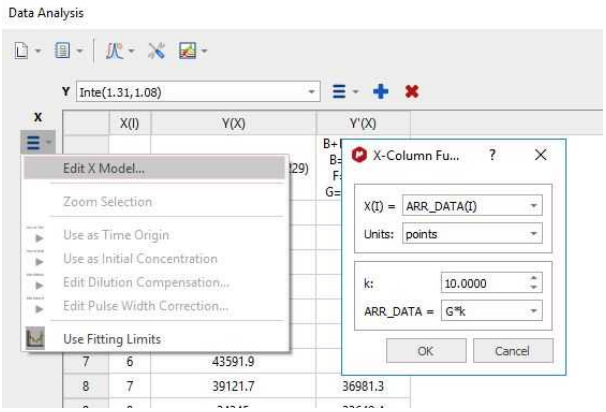


You can also select any existing parameter of the parameters table to be parsed to the Data Analysis panel, just by using a macro like this one:

PARAM(I, "My Parm"); being "My Parm" an existing parameter of the Parameters table (Temperature, Number of Scans, etc):



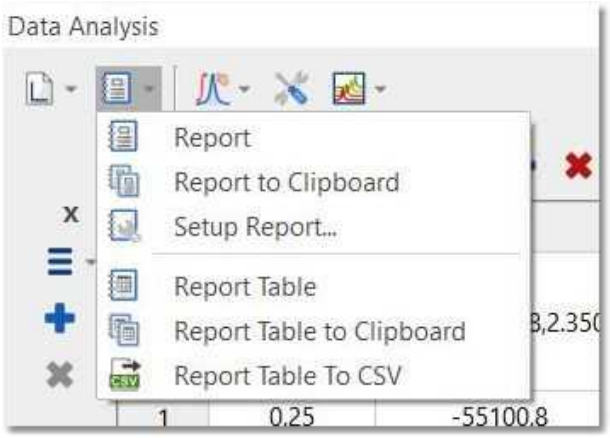
From the same dialog (or by selecting 'Edit X Model'), you can also change the time units and setting the k and ARR_DATA values:



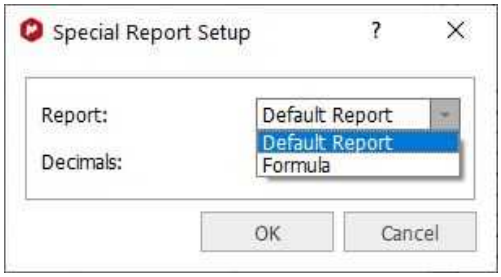
Copy the values from an an Excel worksheet and paste them into the X(I) column by highlight the first cell (in the row 1) and doing ‘Ctrl/Cmd+V’ to paste the values.

© 2024 MESTRELAB RESEARCH

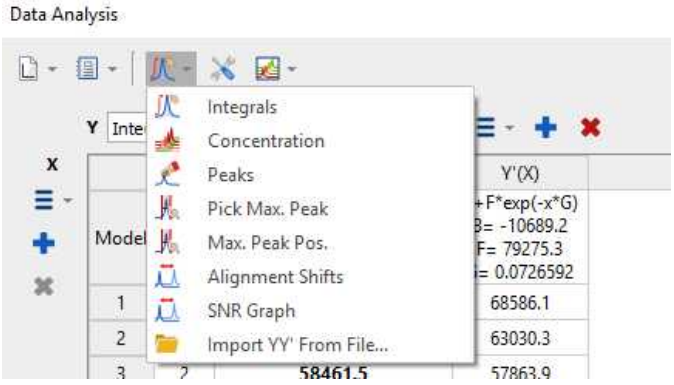
Clicking on the Report scroll down menu, will paste the report or the table to your spectrum, to the clipboard or to a csv file:



Selecting the Setup Report option, will allow you to paste only the formula or the default reportt:

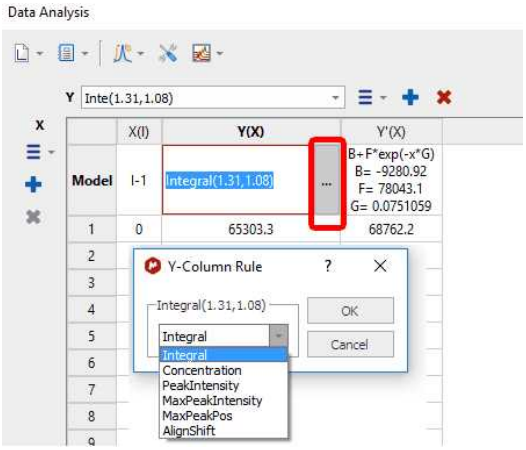


Clicking on the 'Interactive Y Filling' scroll down menu will allow you to select between different peaks selection modes (Peak Integrals, Concentrations, Intensities, or Maximum Peak Intensity, Alignment shifts, SNR Graph or import YY' File), in order to generate the applicable plot.



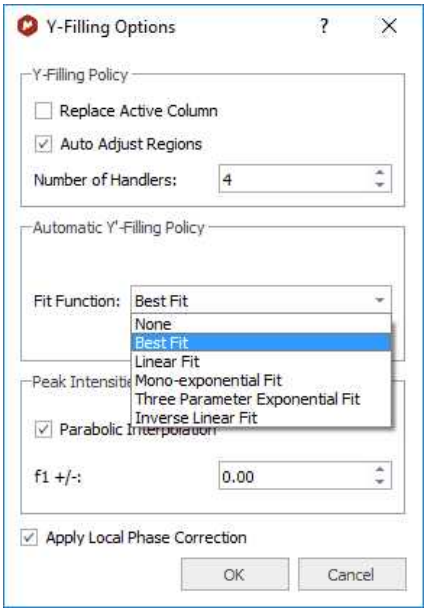
Please note that you can also change those options by double clicking on the '...' button of the Model cell under the Y(X) header of the Data Analysis panel:

© 2024 MESTRELAB RESEARCH



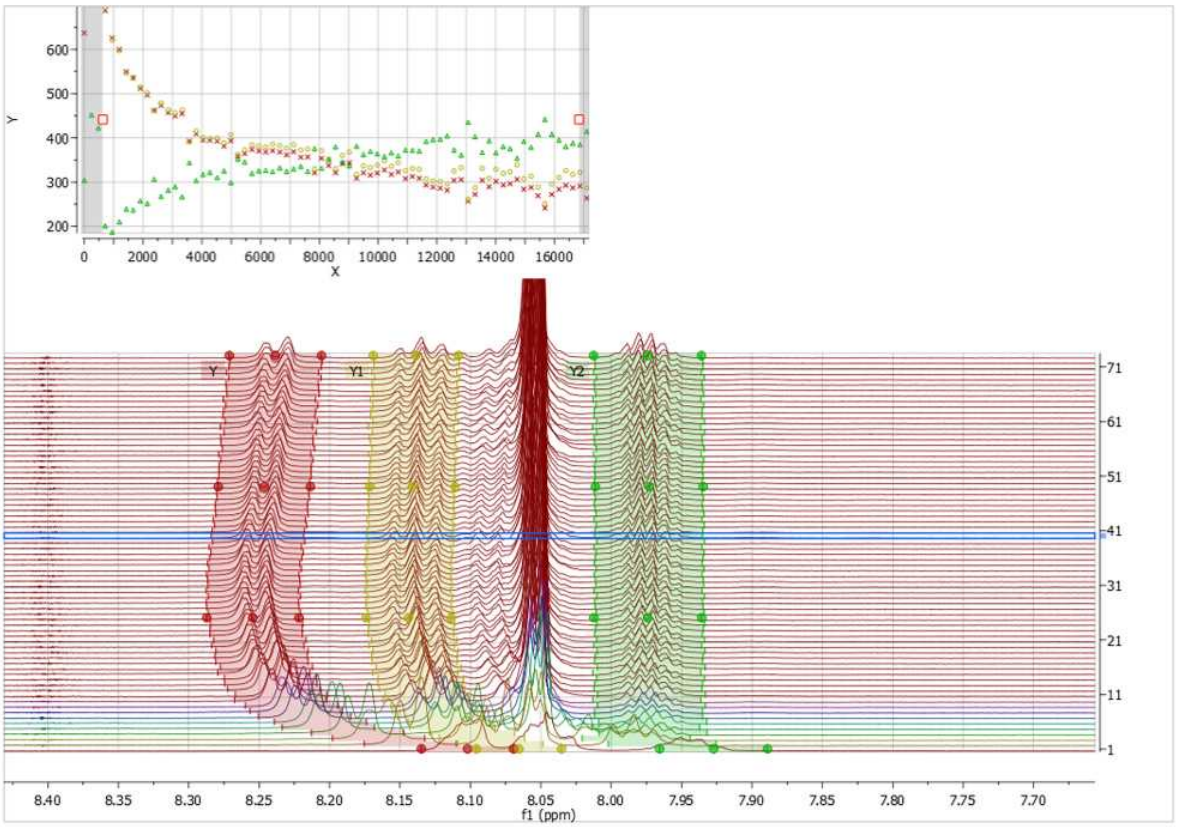
The 'Alignment shift' option will measure the change of chemical shift of the peak across the spectra, after having used the Alignment algorithm to align the peaks of interest.

Clicking on the 'Edit Model Options' button, will allow you to change some defaults, such as the capability to replace the active column (or to create a new one); number of handles of the selected regions, the Fit function for the Y'-Filling column or use a 'Parabolic Interpolation for the 'Peak intensities'.



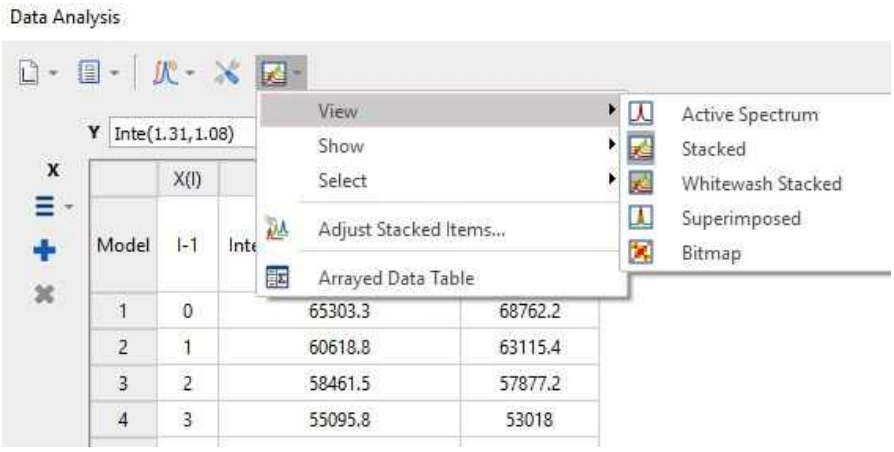
The 'auto adjust regions' option will automatically track the 'move' of the peaks/multiplets through the time course of a reaction.

© 2024 MESTRELAB RESEARCH



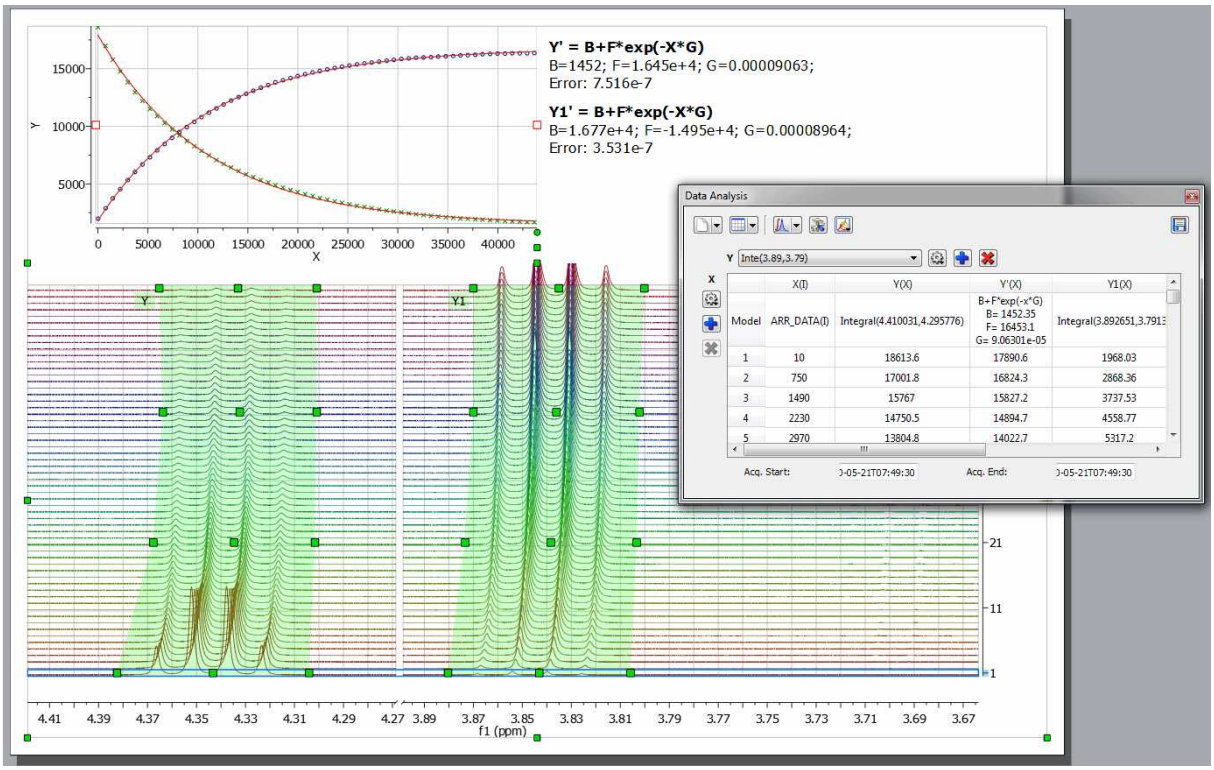
See this blog entry for the 'auto adjust regions' feature:
http://mestrelab.com/blog/automatic-peak-tracking-in-arrayed-nmr-data/

In this panel; you will also find a button ("Reaction Monitoring") which contains all Show and Select options from Stacked Items table, and options to show/hide Stacked Spectra and Arrayed Data tables. The button is only visible when RM plugin is installed and licensed:



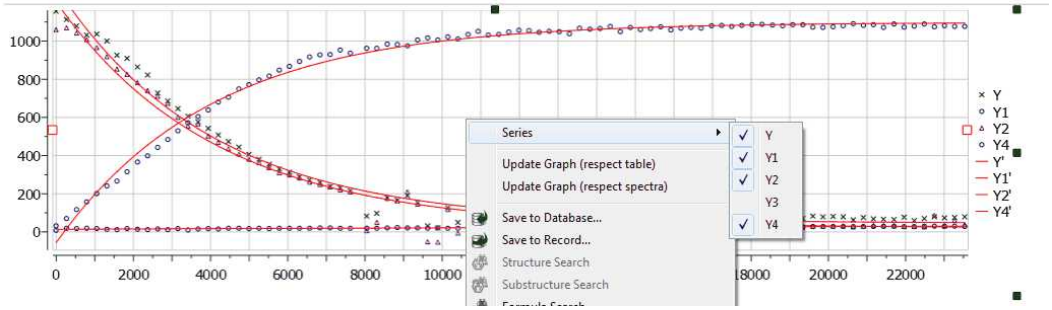
You can add additional curves to the representation by clicking on the 'Add New Data Serie' icon (of the 'Data Analysis' dialog box) and integrating (or applying any other analysis) the desired region of the spectrum:

© 2024 MESTRELAB RESEARCH



- To export the graphical dataset to an ASCII file, select the 'Data Analysis graphic' and follow the menu 'File/Save as/ASCII Text File (*.txt).
- Rows corresponding to spectra which have been removed from the dataset will be marked with **red** color in the Data Analysis table.

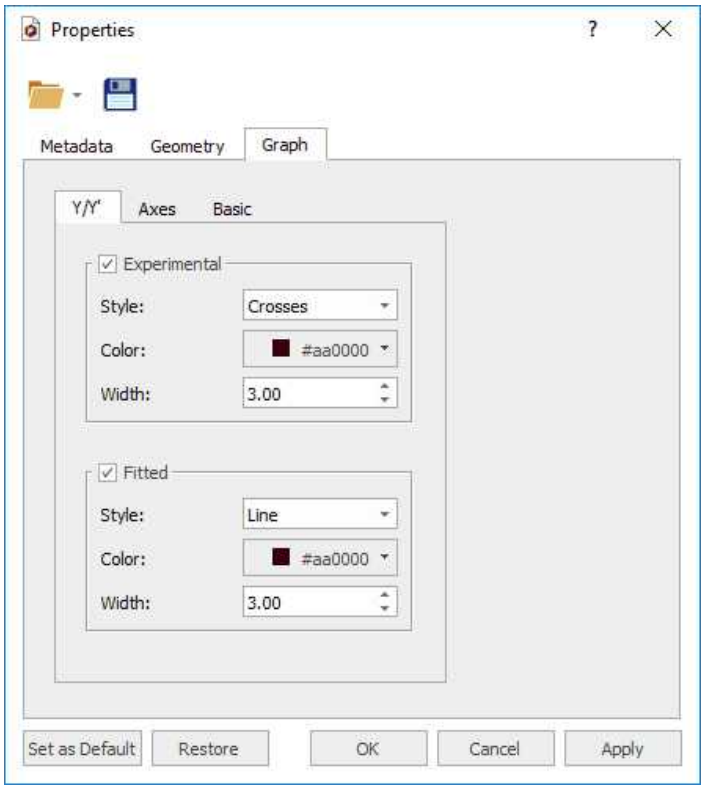
Right clicking on the Data Analysis Graphic, will allow you to update the graph after having made changes in the series of the 'Data Analysis Table' or directly on the stacked spectra (very useful, for example if you invert the order of the spectra). If you have more than one curve, you will be able to select/deselect available "series" (curves) on the plot.



Follow the menu 'Processing/Blind Regions/Add Blind Regions' to add blind regions to your arrayed experiment.

9.5.1.2 Data Analysis Properties

Modify the properties of the graphic representation, just by double clicking on it to display the 'Properties dialog box' which contains three labels (Y/Y', Axes and Basic).
In the first label you will be able to change the style, color and width of the Experimental and Fitted lines



Selecting the Axes tab will allow you to change the color, font and margin of the axes and also to select the units scale (seconds, minutes, hours, points) and the capability to select Logarithmic values or to change the label of each axis.

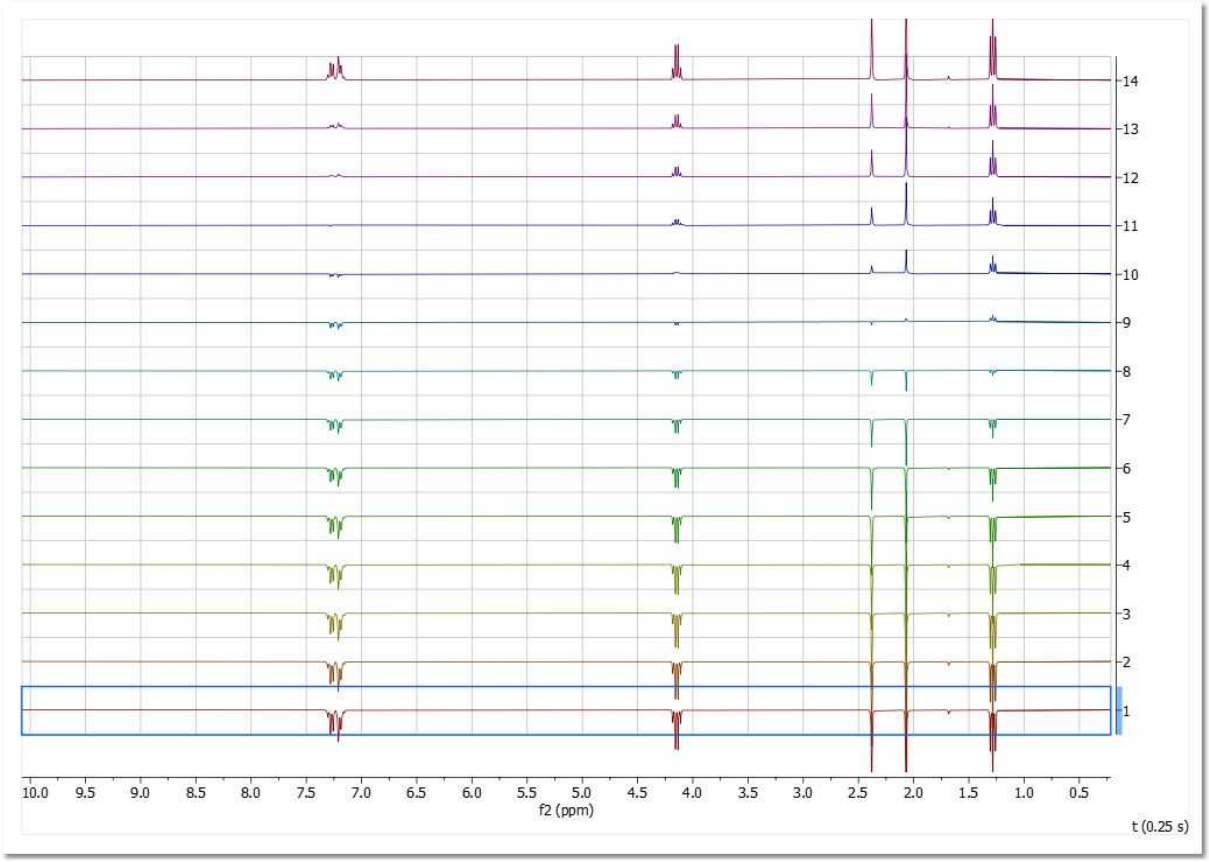
Clicking on the Basic tab will allow you to change the background, the grid and also to show the corresponding legend.

9.5.1.3 T1 Analysis

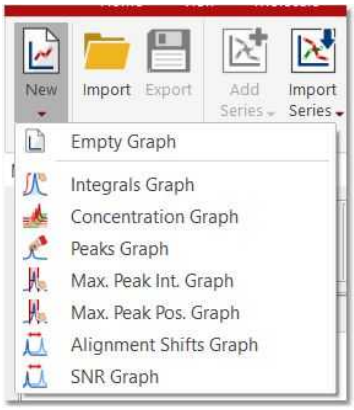
Let's see how to use the Data Analysis feature to calculate the T1 value of a 1H-NMR dataset.

Firstly, load your dataset into Mnova and make sure that the phase is right (if not correct it by using the 'Manual Phase Correction tool):

© 2024 MESTRELAB RESEARCH

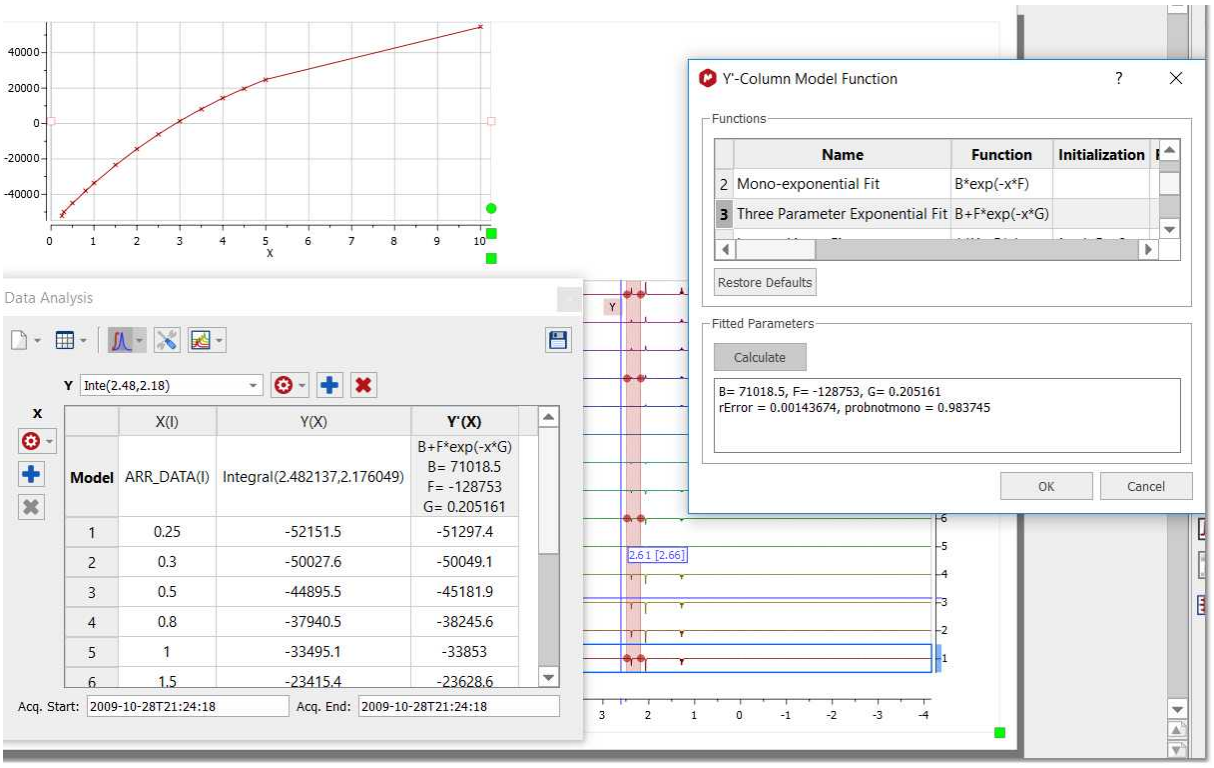


Follow the menu 'Data Analysis/New/Integrals Graph' and select the desired signal to analyze (i.e. the singlet at 2.38 ppm). You will notice how the graphical plot will be updated with the integral values:

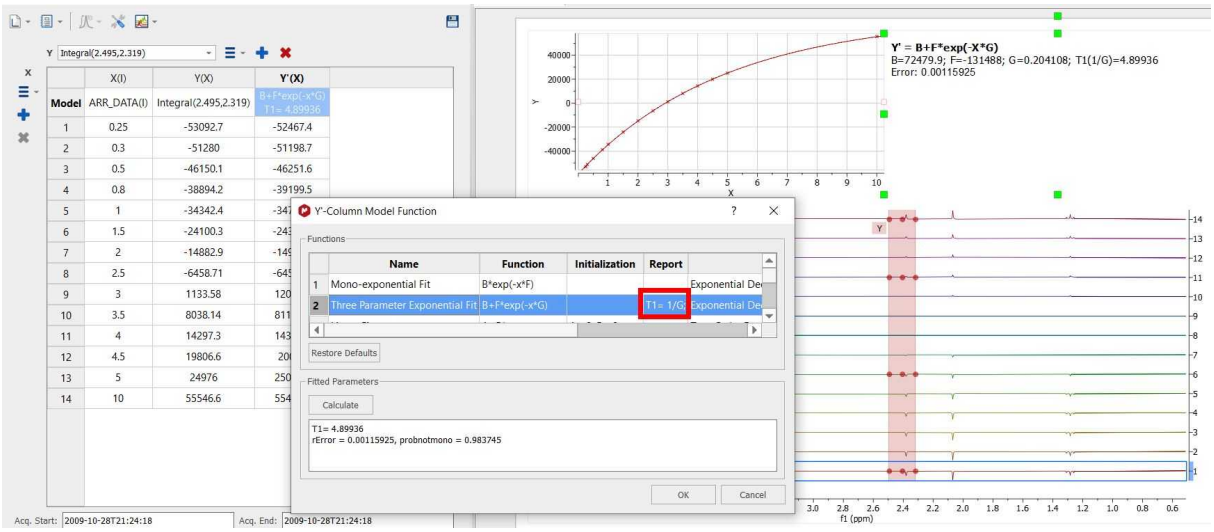


Finally double click on the Y'(X) cell, select the 'Three parameter exponential Fit' (**B+F*exp(-x*G)**) and click on the Calculate button

© 2024 MESTRELAB RESEARCH



The T1 value will be the inverse of the G value, so in that case the T1 value for the proton at 2.38 ppm will be 4.90 seconds. Here you can see the result:



9.6 DOSY/ROSY Transform

(Not available in Mnova Lite)

NMR diffusion experiments provide a way to separate the different compounds in a mixture based on the differing translation diffusion coefficients (and therefore differences in the size and shape of the molecule, as well as physical properties of the surrounding environment such as viscosity, temperature, etc) of each chemical species in solution. In a certain way, it can be regarded as a special

© 2024 MESTRELAB RESEARCH

chromatographic method for physical component separation, but unlike those techniques, it does not require any particular sample preparation or chromatographic method optimization and maintains the innate chemical environment of the sample during analysis.

The measurement of diffusion is carried out by observing the attenuation of the NMR signals during a pulsed field gradient experiment. The degree of attenuation is a function of the magnetic gradient pulse amplitude (G) and occurs at a rate proportional to the diffusion coefficient (D) of the molecule. Assuming that a line at a given (fixed) chemical shift f belongs to a single sample component A with a diffusion constant D_A, we have

$$S(f,z) = S_A(f) \exp(-D_A Z) \qquad (1)$$

where $S_A(f)$ is the spectral intensity of component A in zero gradient ('normal' spectrum of A), D_A is its diffusion coefficient and **Z** encodes the different gradient amplitudes used in the experiment.

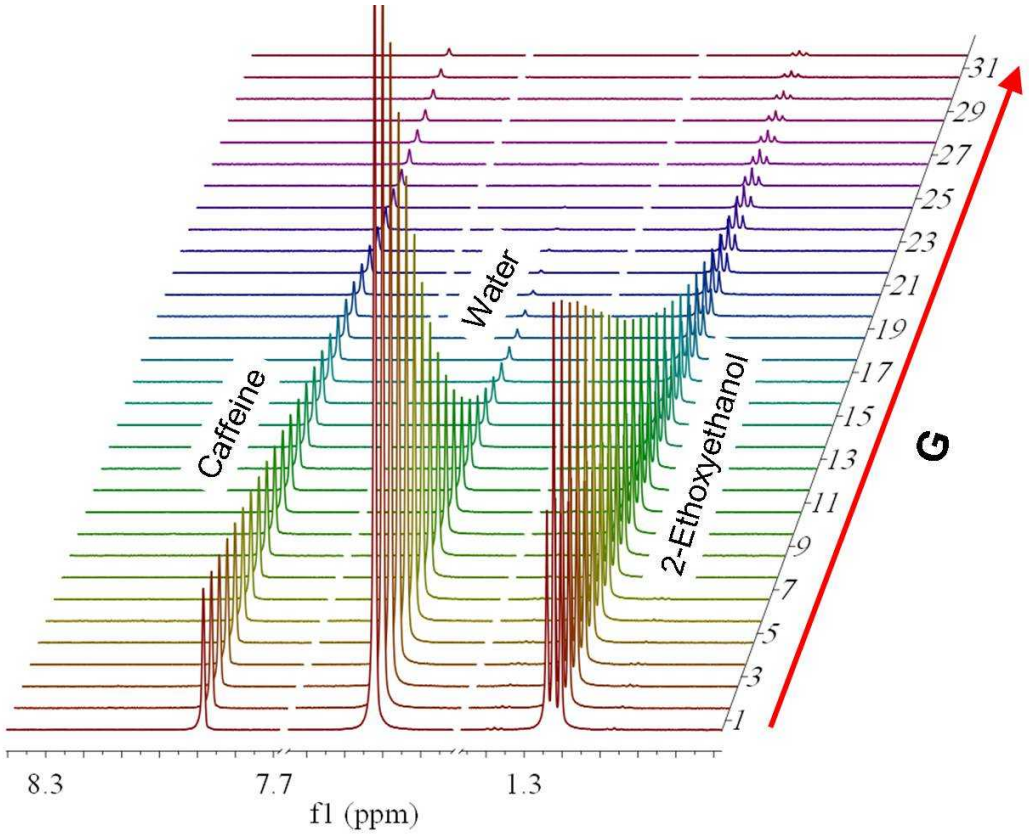
Depending on the type of experiment, there are various formulae for **Z** in terms of the amplitude **G** of the applied gradient and one or more timing parameters such as Δ (time between two pulse gradients, related to echo time) and δ (gradient pulse width). In the original Tanner-Stejskal method using two rectangular gradient pulses, for example,

$$Z = \gamma^2 G^2 \delta^2 (\Delta - \delta/3) \qquad (2)$$

The above equation holds strictly for simple PFG-NMR experiments, and is modified slightly to accommodate more complicated pulse sequences.

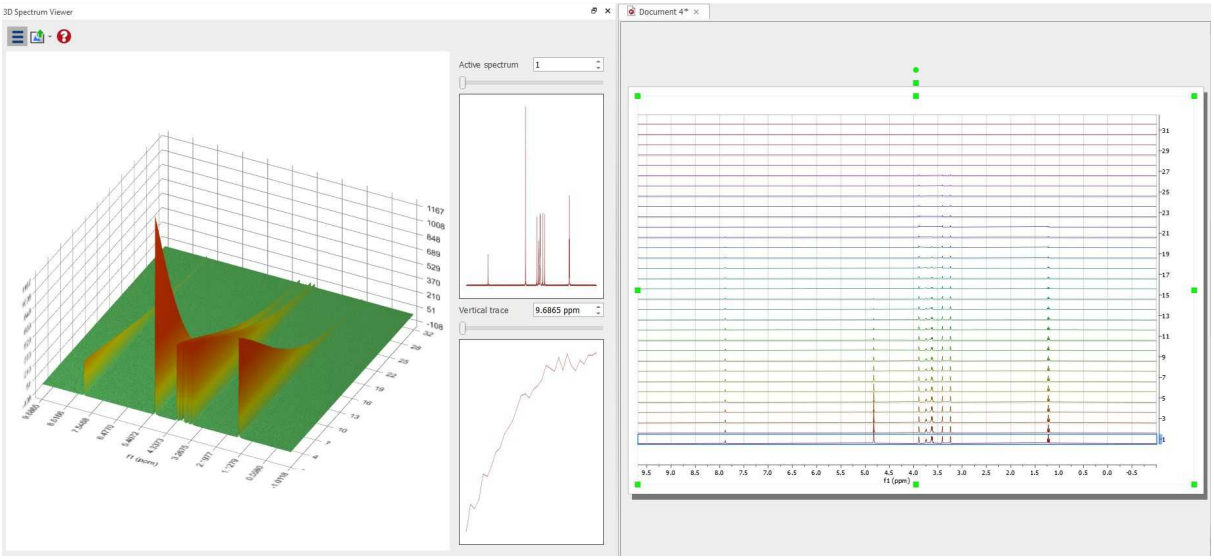
In practice, a series of NMR diffusion spectra are acquired as a function of the gradient strength. For example, the figure below shows the results of a series of 1H-NMR diffusion experiments for a mixture containing caffeine, 2-Ethoxyethanol and water.

© 2024 MESTRELAB RESEARCH



It can be observed that the intensities of the resonances follow an exponential decay. The slope of this decay is proportional to the diffusion coefficient according to equation (1). All signals corresponding to the same molecular species will decay at the same rate. For example, peaks applicable to water decay faster than the peaks of caffeine and 2-Ethoxyethanol.

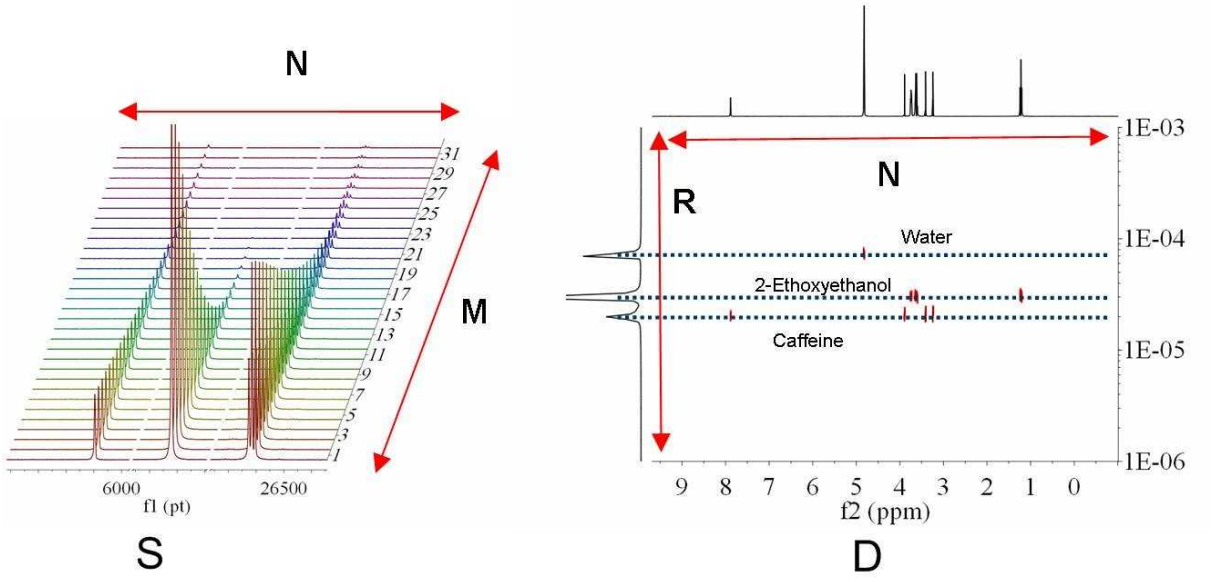
Please note that loading a stacked plot of 1D NMR spectra will also display the result in the 3D viewer:



The DOSY transformation

© 2024 MESTRELAB RESEARCH

As far as data processing of raw PFG-NMR spectra is concerned, the goal is to transform the NxM data matrix S into an NxR matrix (2D DOSY spectrum) as follows:



The horizontal axis of the DOSY map D is identical to that of S and encodes the chemical shift of the nucleus observed (general 1H). The vertical dimension, however, encodes the diffusion constant D. This is termed **Diffusion Ordered Spectroscopy** (DOSY) NMR. In the ideal case of non-overlapping component lines and no chemical exchange, the 2D peaks align themselves along horizontal lines, each corresponding to one sample component (molecule).

The horizontal cut along such a line should show that component's 'normal' spectrum. Vertical cuts show the diffusion peaks at positions defining the corresponding diffusion constants.

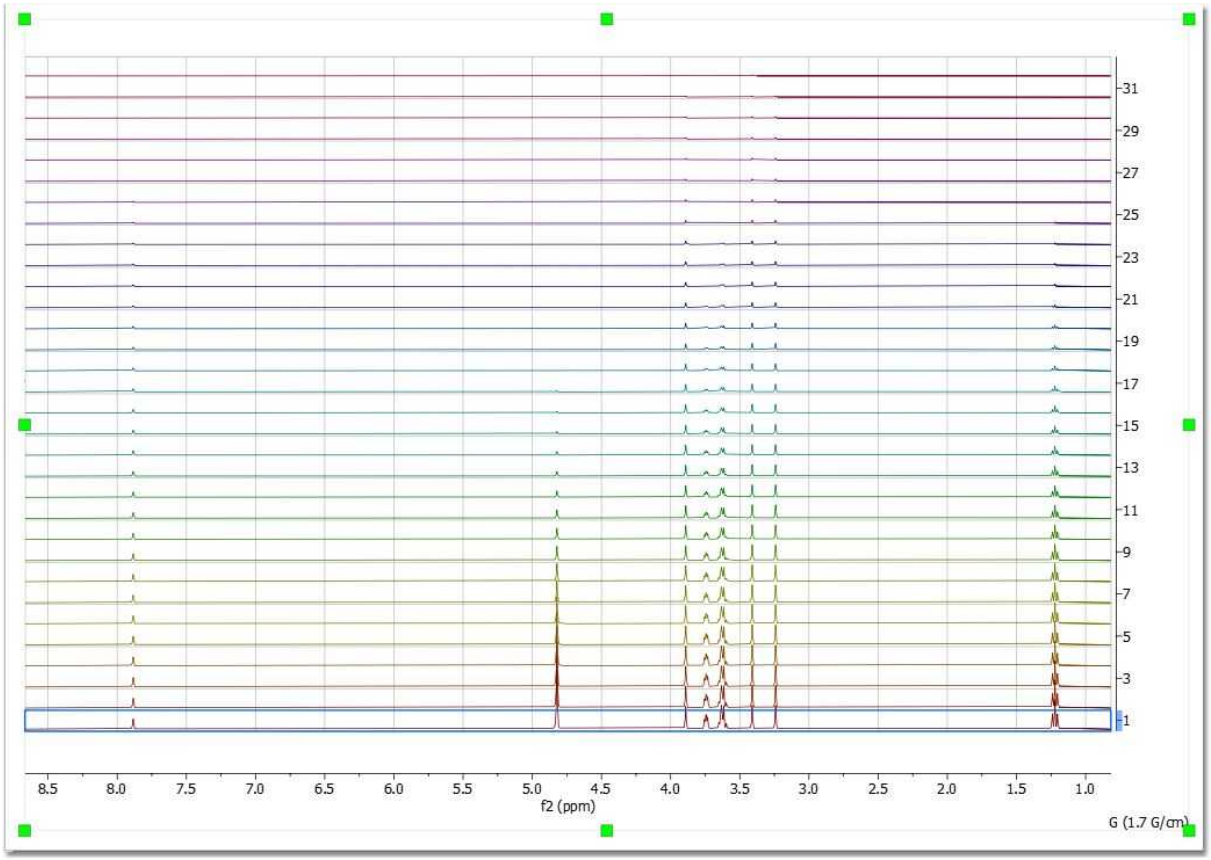
The **mapping S=>D** will be henceforth called the DOSY transformation. This transformation is, unfortunately, far from straightforward. Practical implementations include mono and biexponential fitting, Maximum Entropy, and multivariate methods such as DECRA.

We have recently developed a brand new method for DOSY processing which has been included in **Mnova**.

Bayesian DOSY Transform

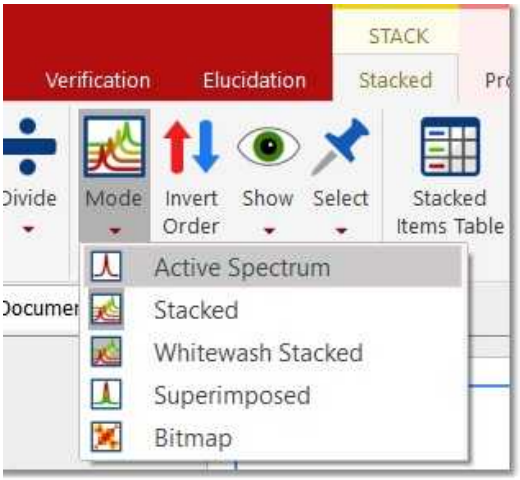
Let´s see how this feature works step by step:

1. **Import your raw data:** for example by dragging the spectrum folder into Mnova:



2. **Process the spectrum:** Mnova processes the spectrum automatically for you. However, it might be necessary to apply some further processing, usually manual phase and baseline correction

You can do that directly on the stacked plot mode, but it's recommended to use the 'Active Spectrum' mode as depicted below:

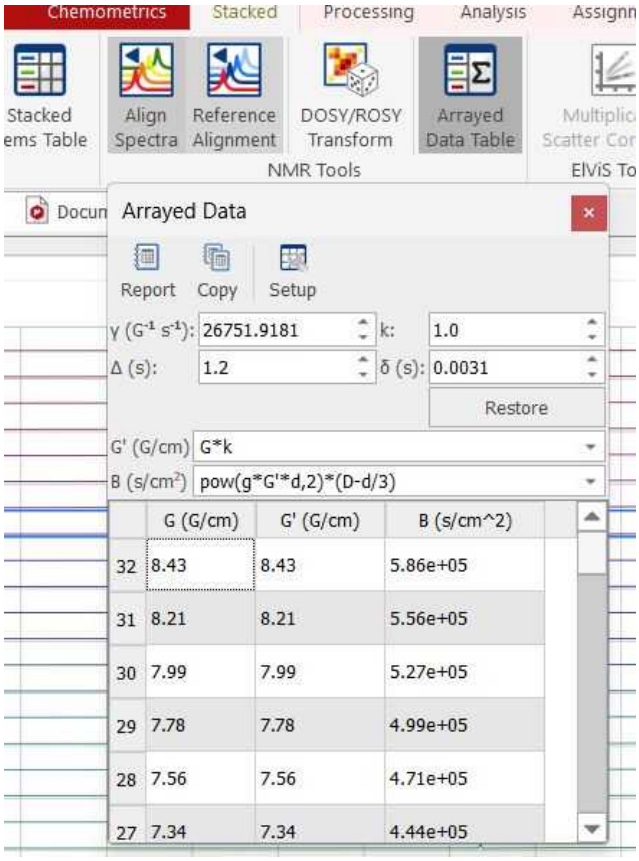


Within the 'Active Spectrum' mode, correct any phase distortion and apply an automatic baseline correction (use Berstein polynomials). These corrections will be applied to the whole dataset. You can also apply the Align tool (under the Stacked ribbon) if the traces are not aligned.

© 2024 MESTRELAB RESEARCH

3. **Setup Diffusion-related exp. values:** Make sure that the big and little delta values have been correctly read from the experiment files (in seconds) by following the menu 'View/Tables/Arrayed Data' (or Stack 'Arrayed Data Table').

> **NOTE**
>
> The gradient values are read from the difflist, whilst the small delta values are taken from Pulse Width (from P30 and PULPROG) in acqus file. A formula has to be used to calculate the value: p30*1.0e-6. When the pulprog is bipolar (pulse sequence containing 'bp'), the value is multiplied by 2.



By means of the bayDOSY transform (BDT) we are fitting a model of the type

$$S(f,z) = S_A(f)\ exp(-d_A z)$$

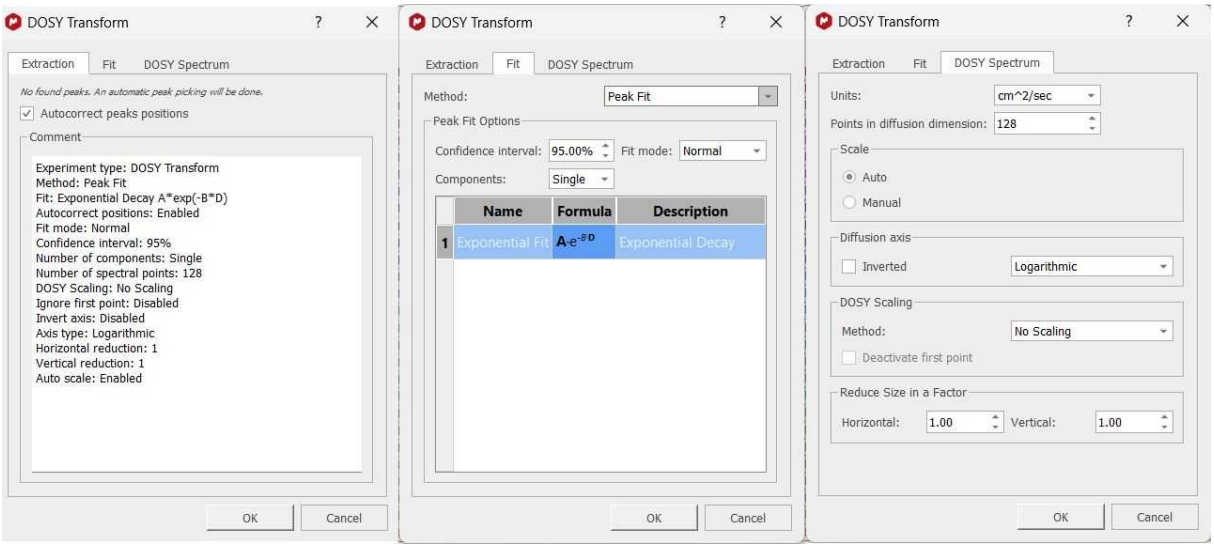
where $S_A(f)$ is the spectral intensity of component A in zero gradient ('normal' spectrum of A) and d_A is its diffusion coefficient. Z values corresponding to the expression above are displayed in the third column which are calculated according to the formula highlighted in the figure.

It's assumed that the gradient strength comes in G/cm. Note that the values used by the algorithm are those listed in the G' column which are in turn obtained from G column after multiplying these values by the k factor.

© 2024 MESTRELAB RESEARCH

NOTE
Mnova will read the arrayed parameter directly from the metadata. However, in some cases, the units are not in the correct format and thus Mnova gives the user the opportunity to scale the values parsed from the metadata by simply scaling those values by a factor *k*.
So what Mnova reads directly from the metadata is column *G* (or *t* in the case of Reaction-Monitoring or metabonomics examples), whilst column *G'* is the result of scaling *G* by the scaling factor *k*. Column *Z* is the final arrayed parameter, parameter which will be used in several other places such as DOSY transform, Data Analysis, etc.

4. **Run the DOSY Transform:** Just by following the menu 'Stacked/DOSY/ROSY Transform'



The first tab (Extraction) includes a comment that matches the selected options and an option to autocorrect the peaks positions. The Fit tab will display the equation that will be used according to the selected method.

The DOSY Spectrum tab will allow you to select the units. If the gradient strengths have been introduced in G/cm, the resulting diffusion coefficients will be in cm^2/s.Those units could be also changed under the 'Properties' dialog box (or by right clicking on the diffusion scale of the pseudo 2D plot)

You can also change the number of points in the diffussion dimension, scale (automatic or manual), the diffusions axis, DOSY scaling (not available for ROSY) and to reduce the size in a factor.

DOSY Scaling: you can apply 'no scaling', 'scale consecutive' or 'scale halves'.

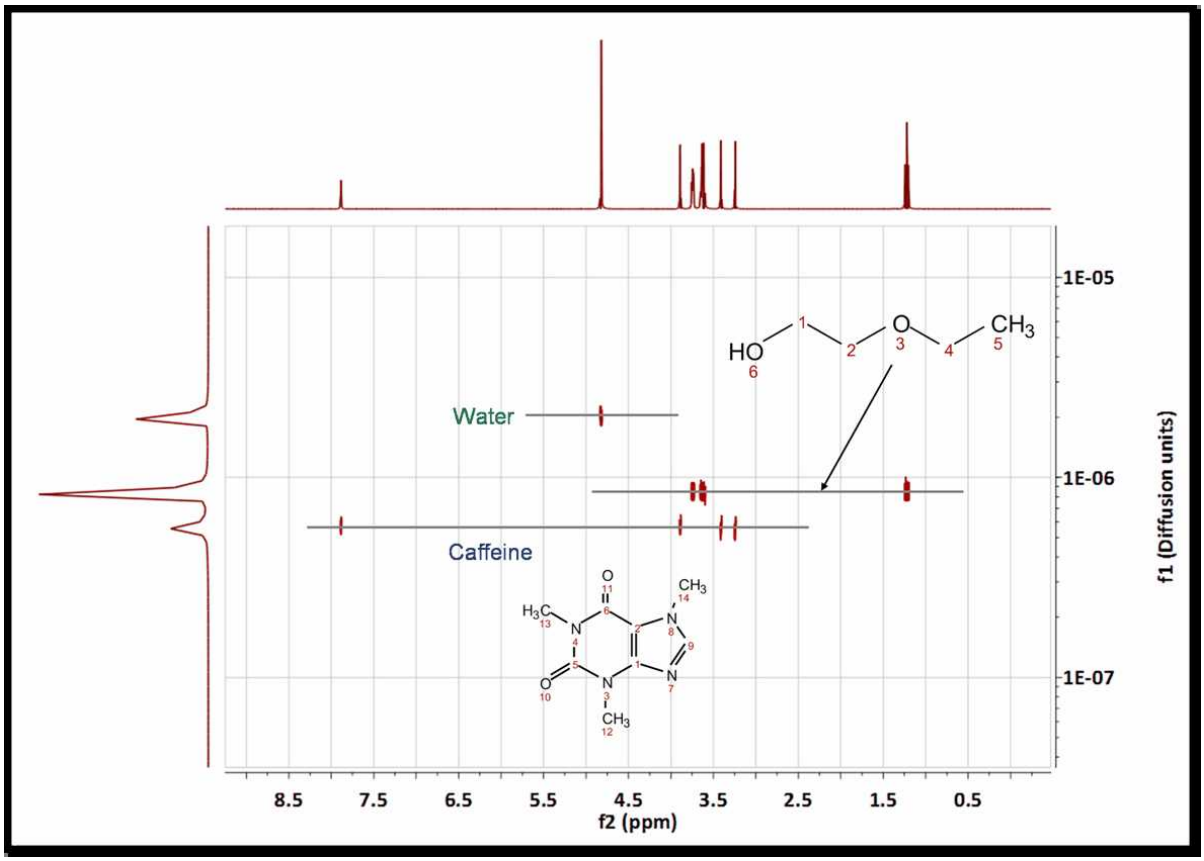
Scale Consecutive: it is often used in experiments where big delta is varied and serves to minimize the influence of T1 relaxation to the data.

Scale Halves: only the first half of the spectral set in the stack is shown in the result.

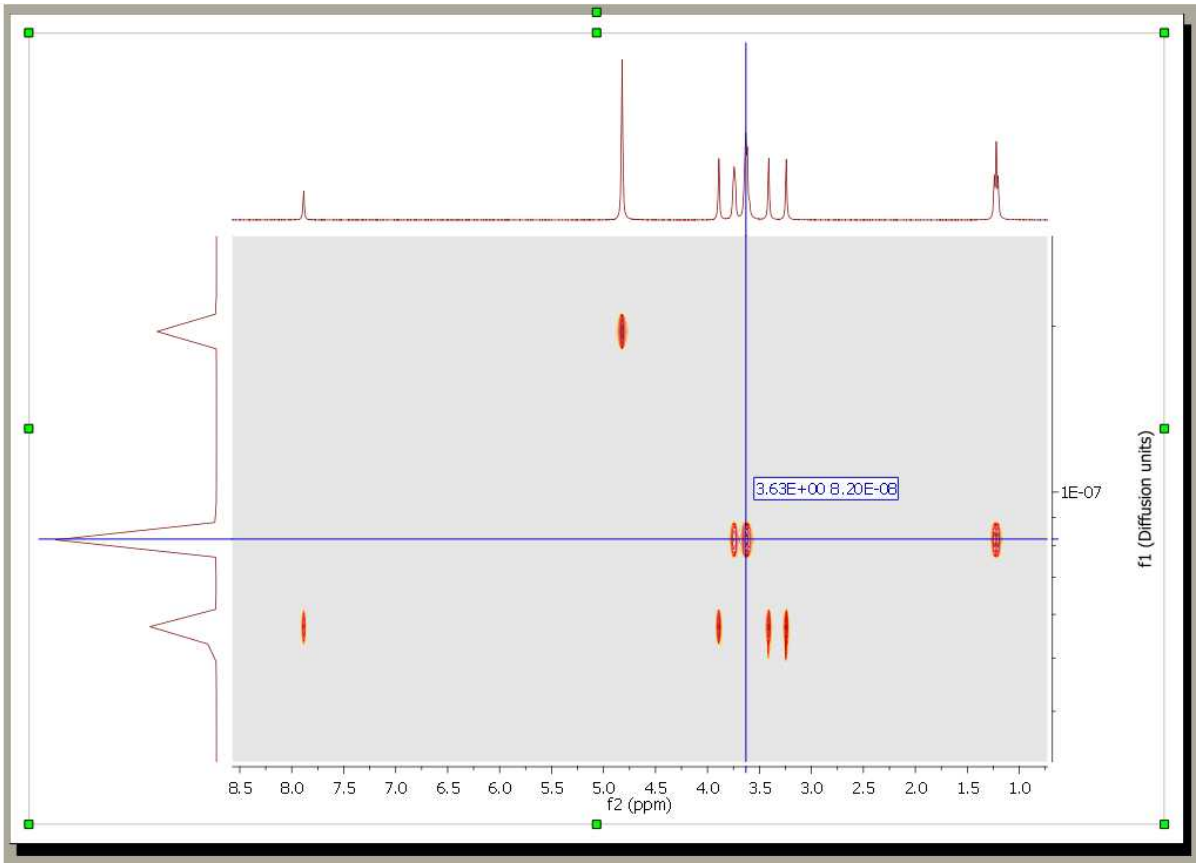
Check the 'Deactivate first point' box if you want to discard the first spectrum of the stacked plot, making sure that you end with an odd number of traces.

© 2024 MESTRELAB RESEARCH

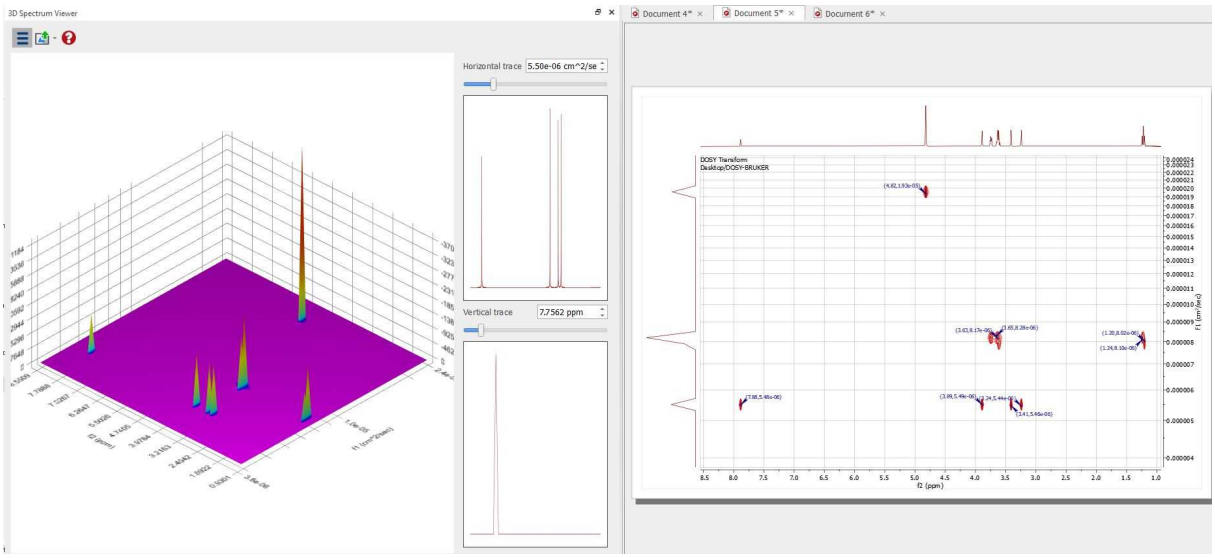
This is the result obtained. As you can see the 3 compounds (water, ethoxyethanol and caffeine) are perfectly separated.



You can use the 'Crosshair' to measure the Difussion values.

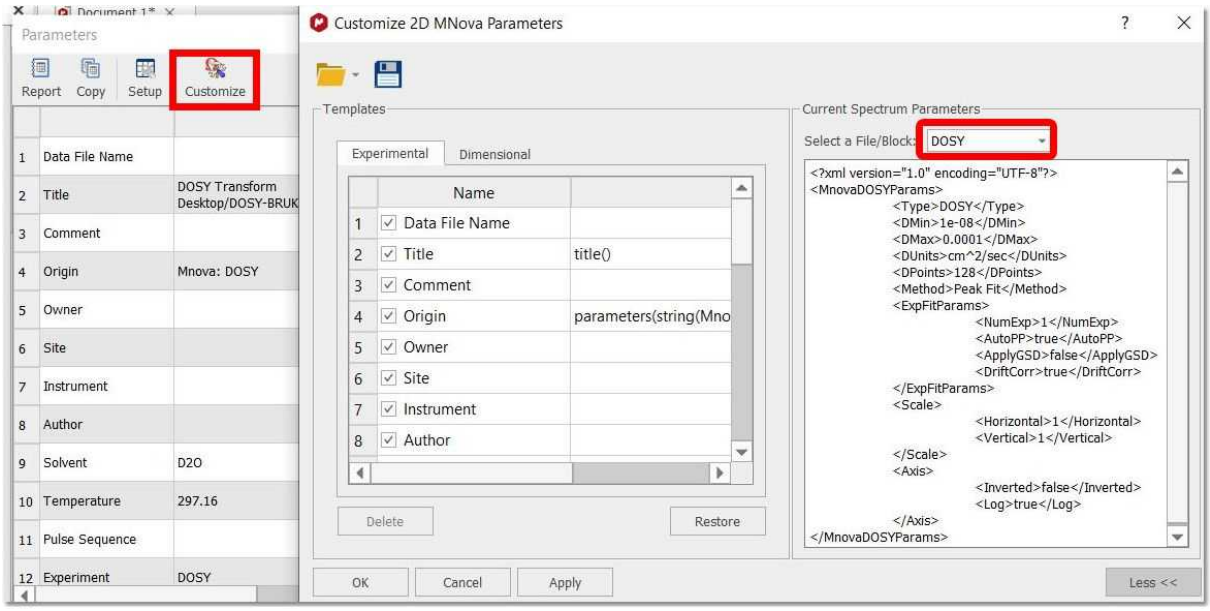


The result can be displayed in the 3D viewer:

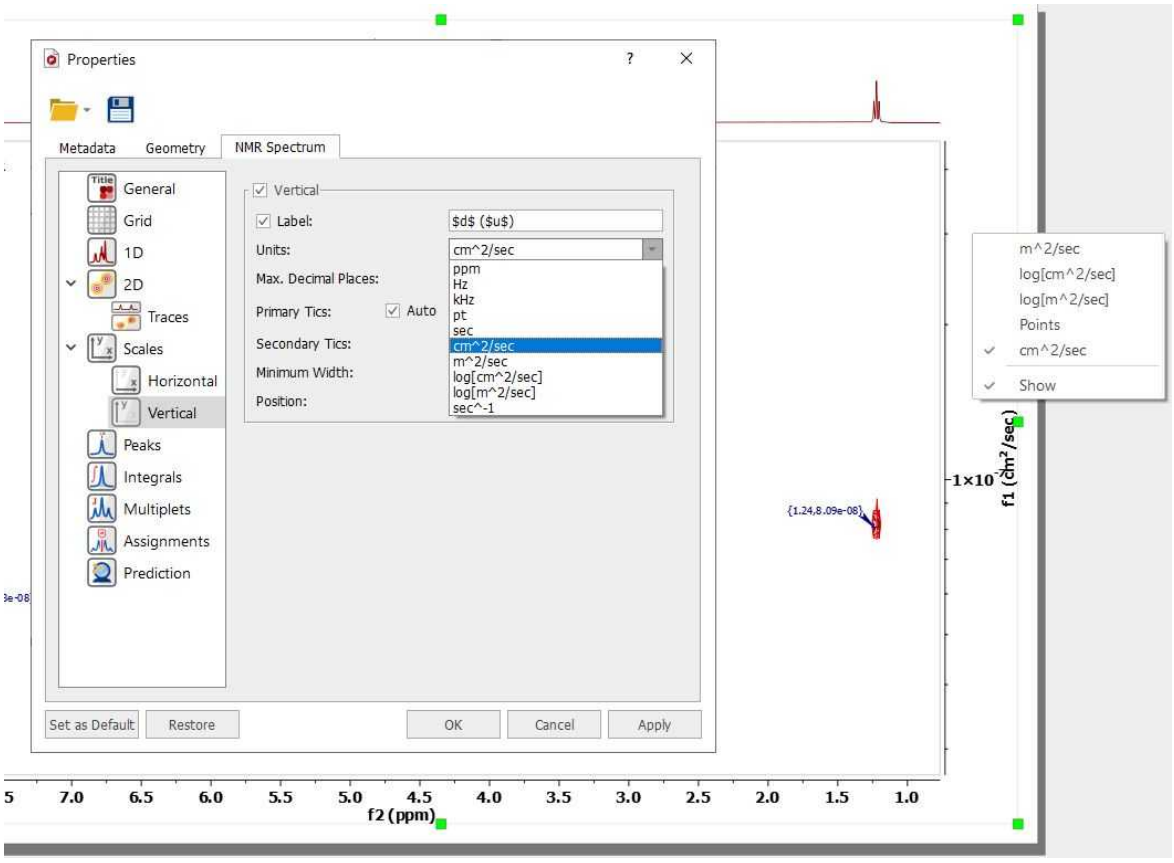


You can check the setting used for the DOSY transform by following the menu 'View/Parameters/Customize' and selecting DOSY block from the scroll down menu:

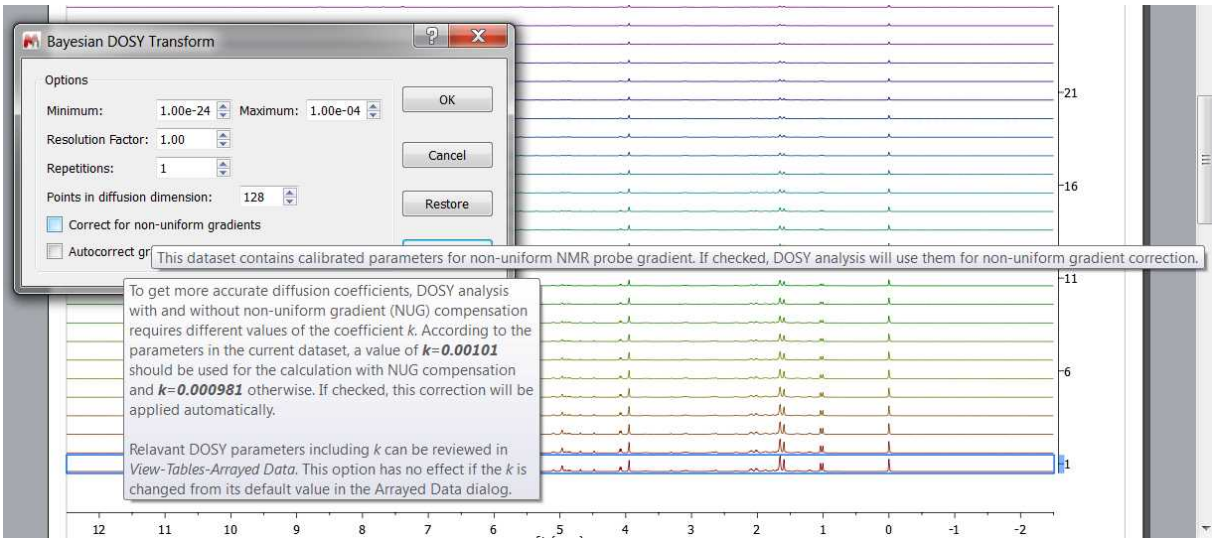
© 2024 MESTRELAB RESEARCH



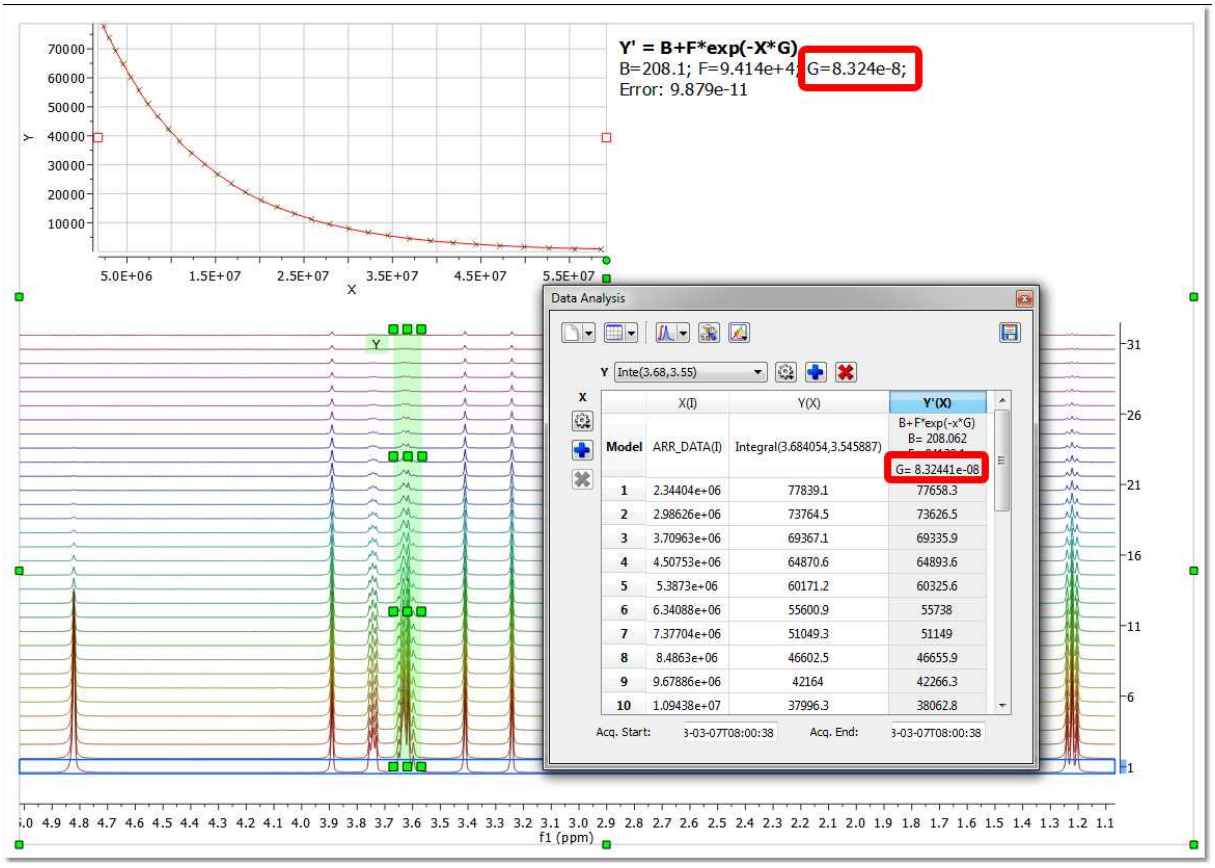
The diffusion units can changed under the 'Properties' dialog box (or by right clicking on the diffusion scale of the pseudo 2D plot):



Non-uniform gradient (NUG) correction can be applied by checking the option "Correct for non-uniform gradients" in Bayesian DOSY Transform dialog (the options only appear if the dataset requires them):



You can also use the 'Data Analysis' feature to get your **Diffusion coefficient** in the traditional way (given by the value of the parameter G):

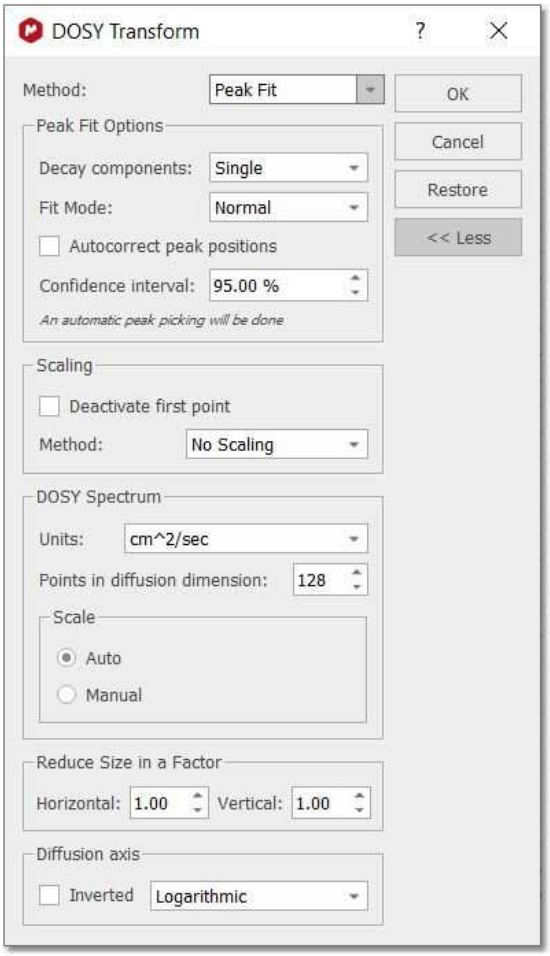


You can display the diffusion axis in logarithmic scale (even with vertical scale inverted), just by checking the applicable options obtained after having clicked on the 'advanced' button.

Peak Fit Method

© 2024 MESTRELAB RESEARCH

The "Peak Heights Fit" method was added to DOSY processing capabilities. The existing Bayesian DOSY routine provides a full continuous map of diffusion coefficients found in the system. To provide a more "filtered" DOSY 2D output and improve consistency with other spectrometer software a new DOSY processing technique is implemented.



After data preparation the ***Peak Fit*** method fits the extracted peak intensity values using exponential decay. In case if Varian non-uniform gradients (NUG) calibration is detected, the calibrated NUG decay is used instead of the exponential decay. Initially only monoexponential, two-parameters (I_0 and R) exponential decay was used which produce good quality results for majority of DOSY experiments. The new feature ***Decay components: Auto*** allows to apply multi-exponential (up to n=3 exponential components):

$$I(t) = \sum_{k=0}^{n} I_k exp(-t * R_i)$$

The number of components is determined automatically:

- First, the decaying peak intensities are fitted using monoexponential function.

© 2024 MESTRELAB RESEARCH

- The residuals of the optimized solution are analysed. If residuals exceed the experimental noise level significantly, the fit is considered to be unsatisfactory. In this case another exponential component is added to the fitting model and fit is optimised once again.
- The cycle is repeated till either the maximum number of components (up to 3) is reached or the satisfactory residuals are obtained.

To choose automatic multiexponential fitting in DOSY/ROSY transform dialog select ***Method: Peak Fit*** and option ***Decay components: Auto***. The option ***Decay components: Single*** performs old monoexponential decay analysis. Both, mono- and auto multi-exponential analysis support NUG-calibrated decay functions for DOSY.

Fit Modes:

"Weighted fit": it uses the noise in the spectrum to set an error for each point in the fit.

"Montecarlo": estimates the fit error by using a Montecarlo simulation using the spectrum error as standard deviation.

Options:

If there are user-defined peaks at the start-up of the procedure, these peaks can be used instead of applying a fresh peak picking.

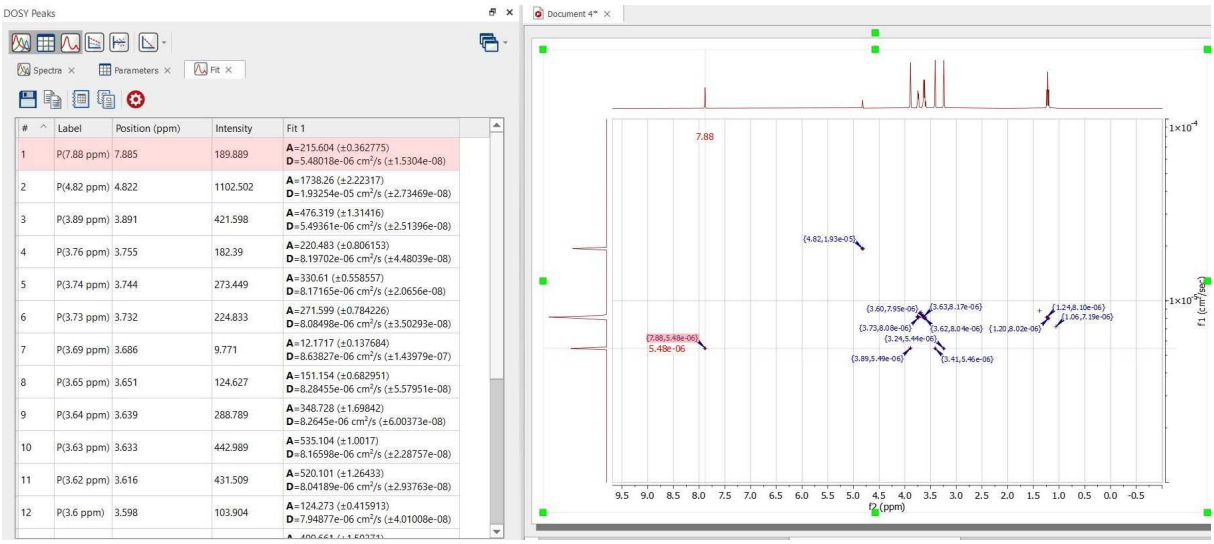
GSD analysis: If you want to apply a GSD analysis, you will need to apply a GSD Peak Picking before applying the DOSY transform. That will apply GSD on every spectrum in the original stack and estimate diffussion coeficients for all peaks individually using GSD peak areas and correction minor peaks position drifts. This option can be slow for very complex spectra. If ***GSD analysis*** is applied, the deconvoluted peak area is used instead of the default peak height (standard Mnova peak intensity). This should help in situations where peaks are changing their widths along the DOSY/ROSY experimental array and the peak area is a better representation of the intensities. The peak drift correction is automatic when ***GSD Analysis*** is used, so ***Autocorrect peak positions*** will not be needed in this case.

Autocorrect peaks positions: It could be a slight variation in the peak positions going from row to row of the arrayed DOSY experiment. The peak position can be adjusted to compensate the effect.

The procedure support NUG correction. For data without NUG coefficient a highly efficient exponential fitting routines is used. If NUG calibration exists, then D values found in this procedure are used as starting points for non-linear Levenberg–Marquardt fitting using NUG-corrected decay model.

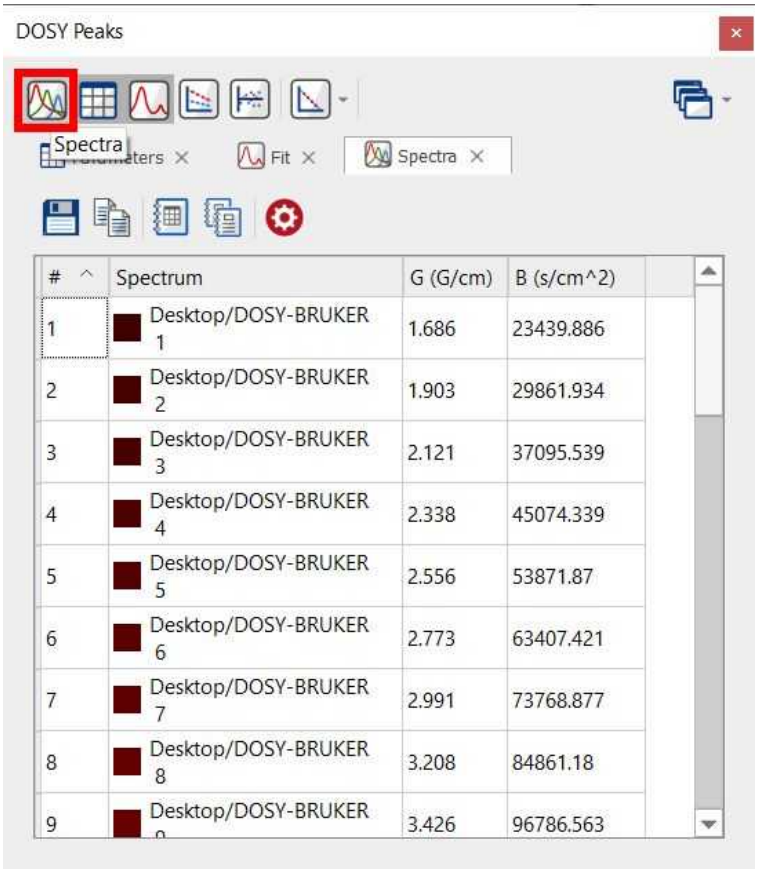
Once you have run the analysis, you will get the pseudo 2D plot in your spectral viewer and the 'DOSY Peaks' panel will be displayed:

© 2024 MESTRELAB RESEARCH



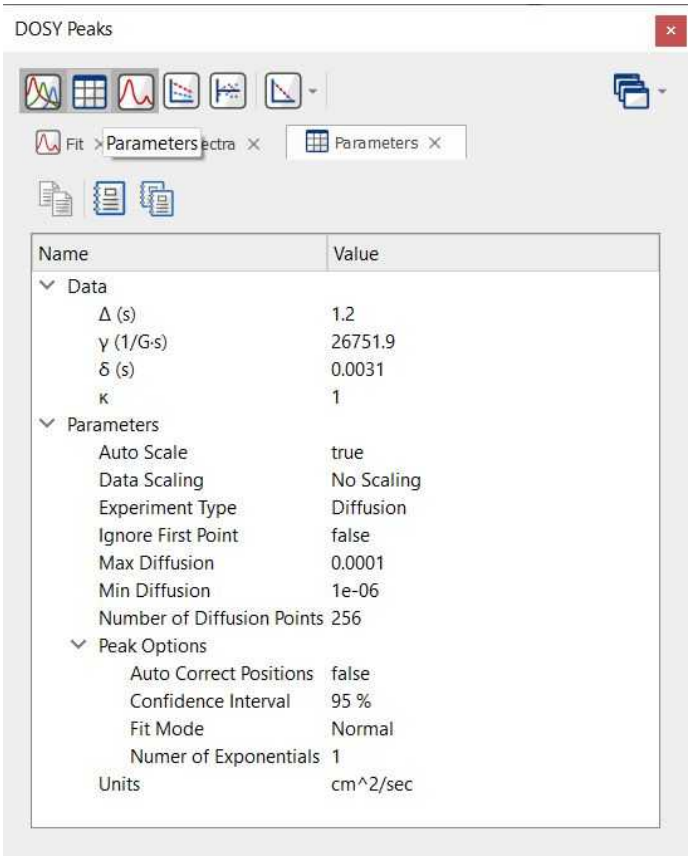
From that panel, you will be able to get information about each peak and the diffusion values from the fit analysis.

Clicking on the 'Spectra' button will display information about each trace of the stacked plot:

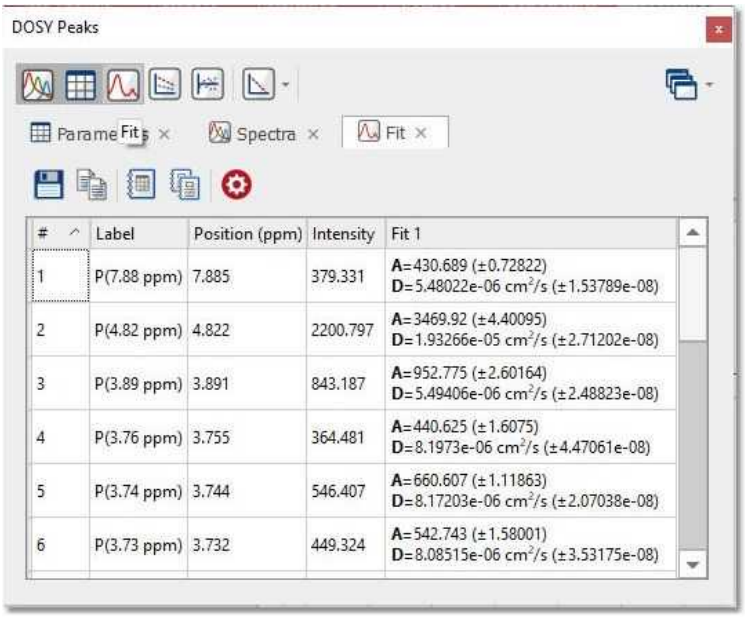


#	Spectrum	G (G/cm)	B (s/cm^2)
1	Desktop/DOSY-BRUKER 1	1.686	23439.886
2	Desktop/DOSY-BRUKER 2	1.903	29861.934
3	Desktop/DOSY-BRUKER 3	2.121	37095.539
4	Desktop/DOSY-BRUKER 4	2.338	45074.339
5	Desktop/DOSY-BRUKER 5	2.556	53871.87
6	Desktop/DOSY-BRUKER 6	2.773	63407.421
7	Desktop/DOSY-BRUKER 7	2.991	73768.877
8	Desktop/DOSY-BRUKER 8	3.208	84861.18
9	Desktop/DOSY-BRUKER	3.426	96786.563

Selecting the Parameters button will display some useful information from the metadata:

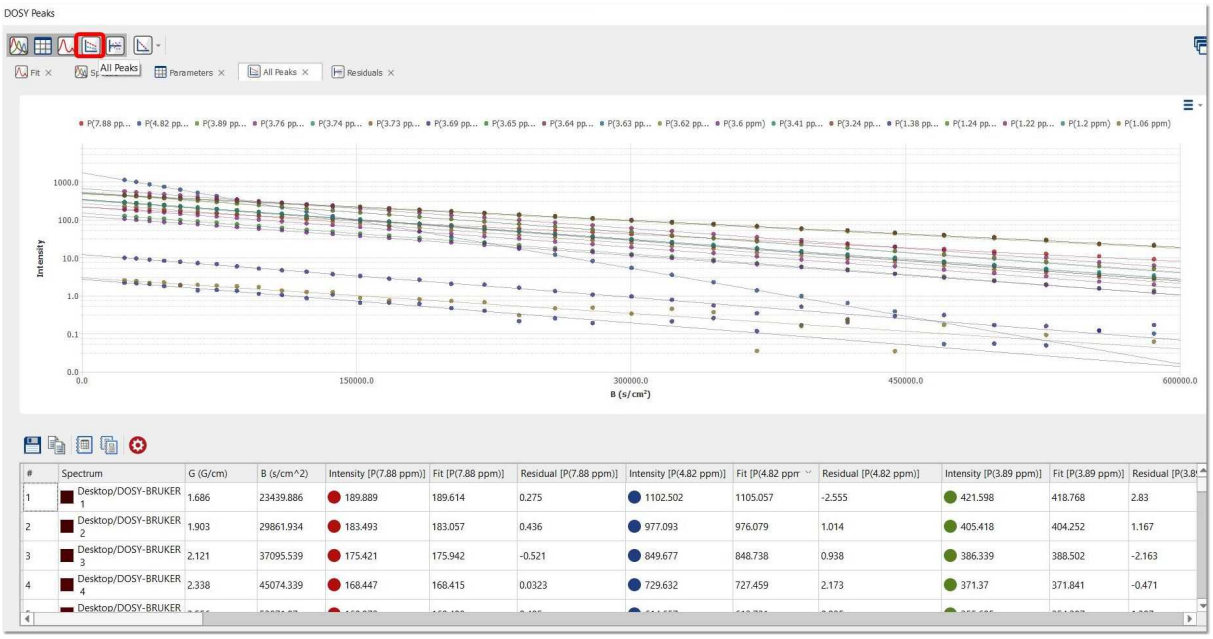


Selecting 'Fit' will display a table with information about the fitting values for each peak:



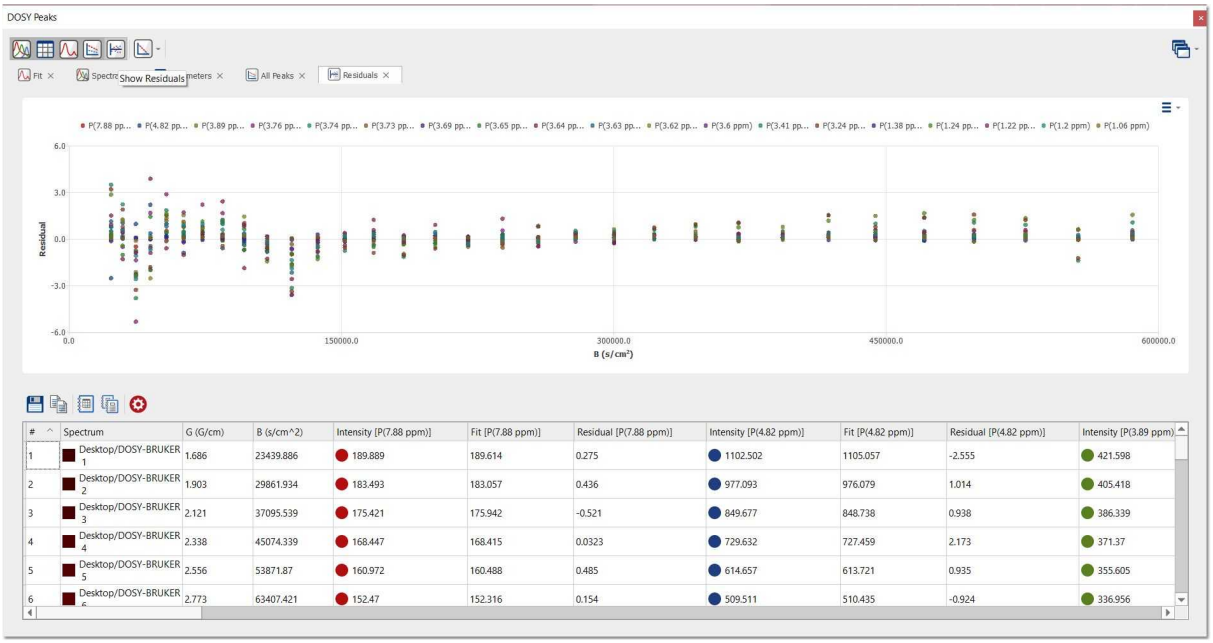
Click on 'All Peaks' button to display the Intensity/B graph of each peak of the applicable trace of the stacked plot:

© 2024 MESTRELAB RESEARCH



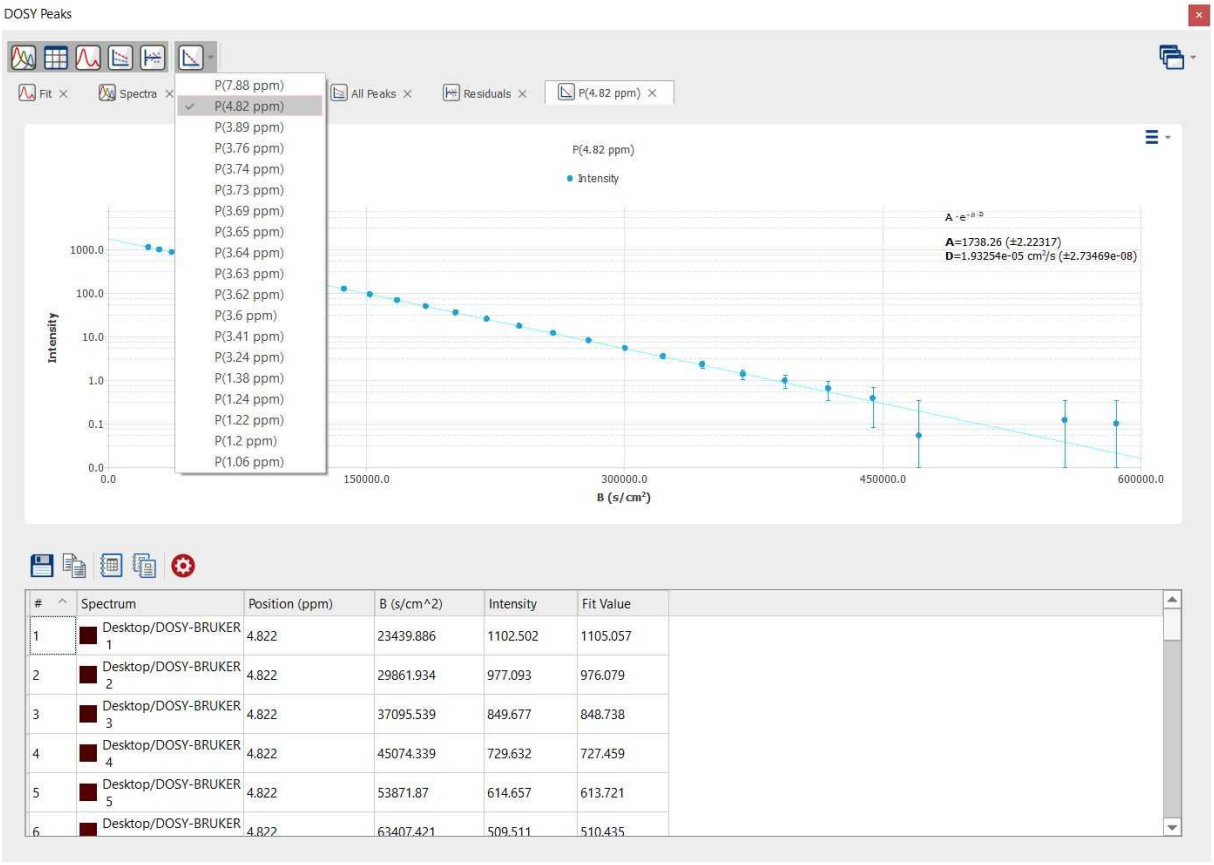
#	Spectrum	G (G/cm)	B (s/cm^2)	Intensity [P(7.88 ppm)]	Fit [P(7.88 ppm)]	Residual [P(7.88 ppm)]	Intensity [P(4.82 ppm)]	Fit [P(4.82 ppm	Residual [P(4.82 ppm)]	Intensity [P(3.89 ppm)]	Fit [P(3.89 ppm)]	Residual [P(3.8
1	Desktop/DOSY-BRUKER 1	1.686	23439.886	189.889	189.614	0.275	1102.502	1105.057	-2.555	421.598	418.768	2.83
2	Desktop/DOSY-BRUKER 2	1.903	29861.934	183.493	183.057	0.436	977.093	976.079	1.014	405.418	404.252	1.167
3	Desktop/DOSY-BRUKER 3	2.121	37095.539	175.421	175.942	-0.521	849.677	848.738	0.938	386.339	388.502	-2.163
4	Desktop/DOSY-BRUKER 4	2.338	45074.339	168.447	168.415	0.0323	729.632	727.459	2.173	371.37	371.841	-0.471
	Desktop/DOSY-BRUKER											

Click on the 'Show residuals' button to display the residuals of each fitting:



#	Spectrum	G (G/cm)	B (s/cm^2)	Intensity [P(7.88 ppm)]	Fit [P(7.88 ppm)]	Residual [P(7.88 ppm)]	Intensity [P(4.82 ppm)]	Fit [P(4.82 ppm)]	Residual [P(4.82 ppm)]	Intensity [P(3.89 ppm)]
1	Desktop/DOSY-BRUKER 1	1.686	23439.886	189.889	189.614	0.275	1102.502	1105.057	-2.555	421.598
2	Desktop/DOSY-BRUKER 2	1.903	29861.934	183.493	183.057	0.436	977.093	976.079	1.014	405.418
3	Desktop/DOSY-BRUKER 3	2.121	37095.539	175.421	175.942	-0.521	849.677	848.738	0.938	386.339
4	Desktop/DOSY-BRUKER 4	2.338	45074.339	168.447	168.415	0.0323	729.632	727.459	2.173	371.37
5	Desktop/DOSY-BRUKER 5	2.556	53871.87	160.972	160.488	0.485	614.657	613.721	0.935	355.605
6	Desktop/DOSY-BRUKER	2.773	63407.421	152.47	152.316	0.154	509.511	510.435	-0.924	336.956

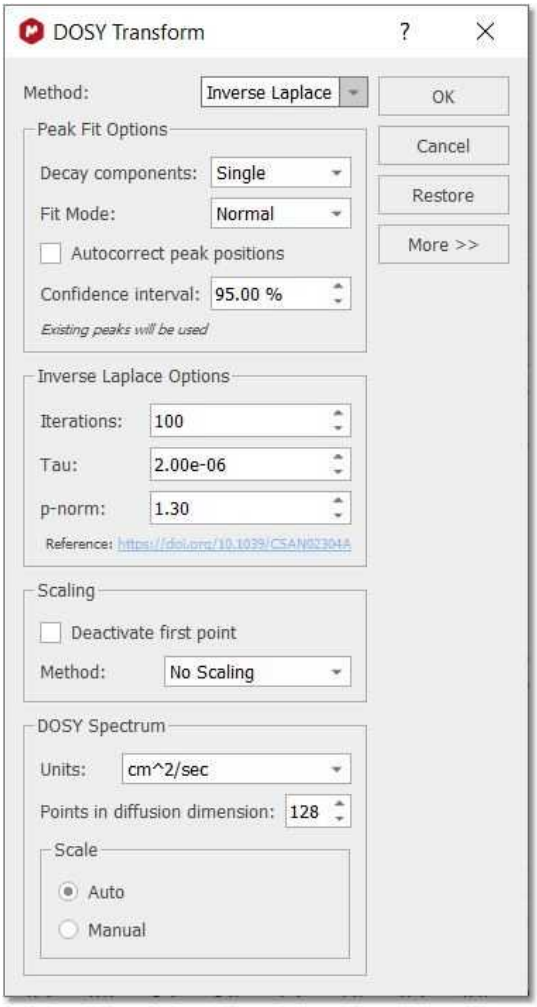
You can also select the individual fitting curves per each peak by selecting them from the scroll down menu:



Inverse Laplace Method

Inverse Laplace transform is a technique, which gives a continuous distribution of the diffusion coefficients/relaxation rates. It's an alternative technique to ***Peak Fit*** which rather uses discrete number of components in the decay. Finding a distribution of decay constants from a data with discrete number of points, affected by experimental noise is an ill-defined mathematical problem which may have multiple solution, valid mathematically, but not necessarily meaningful in the chemical nature. A lot of efforts has been done to tackle the problem: usually some sort of regularization is used to restrict the distribution search. For example, the distribution is assumed to be "smooth", has the minimal "entropy" or so on.

© 2024 MESTRELAB RESEARCH



There is a number of options which affects the method performance and final results. They are available for user modification if ***Method: Inverse Laplace*** is selected.

- ***Iterations***: the number of iterations steps, used for iteratively re-weighted least-square algorithm. The number should be sufficiently large to solutions to converge. Default value is 100 but value around 20 should give solutions with reasonable quality. The computation time is roughly proportional to this parameter.

- ***Tau***: the ratio between root-mean-squared deviation and the regularization term in objective function used for optimization. Very small values of ***tau*** make the root-mean-squared deviation dominant, but the lack of regularization can cause instability of solutions and solutions with many unnaturally sharp peaks. Very large value can cause “over-smoothening” of the solution. See original paper and the supplementary materials for the examples how ***tau*** affects the results.

- ***P-norm***: coefficient p for l_p-norm regularisation. The value is in the limits $1 \le p \le 2$. Values of $p>1$ produces “smoothed” solutions. See the original paper for the discussion of l_p-norm regularisation. The original paper proposes a method for automatic determination of the p value. However, the procedure is too time-consuming to be used for transforming to 2D map with many point in chemical shift dimension. Instead, the parameter p is adjustable by the user.

© 2024 MESTRELAB RESEARCH

Parallel CPU computation is used on supported platforms.

The diffusion or relaxation scale can be represented either as logarithmic or as a linear one. The direction of values in the scale (ascending or descending) also can be changed. These changes are made for compatibility with external NMR software (e.g. Bruker TopSpin) as well as following preferences of several users. The features can be found in the ***More*** section of the ***DOSY/ROSY transform*** dialog.

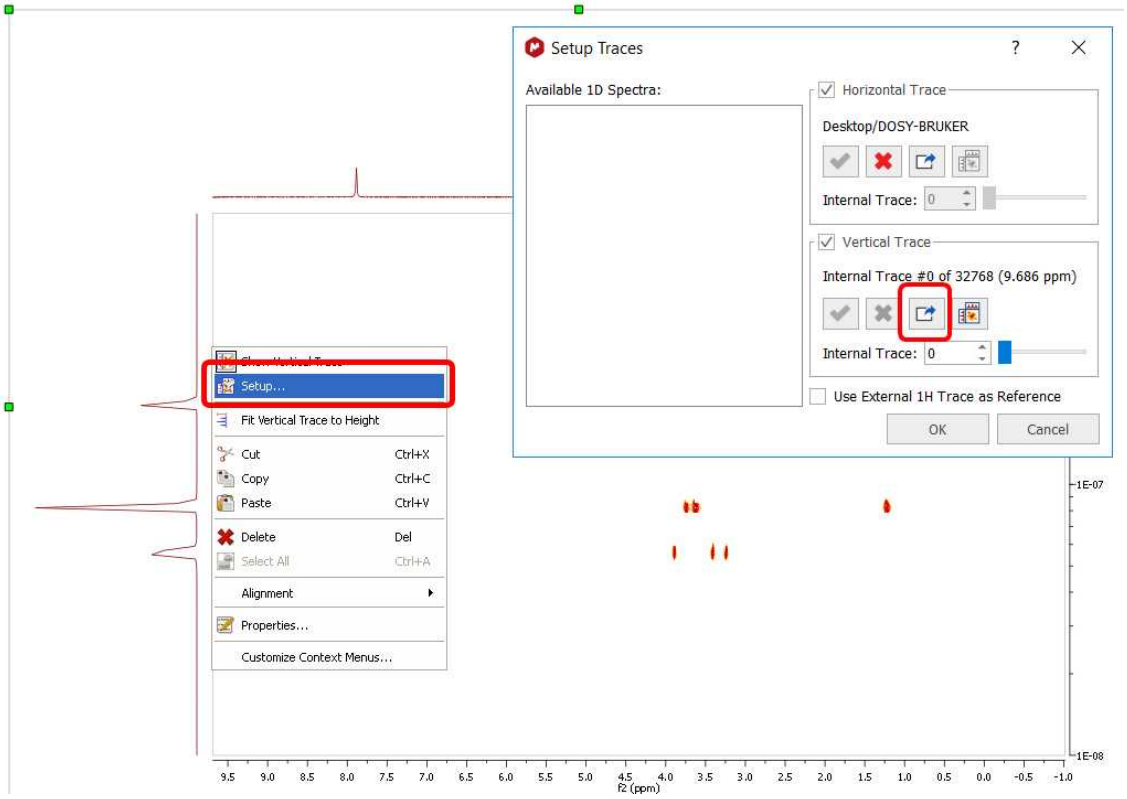
More information can be found here: https://doi.org/10.1039/C5AN02304A

Bayesian DOSY and quantification

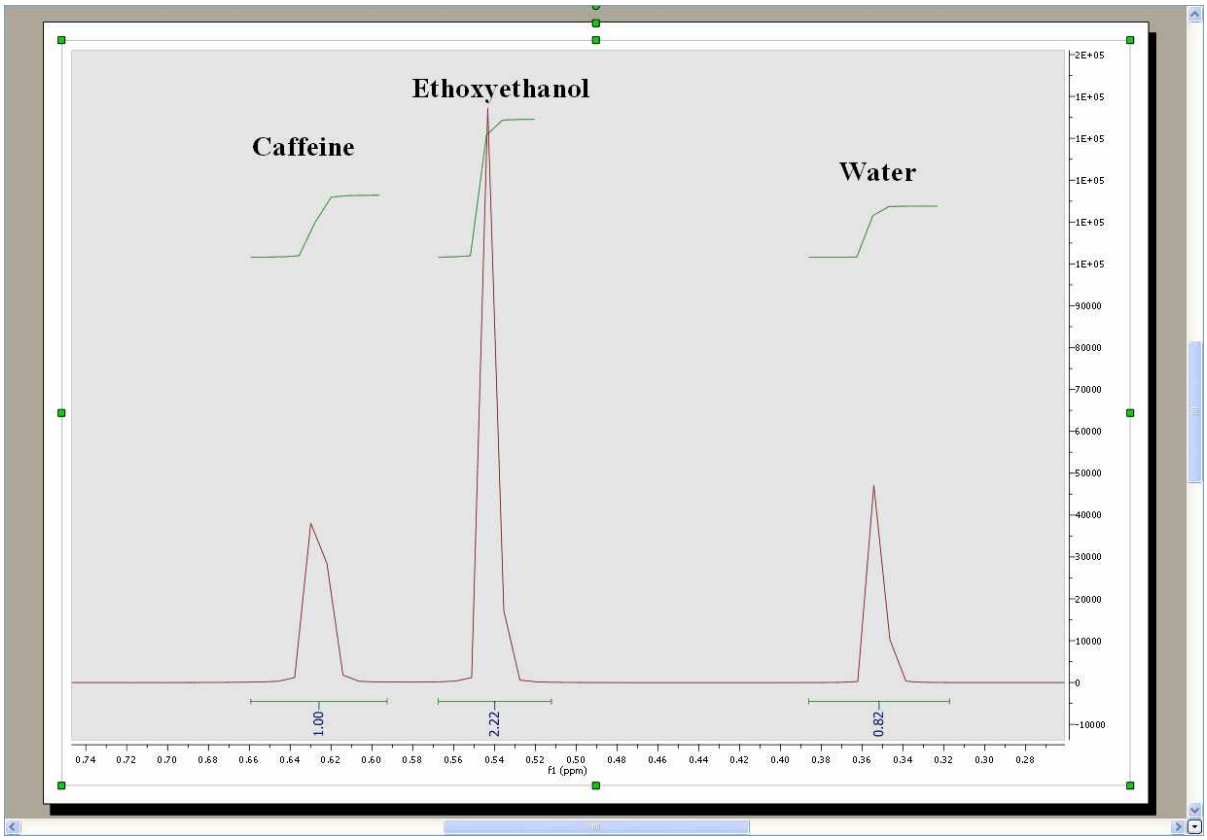
The Bayesian DOSY-data transform algorithm (BDT) in Mnova is inherently linear so amplitudes of "responses" are proportional to "inputs". However, that is not the whole story: The Bayesian approach always involves a final normalization which is pretty much a matter of choice and in BDT leaves a lot of degrees of freedom to play with. We normalize each column in the resulting 2D map so that it matches the point in what would be the 1D spectrum corresponding to zero gradient. Such column normalization might look as involving a series of integrals along the D-axis, but it is not, because an "integral along the D-scale" makes no sense (at best it might be viewed as a kind of Stieltjes integral, with properly defined D-scale interval weights). It is a trick, an intuitive hack, which however greatly alleviates the quantitation problem. Ratios of peak integrals in the projection therefore come quite close to ratios between component quantities. However, given the inherent impossibility to justify an "integral along D or logD", we tend to avoid the concept, at least for the time being, especially should somebody integrate over very broad intervals of D's, or compare two integrals around very different D values. From this point of view, given the kind of normalization we have adopted, peak heights are more rigorous quantities - unless the peaks of a component get misaligned on the D axis because of artifacts (for that reason a moderate integration including just very few D-scale points might be acceptable).

So, BDT will help you to obtain the relation of each compound by just integrating the diffusion trace. To do that, just right click on the trace and select 'Setup' in the contextual menu to display the 'Setup Traces' dialog box. Once there click on the 'blue arrow icon to extract the 'Vertical Trace'. Please make sure that this trace is the 'Internal Projection' (Sum):

© 2024 MESTRELAB RESEARCH



This will extract the trace to a new page. Finally integrate the signals of this trace to get the applicable ratio of the compounds:

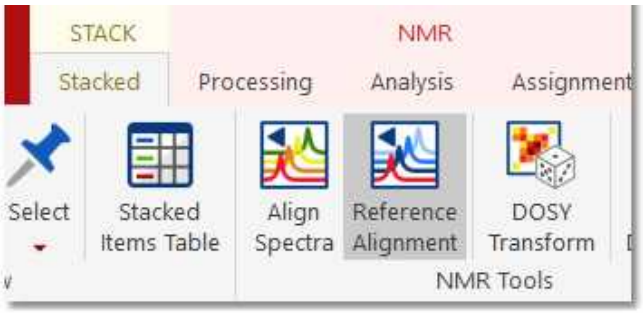


See this webinar for further information about DOSY processing and this video: https://youtu.be/UlJsTFdB6qI

9.7 Reference Alignment

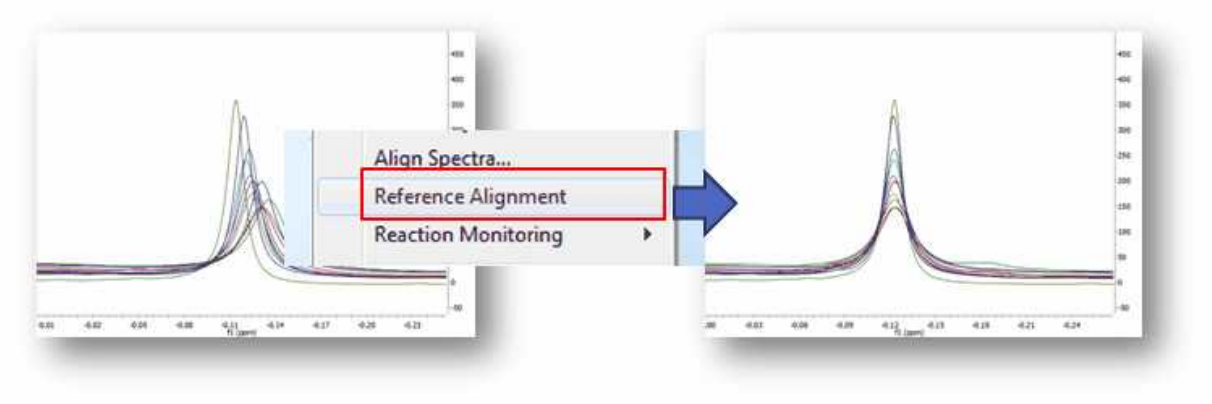
This feature will be used to manually align the chemical shift of 1D or 2D spectra (very helpful in Metabonomics), that is, to correlate signals of 1D or 2D spectra.

Just select, in the page navigator, the spectra that you want to align and follow the menu 'Stacked/Reference Alignment'. All the spectra will be aligned with the first one.



You can also use this feature to align stacked spectra. In that case, the method uses the active one and then aligns all remaining spectra by maximizing the cross-correlation between the reference spectrum and the spectra to be aligned:

© 2024 MESTRELAB RESEARCH



The idea of global alignment is very simple and corresponds to the well-known chemical shift referencing method in which the user sets the internal reference peak (e.g. TMS, DSS, TSP, etc) of each spectrum to e.g. 0 ppm. In order to cope with small fluctuations in chemical shifts, this method seeks for the highest peak within a narrow (user-defined, auto-tuning option in Mnova) interval, as depicted in the figure above.

See also Align Spectra**.**

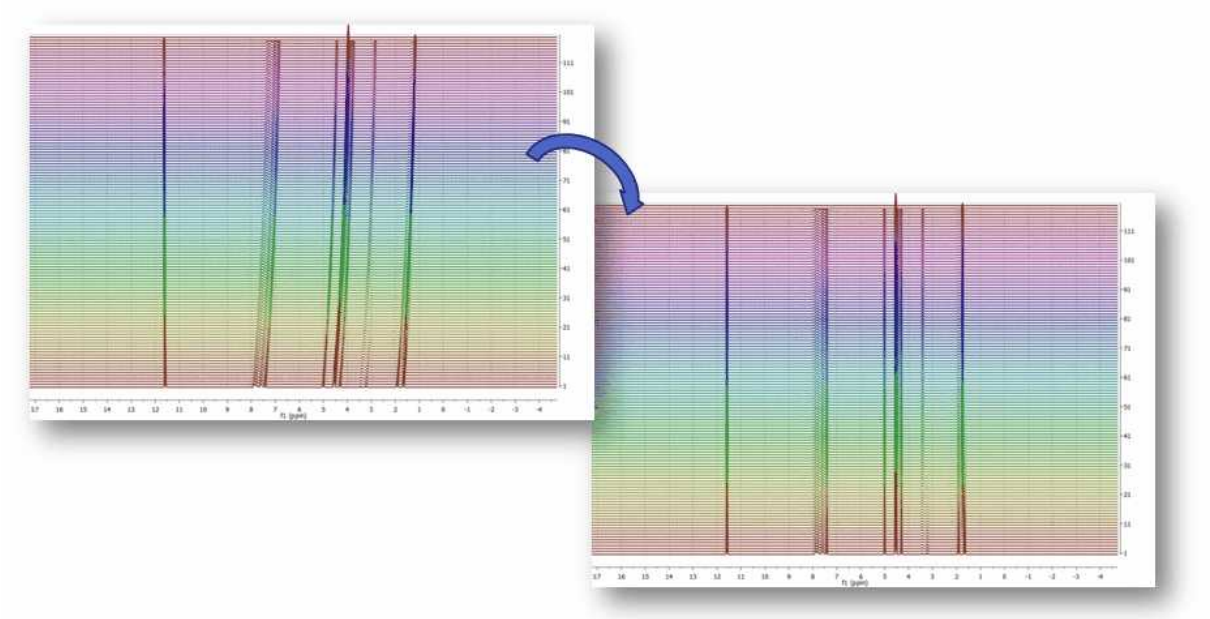
9.8 Align Spectra

The **chemical shift** is of great importance for NMR spectroscopy because it reflects the chemical environment of the nuclides under observation providing detailed information about the structure of a molecule.

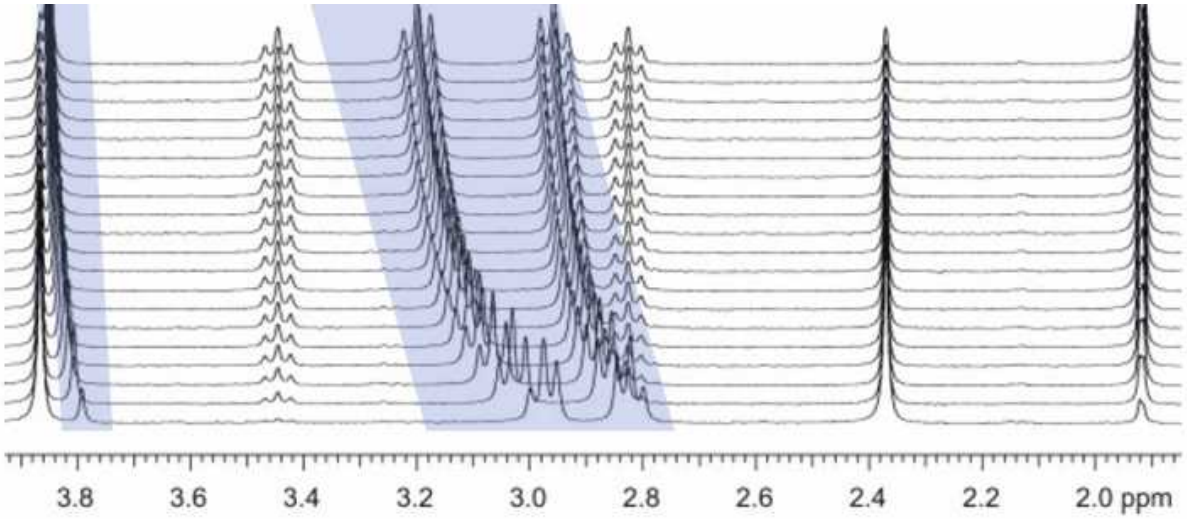
Although the chemical shift of a nucleus in a molecule is generally assumed to be fairly stable, there are a number of experimental factors (pH, salt concentration, solvent, field inhomogeneity –bad shimming, etc) which might produce slight or even quite significant variations in chemical shifts.

This is particularly important in **metabonomics/metabolomics** NMR where shifts of NMR peaks due to differences in pH and other physico-chemical interactions are quite common in NMR spectra of biological samples. For example, some important metabolites, such as citrate or taurine, have peaks whose chemical shifts fluctuate in an uncontrolled way from sample to sample. These variations can cause spurious grouping of samples in chemometric models.

© 2024 MESTRELAB RESEARCH



This problem is also very common in **kinetics** or **reaction monitoring** experiments by NMR. For example, consider the following reaction monitoring example:



It can be appreciated that during the course of the reaction, the chemical shifts of highlighted signals change as a result of the change in pH (in this case, as a hydrolysis proceeds).

Although characterizing these chemical shifts fluctuations can be sometimes important (pH or drug binding-induced chemical shifts, for example) in general they obscure the process of pattern recognition and impede the performance of data analysis (e.g. selection of the peaks whose intensities/heights need to be monitored can be more difficult).

Whilst it is critical to setup the experimental conditions in the best way to minimize these chemical shift fluctuations (for example by using an appropriate buffer); spectral misalignments may still occur and special post-processing methods have to be employed.

In order to cope with this misalignment issue, Mnova includes several methods, namely:

© 2024 MESTRELAB RESEARCH

- Manual (semi-automatic) global spectral referencing
- Fully automatic global spectral referencing
- Spectral alignment (including global and regional-wise spectral alignment)

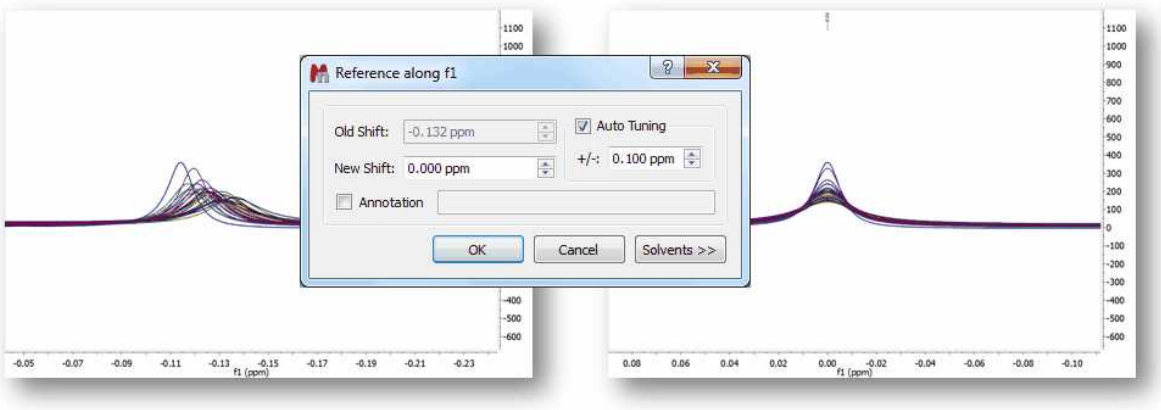
The first two methods can be regarded as simple, global methods for peak alignment as they shift the entire spectrum by some constant amount, whilst the third method is much more sophisticated making possible both the global and local alignment of different spectral regions

These three methods are explained below:

1) Manual (semi-automatic) spectral referencing

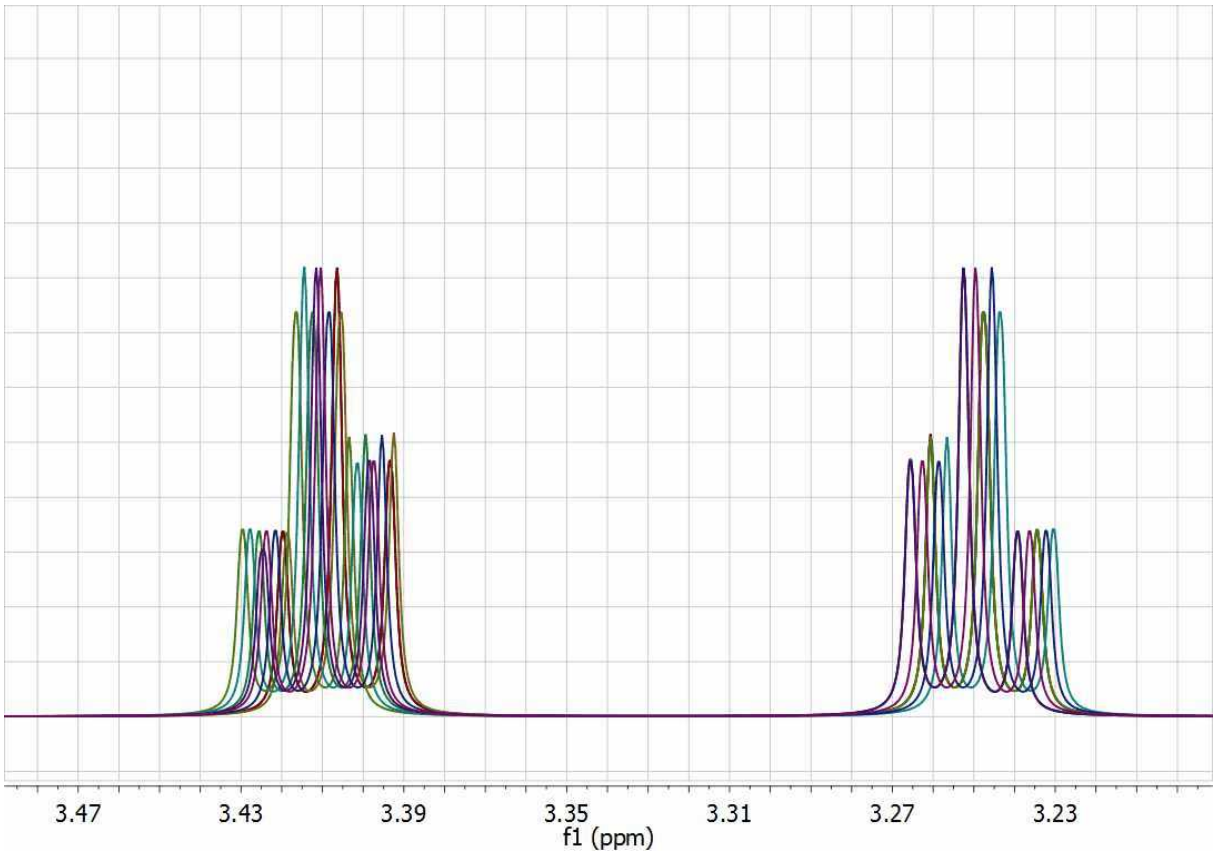
This method corresponds to the well-known chemical shift referencing method in which the user sets the internal reference peak (e.g. TMS, DSS, TSP etc) of each spectrum to e.g. 0 ppm. The particularity of Mnova is that it contains an **auto-tuning** parameter which makes it possible the automatic referencing of many spectra by simply seeking for the highest peak within a narrow (user-defined) interval.

The figure below (left) shows 26 superimposed misaligned peaks. Manual Referencing using an auto-tune parameter of 0.1 ppm yields the result depicted in the figure on the right:



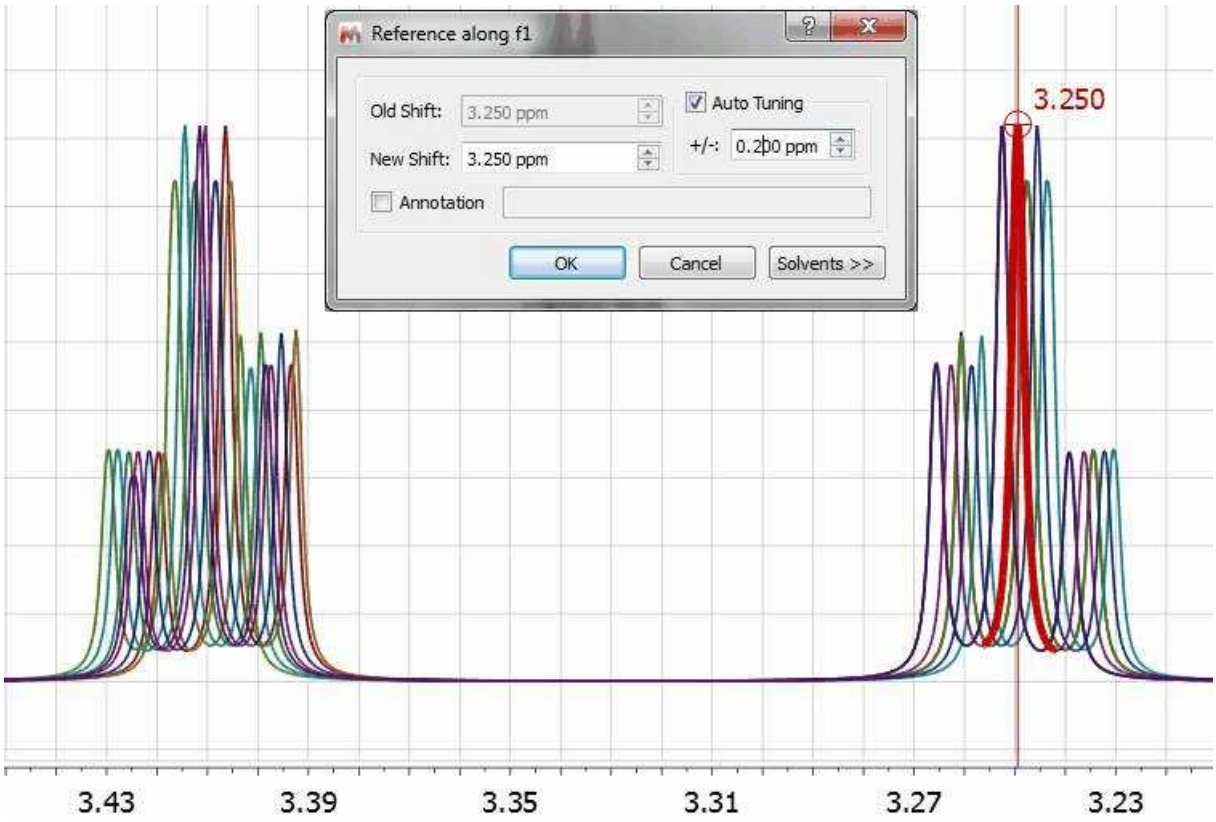
Clearly, this method will not work properly in those data sets with local misalignments, that is, when signals of one metabolite fluctuates in one direction whilst the peaks of a different metabolite move differently). As an example, let's consider a simulated data set of Taurine:

© 2024 MESTRELAB RESEARCH

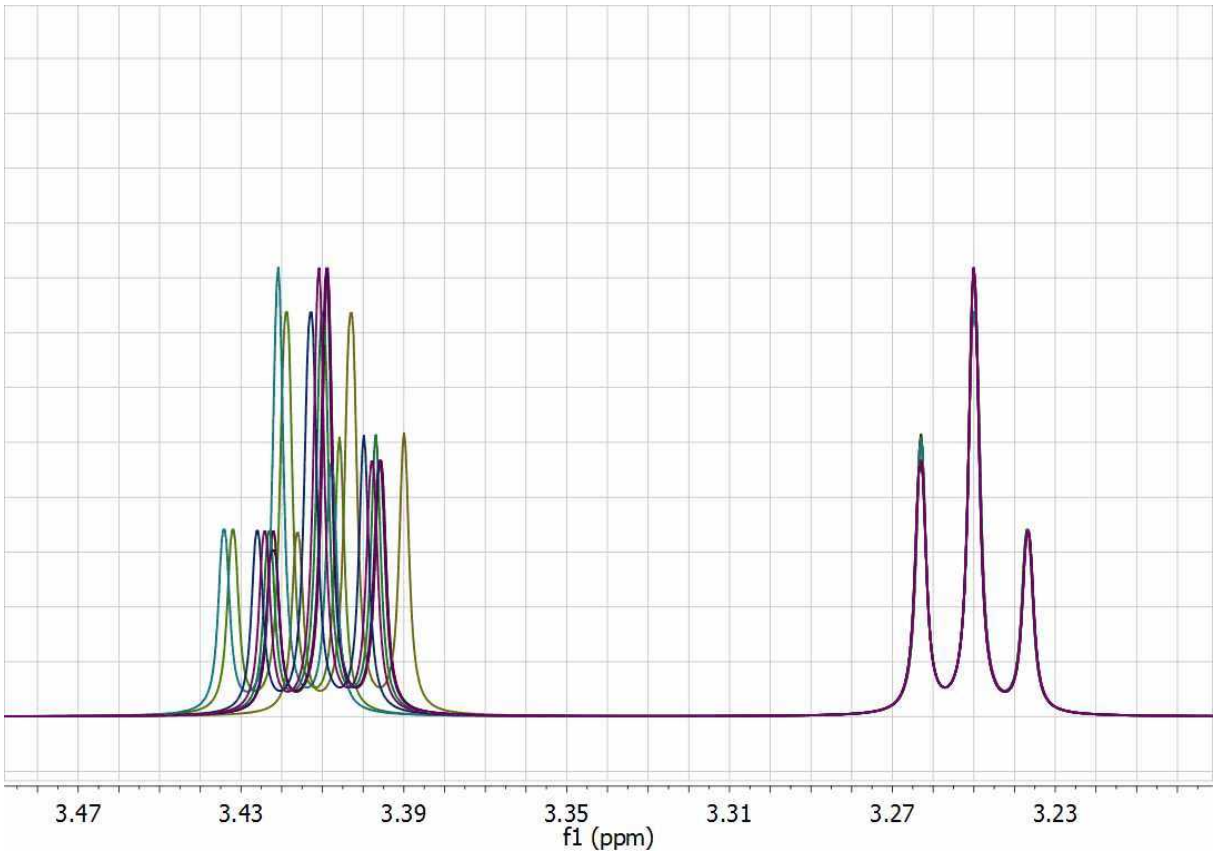


This data set has been generated by randomly changing the chemical shifts of the two CH2 groups. Now, let's apply the global alignment procedure using as chemical shift reference at a value of 3.25 ppm as shown in the picture below:

© 2024 MESTRELAB RESEARCH



As expected, all peaks corresponding to the triplet at 3.25 get perfectly aligned, but the other multiplet remains misaligned:

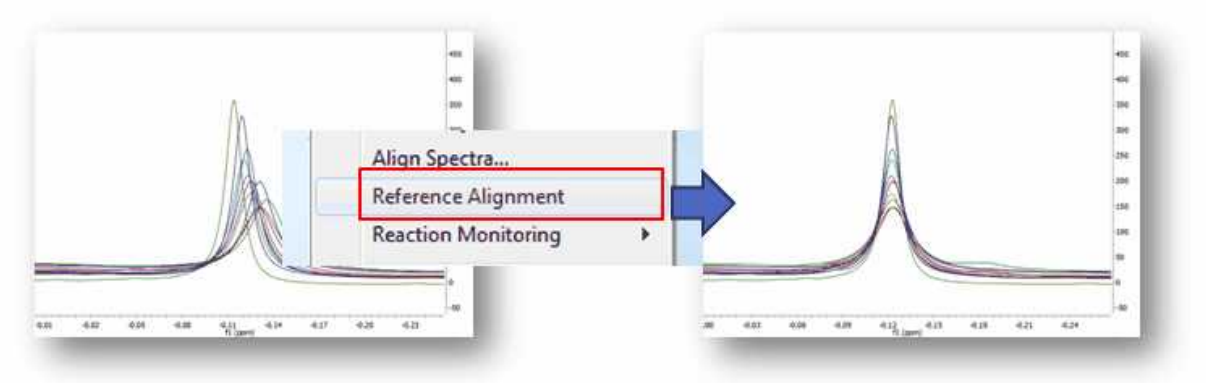


One could devise an extension to this global alignment procedure in which the same procedure is applied to different segments of the spectrum. In this particular case, one could select two different windows, one for each triplet and apply the same algorithm locally to each segment. However, having to manually select the chemical shift reference for each segment is not very practical and, in addition, relying only on the simple search of the maximum peak within each segment is not a very robust method for automatic alignment.

2) Fully automatic global spectral referencing

In the previous method, the user needs to select a peak as a reference for the alignment process, together with the window width for the auto-tuning parameter.
Mnova provides an alternative, fully automatic method which aligns, ***globally***, all spectra in a stack. This method uses one spectrum as a reference (the active spectrum) and then aligns all remaining spectra by maximizing the cross-correlation between the reference spectrum and the spectra to be aligned:

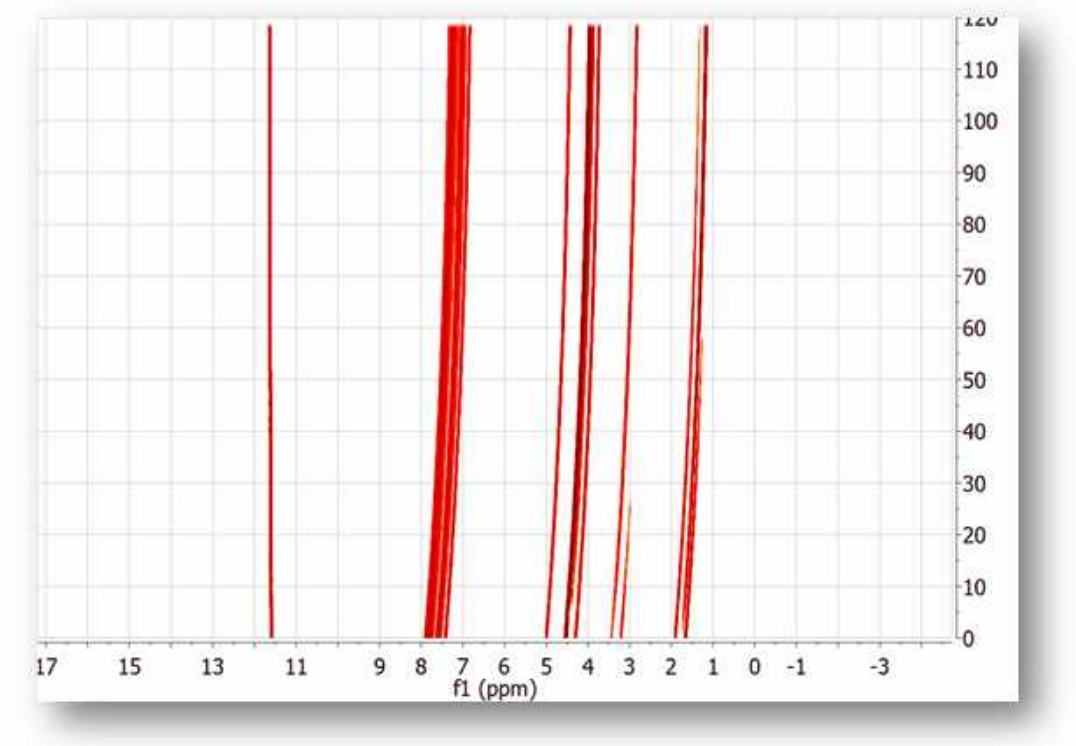
© 2024 MESTRELAB RESEARCH



3) Spectral alignment (including global and regional-wise spectral alignment)

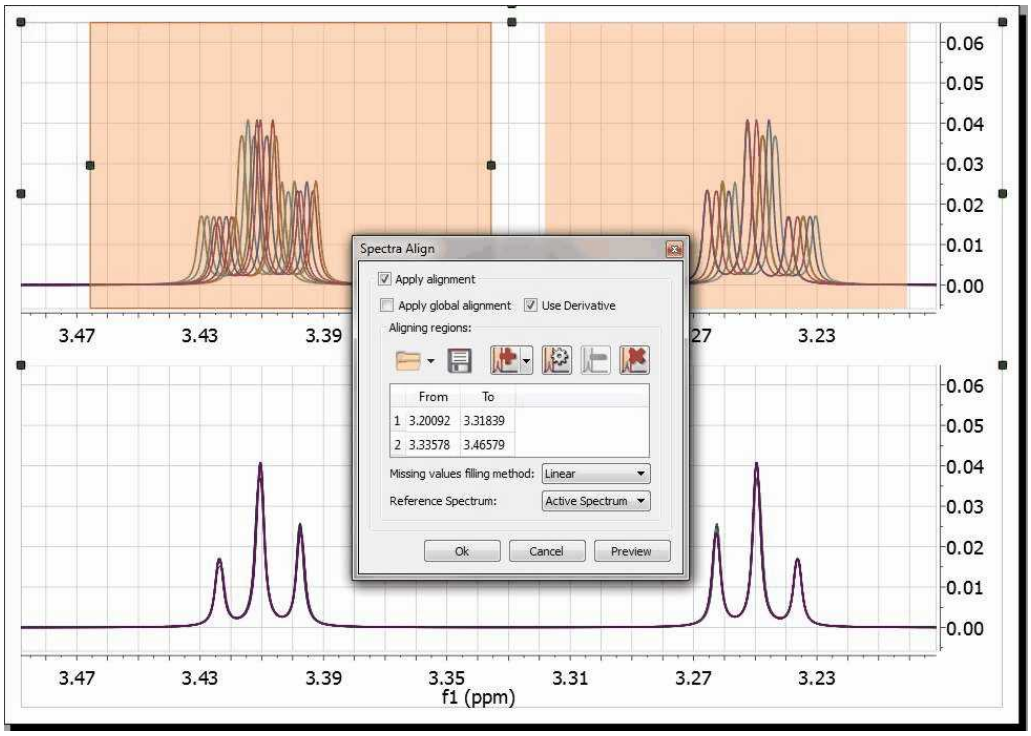
The magnitude of ***misalignments*** within a single sample may be ***nonlinear***, making it challenging to apply a simple alignment correction across an entire spectrum.

An example of this nonlinear misalignment is shown in the spectrum below (intensity map or bitmap plotting mode). It can be noticed that the peaks at about ca 11.6 ppm do not move whilst the peaks at higher field move very significantly.
It's evident that correcting the data using a single reference peak (or a global shift) is not sufficient:

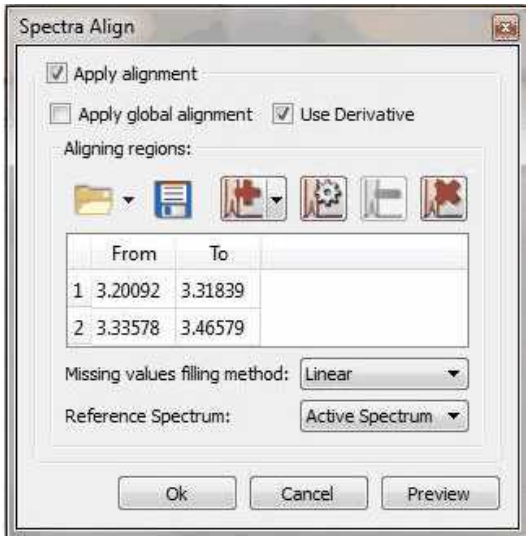


Resorting back to the synthetic data set used in the previous section, let´s see a semi-automatic method designed specifically to align spectra having local chemical shift variations. From a practical point of view, the user needs to select the spectral regions to be aligned and then the program will automatically align those regions separately The picture below shows the spectrum before alignment and the two selected regions (top) and the result obtained after applying the alignment algorithm (bottom):

© 2024 MESTRELAB RESEARCH



Mnova provides a powerful and flexible approach to dealing with misalignments based on a segment-wise peak alignment with the option to apply a global alignment as a pre-processing stage.
The algorithm allows the user to select the boundary locations using an interactive display, but the process is also amenable to automation.



The 'Missing values filling method' option is applied when you have ‘Aligning Regions’ in order to reconstruct those parts of the spectra that are shifted when you apply the alignment. The default method is Linear and there seems to give good results, but ‘Ending Values’ and ‘Keep old data’ also work fine. The ‘zeroes’ method is filling the holes with zeroes.

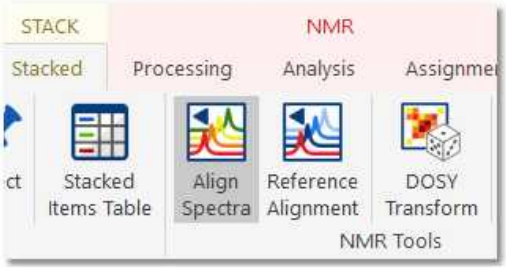
Use the applicable buttons to load and save alignment regions in XML format.

© 2024 MESTRELAB RESEARCH

We have improved the traditional cross-correlation algorithm by working on the first **derivative** domain calculated using an improved Savtizky-Golay routine in which the order of the smoothing polynomial is automatically calculated. The idea is to minimize potential problems caused by baseline distortions or very broad peaks.

We have found this method to be very useful not only in the context of metabonomics, but also in the alignment of Reaction Monitoring data sets.

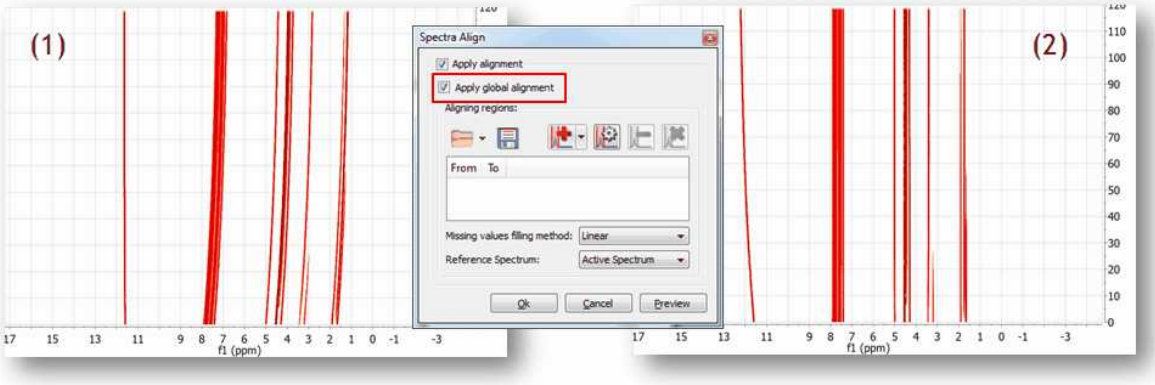
This comprehensive spectral alignment toolkit is available in Mnova in the 'Stacked' menu. It can work on the entire spectrum (Global Alignment) as well as on intervals of arbitrary length in sequence. Based on these options, different strategies can be employed.



Two examples (strategies) will be shown below:

Strategy 1

Starting with raw spectrum (1), to perform a full-spectrum correction before the single intervals are aligned



It can be appreciated that after applying the global alignment, most of the peaks in (2) are now properly aligned, except the peaks at the left which were previously aligned but after this operation get misaligned. This problem will be cured in the next step.

Strategy 2

A different, although analogous strategy, would consist in aligning two different spectral intervals separately without resorting to a global alignment as shown in (1). Note that the peaks in the interval in the left are already well aligned.

© 2024 MESTRELAB RESEARCH

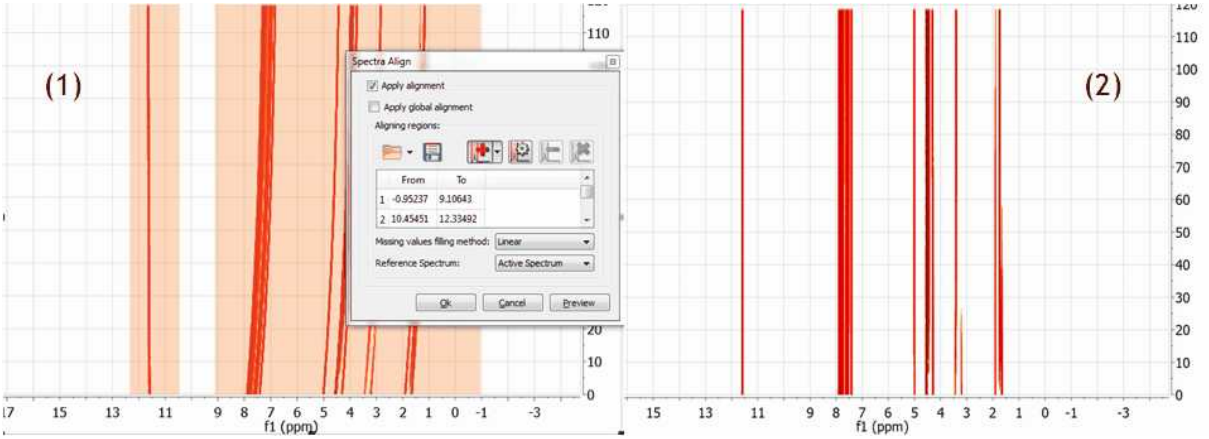
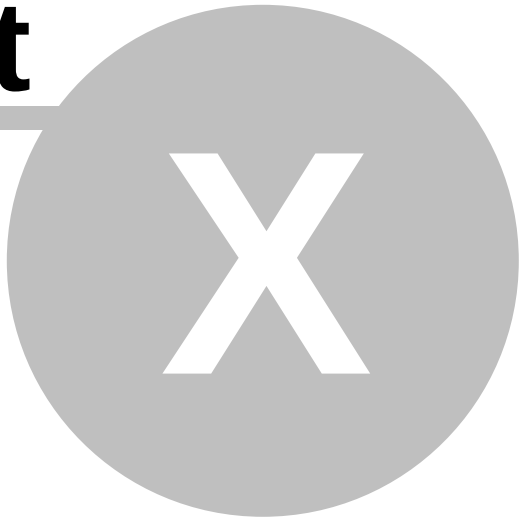


Figure (2) shows the final result after the two interval have been aligned. It's completely equivalent to the result obtained with Strategy 1

[See also our blog]

© 2024 MESTRELAB RESEARCH

Part



10 Mnova Verify

Mnova Verify is a new developing approach for structure verification by NMR and MS.

The pharmaceutical and biotechnology industries are currently facing very significant challenges which are resulting on major organizational and operational changes. The trend is for pharmaceutical companies to outsource many of their Research and Development operations to third parties with specific specialties and areas of expertise, in order to focus their own resources on areas where they are in a position to maximize value.

As a result, where many operations such as data mining, information sharing and decisions on chemical entities used to have to be carried out at an internal company level, now these same functions need to encompass the entirety of the supply chain and all partners involved in the company´s R&D effort. In addition, it is desirable to standardize data formats and data handling as much as possible, to keep tight controls on quality, particularly when compounds are being transferred between organizations and to optimize the sharing of information to maximize productivity.

A further challenge, which affects, among others, the domain of Analytical Data, is the reduction in the resourcing of internal support teams. In the case of Analytical Data, this means that, whilst progress in hardware capabilities and huge steps forward in automation allow us to generate very large amounts of analytical data in high throughput, a bottleneck is being created by the capability of Analytical Departments to get eyes on data. In this situation, overseeing also the data and proposed chemical structures proposed by partners outside the organization, and typically received in large numbers, is becoming harder and in most cases has been abandoned as an achievable objective.

Mnova Verify evaluates a series of elements (GSD, solvent recognition, novel similarity measure, multiplets and chemical shift predictions) and applies a scoring system to return a Yes/No/I don´t know answer. This makes it a very powerful screening system useful for chemists who need to validate decisions as to the structure corresponding to their experiments, either in single or batch mode.



© 2024 MESTRELAB RESEARCH

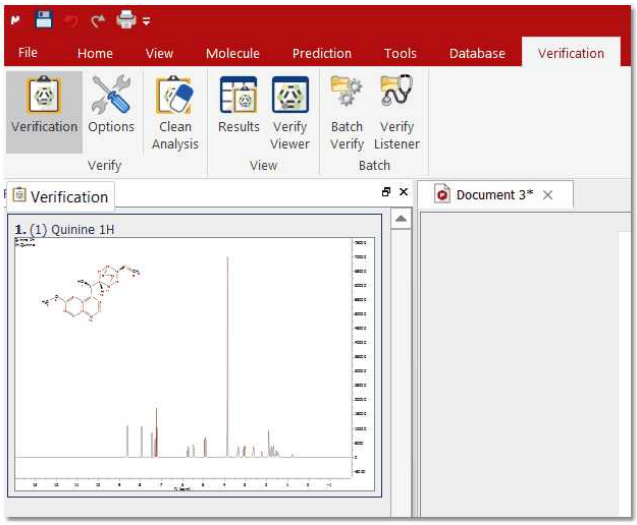
10.1 Single Mode

To run a verification in single mode, import into Mnova (by using the File/Open dialog or by dragging and dropping from the OS browser) a set of spectra (any combination of one 1H spectrum, one HSQC, 13C and one LCMS or GCMS) and a single structure (.mol or .sdf).

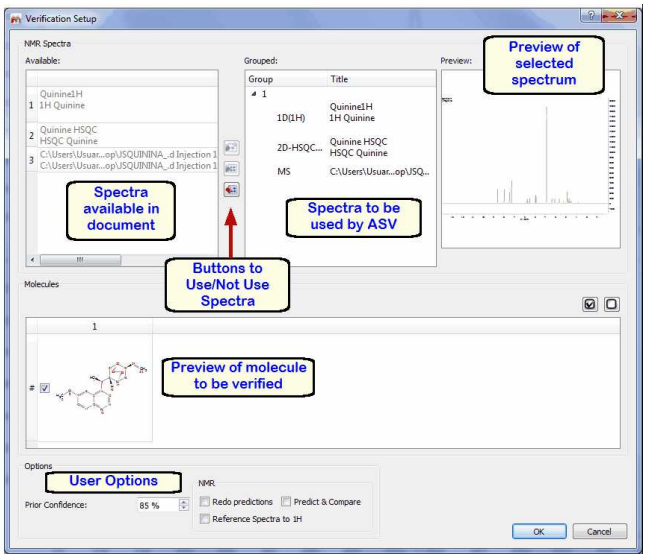
Please note that our preference is to work with fid and ser files as opposed to processed data, as the optimal processing for the verification may differ from processing used for visualization, etc.

Optimally, prior to running the verification, 1H NMR should be zero filled to 64K or 128K points and should have no line broadening applied.

Once the data have been imported, invoke the 'Verification' menu.



Once you have selected 'Verification'; the 'verification setup' dialog box will be displayed:



The document spectra panel displays the spectra which are available in the document.

The verification spectra panel displays the spectra which will be used for the verification (you could have some more spectra in the document which you do not want to use for verification, so this allow you to use only those which are relevant to you at that stage)

The spectral preview just shows you an image of the spectrum you have selected in the verification spectra panel (this could be handy when you are trying to decide maybe between 2 different 1H spectra to do the verification on)

The buttons to Use/Not Use spectra allow you to move spectra which are available on the document to the list of spectra to be used for verification or viceversa. To do so, just select the relevant spectrum and click on the relevant arrow to move from the list on the left to the list on the right or from the list on the right to the list on the left.

The molecule panel allows you to visualize the molecules you have available in the document and to select the one you want to verify by ticking it.

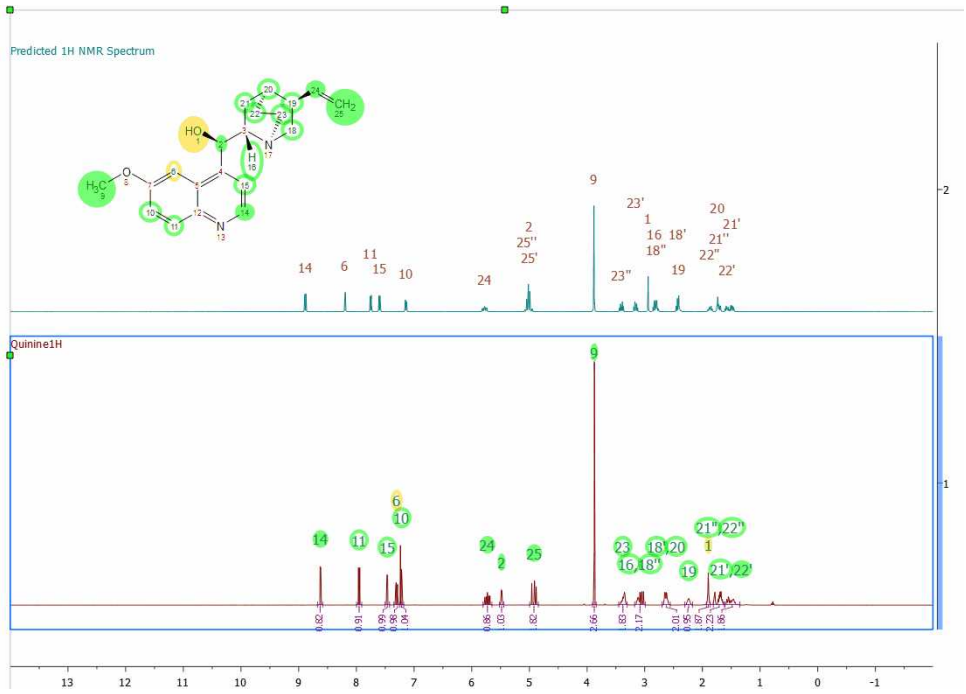
The **User Options** do the following:

Prior Confidence: If the prior probability is 0.5, that means that we don't have any prior knowledge about the quality of our structures so that the new score will be exactly the same as if the prior probability concept is not applied.
On the other hand, higher values of the prior confidence (i.e. 0.95) will result in higher scores (the significances are untouched). This is like saying: I know that 95% of my molecules are always correct, so please use this prior knowledge to scale the scores. Values of prior confidence below 0.5 will drop the scores (I know that most of my molecules are wrong, so take this into account).

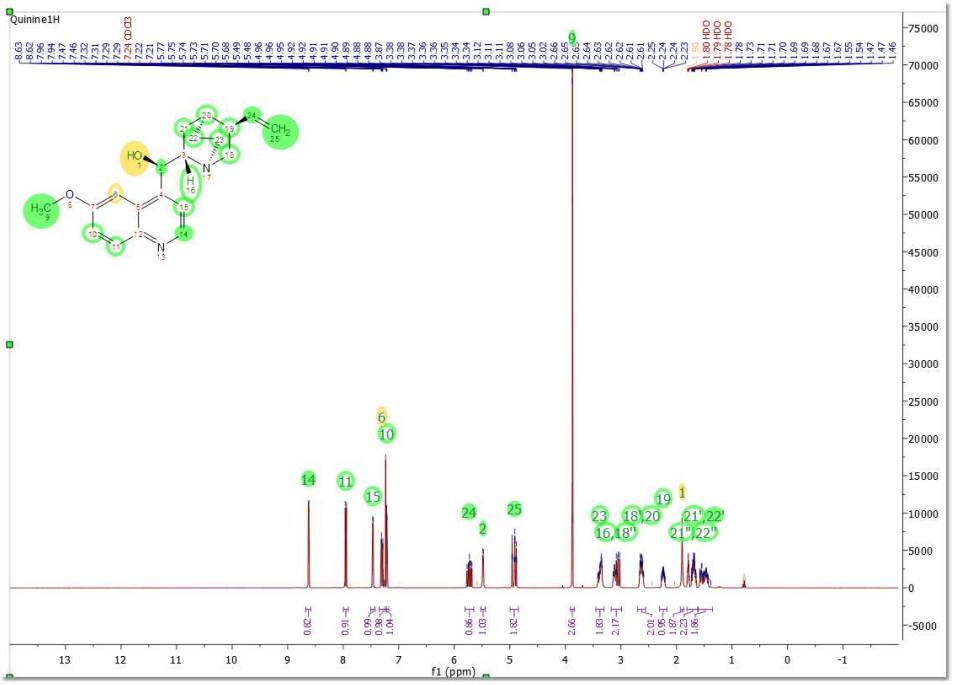
Redo Predictions: to be used in cases where the prediction failed and the user manually corrected the values. See this chapter (example 2) for further information

Predict and Compare tick box allows you to select how you want the spectra to be presented after verification. If you tick the box, you will be presented with the experimental and predicted spectra in a stack:

© 2024 MESTRELAB RESEARCH



If you untick the Predict & Compare box, then you will be presented with just the Experimental spectrum:



Reference Spectra to 1H: to apply Absolute Reference to the document

Once you have selected the desired options, click OK and the verification will run.

Once you have run verification, you will be presented with the following:

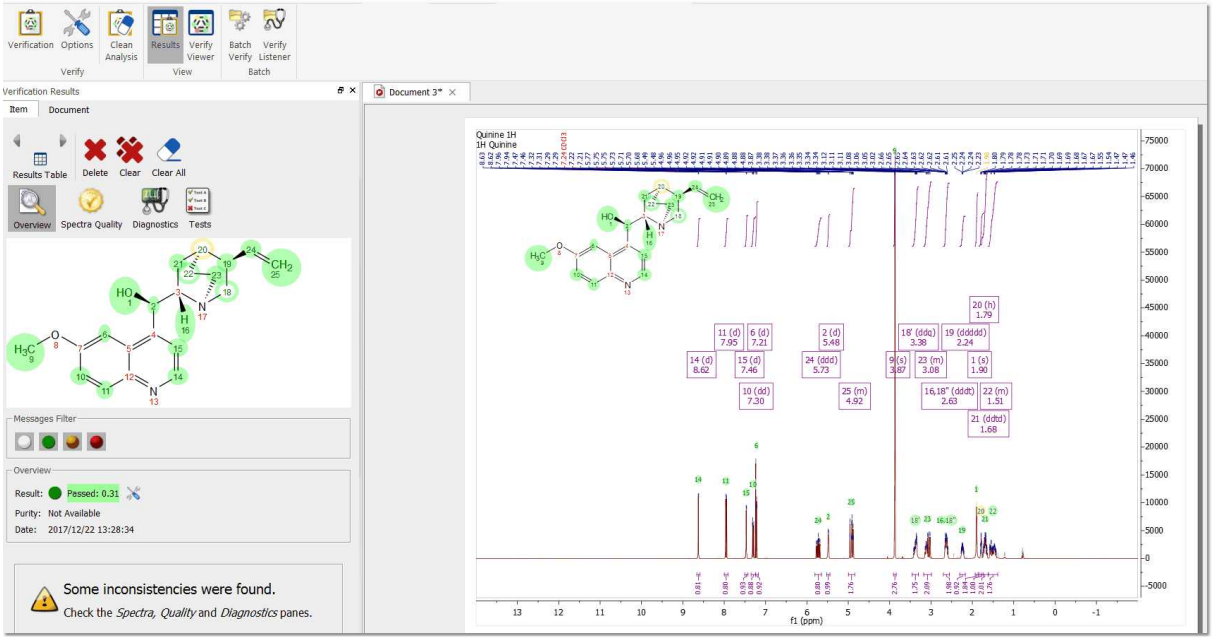
© 2024 MESTRELAB RESEARCH

1. Spectrum/spectra, in your chosen view mode (Predict & Compare or Active)

2. A table of verification results which should appear after you run. If not, turn it on by using the ´View/Tables/Verification Results´ menu.

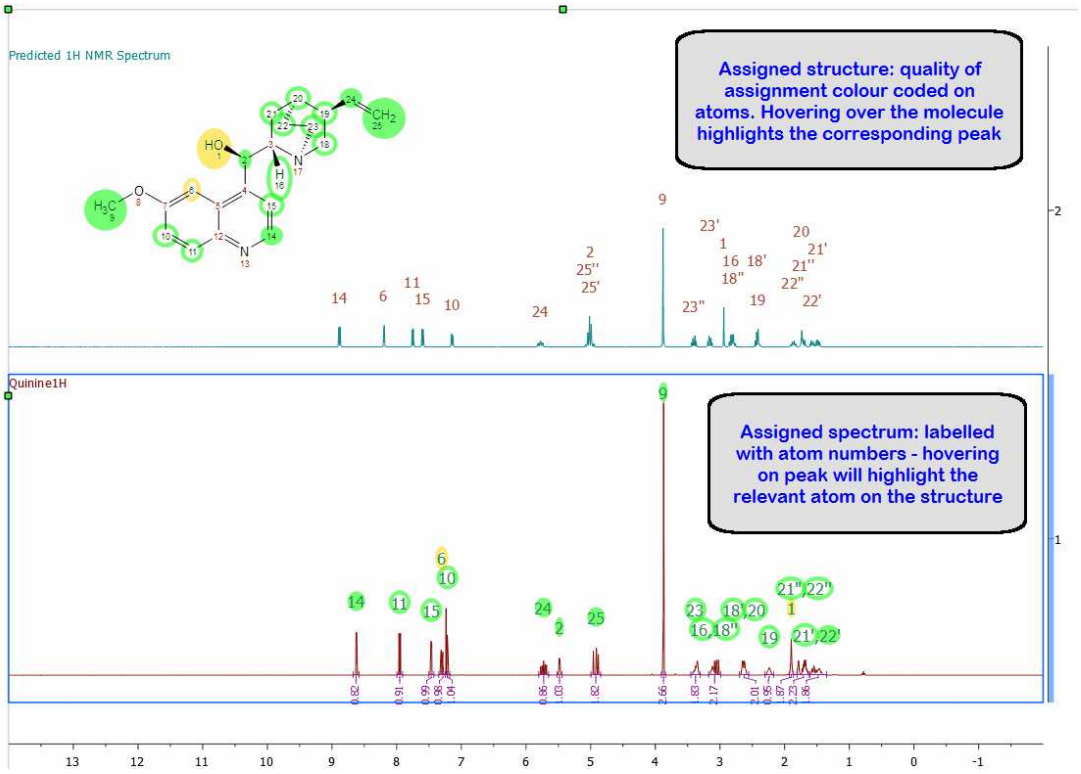
3. The structure, annotated with colour coded assignments.The color coding of the elementary assignments is made according to their quality (Green = High Quality; Yellow = Medium quality; Red = bad quality; Gray = worse quality).

However, this quality parameter is not the only descriptor that we can use to show the outcome of a given assignment. There is another very useful parameter that is called 'Stability'. A stable elementary assignment means that this remains stable in all the assignments solutions whose score is higher than a given positive threshold, that is, that assignment is found in all the assignments solutions. This is another way to say that such assignment is very likely to be correct. So the stable assignments are displayed as filled disks, whilst normal assignments are displayed with unfilled disks.



The paper area will present the spectra annotated with the assignments the software has made to the molecule and the molecule with each atom colour coded by quality of assignment (green is a good assignment, yellow is poorer assignment and red is a bad assignment but which still allows the software to make a total assignment of the structure).

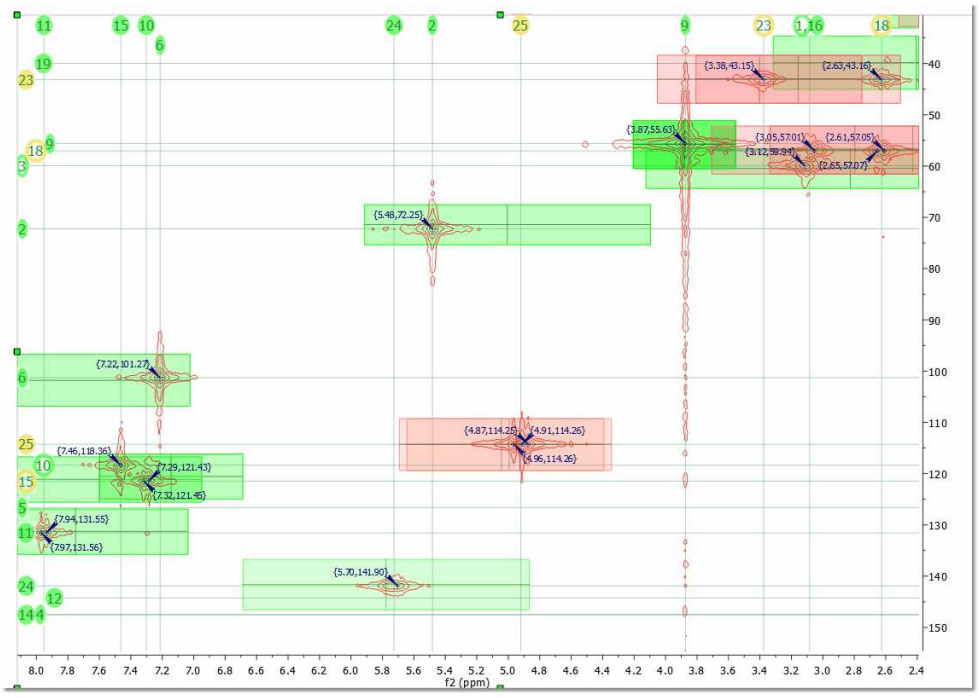
© 2024 MESTRELAB RESEARCH



In the case of HSQC, the software will present you with the assigned molecule and assigned HSQC spectrum, with the assignment labels for the spectrum placed at the top of the paper. In addition, the spectrum is marked with colour coded rectangles as follows:

- A green rectangle represents a predicted 2D range for a peak, which does have a real peak within it, therefore indicating a good match between experimental and predicted peaks.
- A yellow rectangle represents a predicted 2D range for a peak, which has a real peak very close to its edge, therefore indicating an acceptable but less reliable match between experimental and predicted peaks.
- A red rectangle represents a predicted 2D range for a peak, which does not coincide with any experimental peaks, and therefore indicates a significant difference between a predicted and experimental peak or the complete lack of an experimental peak.
- A peak without a rectangle means that there is an experimental peak present where the prediction does not expect any peaks

© 2024 MESTRELAB RESEARCH



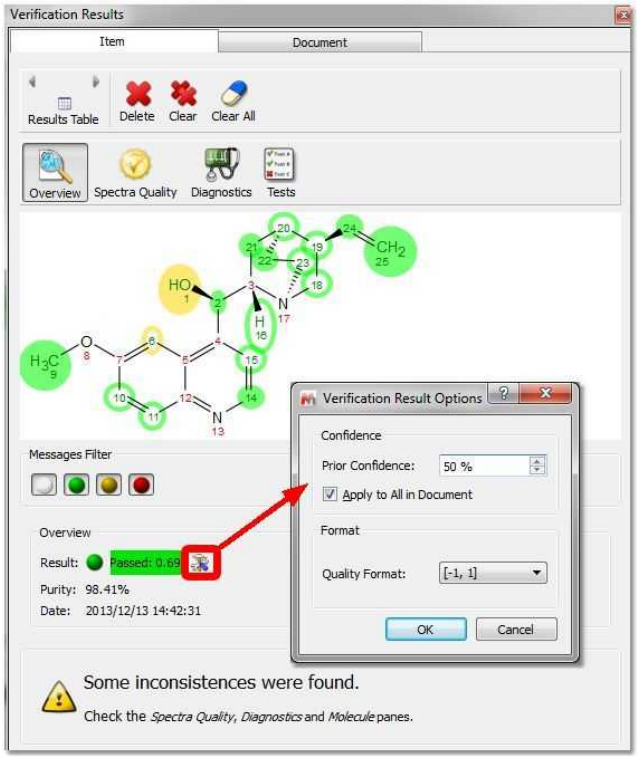
10.2 Verification Results panel

In the Verification Results table, the information is distributed in different pages (Overview, Spectra Quality, Diagnostics and Tests). The messages can be filtered depending on the type of inconsistency:



In the **Overview** mode, we will get the result, purity value, date and the verification results options. From that dialog, you can change the 'prior confidence' to one spectrum or to all in the document (which can be useful for example if you had run a Verification with 100 spectra and then you want to see how the results would be with different prior confidence value, without needing to re-run the Verification) and also the quality format:

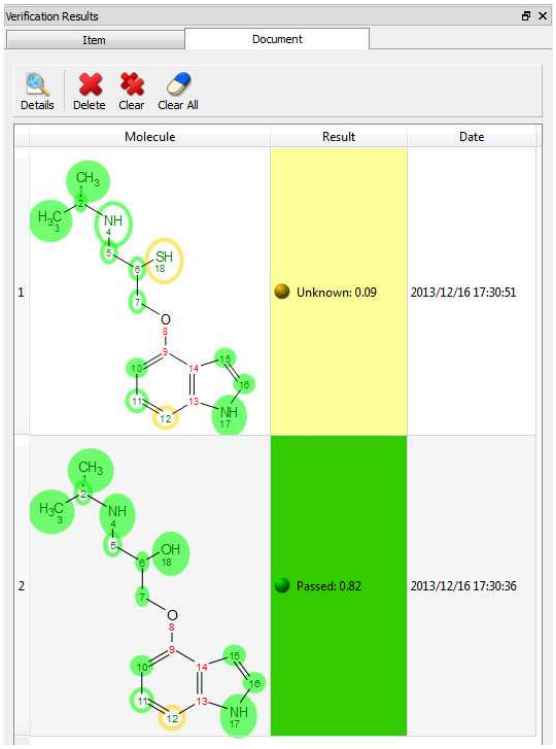
© 2024 MESTRELAB RESEARCH



If the document contains more than on molecule, you can use the 'Result Table' arrows to navigate through the results:

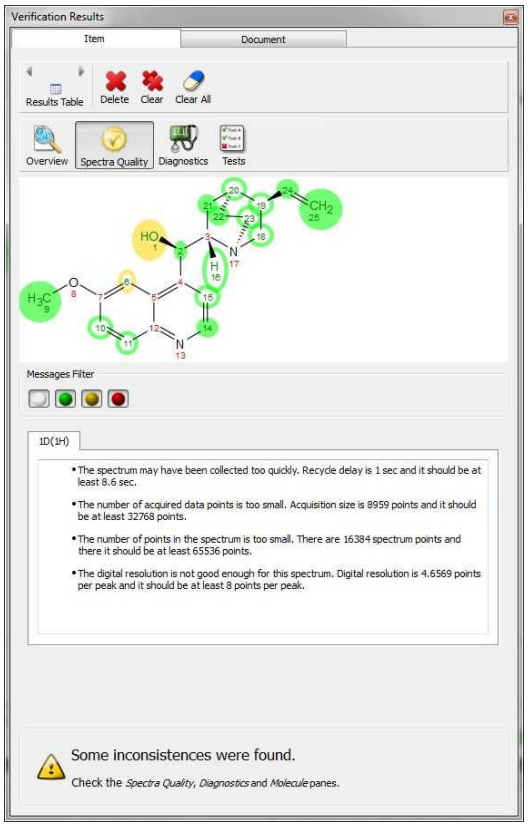


Clicking on the 'Results Table' button will display the verification results in a table:

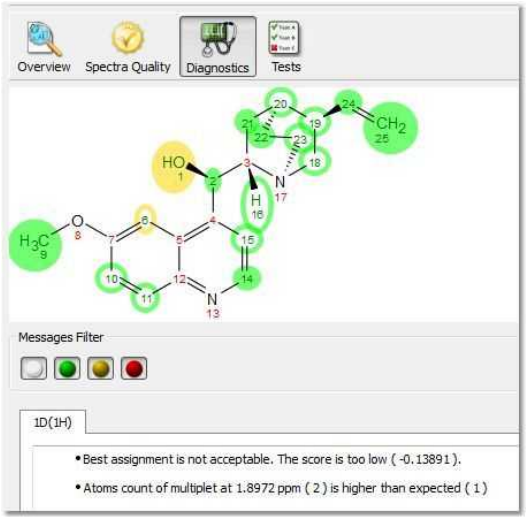


Clicking on the Spectral Quality button, will display the report any problem detected concerning the quality of the experimental data, which you can filter with the coloured buttons:

© 2024 MESTRELAB RESEARCH

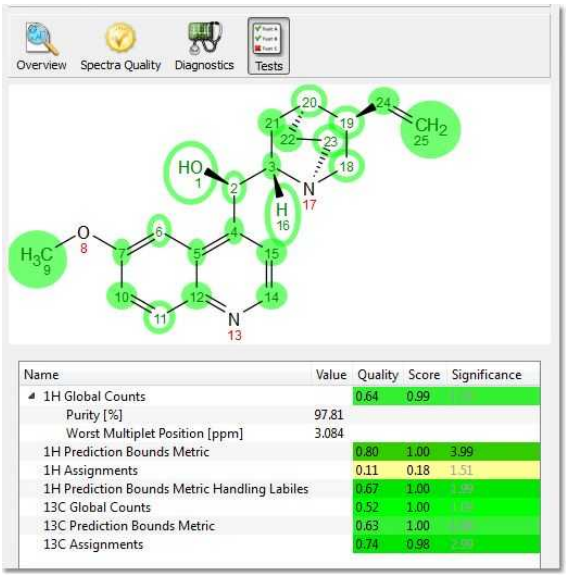


Clicking on the 'Diagnostics' button, will display some warning messages:



Clicking on the 'Tests button', will display the Score, Significance and Quality values for each individual test, which is designed to help you identify quickly possible reasons for a failure:

© 2024 MESTRELAB RESEARCH



- **Global Counts:**

Purity: this is an estimation of the purity of the sample calculated by NMR. Basically, it is the ratio of Compounds Vs (Impurity+Artifacts) peaks. Purity can be displayed in Peaks table by selecting the checkbox "Show Purity Label" in Setup Table (disabled by default).

Worst Multiplet position: this is the location of the real multiplet which is furthest away on the chemical shift scale from its predicted counterpart

- **Prediction Bounds Metrics:** this is the location of the predicted multiplet which is furthest away on the chemical shift scale from its experimental counterpart. Each atom in the molecular structure is given a chemical shift and an allowable range based on the quality of the prediction for its specific environment. The software then checks whether a multiplet with the right number of nuclides is present within that allowable range. There will be a specific test for multiplet labeled as labiles by the Autoedit analysis step.

- **Assignments Test:** Effective number of missing peaks and effective number of extra peaks (1H, 13C and HSQC). The full spin system for the proposed molecular structure is built and compared to the experimental data. Every possible assignment of the multiplets in the spectrum to the spin system (and through it to the atoms) is enumerated and its likeability calculated by a merit function. The highest positive result of the merit function will be reported as both as verification test result and as automatic assignments made on the structure/spectrum pair. In the case of HSQC, the 2D spectrum for the structure is predicted and each predicted peak is given a 2 dimensional allowable range. Each of these ranges is then evaluated as to whether a real peak in present within them, and whether there are ranges with missing peaks or real peaks which do not correspond to allowable predicted ranges.

When running the verification of a HSQC against several molecules, the user has the ability to show the rectangles in the HSQC for each molecule, by selecting the molecule from the ASV Results table and checking/unchecking the 'Highlight' box for the HSQC assignments in the table:

© 2024 MESTRELAB RESEARCH

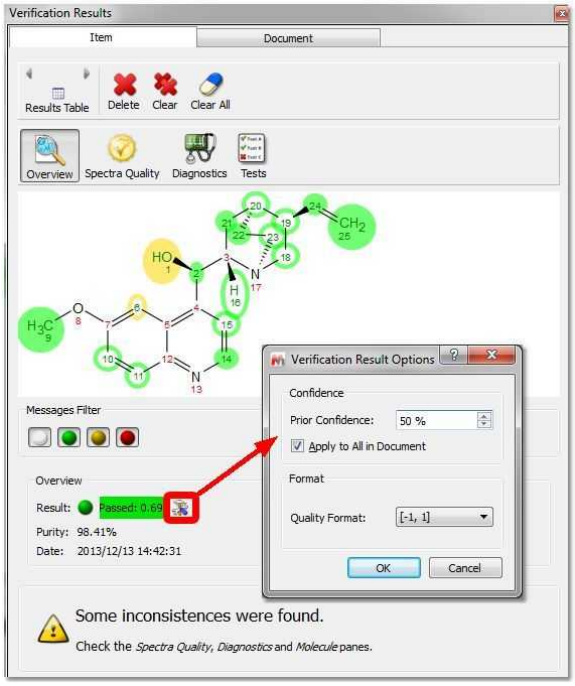


- **MS Molecule Match Score:** For each proposed structure's molecular formula, with the given ionization mode (such as protonation), a molecular ion cluster spectrum is computed. Each molecular ion cluster spectrum is then compared to every spectrum in the LC- or GC- MS run, and ranked according to spectral similarity, where 1.0 is identical within the m/z region of the molecular ion cluster. This ranking is further refined by taking into account the response of a molecular ion chromatogram (or chemical chromatogram) , which also has a maximum value of 1.0. The final match score is the product of the two metrics. The MS Molecule Match similarity measurements are derived from traditional dot product calculations and take into account a user specified m/z accuracy, to make full use of accurately mass measured mass spectral data. If MS/MS spectra are also present in the data set, the structures are used to predict fragment spectra, and these predicted fragment spectra are compared with the acquired MS/MS spectra.

Once the data is processed and made ready for the verification, a series of tests of the hypothesis that the proposed structure is the major component of the sample are carried out. For an effective automatic structure verification system when many of the parameters being considered by it may well be imperfect (integration, spectral analysis, predicted chemical shifts, etc.) it is fundamental that the system has the capability to weigh different results in different ways and to auto-evaluate the importance of specific tests in specific conditions. This allows the system to become robust to a level of uncertainty in the quality of the inputs. Therefore, the single tests may have not only a score but a different significance which, in addition, may correlate with the principle outcome. Each test generates a [quality, score, significance] result:

- **Quality**: The Quality Score corresponding to each individual test is generated by combining the Score and the Significance for that test. The objective of this Quality Score is to generate one single number to allow easy comparison when handling large volumes of data. The format of Quality values can be selected from 'Verification Results table' after having clicked on the 'Options' button:

© 2024 MESTRELAB RESEARCH



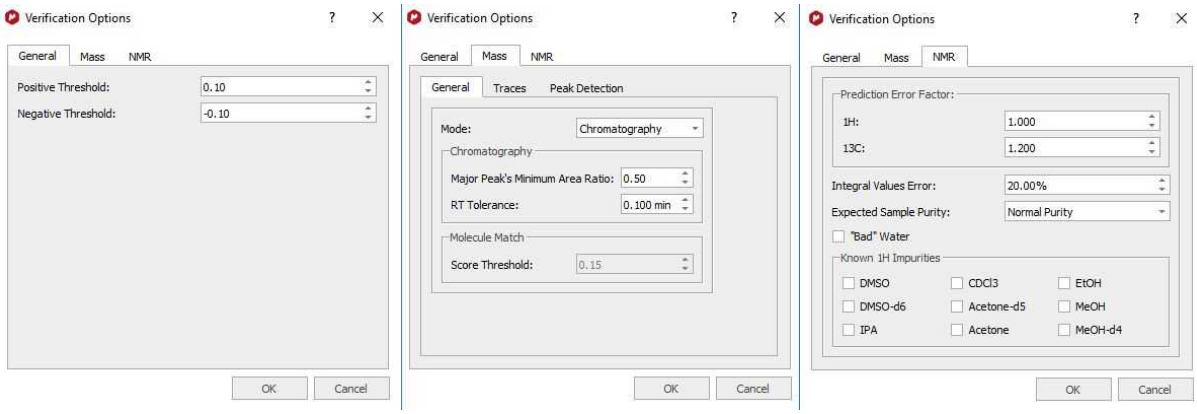
- **Score**: This is the result of the test, where a value of 1 means that the hypothesis fits perfectly with the experimental data, whilst a score of -1 means that the hypothesis is a perfect failure and therefore definitely wrong. The score value will include three digits (Score taking into account the prior confidence, verification score (without taking into account the prior confidence) and 'prior confidence')

- **Significance**: Each test, depending on the characteristics of the data, the proposed molecular structure, the prediction quality for the given HOSE codes or chemical environments present on the molecule, etc., gets given a significance. Thus, a high level of impurities in the spectrum may reduce the significance of tests which rely heavily on integration, whilst a poorly predicted chemical environment may reduce the significance of a test relying heavily on chemical shift prediction. Significances of 0-2 are low, 3-5 are medium and >5 are highly significant. There is no theoretical limit to the value of significance, if a test could be devised which was fully deterministic and completely infallible, this could have infinite significant within this concept.

© 2024 MESTRELAB RESEARCH

10.3 Options

Following the menu 'Verification/Options' will display the dialog box below:



From here, you will be allowed to change some general settings (the thresholds for positive and negative verification results) and also for the MS and NMR verifications (i.e prediction error factors, to select a threshold for the integration values, the percentage of impurities, etc).

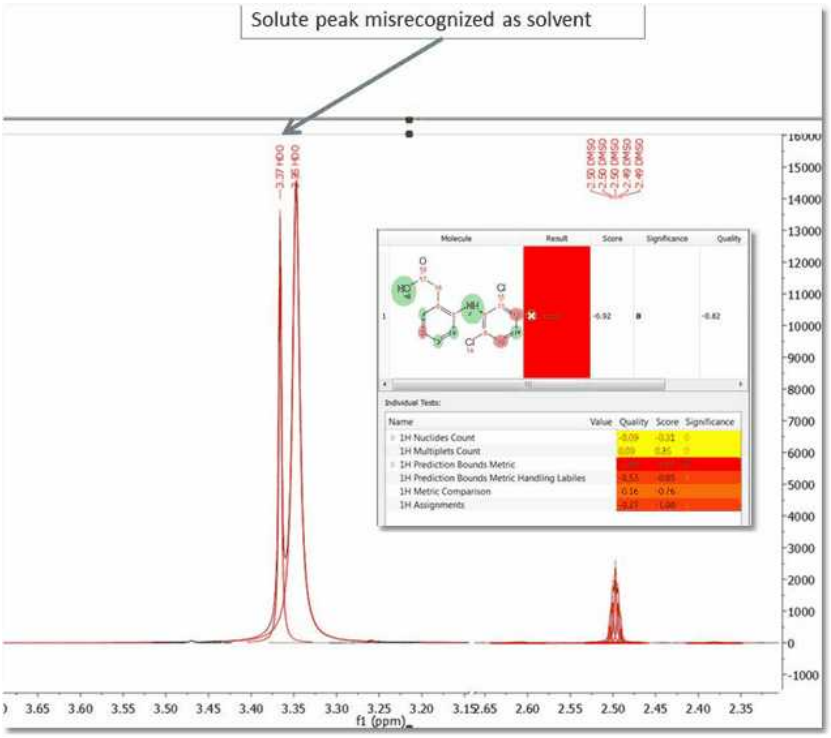
NOTE: The option to 'clean analysis' (under the Analysis menu) could be useful to rerun a verification after having changed the options.

10.4 Troubleshooting a negative result

What happens if I get a negative verification result?

There are several reasons to get negative results in the verification. Let´s take a look at some of them

1. **All or most of the NMR tests fail with bad scores**: this could be an indication of either the wrong structure, a very poor quality spectrum or **solvent misrecognition, which will affect most tests very significantly**. The recommended Operating Procedure is to check the solvent recognition first. To do so, make sure you are in ´Active Spectrum´ mode and that your graphical properties (Home/Properties/NMRSpectrum/Peaks/Curve) has the ´Peaks´ box ticked. This will display colour coding for the autoediting carried out by the software on the spectra. You may identify this way that a solute peak has been considered as solvent or viceversa.

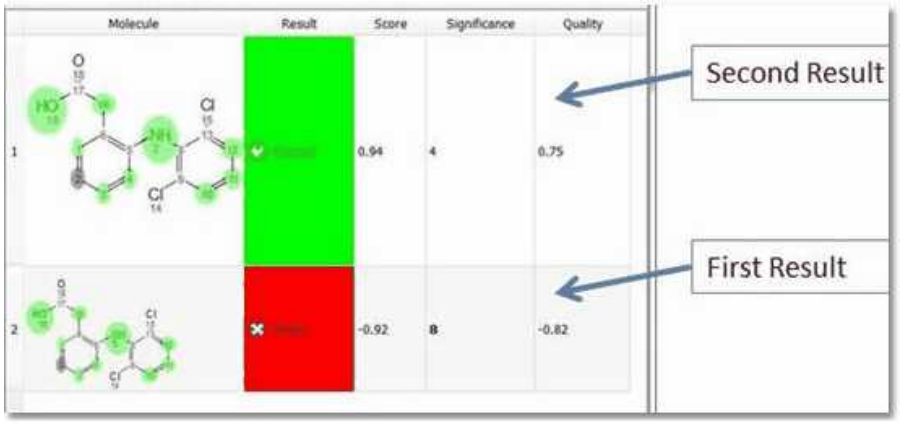


In a case in which this happens, this can be corrected by right clicking on the peak label (pointed at by the arrow on the figure) and selecting Edit Peak Type, then marking this peak correctly (in the case above would be marked as compound and with the Flags unticked…)

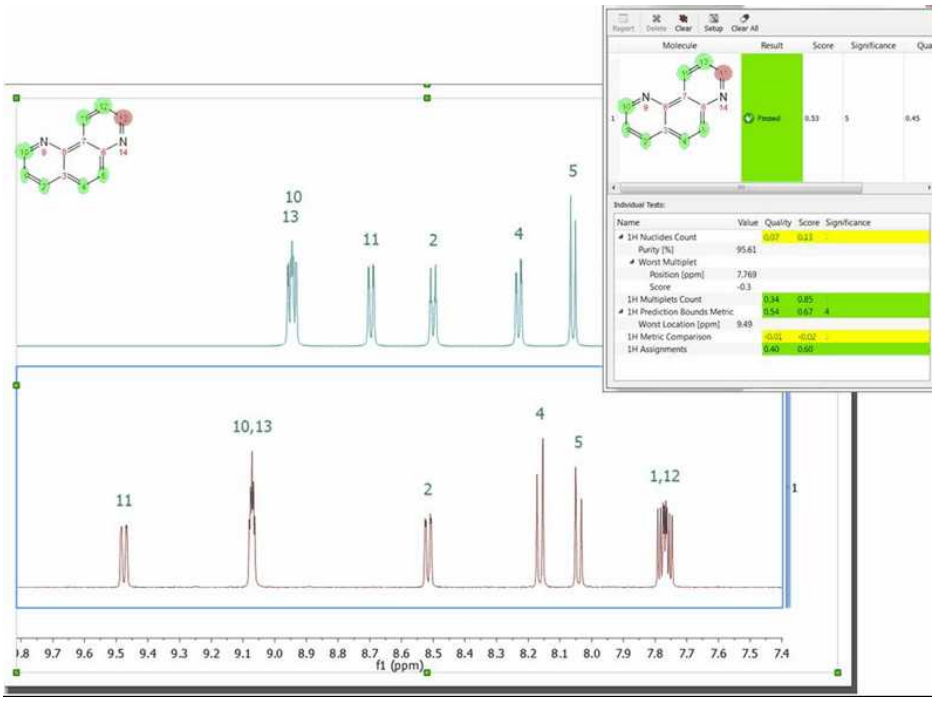


Once GSD/AutoEdit has been modified, you can just re-run Verification and your GSD modifications will be respected. In this case, after running again, the result is totally different, as can be appreciated in the figure below:

© 2024 MESTRELAB RESEARCH



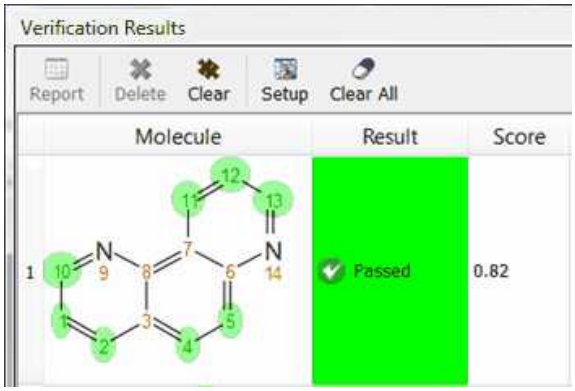
2. **Should you find that the score is reasonable but that some atoms are showing assigned in red and that either the PBM or NN tests have low values**, then this could be due to an ***inaccurate prediction*** or to an atom which is not where it is supposed to be (in the latter case this would be a true negative result). In this case, check the ´Worst Multiplet´ and ´Worst Location´ feedback, as this might indicate where you should look first. In the case of the figure below, ´Worst Location´ is 9.49 ppm, and there we can see that there is a peak, which is being assigned to atom number 11, but the predicted value for atom number 11 is at 8.75 ppm. The user can decide here whether it is reasonable that the prediction is poor and that the real value of atom 11 could indeed be 9.49.



In this case, the user can grab the prediction label (the same could be done with the rectangles in a HSQC) by clicking on it (the number 11 label on the blue spectrum) and moving it to the 9.49 ppm position. Once this has been done, the verification can be carried out again, making sure that the ´**Redo Predictions**´ tickbox in the verification dialog is unticked. This will allow the user to see what the result of the verification would be in case the prediction was being made at 9.49 ppm, rather than 8.75

© 2024 MESTRELAB RESEARCH

ppm. In this case, as the figure below shows, the verification ´Score´ has improved from 0.56 to 0.82 and the atom which was badly assigned (colour coded in red on the molecule previously) is now well assigned. Should the user decide that the presence of atom 11 at 9.49 ppm is possible given its chemical environment, then this is a likely indication that the reason for the previous lower score was simply a poor prediction.

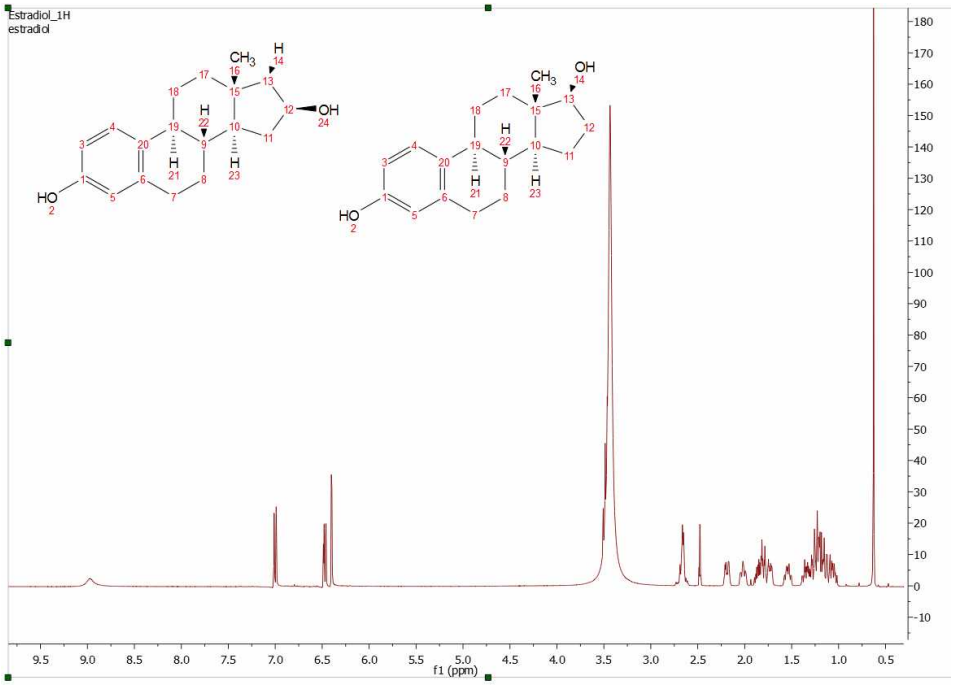


3. **A low result in 1H Nuclides Count** could also be indicative of poor integration due to either impurities or a very short relaxation time. It is to be noted that a very short relaxation time can affect integration and that poor integrals will have a negative impact on the verification result. This can be thought of as a false positive but in fact the software is reporting a problem or incompatibility in the data with the proposed molecule.

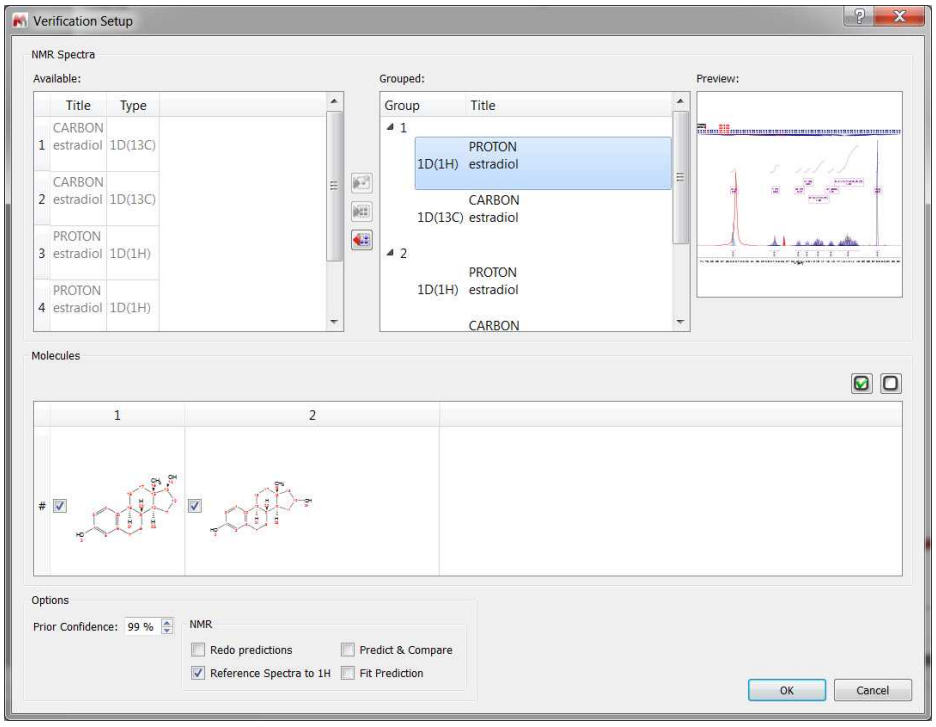
10.5 Rank potential molecular candidates

Mnova Verify can also be used to run one set of spectra against more than one potential structural candidate (or viceversa). Taking as an example a situation where we have one single spectra set (1H only, for example), as we can see in the figure below:

© 2024 MESTRELAB RESEARCH

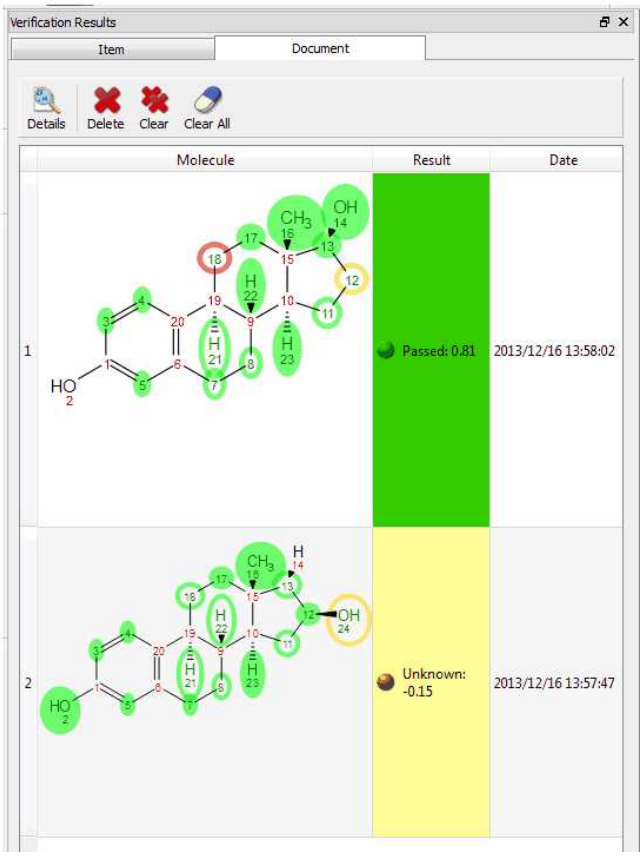


Mnova Verify will be able to rank these 2 structural candidates on the basis of their NMR. To do so, we would need to duplicate the NMR datasets inside the same document and run the Verification against the two molecules:



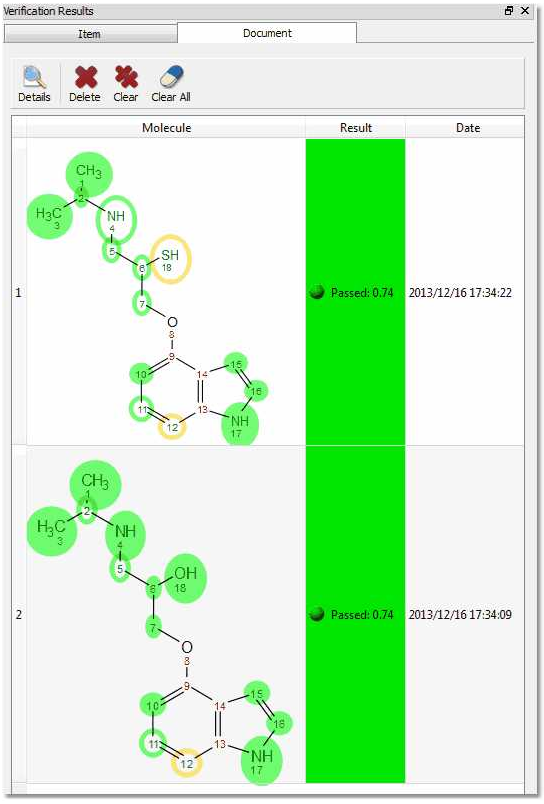
The software will run the spectral set against both structures and present the user with a score for each structural proposal, which may in many cases allow the user to differentiate between 2 possible candidate structures.

© 2024 MESTRELAB RESEARCH

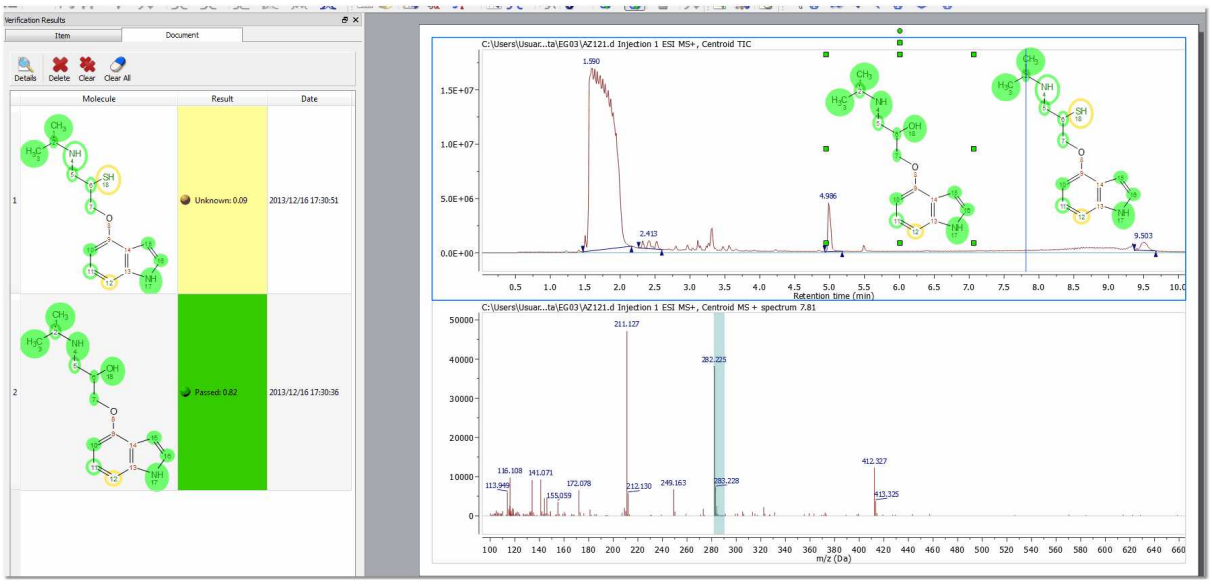


This is one of the areas where having the ability to combine NMR and MS data can prove very powerful. In the following example, 1H NMR alone does not distinguish between the structures on the total score, as the only difference is in a different nuclei (S or O) (although there are already some indications that one structural proposal has greater merit than the alternative in the quality of the assignments made by the verification module and reflected in the colour coding).

© 2024 MESTRELAB RESEARCH



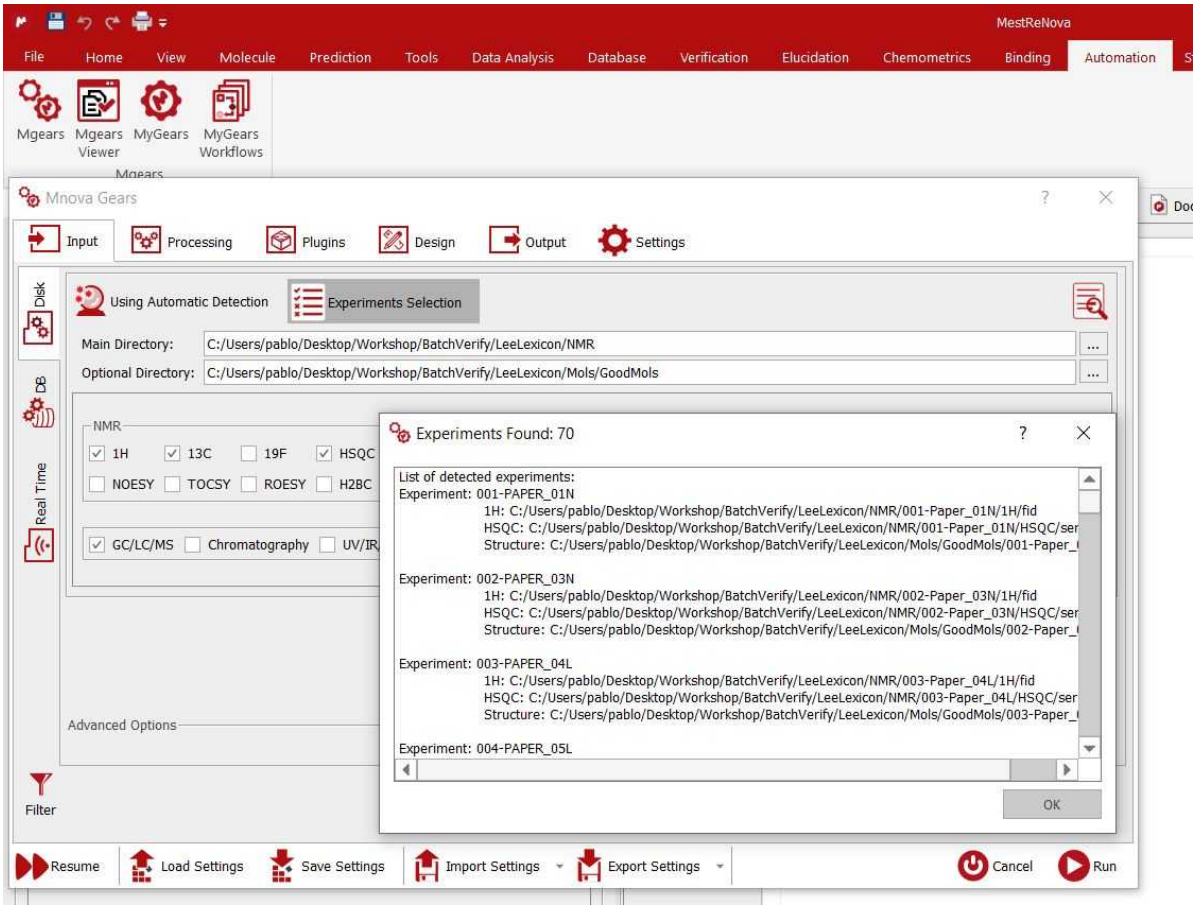
Should the user have MS data in this example, however, the difference between both proposals would be immediately obvious:



10.6 Verification in batch mode

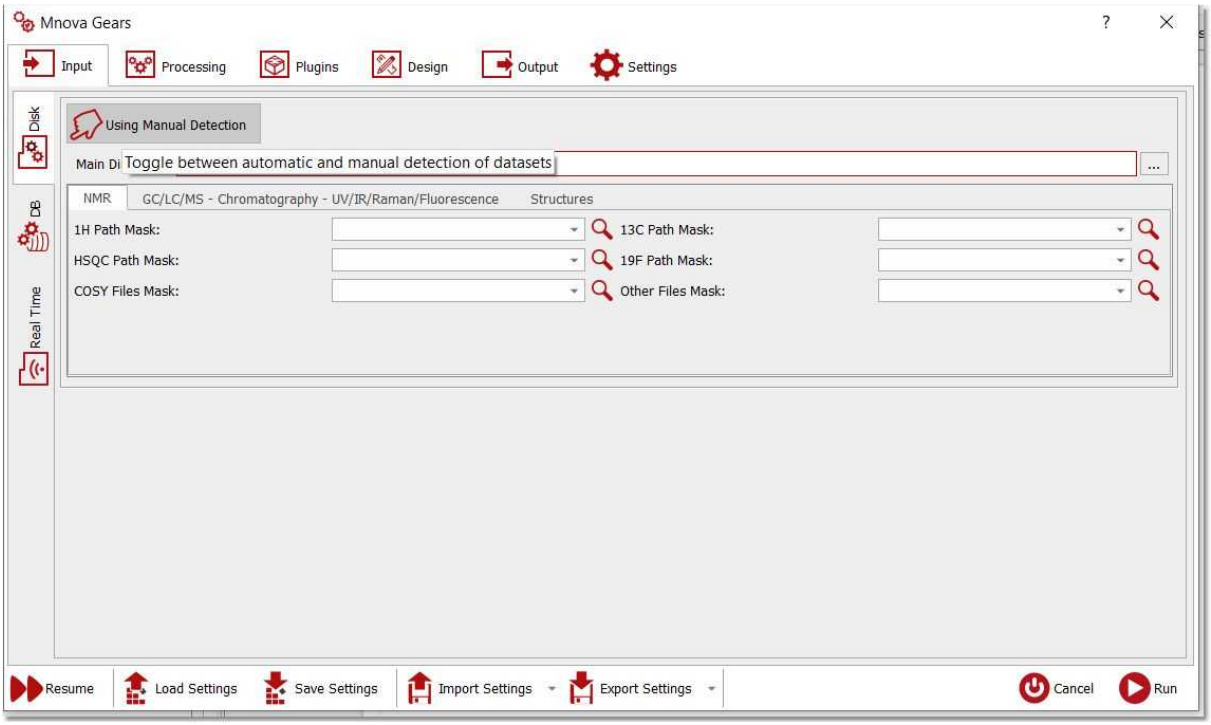
Mnova Batch Verify evaluates analytical data and makes a judgment as to whether they are compatible with the structures proposed by the user in full automation mode (from libraries or at the point of data acquisition by using the Listener). This plugin is sold apart and an specific license is needed. You will need to install Mgears and the Batch Verify brick by following the menu 'File/Advanced Plugins' and clicking on the 'blue plus' button or just by dragging&dropping the XML file in Mnova

With this plugin licensed, you can run verifications in Mnova in batch mode by following the menu 'Automation/Mgears'. From Input/Disk tab, you will be able to select the file locations in your hard disk (for your datasets and molecules). Clicking on the 'Automatic Inspection' button will check the datasets included in the Base Directory:



If you click on the 'Toggle' button, you could select the masks for the spectra and molecules (instead of an automatic detection):

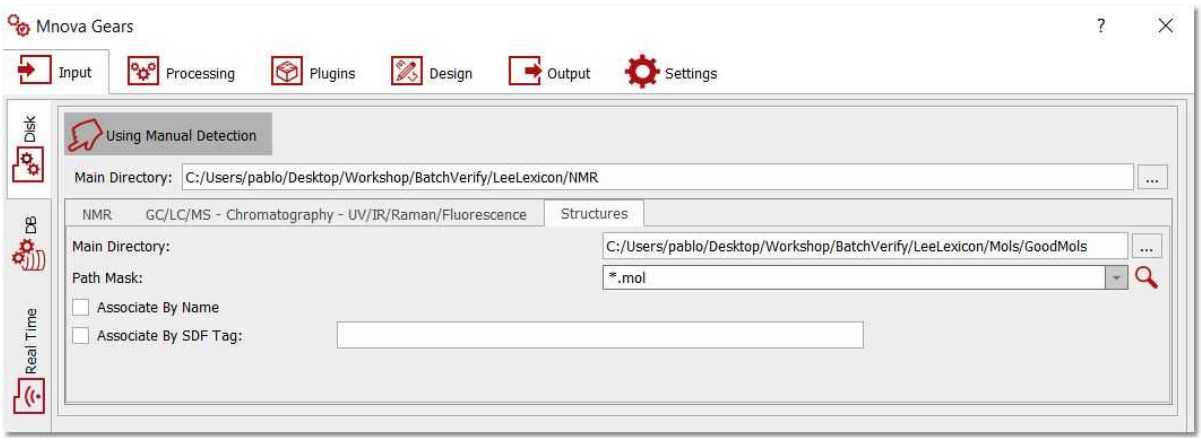
© 2024 MESTRELAB RESEARCH



Mask: Should you desire to use 1H spectra as part of the verification, a file mask describing how to open each of the spectra must be entered here. All Operating System standard conventions apply (*, ? , !, etc.).

The scroll down menu of the Masks will hold the last 5 used masks. Please bear in mind that you can use more than one mask at a time by separating the masks with the character |. This separator will be inserted automatically if the mask is chosen from the list.

The same can be done for the molecules:



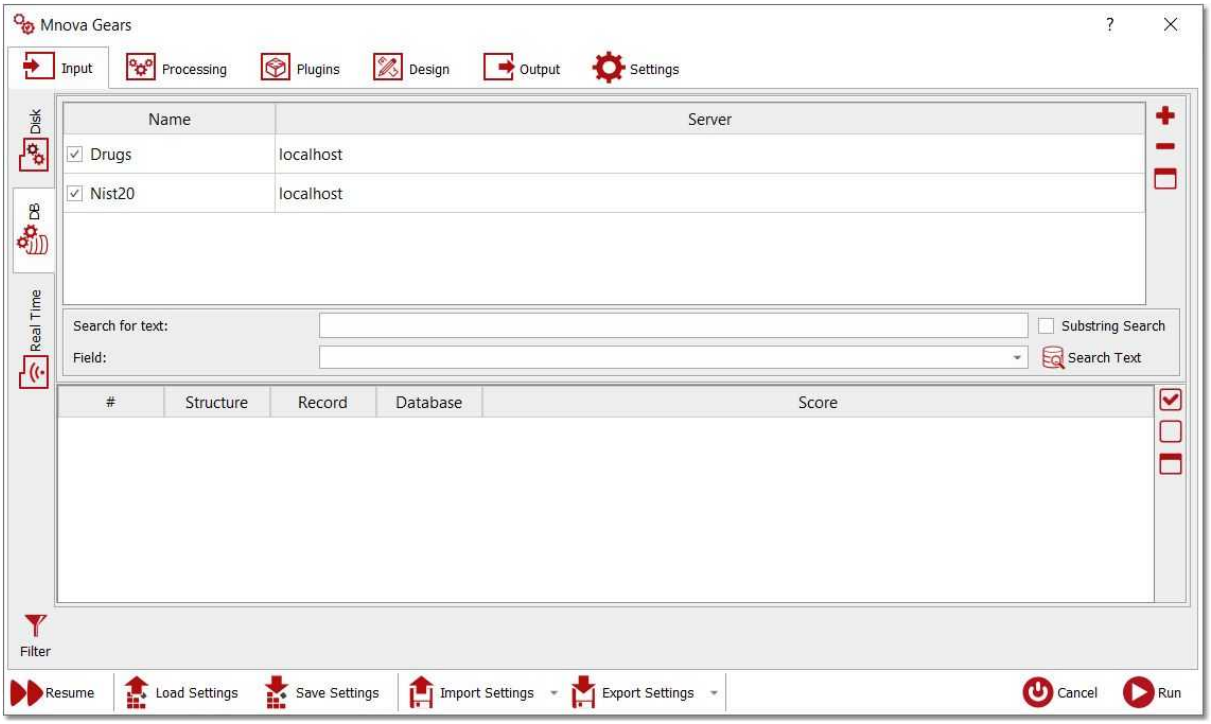
Main Directory: The location of the .mol (or .sdf) files to verify against can be entered here, as well as the File Mask to each one of the individual files.

The automation has the options to run spectra and molecules with the same name (therefore user should rename the mol or sdf files to the same name as the spectra) or in the same order (in which case user should make sure that the spectra and the molfiles are in the same order in their respective locations). If

© 2024 MESTRELAB RESEARCH

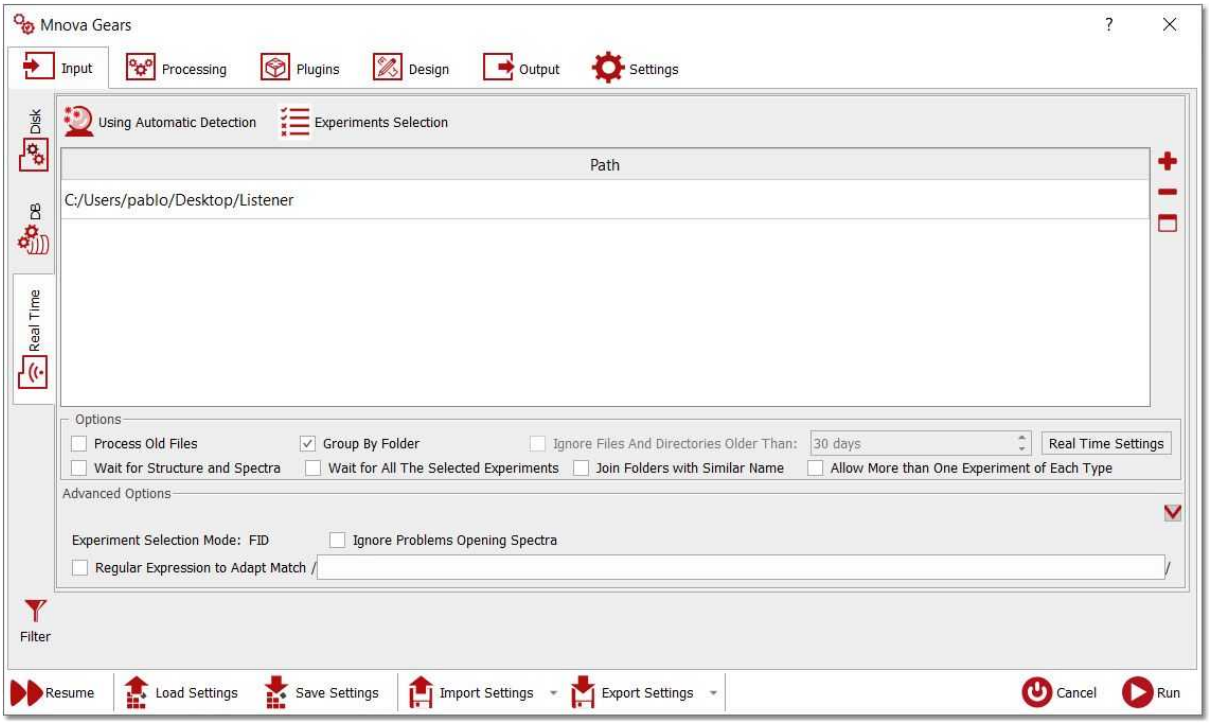
you are working directly with Mnova documents (instead of raw data) containing the molecular structure inside, you will not need to fill the molecule file location.

Selecting the Database tab will allow you to choose the input from existing Mnova databases:



You can also run Batch Verifications as a **listener**. Mnova can ´listen´ to a series of folders or drives and can grab data as is acquired and saved, and run the verification analysis automatically, saving the result in the selected folder. The settings for the Verify listener can be selected under the 'Real Time' tab:

© 2024 MESTRELAB RESEARCH

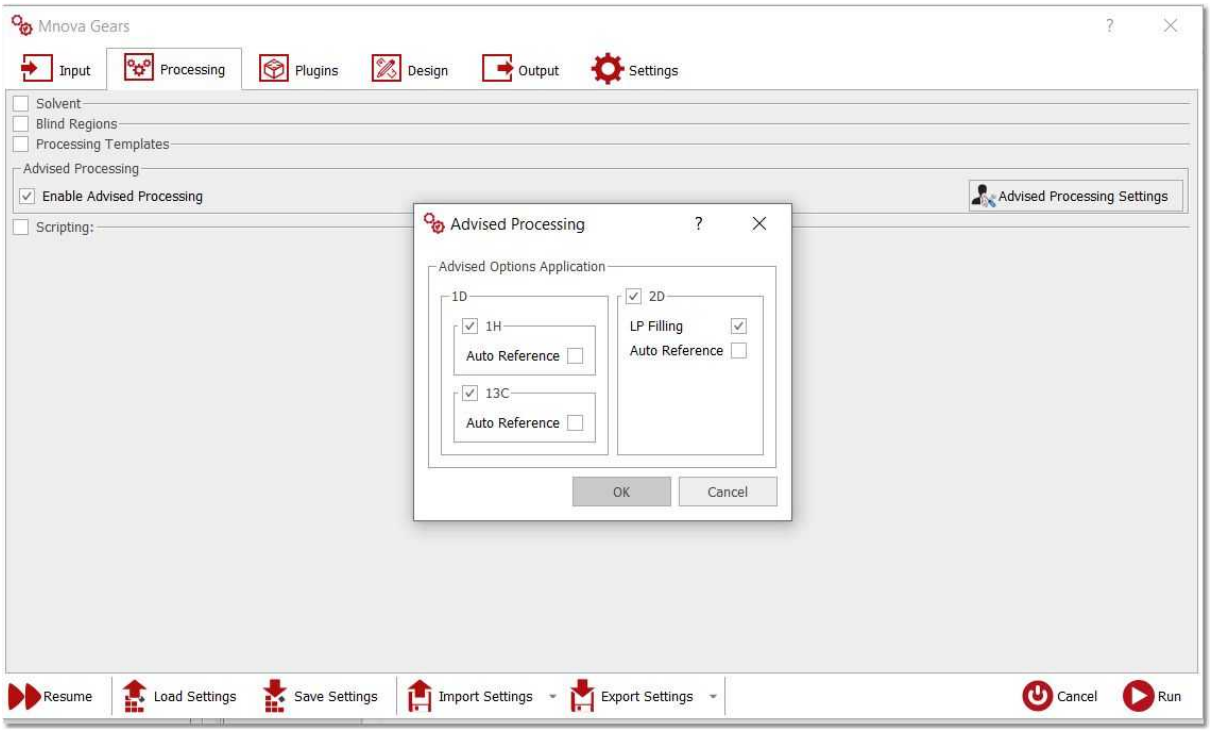


In that case, you will need to select the path of the datasets folder (and click on the 'red plus button' to add it to the list of 'Listened Folders'), the experiment types that you want to import and the path folder of the molecules. Please bear in mind that if you only use one listened folder; the **name of each spectral folder** must be the **same** with the name of the applicable **molecule file** and the molecules folder must be loaded previously in the listener folder (before running the listener).

If you select a specific listener folder for the spectra and another for molecules; then molecules and spectra will get grouped according to the name of the mol files and the first level folder where the spectra are transferred.

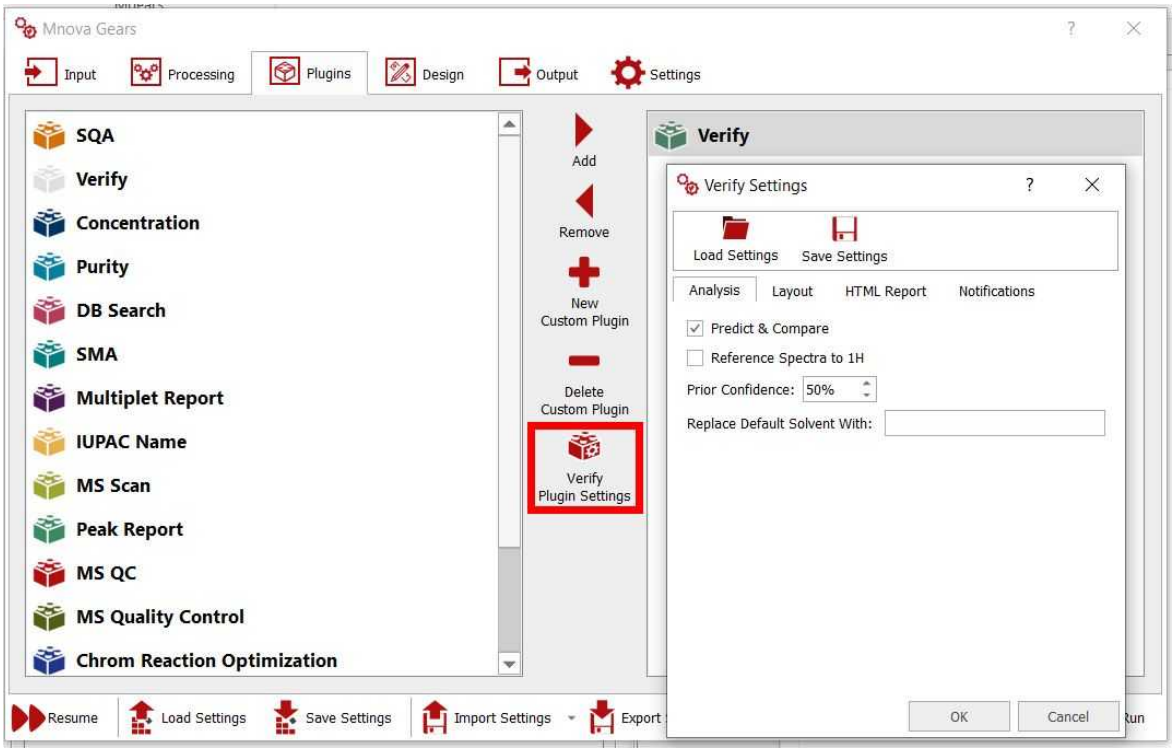
From the 'Processing' tab, you could replace the default solvent (this allows you to handle situations in which the solvent may be wrong in the parameters), add blind regions, use processing templates or enable your Advised processing settings:

© 2024 MESTRELAB RESEARCH



Checking the scripting box will allow you to run a script when the automation is started (starting script) or a script to be executed once per dataset after having opened it.

Under the Plugins tab, you could select your Verification settings for the analysis, select layout templates and customize your reports and notifications:



© 2024 MESTRELAB RESEARCH

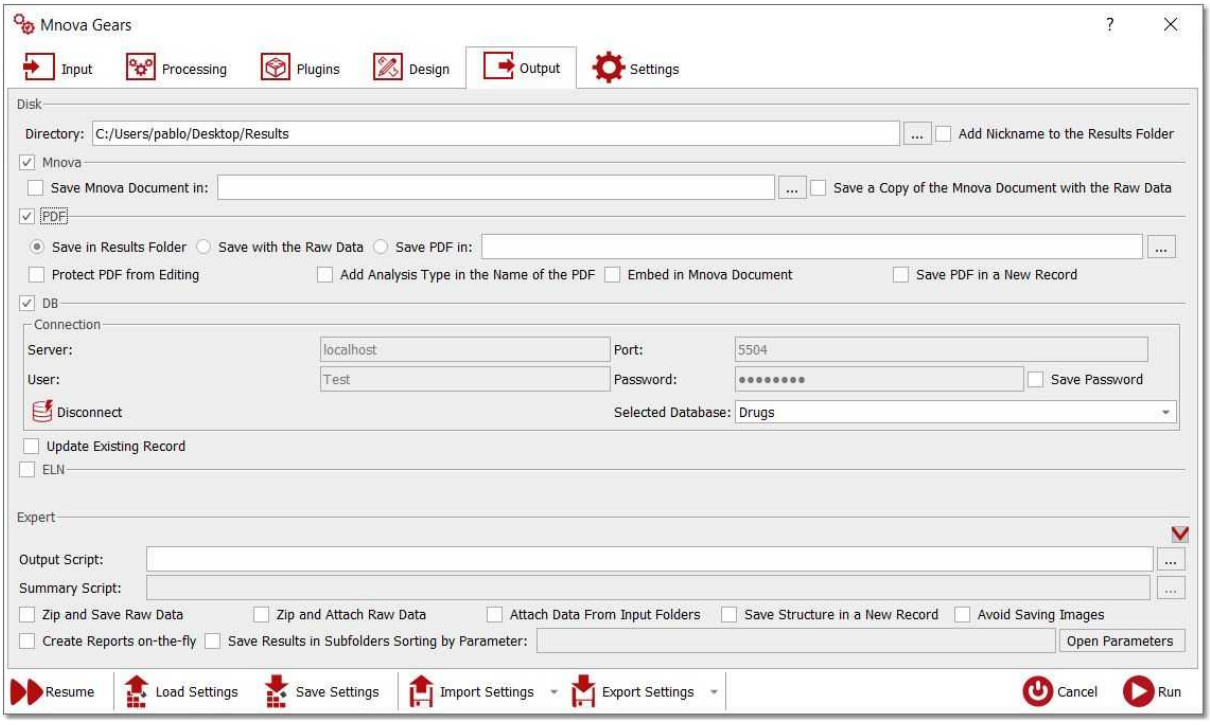
Predict&Compare: as with the single mode verification, this just sets the way in which the data are presented to the user after the verification has been completed. If the option is selected, the user will be presented with a stack of the experimental and the predicted 1H spectrum, if the option is unselected the user will get presented with just the experimental spectrum.

Reference Spectra to 1H: You can Absolute Reference the spectra to the 1H-NMR spectrum

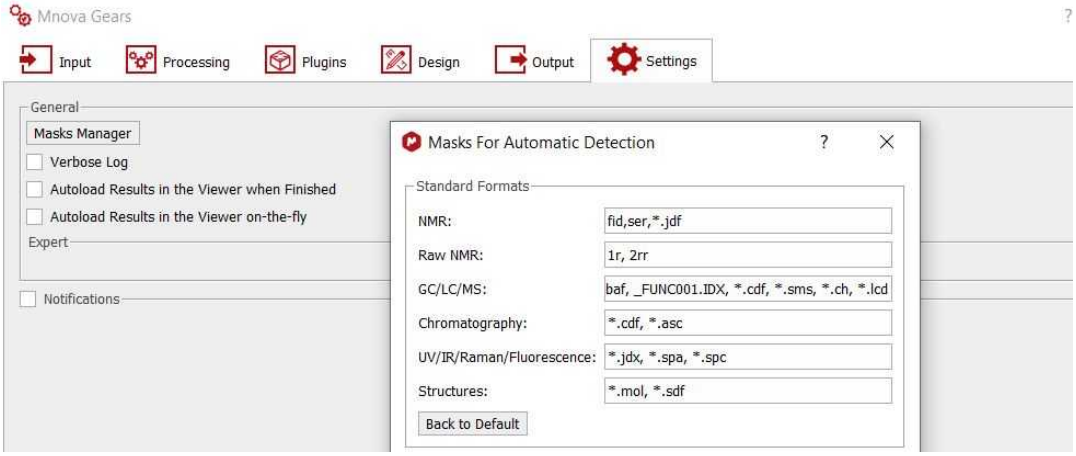
Prior Confidence: If the prior probability is 50%, that means that we don't have any prior knowledge about the quality of our structures so that the new score will be exactly the same as if the prior probability concept is not applied.
On the other hand, higher values of the prior confidence (i.e. 95%) will result in higher scores (the significances are untouched). This is like saying: I know that 95% of my molecules are always correct, so please use this prior knowledge to scale the scores. Values of prior confidence below 50% will drop the scores (I know that most of my molecules are wrong, so take this into account).

Replace Default Solvent With: This allows users to handle situations in which the solvent may be wrong in the parameters. It can be the case that a certain batch has been run on a specific solvent but that the user left the default solvent on the spectrometer data entry and therefore the solvent could be wrongly entered. Since the target solvents will affect the performance of the Autoedit function in Mnova Verify, it is important to be able to correct for this at the script level.

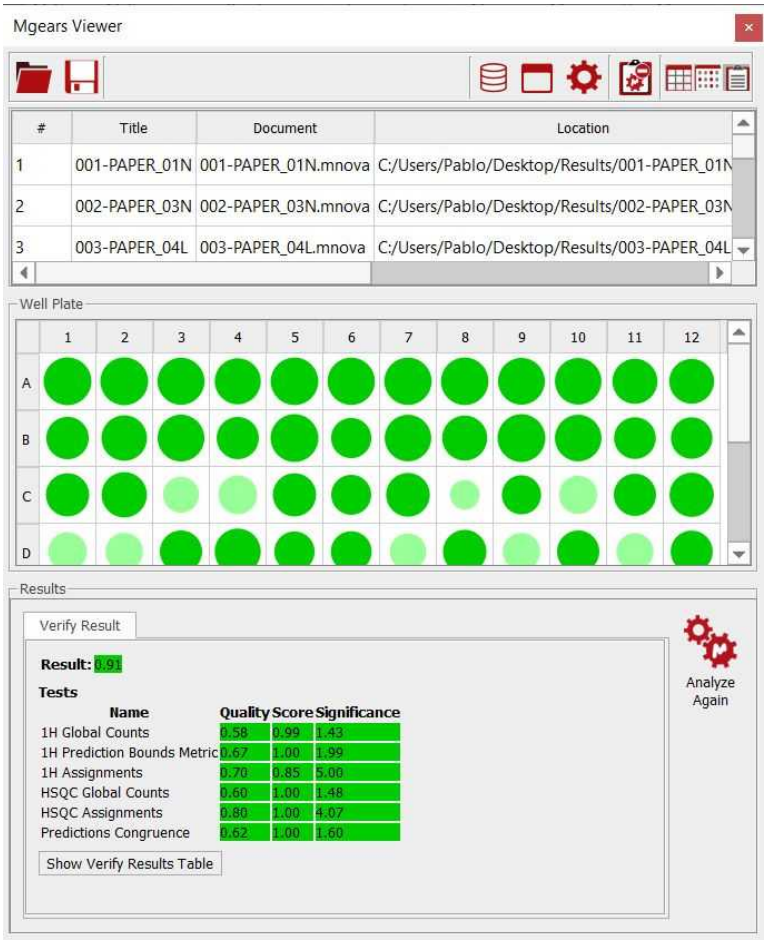
The Design tab will allow you to use a Layout template, whilst the Output tab will be used to select the folder containing your results (with the option to save them as Mnova documents, PDF, or to an existing Database):



The settings tab will be useful to select the masks for the automatic detections of your input (datasets and molecules):



Once the analysis has done the Mgears Viewer can be used to check the results. You would need to navigate to the “results” directory and select the 'data' subfolder”:

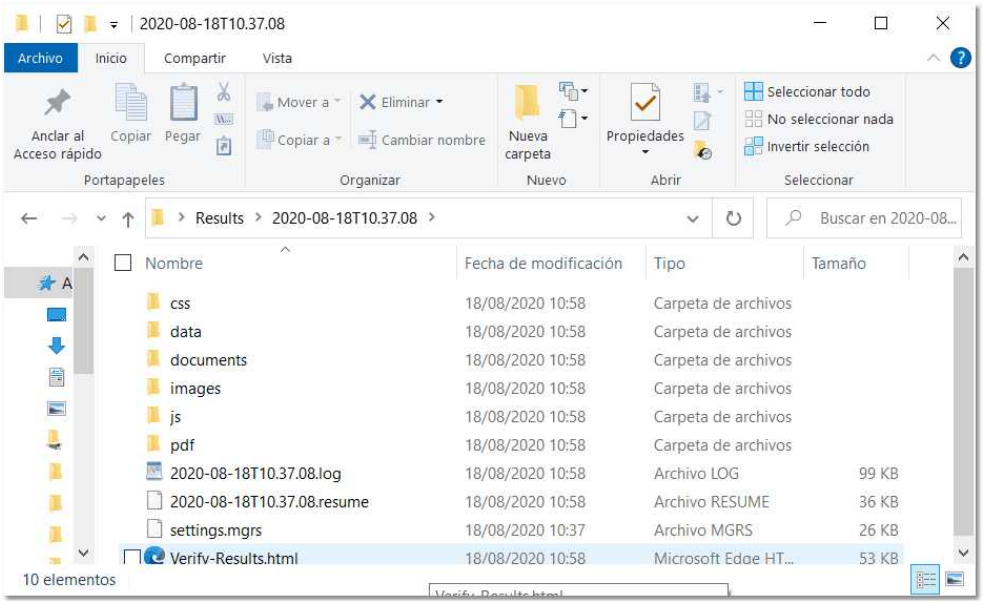


Clicking on each row or well plate, will load the dataset in Mnova to allow you to make changes if necessary. The changes made in the Mnova document will be automatically updated in the Viewer.

From the viewer, you can load/save/clear, copy the report to the clipboard (by default) and save to the results to your Database.

© 2024 MESTRELAB RESEARCH

Mgears Verify will also generate a log file with some basic information about the verification that has been run (time it was run, number of datasets, etc.), the Mgears settings used for the verification and a summary html file which can be opened in any web browser and which will contain a table showing all the verification results for all the sets.

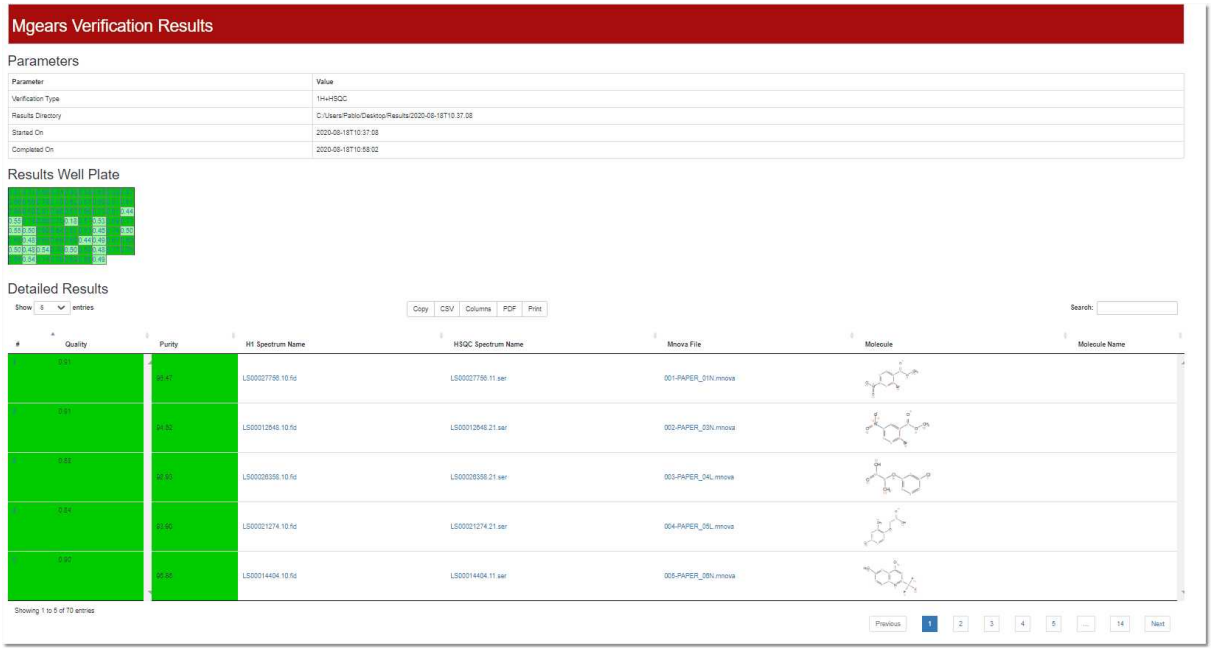


In the documents subfolder, you will have the Mnova documents to allow you to interrogate the data further or look at the detail behind, for example, unexpected failures.

For results interrogation and to gain understanding of the results, user should open the ´result´ html file, with any web browser. This file will present, in one single view:

- Some basic verification parameters (time run, type of data)
- A well plate with the summary results for all datasets
- An extended table with the final result for each dataset as well as all the individual test results for each individual dataset
- Links to the .mnova, .csv and .pdf files generated, as well as to original files (fid, mol, etc.) to allow user to quickly drill into specific results which need to be check or looked at further.

© 2024 MESTRELAB RESEARCH



Mgears Verification Results

Parameters

Parameter	Value
Verification Type	1H+HSQC
Results Directory	C:/Users/Pablo/Desktop/Results/2020-08-18T10.37.08
Started On	2020-08-18T10:37:08
Completed On	2020-08-18T10:58:02

Results Well Plate

Detailed Results

Show 5 entries — Copy | CSV | Columns | PDF | Print — Search:

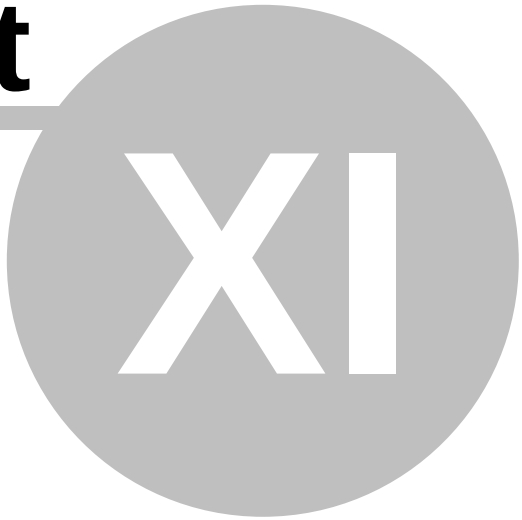
#	Quality	Purity	H1 Spectrum Name	HSQC Spectrum Name	Mnova File	Molecule	Molecule Name
1	0.91	91.47	LS00027766.10.fid	LS00027766.11.ser	001-PAPER_01N.mnova		
2	0.91	94.82	LS00012646.10.fid	LS00012646.21.ser	002-PAPER_03N.mnova		
3	0.82	92.50	LS00026368.10.fid	LS00026368.21.ser	003-PAPER_04L.mnova		
4	0.84	93.60	LS00021274.10.fid	LS00021274.21.ser	004-PAPER_05L.mnova		
5	0.90	95.66	LS00014404.10.fid	LS00014404.11.ser	005-PAPER_08N.mnova		

Showing 1 to 5 of 70 entries

Previous | 1 | 2 | 3 | 4 | 5 | ... | 14 | Next

© 2024 MESTRELAB RESEARCH

Part

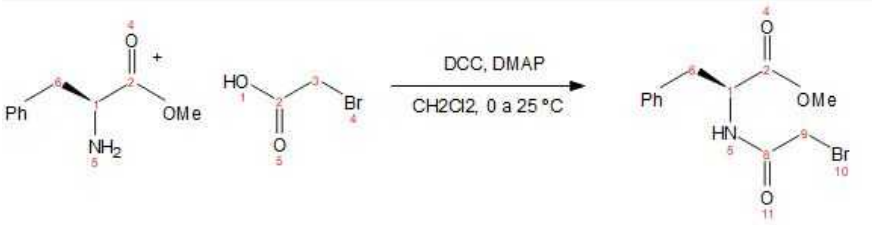


11 Molecular Structure

Mnova allows the user to import Molecular Structures, and to interact with them.

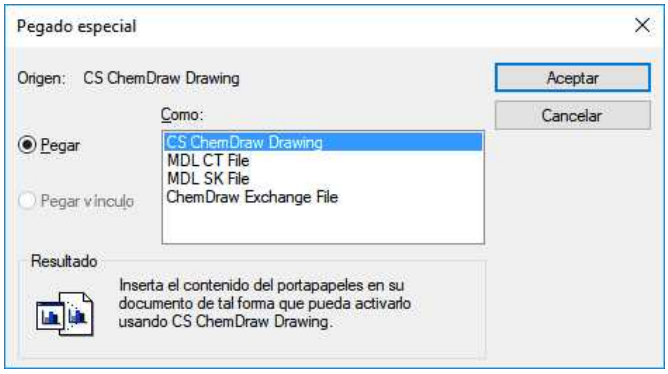
Currently, molecular structures can be imported as a **.mol, .cdx, .cdxml, .json, .mrv or .sdf** files by simply using the Open command (or by dragging & dropping the file to the Mnova icon or interface) exactly in the same way as this would be applied to a spectrum. Support to 'drag & drop' operations are currently limited to **ACD ChemSketch, Marvin and ChemDraw**.

You can also import reactions in cdxml format:



If you have OpenBabel installed, you will also be able to load (or copy&paste) molecular structures in INCHI and SMILE formats. For example you can copy to clipboard each line above (molecules in SMILE and INCHI formats) and then run Mnova and paste them as molecular structures:
COc1cc(ccc1)C#N
InChI=1S/C9H7F3O2/c10-6-2-1-5(7(11)8(6)12)9-13-3-4-14-9/h1-2,9H,3-4H2

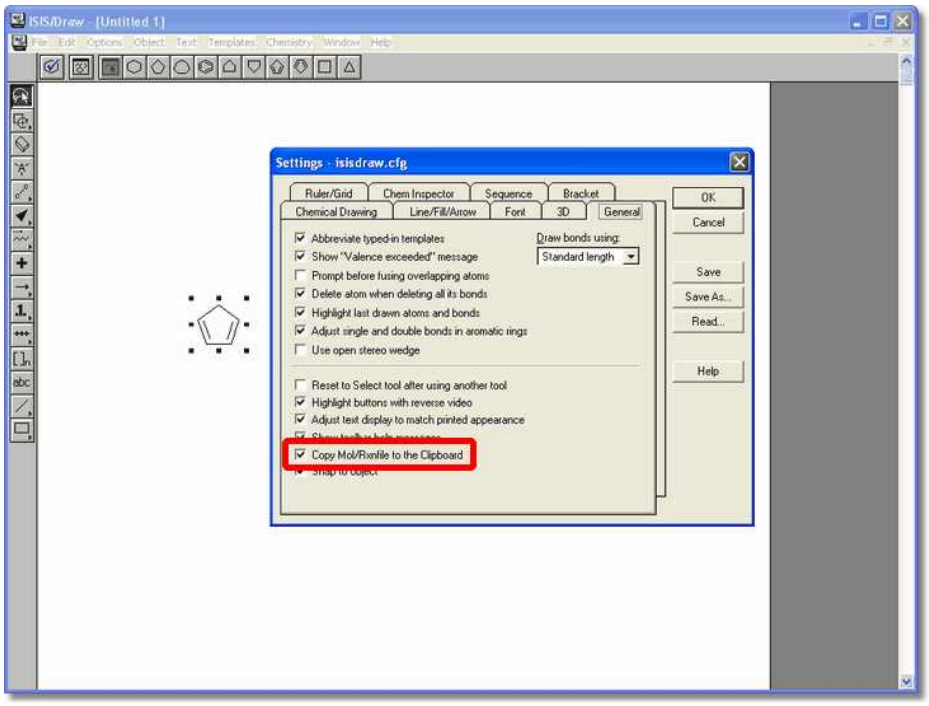
You can also copy structures as MDL SK format (with abbreviated groups), to do that just copy the molecular structure to the clipboard (from ChemDraw for example) and follow the menu 'Edit/Paste Special':



You can keep the numbering from ChemDraw or the abbreviated structures by using the 'ChemDraw Exchage file' from the dialog box above.

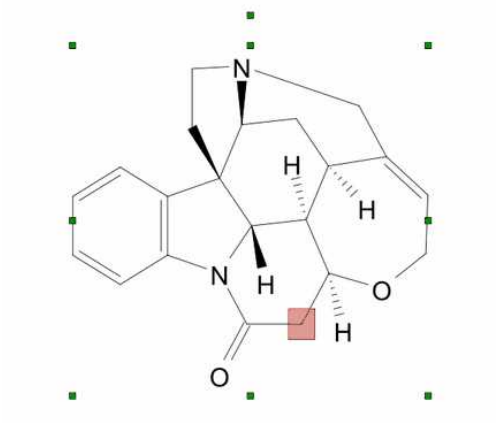
If you are using IsisDraw, please keep in mind that you will need to select the capability to copy your molecules as mol files in the 'Options/General Settings' menu.

© 2024 MESTRELAB RESEARCH



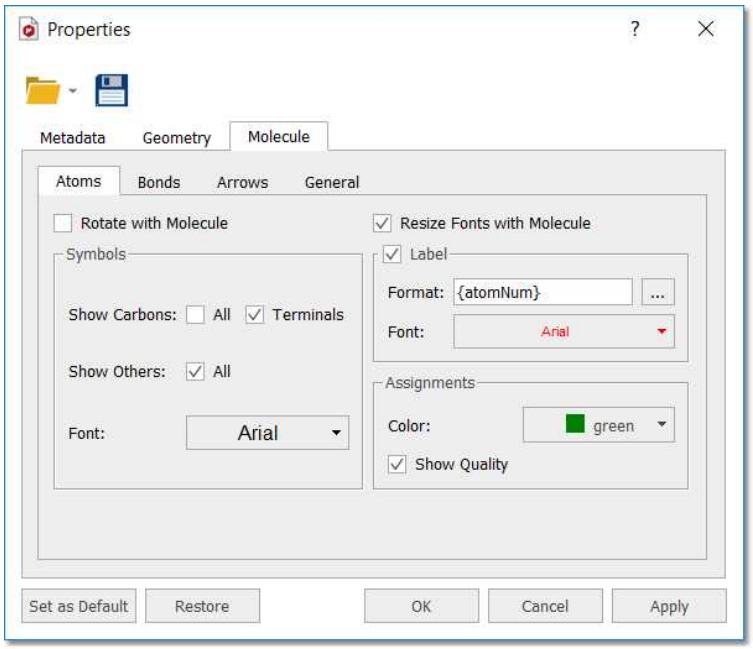
The molecular structure can be handled (resized, moved, aligned, copied, pasted, etc) exactly in the same way as all other Mnova objects. Molecular structures can be imported in a separate page or into a page already occupied by another object, such as for example its applicable spectrum.

The first thing you will notice when interacting with molecular structures, is the fact that atoms will become highlighted in a red transparent box when the mouse hovers over them. Clicking on an specific atom will make it the active atom and highlight in a red box with pink background, as can be seen in the figure below. This atom selection will become essential when interacting with the molecule, as will be explained in the following chapters.

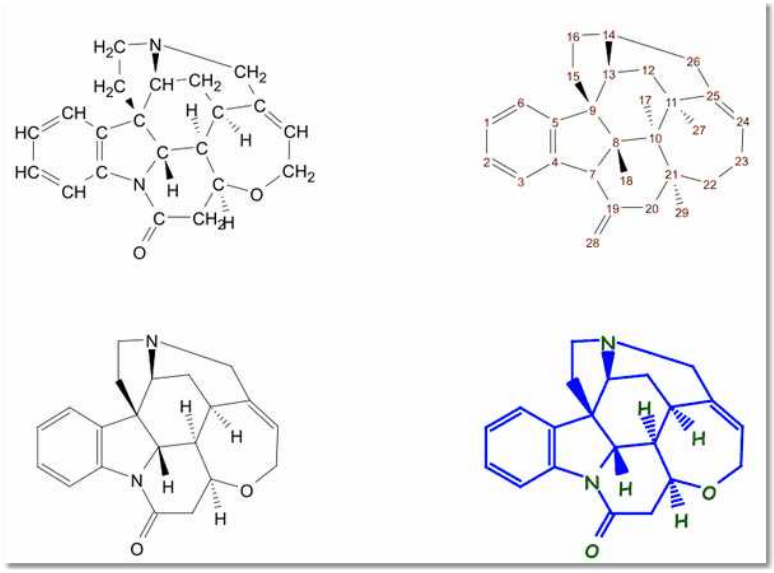


Mnova will allow you to customize the appearance of the molecule, by using the object 'Properties' dialog box. This box can be accessed by double clicking on the molecule, or by right clicking and then scrolling down to 'Properties'.

© 2024 MESTRELAB RESEARCH

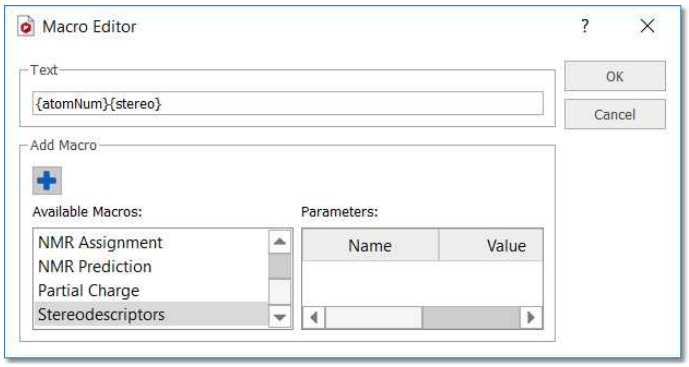


The 'Properties' dialog box will allow you to customize the size of the molecule, its angle with respect to the paper (geometry properties) and all representation properties, such as whether or not to show explicit carbon and hydrogen atoms, whether to show atom numbers, the length and spacing of bonds, etc. It is therefore possible to achieve many different representations of the same molecule, as the figure below shows.

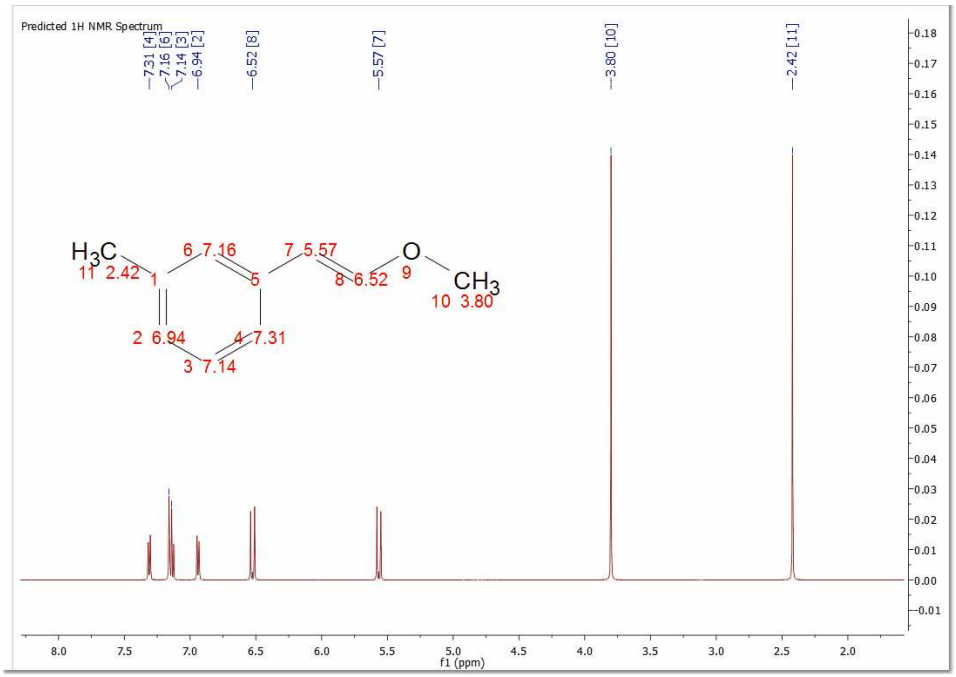


To customize the appearance of all the atom labels of the molecule, just double click on this button . From the 'Macro Editor' dialog box, you can add information about 'annotations', 'atom number', 'assignments', 'predicted chemical shift', etc.

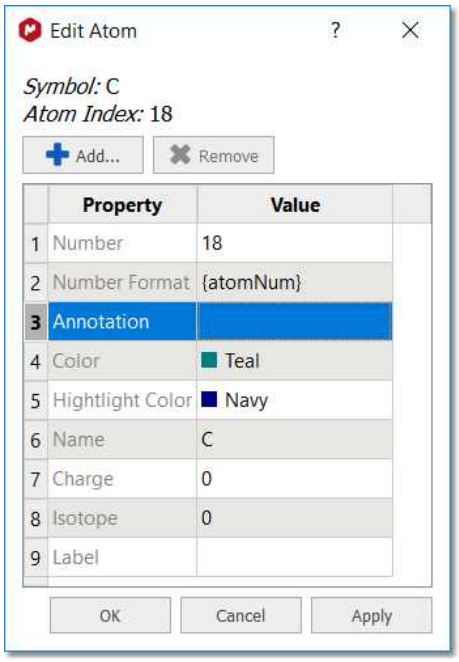
© 2024 MESTRELAB RESEARCH



In the example below, it is shown an atom label which contains information about the 'atom number' and the 'predicted chemical shift':

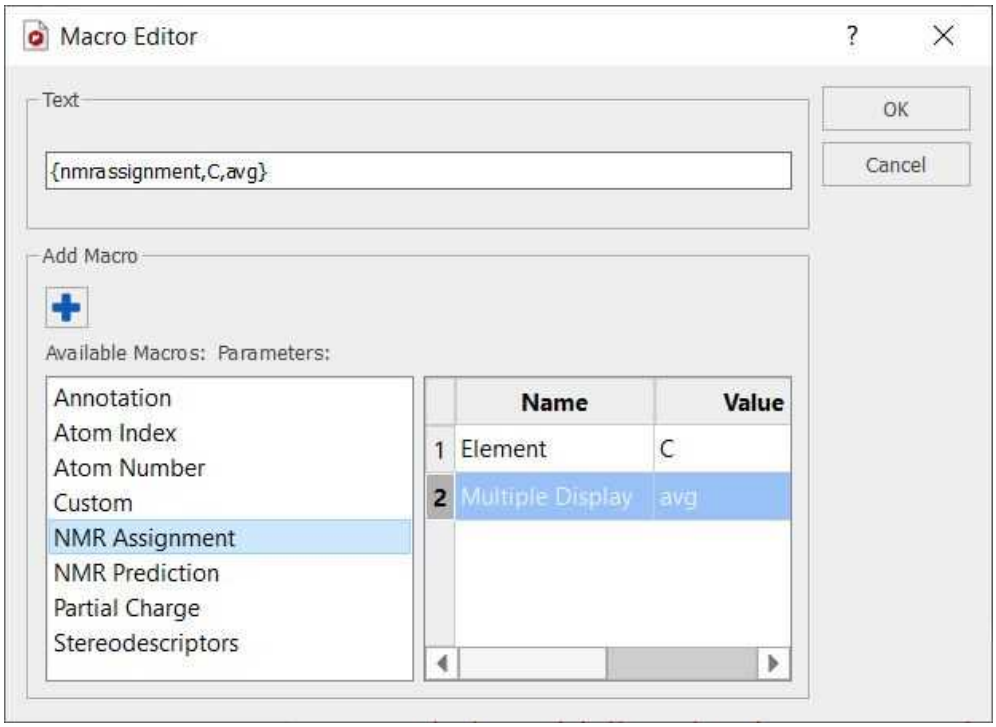


Double clicking on the applicable atom to edit its label:



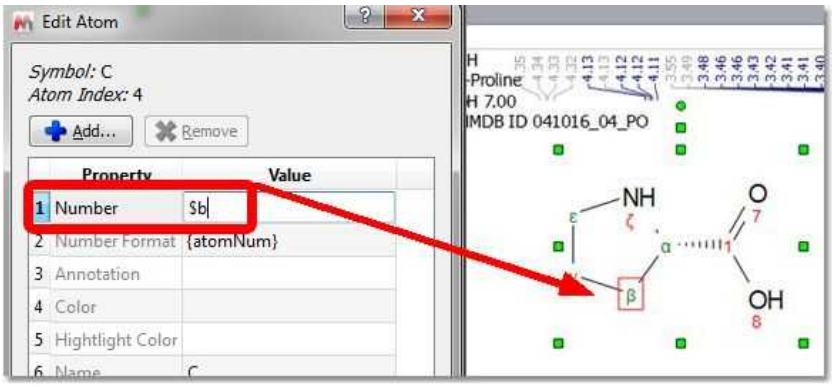
You can add a macro for the atom label (as it is shown a few lines above) in order to include information such as 'annotations', 'atom number', 'assignments' (chemical shift list, range or average), 'predicted chemical shifts' etc...

When you have more than one assignments to the same atom, you can use the 'Multiple Display' parameter to show the assignments as a list (when no value is added), as a range (when settings rng) or as an average value (when setting avg).

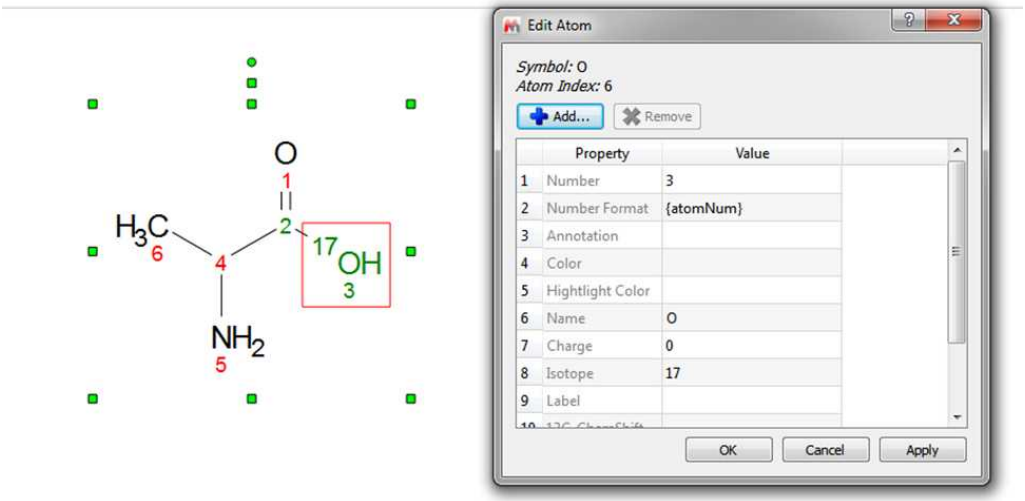


© 2024 MESTRELAB RESEARCH

Typing the $ prefix to add greek letters to the number; for example $a, 4$b, 22gd21 will be converted into α, 4β, 22γδ21:



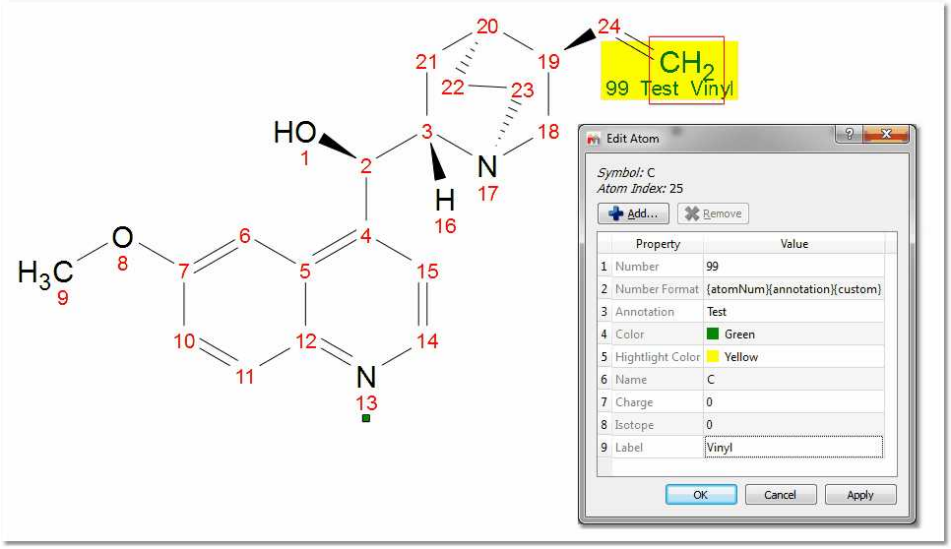
To define an isotope, you only need to enter the value of the mass number in the "Isotope" field. For example, in case of a C atom you can type 12, 13 and 14. The isotope number (and the charge) of an atom can be deleted by typing 0 or by deleting the number of the field.



You can type any annotation, such a comment or a different numbering (just by changing the value of the 'Number' cell). You can change the color of the atom or of the highlight or even to add any property just by clicking on the this button 

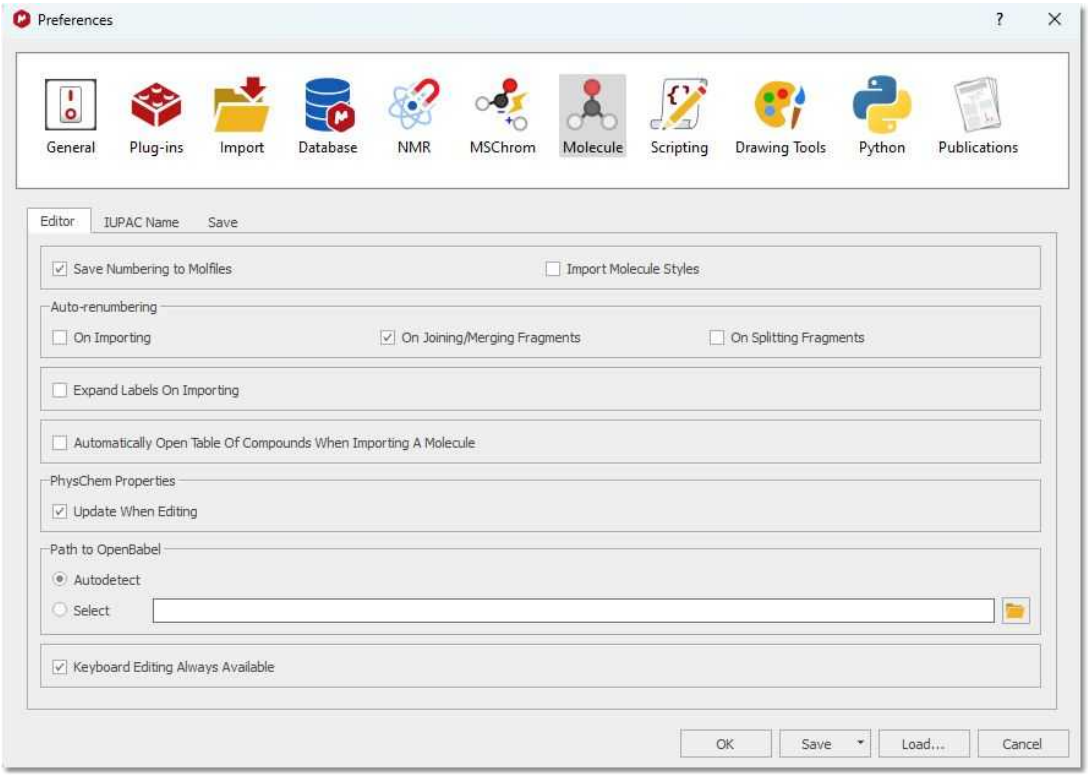
.

In that case we have changed the number to the atom 25 (the new number is 99) and added a macro to show the 'Atom Number', the 'Annotation' (Test) and the 'Custom Property' (Vinyl), changing the color to green with a yellow highlight:



Highlight an atom and type the desired number to renumber it. If the new number already exists, the symbols # will appear on the duplicated numbers.

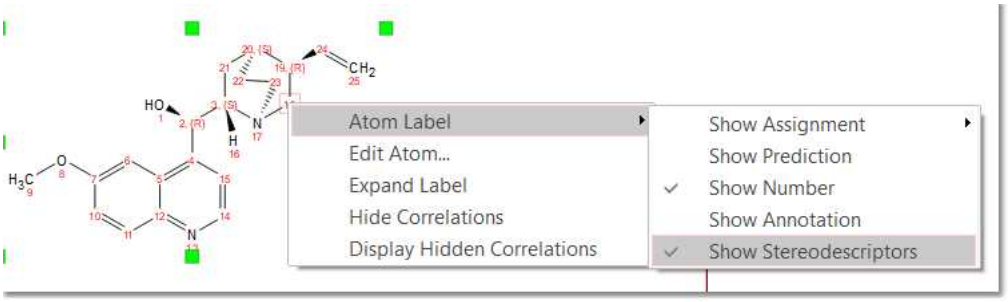
The new numbering can be exported in a mol file and you can 'import molecule styles' (for the 'Edit/Paste Special' feature) by selecting the applicable option in the File/Preferences/Molecules menu.



The "***keyboard editing always available***" preference, will allow you to type any letter when hovering the mouse over an atom.

© 2024 MESTRELAB RESEARCH

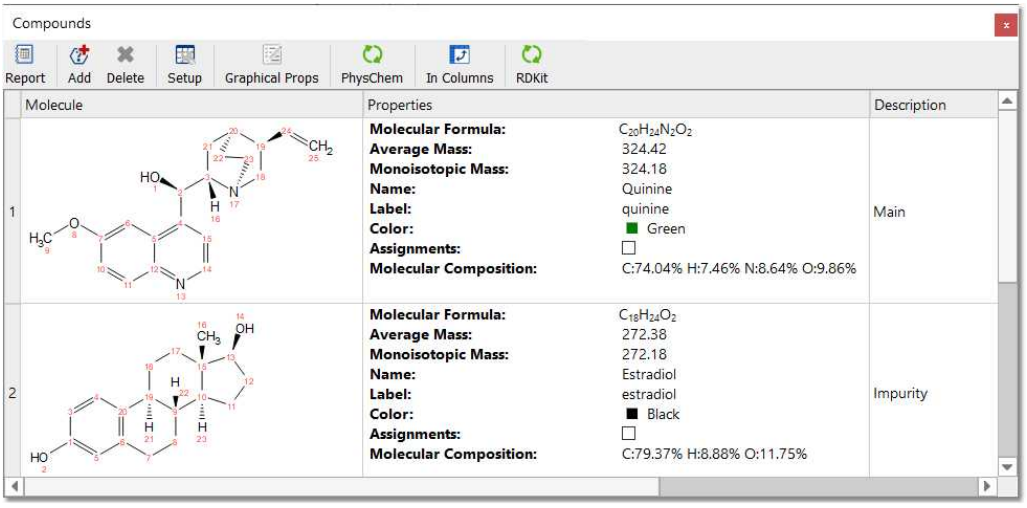
Right clicking on any atom number of the molecular structure will display a context menu which will allow you to show the assignments, prediction, number, annotations or stereodescriptors as atom labels:



You can export your molecules as .mol, mol file V3000, .cdxml (even reactions), .jcamp, .json or.sdf or by following the menu 'File/Save as' and selecting the desired format from the scroll down menu.

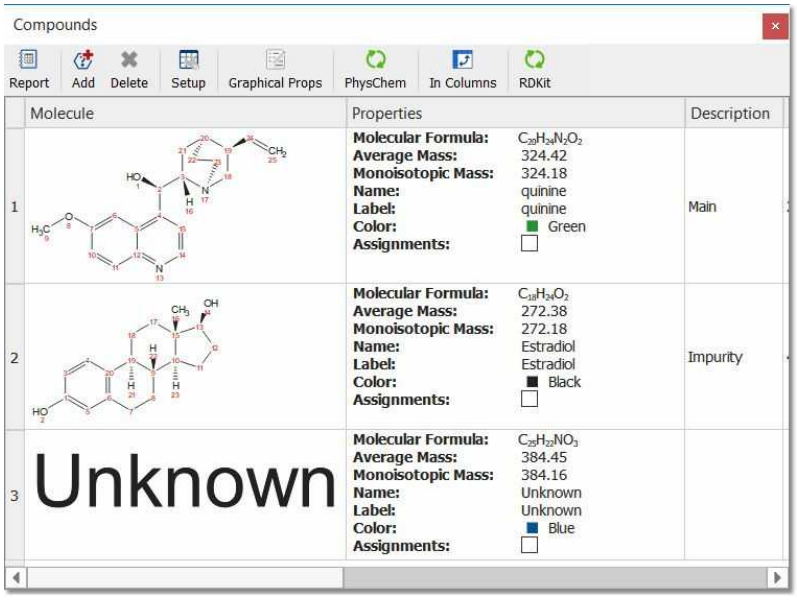
11.1 Compounds Table

This table will show all the molecules present in the document, with information about the chemical formula and molecular weight, name, label, description, color, molecular composition, double bond equivalence, etc.



You can copy any molecule from this table to another page just by selecting it and clicking on the 'Report' button. Please bear in mind that the information about predictions and assignments is linked to the molecule and will be kept after having pasted it to another slide.

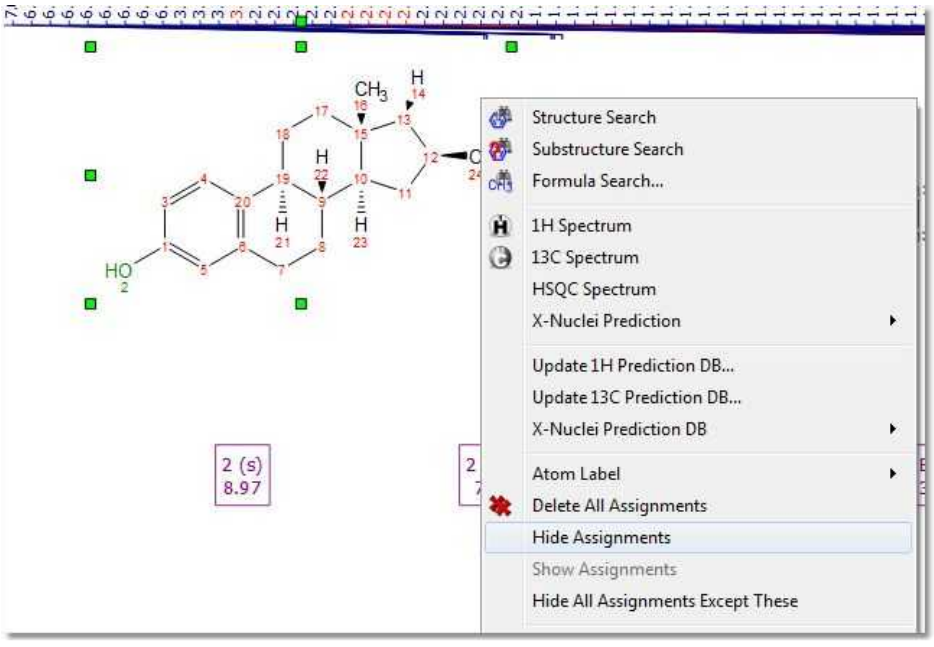
Clicking on the 'Add' button of the table, will allow you to add a new 'unknown structure', very useful to start assignments of unknown compounds.



The user can type the name, label (to be displayed in the assignments) and molecular formula when creating a new unknown compound from the Compounds Table.

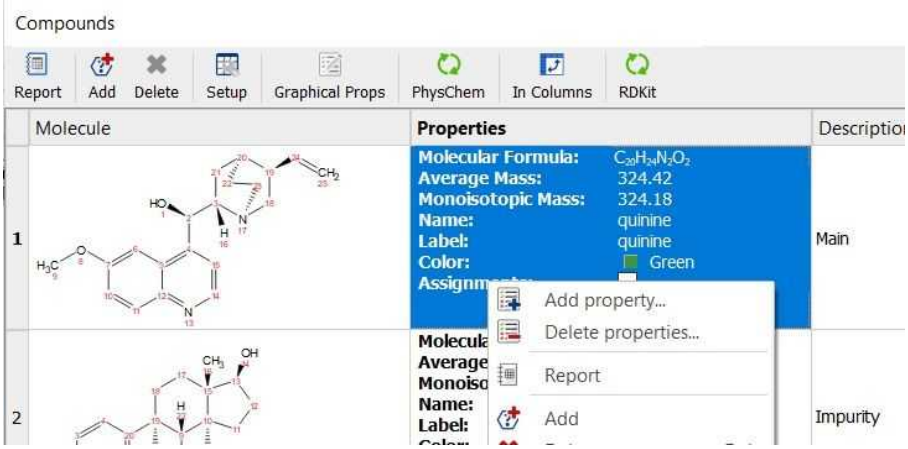
This compound can be assigned in the same way as a molecule, but it's represented as a text item, only the label is shown (the molecular structure is not shown).

By default, all the assignments (of all compounds) will be displayed on your spectral window, however you can use the toggle of the 'Assignment' check box for each compound of the table to show only the applicable assignments for that compound. When the user checks the checkBox, Mnova hides the assignments of all the molecules except the assignments of the current molecule. When the user unchecks the checkBox, Mnova hides all the assignments of the current molecule. (The same behaviour as in the molecule context menu: Hide Assignments).

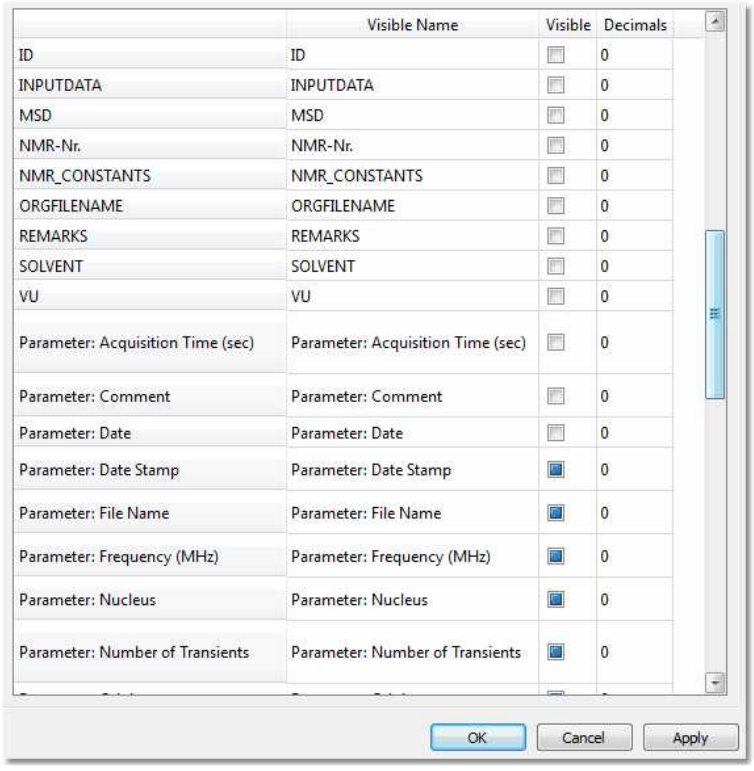


Add a property by right-clicking on the 'Properties' column and selecting 'Add Property':

© 2024 MESTRELAB RESEARCH



The added properties can be deleted in the same way, by selecting 'Delete Properties'. The visibility of these properties can be also customized via Properties button. By default the visibility is setup as automatic.
If you import a SDF file with several tags, you can display them by checking the applicable boxes:

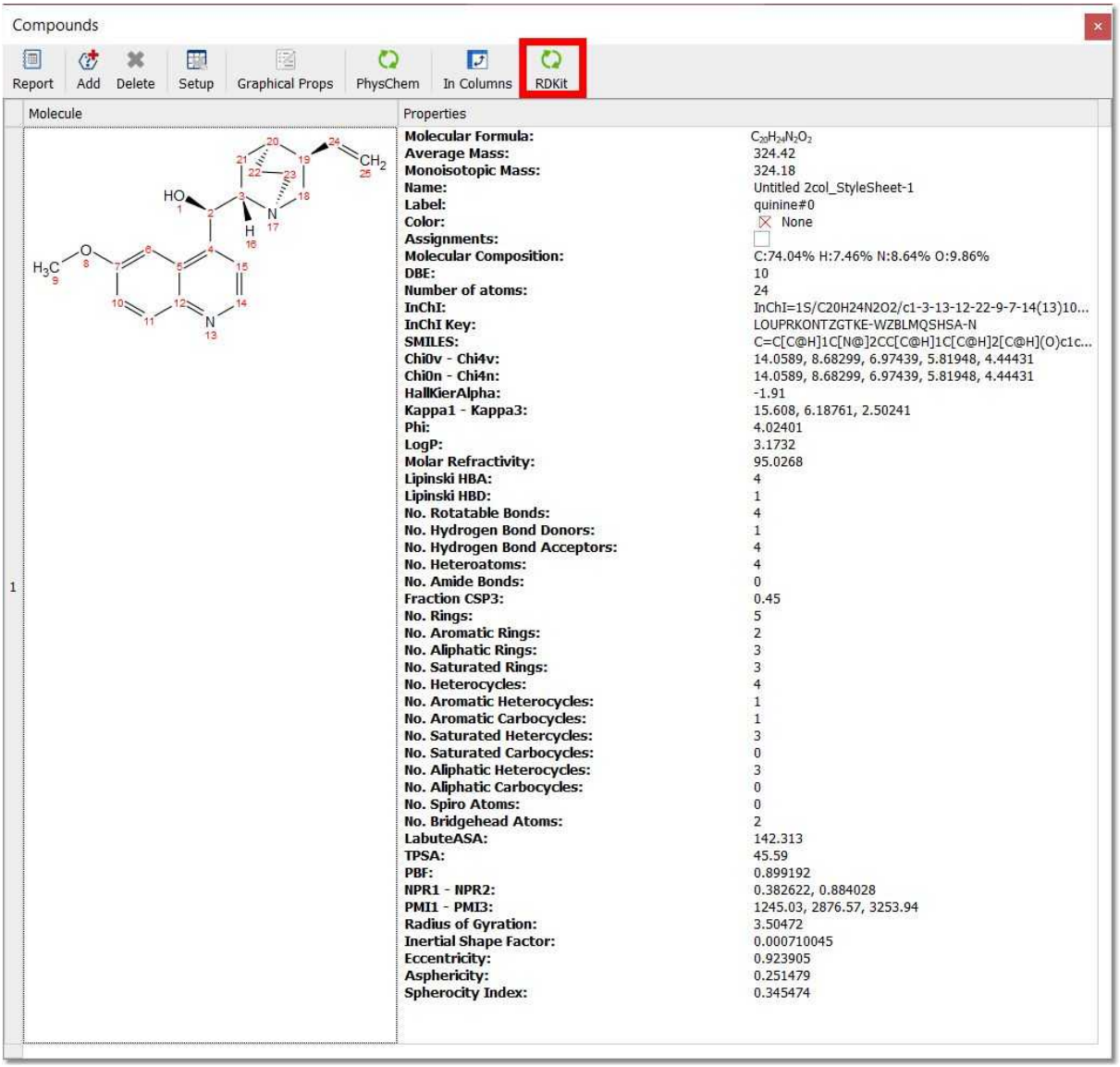


There will be three type of boxes (empty, blue filled and checked):
Empty: the parameter will not be visible
Filled: the parameter will be visible only if it the filed is not empty
Checked: the parameter will be always visible (even when the field is empty)

You can edit any of the properties from the table, by double clicking on it, typing the new value and **pressing the 'enter' key** to update the changes.

© 2024 MESTRELAB RESEARCH

Clicking on the 'RDKit' will display a list of properties for the molecule (in order to display all the properties available; go to "Setup" in the Compounds table and select all the properties available in the "List in properties column"):



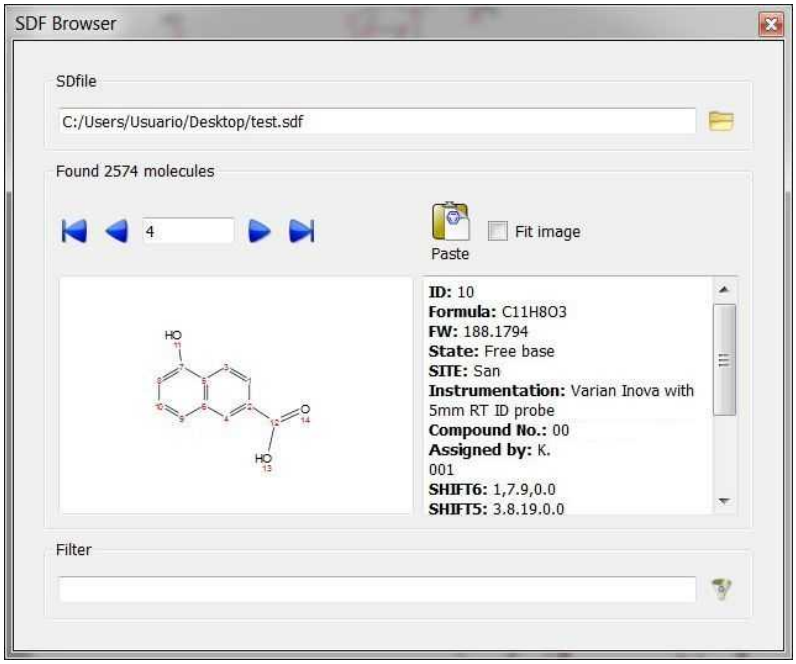
11.2 SDF Browser

The SDFBrowser can be lacunched by following the menu 'View/Tables/Molecule/SDF Browser':

© 2024 MESTRELAB RESEARCH



Clicking on the Load button will allow you to select the desired SDF file:



From there, you can freely navigate through the SDF file, paste the molecule into Mnova and filter molecules by keywords (for example by molecular structure or any other tag contained in the SDF file)

© 2024 MESTRELAB RESEARCH

Part

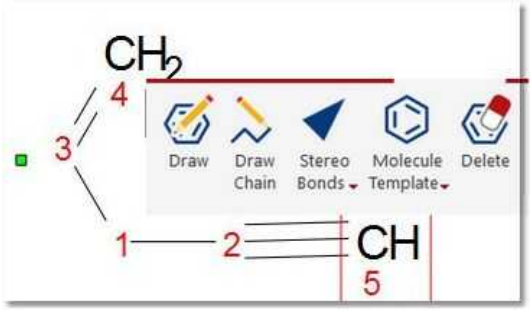


12 Molecules Editor

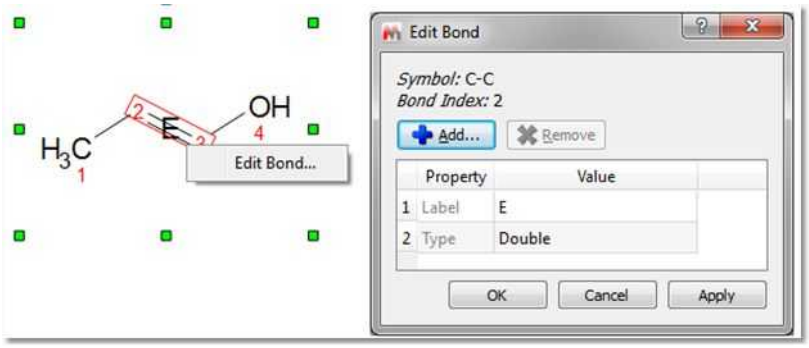
Mnova incorporates a basic 'Molecules Editor' which will be improved in the forthcoming versions of the software. This editor includes a powerful toolbar to draw your molecules:



The **Drawing mode** will allow you to draw a Carbon on your spectral window just by clicking on any empty field. Clicking on any existing atom will add an additional carbon. If you click on a single bond, you will get a double bond (clicking on a double bond will draw a triple bond).



The same result can be obtained from the bond context menu (by right clicking on the bond). The 'Edit Bond' dialog will allow you to change the bond type (single, double, triple, up, down) or to set a label for the bond (to add stereochemistry notation, for example):



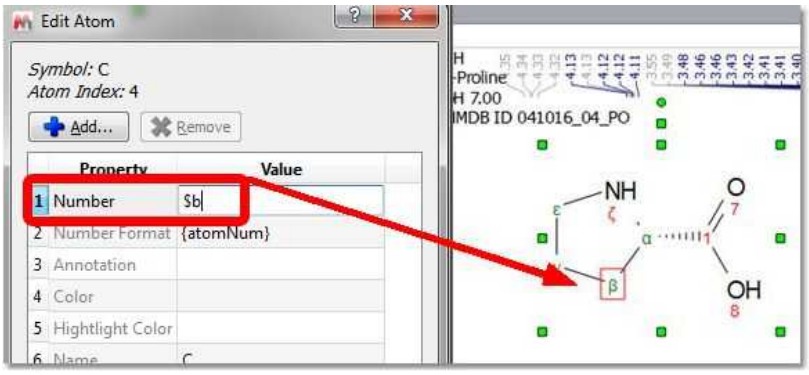
You can **change the atom** just by hovering the mouse over the atom and typing the applicable letter (i.e 'o' for Oxygen, 'n' for Nitrogen, "b" for Boron, 'b' plus 'r' for Bromine, or more complex labels, such as Me, Ph, COOH, etc). Make sure that you have in the 'File/Preferences/Molecule' the option to have the keyboard editing always available.

You can also change the atom by double clicking on it to display the 'Edit Atom' dialog. From here you will be able to change the name (from C to F, for example).

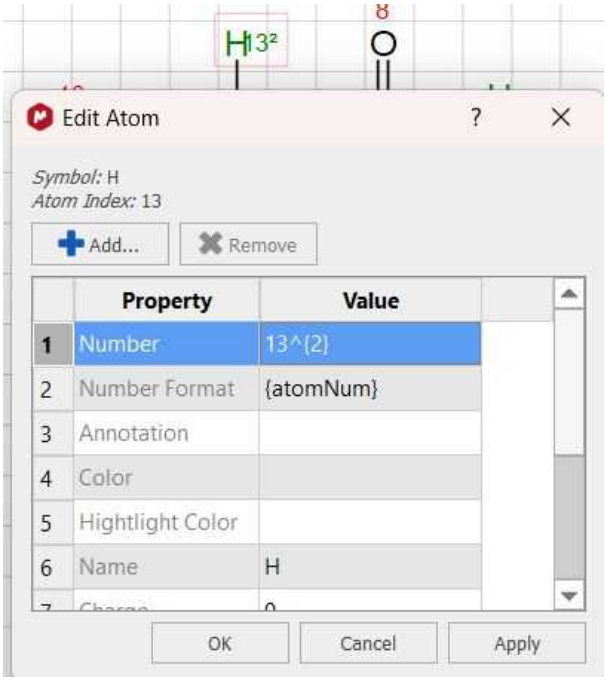
If you have any spectrum loaded together with a molecule; you will need to select any molecular drawing tool (from the molecular sketcher toolbar) before typing the applicable letter (unless you activate a 'Molecule preference" to have the "keyboard editing" always available). See more here.

From the 'Edit Atom' dialog, you can add greek letters to the number by typing the $ prefix; for example $a, 4$b, 22gd21 will be converted into α, 4β, 22γδ21.

© 2024 MESTRELAB RESEARCH

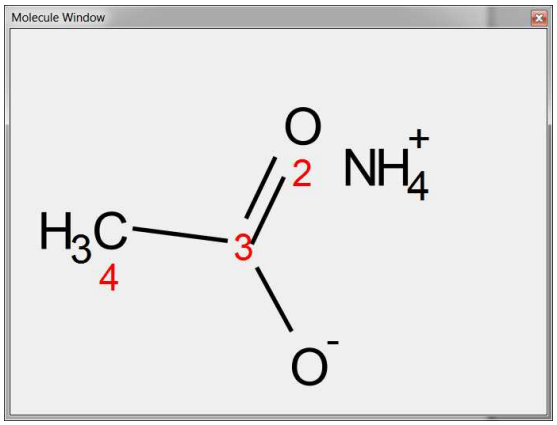


You can also add superscripts by using this type of "X^{N}" constructor:

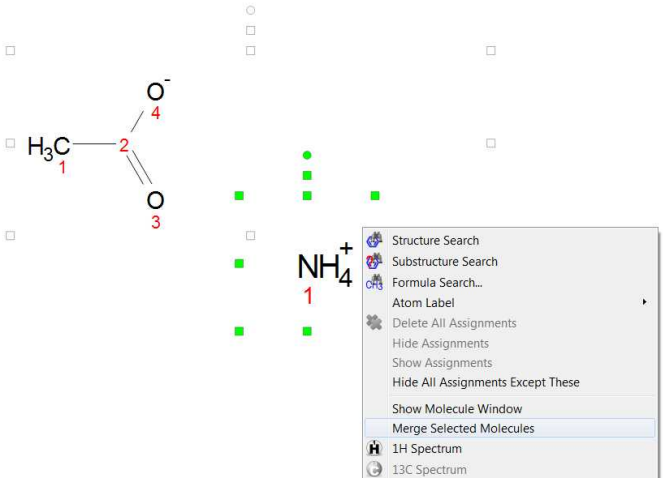


You can **draw salts** from the 'Molecular window' panel (View/Panels/Molecule Windows'), just by drawing both components there. To draw the NH4+ component, you will need to draw a CH4 and then hover the mouse to type the **N** followed by the **+** (to add the positive charge):

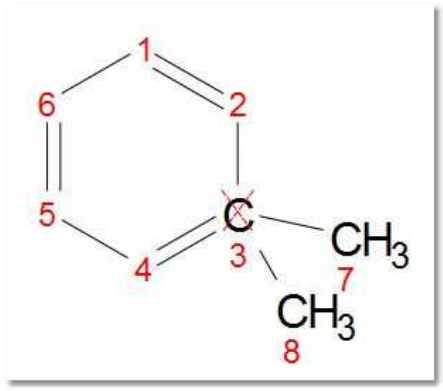
© 2024 MESTRELAB RESEARCH



You will obtain the same result by drawing both components, selecting them, right clicking on any of the molecules and choosing 'Merge selected Molecules':



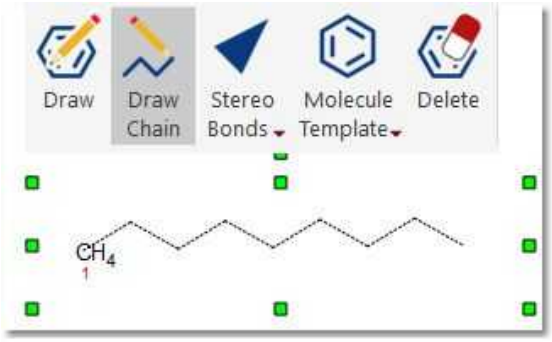
Mnova will not allow to add new atom (clicking on existing one) or change the bond type if that leads to atom valence exceeding, but it will allow you to create a new atom by stretching the bond from existing one. In this case the old atom will be marked with invalid valence cross:



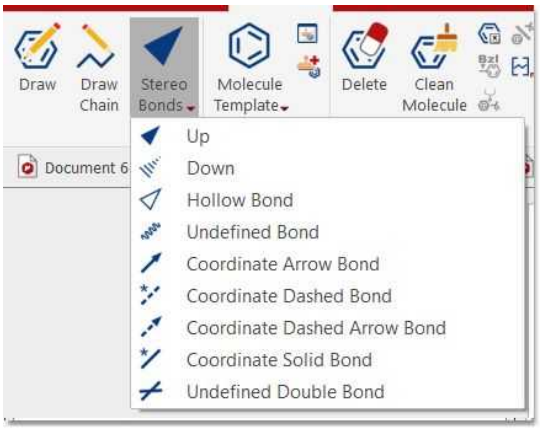
Mnova will also automatically change the atom valence (e.g. 3-5-3 for Nitrogen) when adding/deleting new atoms or changing the bonds type.

© 2024 MESTRELAB RESEARCH

Draw Chains: draw chains by clicking, holding and dragging after having clicked on this button:



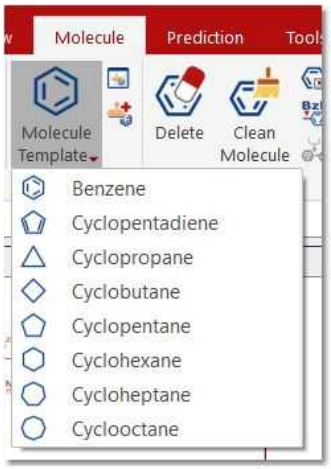
Stereo Bonds: Use this scroll down menu to draw stereo (clicking on any stereo bond will change its direction), coordinate bonds and undefined double bonds.



Use this button to delete an atom. An atom, a bond or multiple atoms can be also deleted by selecting them and pressing the Delete key.

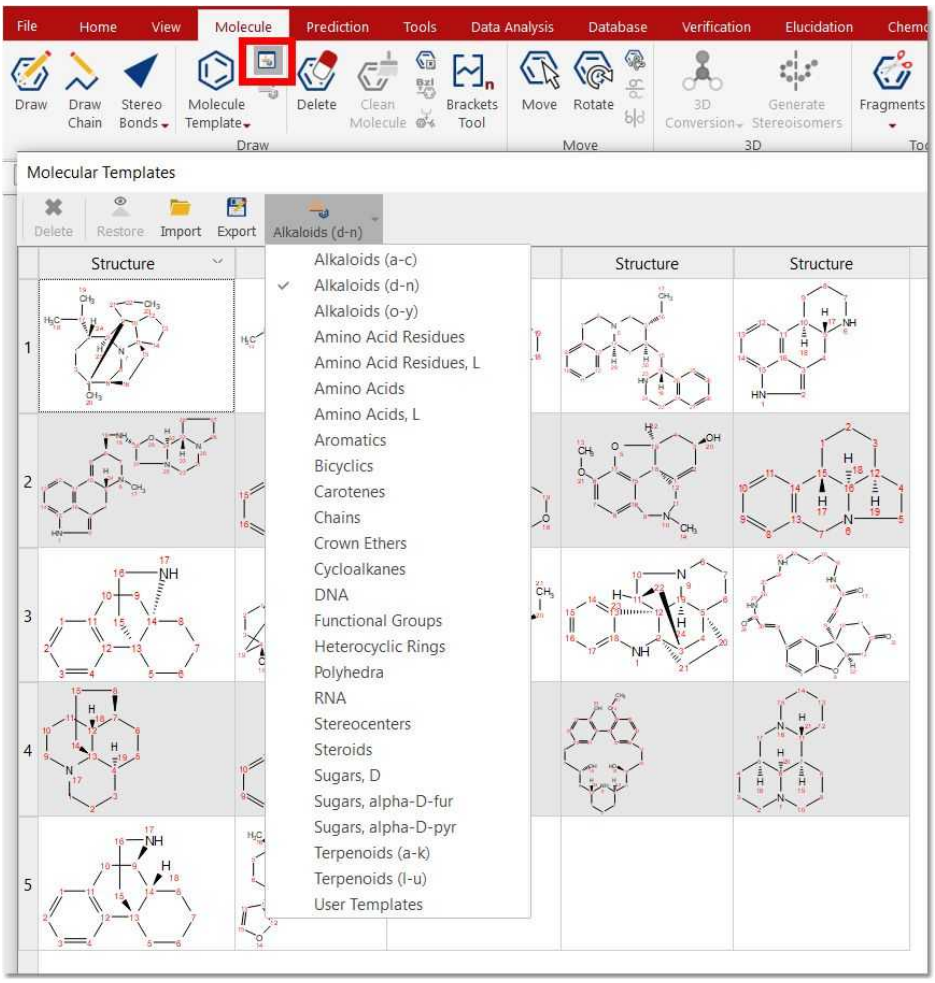
You can delete the bonds keeping the atoms (by holding down the Ctrl/Cmd key). Deleting several atoms selected with lasso while pressing Ctrl/Cmd key allows to keep neighbor terminal atoms.

The Molecule Template scroll down menu, will allow you to draw cycles:

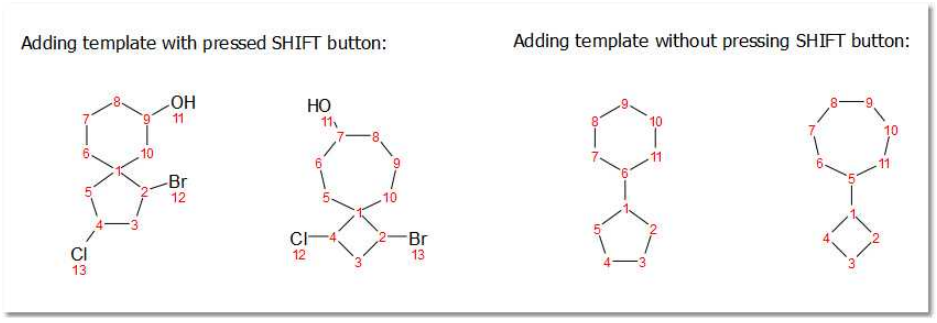


© 2024 MESTRELAB RESEARCH

Clicking on the 'Molecular Templates' button will allow you load more templates. Once you have selected the desired template, you will only need to click on the molecule and click again on your spectral window to paste the molecular structure

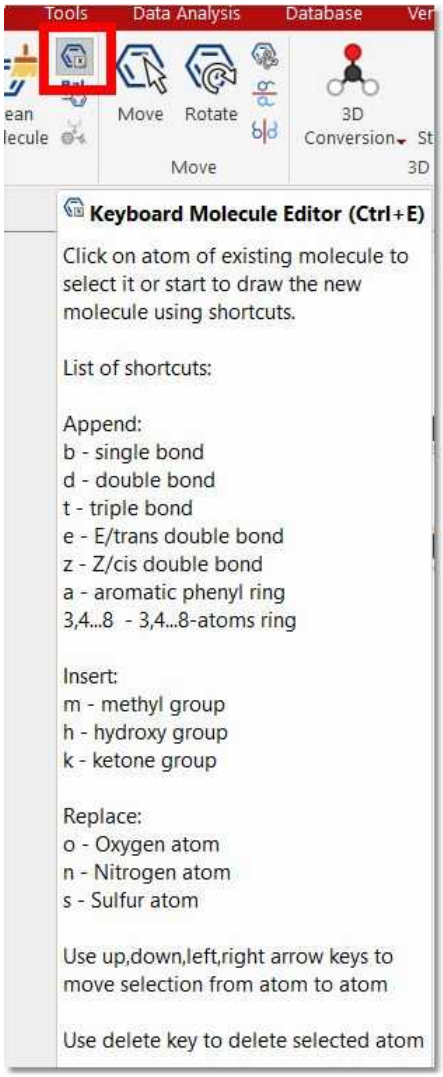


You can add any template as spiro compound by holding down the shift key and clicking on the desired atom.

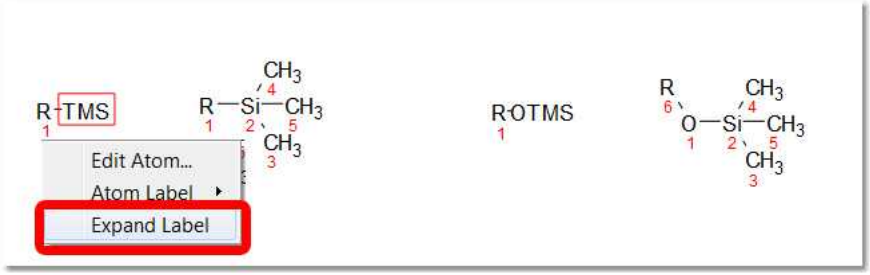


You can also create your own templates by clicking on the 'Import' button and loading a .SDF file containing molecular structures or by clicking on the 'Add Molecule to user Templates' button of the 'Molecule Ribbon'.

Keyboard Molecule Editor (shortcut: Ctrl+E): to draw your molecules without needing to use a mouse. You will find the shortcuts in the tooltip obtained by hovering the mouse over the button:



Expand Labels: to draw molecules with compacted groups and expand them by right clicking on the applicable group:



You can expand them by enabling the "Expand labels on importing" option in 'Preferences/Molecule' and also by clicking on the button "Expand Labels" in the Molecule toolbar.

© 2024 MESTRELAB RESEARCH

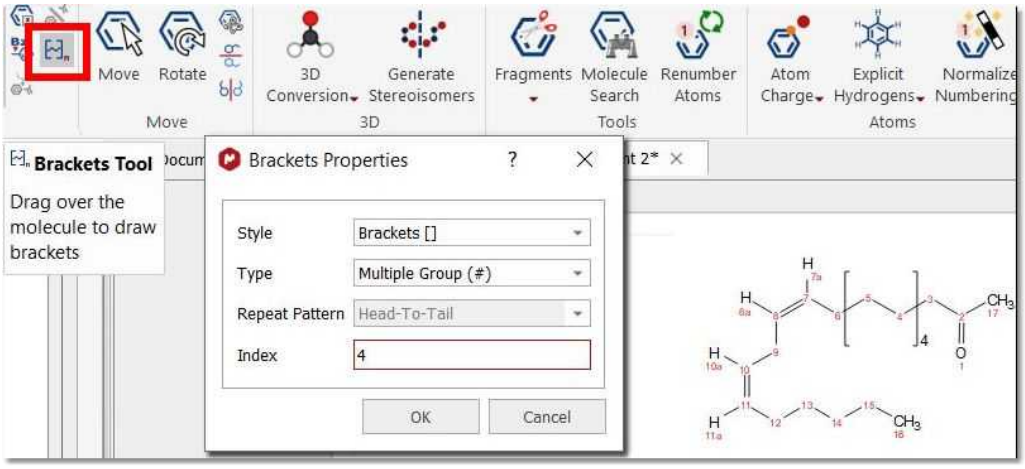
Use the macro editor (under the General tab) to add free text labels and several parameters from the Molecule Properties.

The molecule label is stored in a layout template and also gets applied when applying the template. See also the Properties chapter (for further information about the macro editor)

Merge Selected Molecules: useful to merge several fragments into a unique structure.

Unmerge Molecules: to split a merged molecule into its individuals structures.

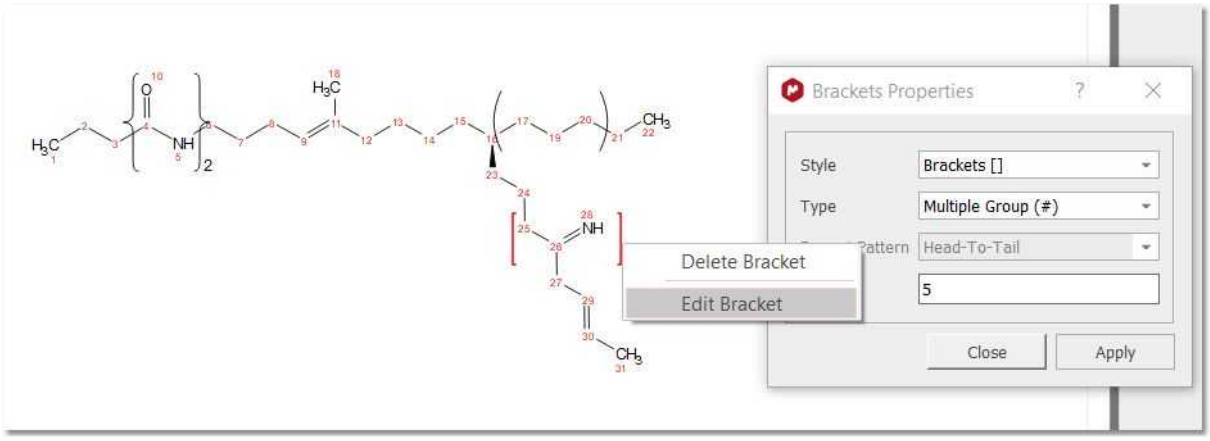
Brackets Tool: to draw polymers. Clicking on this button will display a dialog with different bracket options (style, type, repeat pattern and index).



The brackets can only be drawn while this dialog is opened. We have to select the desired properties and then draw the brackets over the desired region of the molecule. In this way you can draw as many brackets as you want (changing the settings if needed) before closing the dialog.

When drawing several brackets in the molecule, the last drawn is highlighted in red until the "Brackets Properties" dialog is closed or next pair of brackets is drawn:

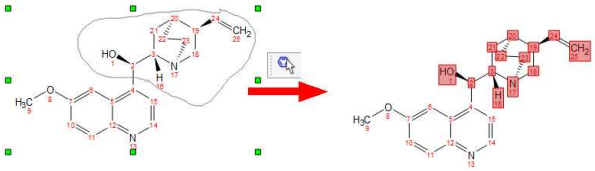
You can edit a selected bracket by right clicking on it to choose "Edit Bracket" from the context menu. The bracket will be highlighted in red and the "Brackets Properties" dialog will be displayed:



Move Mode: This mode will allow you to move an atom or a bond just by clicking&dragging.



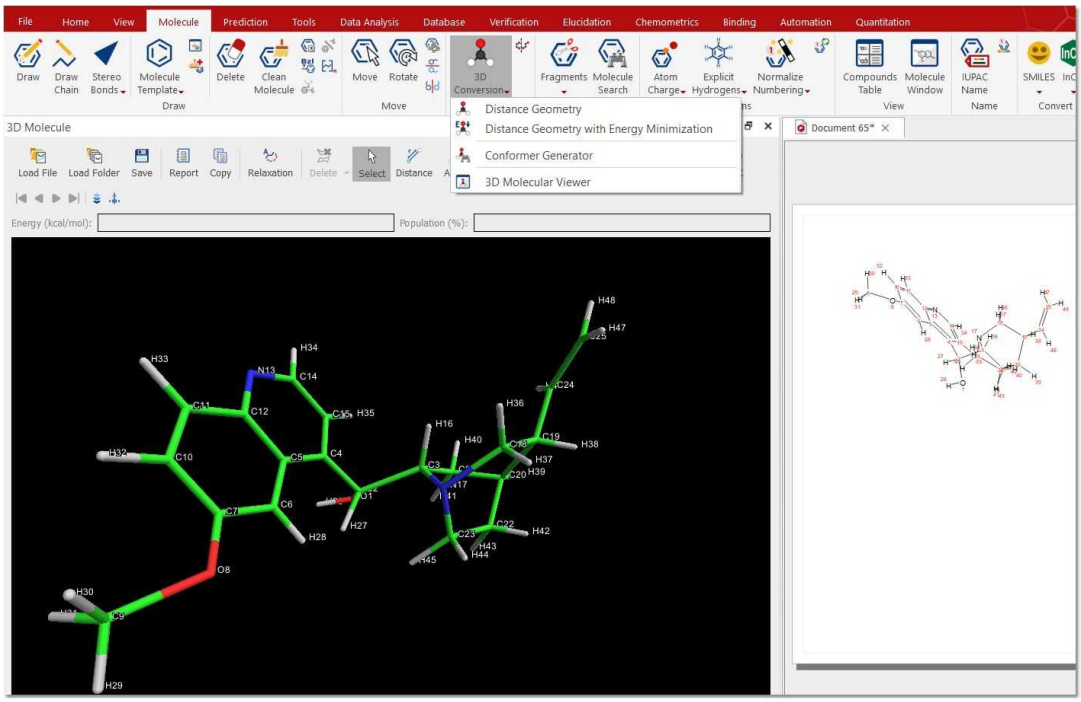
In this mode you can also use the lasso mode (by clicking and dragging the mouse around the desired part of the molecule) to select several atoms (to delete them, for example):



When you select an atom, move it over another one and release the mouse button, only the last atom will stay (except when the first selected atom is non-carbon and the second atom is carbon).

3D Conversion

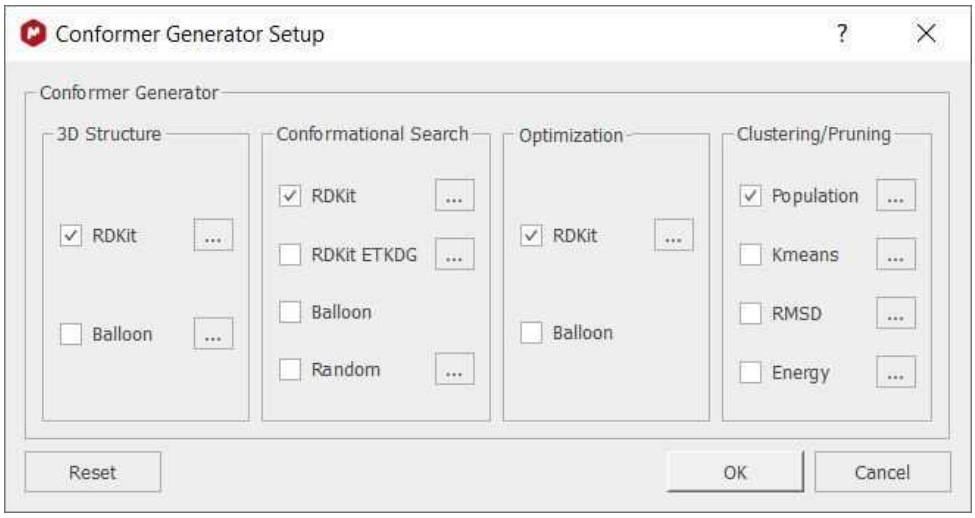
This feature will convert a 2D molecule in a 3D structure, calculating the energy for each conformer and their population.



NOTE: If you are not getting the 3D molecule in the viewer, please change the OpenGL engine from the 'Preferences/Interface' dialog.

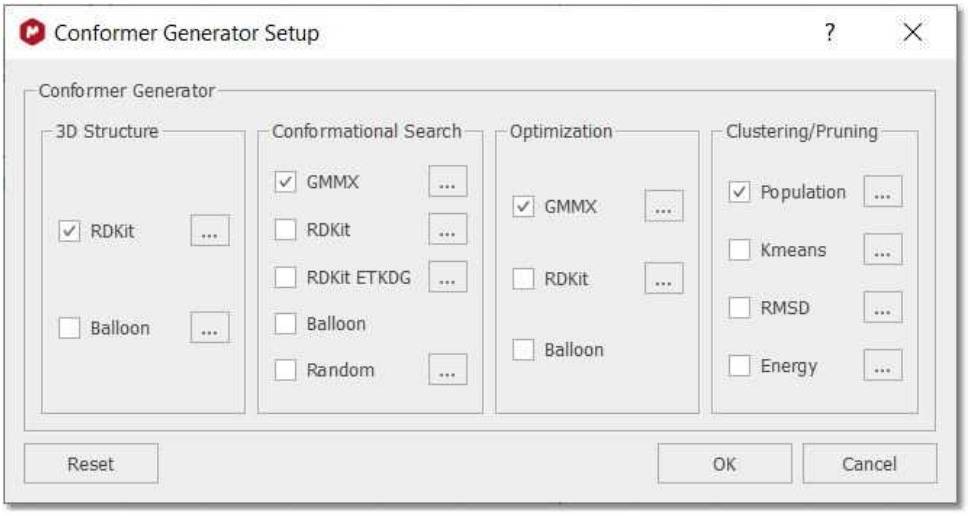
Clicking on the Conformer Generator option will allow you to select the algorithms for the calculation:

© 2024 MESTRELAB RESEARCH



This allows the user to combine different engines, like gmmx, rdkit, and balloon to create list of 3D conformers. These lists can then be reduced via Kmeans clustering or RMSD, to produce a small list of the most relevant conformers.

Users with a Stereofitter license will have GMMX conformation search and optimization capabilities:

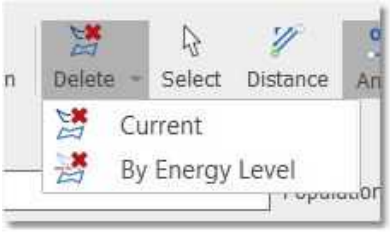


The dialog is set out in four steps: Structure, Conformer, Optimization, and Clustering/Pruning. Each of these steps can be configured to run with different engines and each of these engines can be configured with different options. When clicking the Reset button, the default combination of engines and also the default options for each engine are retrieved

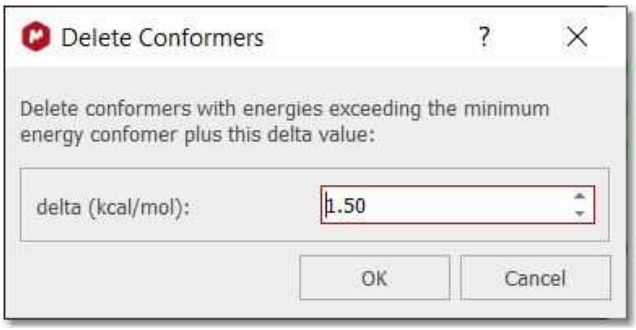
When loading a .mol file and using any of the available options, all possible conformers will be displayed in the 3D Molecule panel, including for each conformer: the Energy (kcal/mol) and Population (%).

You could delete the undesired conformers just by clicking on 'Delete current conformer' button:

© 2024 MESTRELAB RESEARCH

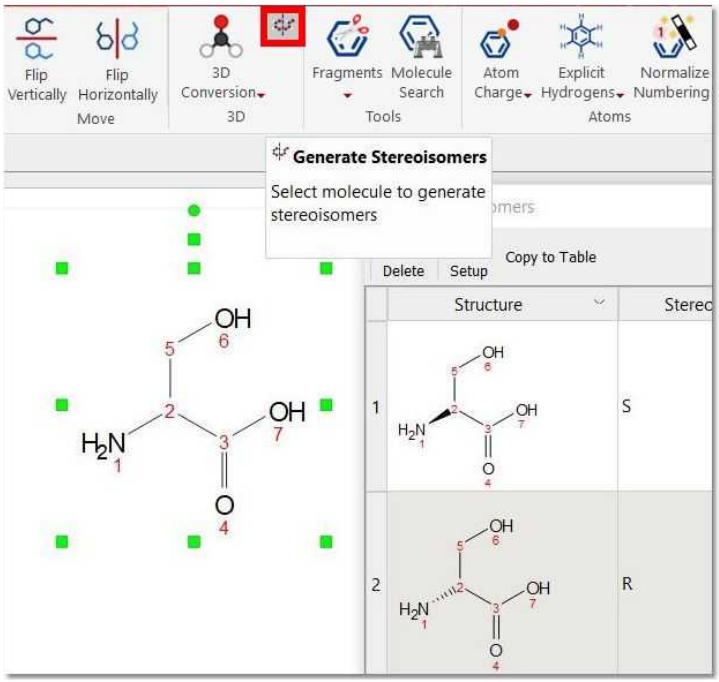


You can also delete conformers by energy level:



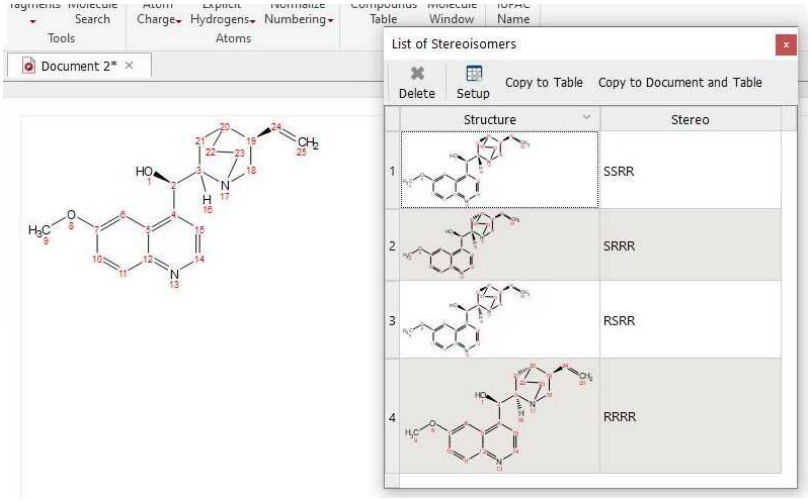
The '3D Conformation Search GMMX' tool will need a valid license for the Stereofitter plugin.

Generate Stereoisomers: it will display all possible stereoisomers for a given molecular structure (drawn with plain bonds).

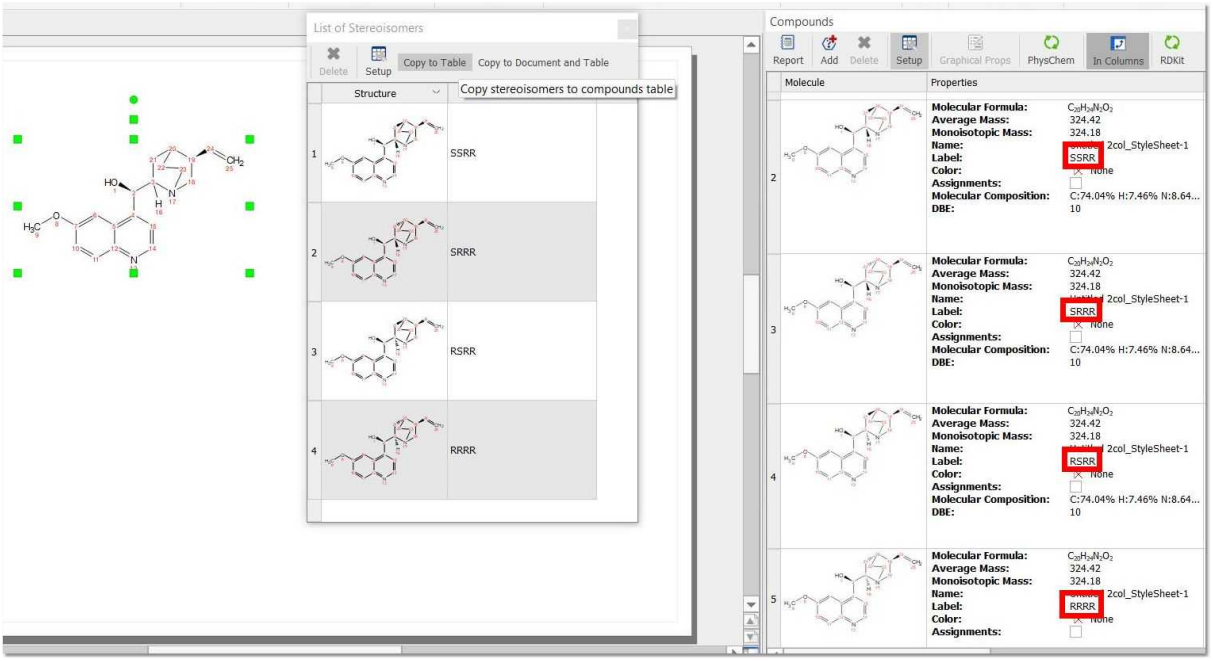


If the given molecule contains any stereobond, that bond will be considered as fixed for the stereoisomers calculation.

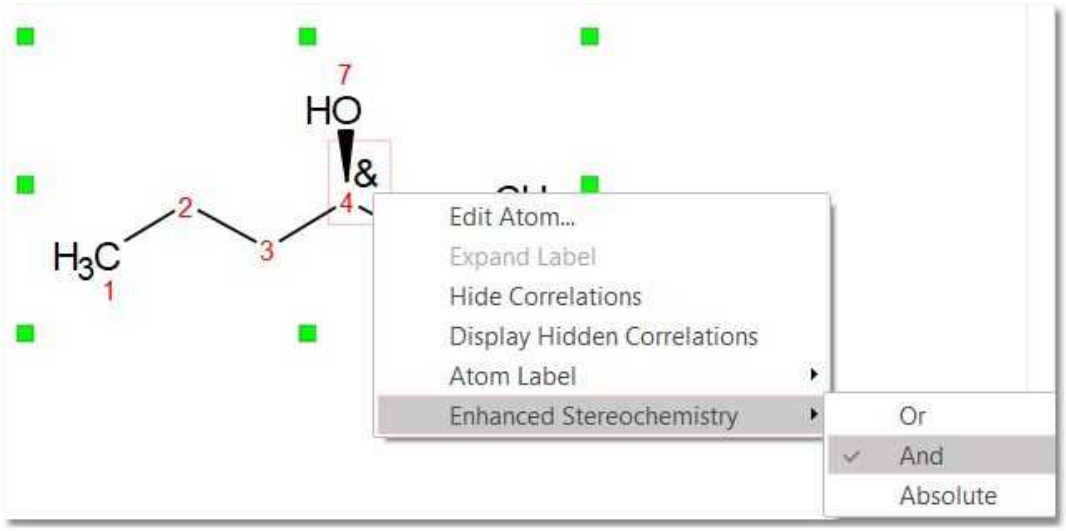
© 2024 MESTRELAB RESEARCH



After having generated the stereosisomers, when copying them to the compounds table, the stereo info for each structure will be added in the "Label" tag:



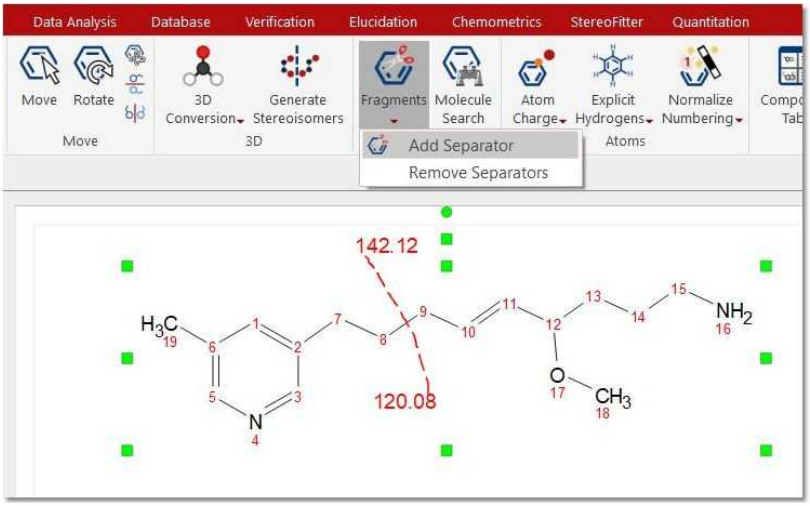
Right clicking on any atom will display the atom context menu which will allow you to set enhanced stereochemistry marks: absolute (abs), relative (or) and racemic (&)



Tools: This ribbon will allow you to add/remove separators and to search for molecules in external Database (eMolecules and BMRB)



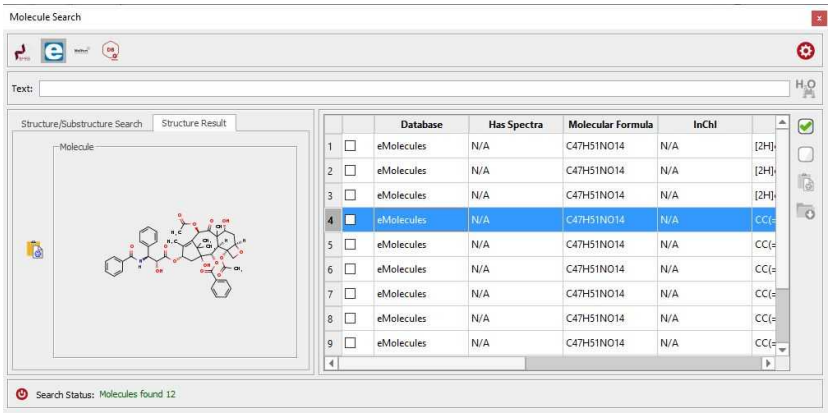
When selecting “Add Separator”, the mouse is changed to the icon of scissors and you can draw a tiny line in a structure (holding left button) to divide it. After that, a red dashed line is displayed over the molecule and the molecular mass of the fragments which divide the line are shown.



You can draw as many fragmentation lines as you want; all of them will be removed at once by selecting the “Remove Separator” tool.

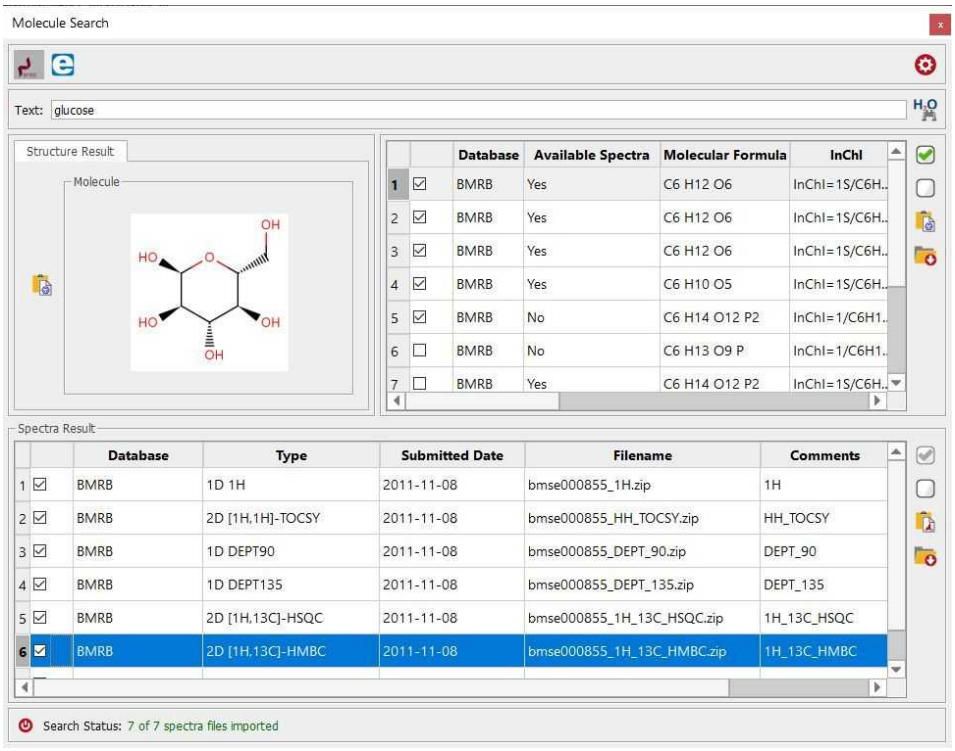
The 'Molecule Search' feature will allow you to search by molecular structure (or compound name) into the eMolecules and BMRB databases:

© 2024 MESTRELAB RESEARCH



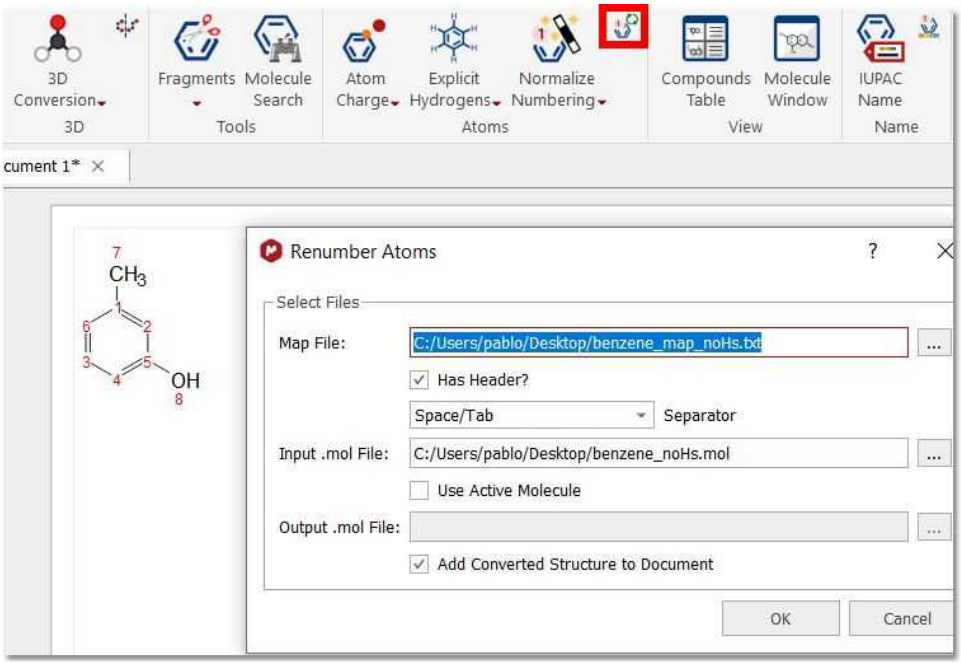
If a hit is found in the database, you can download the molecule and the spectra (if they are available) and work with them in Mnova.

Here you can find an example of the glucose datasets found in the BMRB database:



Renumber Atoms: it will take one map file, one input .mol, and one output .mol. The output will have the original .mol file but with all the atom numbers changed as defined in the map file.

© 2024 MESTRELAB RESEARCH



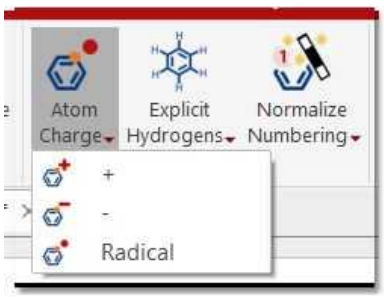
The map file should contain two columns, the first one with the current numbering and the second with the desired one. Here you can see an example of a map file:

input	output
1	1
2	2
3	5
4	4
5	3
6	6
7	7
8	8

Please bear in mind that you do not need a complete list of all of atoms, if you want to swap two atoms, you can use a map file like this:

input	output
3	5
5	3

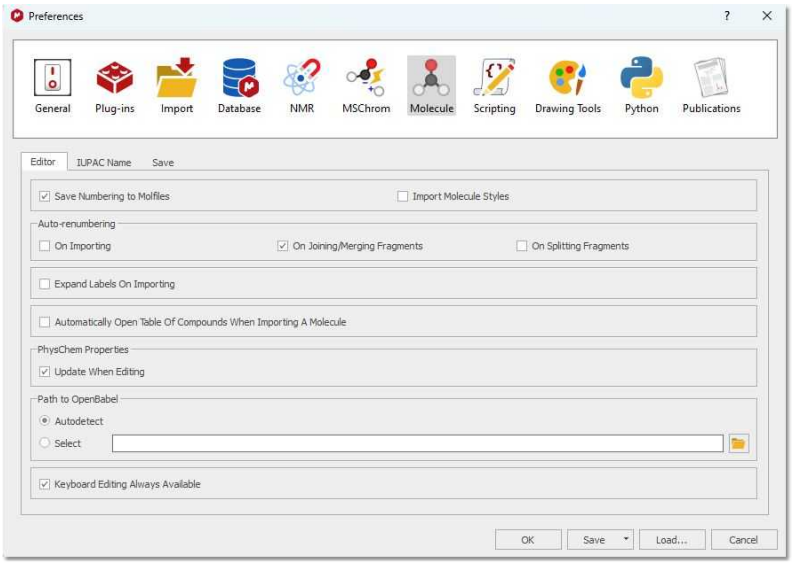
Atoms: This ribbon includes several features to add positive/negative charges (you can set a charge on any atom just by pressing + or - keys), radicals, add/remove explicit H and to normalize, lock or concatenate the numbering.



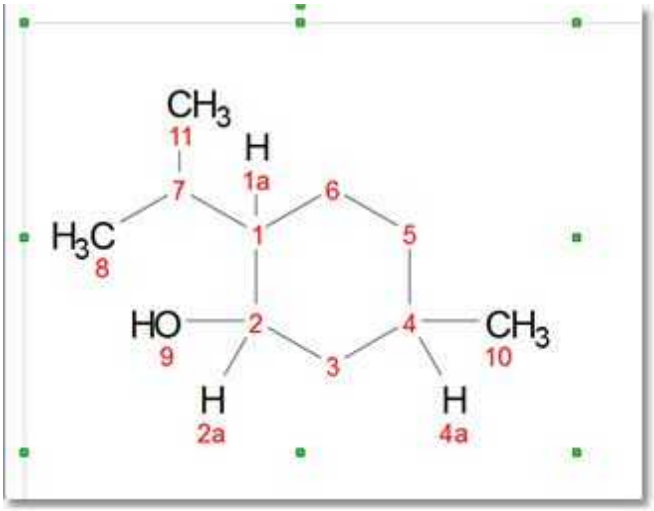
© 2024 MESTRELAB RESEARCH

In the radical mode, after the 1st click on atom, the monoradical symbol will appear (· symbol). After the 2nd click, the diradical (Singlet) will be displayed (·· symbol). After the 3rd click, the diradical (Triplet) will appear (^^ symbol). Finally, after the 4th click, the radical will disappear.

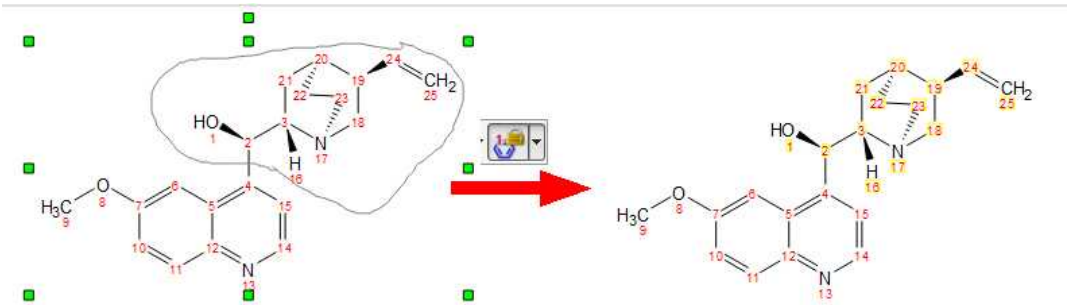
The **Normalize Numbering** button applies re-numbering of the whole selected molecule. From the Preferences you can select if you want to auto-renumber the imported (or pasted) mol files (and also when joining fragments); very useful if you have two equal molecules drawn in different applications or in different order, and you want to normalize the numbering.



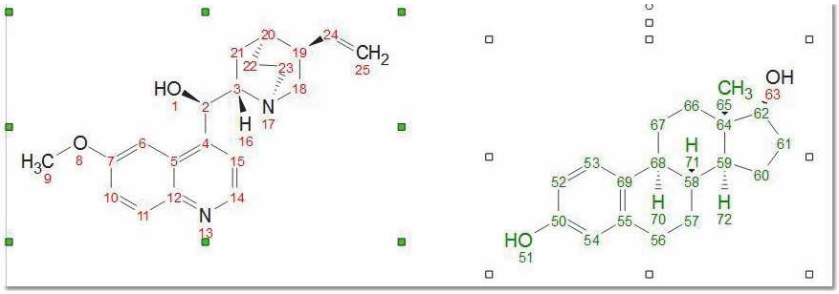
The tool "**Normalize Explicit Hydrogen Numbering**" renumbers only the explicit Hs to match with the heavy atom number (with appended a, b, c... letters):



The **Lock numbering** tool will fix the numbering for the selected part of the structure (using lasso). The fixed atoms will have a yellow background:



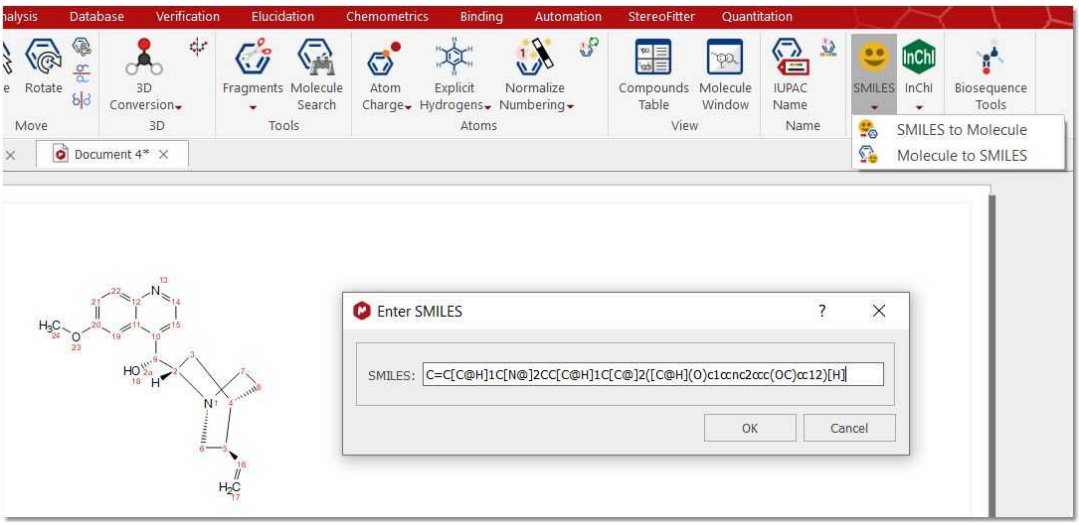
Clicking the '**Concatenate Numbering**' button, will de-duplicate the atom numbers of all the molecules in the document. The highlighted molecules will be renumbered so that the atom numbers are not repeated (leaving a gap from one molecule to another). The order in which molecules get renumbered will depend on the size of the molecule (the biggest molecule will keep its numbering).



The '**Copy Numbering**' feature, will takes the numbering of the selected molecule as template and applies it to all the molecules in the document which are equal to selected one. Very useful for the Stereofitter plugin when working with conformers with different numbering.

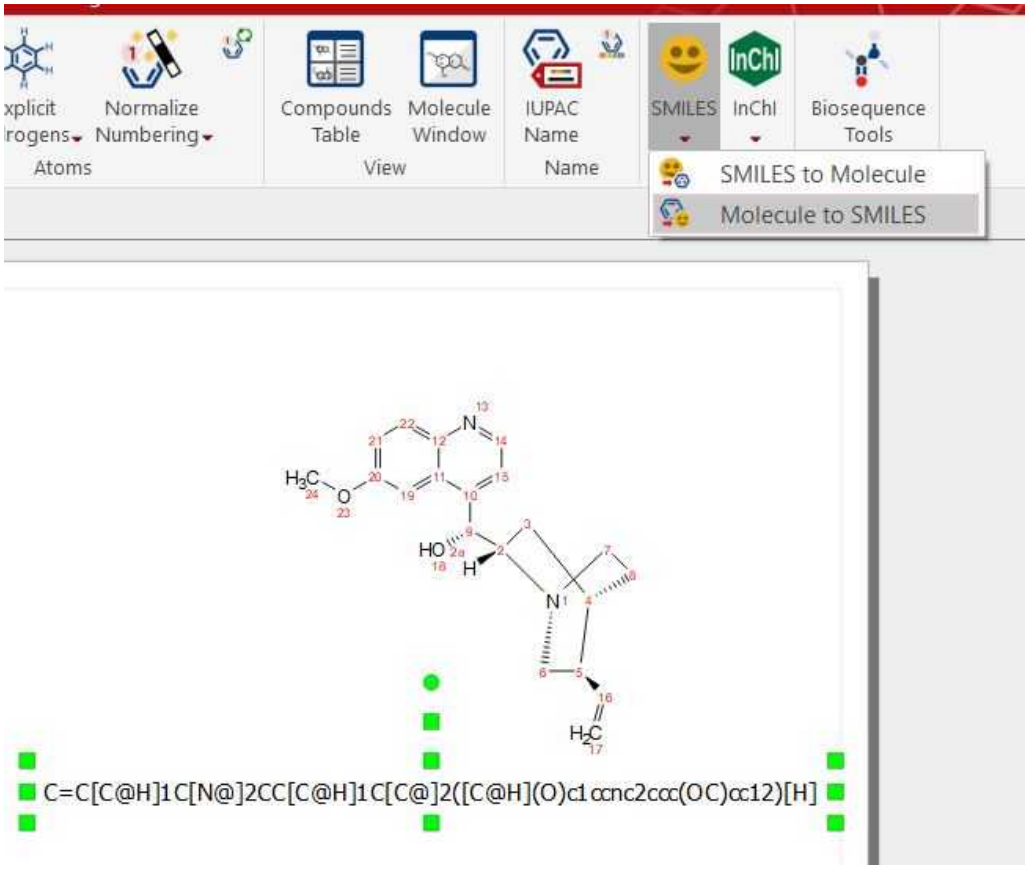
Smiles

Mnova converts the smiles strings into the applicable molecular structure when you paste them from the clipboard or after having clicked on the "Smiles to Molecule" button:

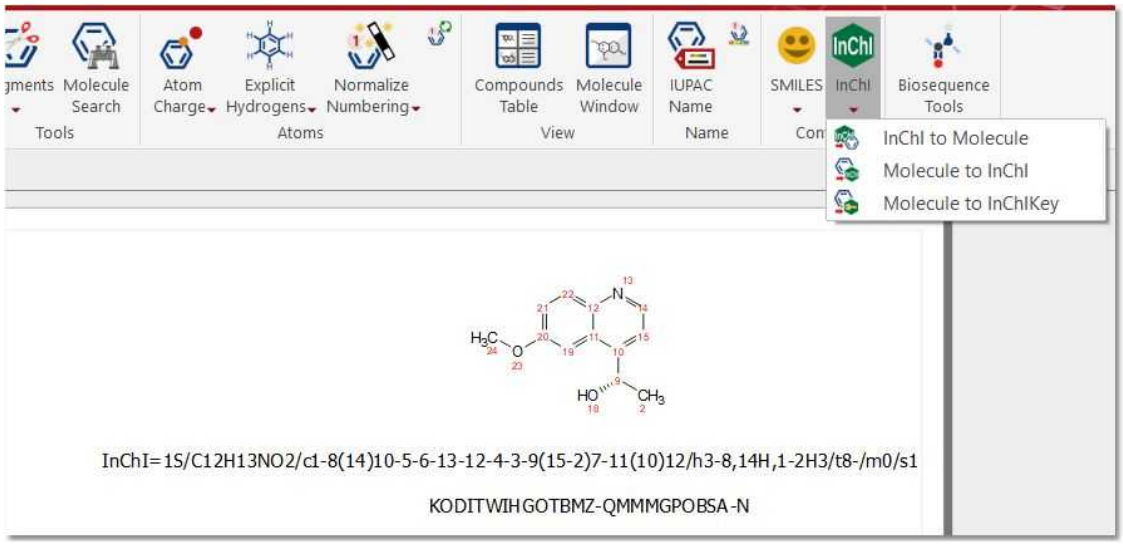


If you have a molecular structure, you can obtain the smile string by clicking on the 'Molecule to SMILES' button:

© 2024 MESTRELAB RESEARCH



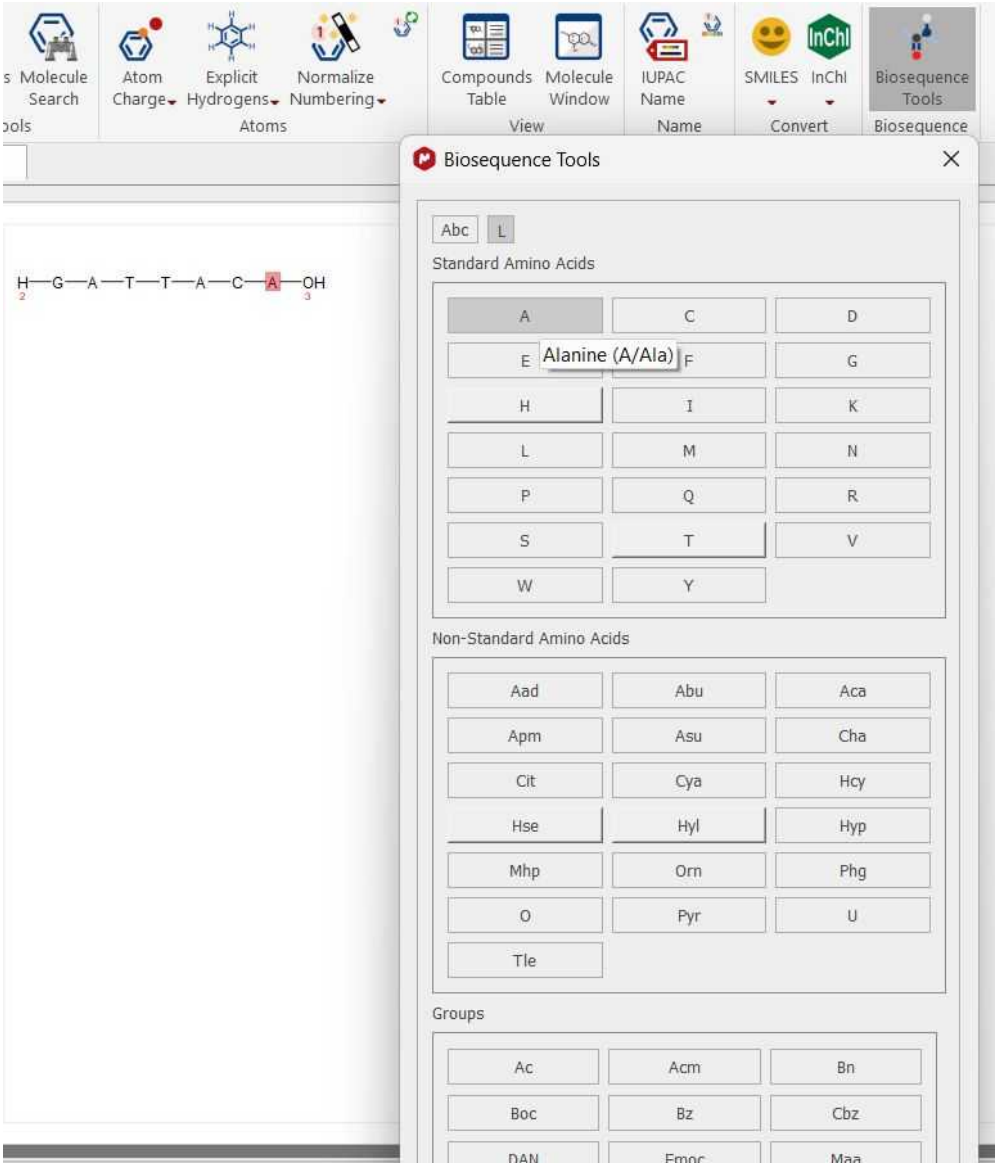
Inchi: You will have the capability to load molecular structure as Inchi, just by loading the applicable .inchi file into Mnova (or by clicking on the 'Inchi to molecule button' and pasting the Inchi string).



You can also convert molecular structures into Inchi and into Inchi Key functions.

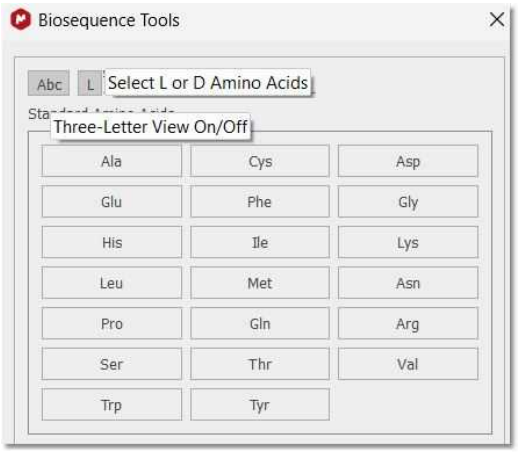
© 2024 MESTRELAB RESEARCH

Biosequence: When clicking on the “Biosequence Tools” button, a dialogue will be displayed to allow you to draw peptides using the 20 standard amino acids, several non-standard and some protective groups:



Once the new “Biosequence Tools” dialogue is opened, the cursor is changed to a peptide mode and only peptides can be drawn by using the 1 or 3 letters code. You can add D or L aminoacids, just by checking the applicable toggle button:

© 2024 MESTRELAB RESEARCH



Once you have drawn the peptide chain, you can toggle the sequence by right clicking and selecting the applicable option from the context menu:

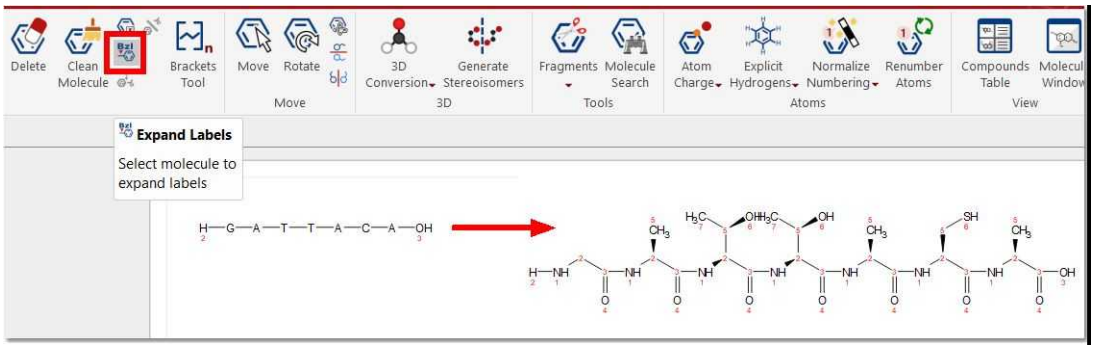


You can add amino acides inside the chain by selecting the AA in the chain and clicking on the desire AA that you want to add. To delete AAs just select the "Delete Atom/Bond" mode from the molecule ribbon and click on the AA (hovering the mouse over the AA and pressing the Delete key will also work).

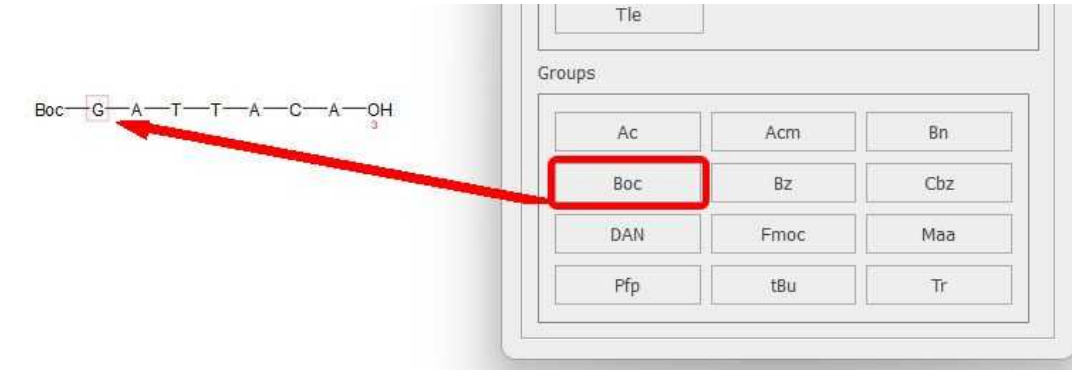
Once you have drawn the peptide chain, you could expand the amino acid just by right clicking on it and selecting 'Expand Labels':



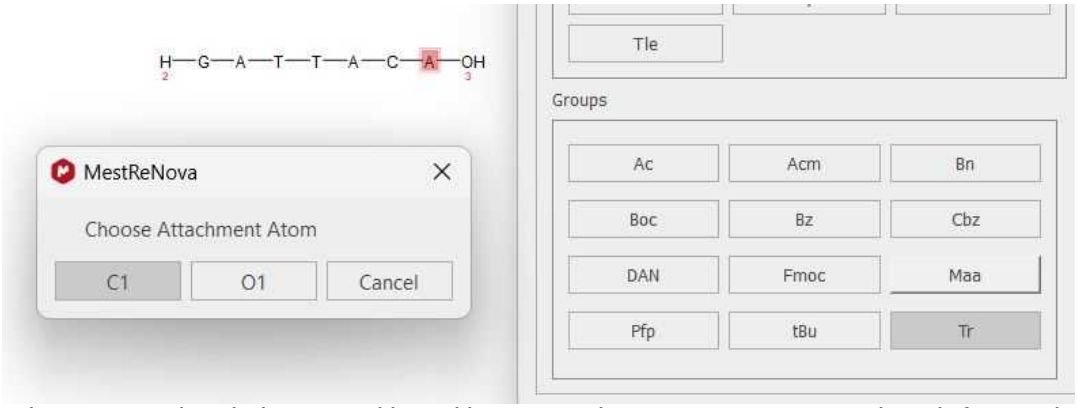
You can expand the entire peptide sequence by clicking on the “Expand Labels” button from the molecule ribbon:



To add protective groups, just select the desired group and click on the applicable AA:

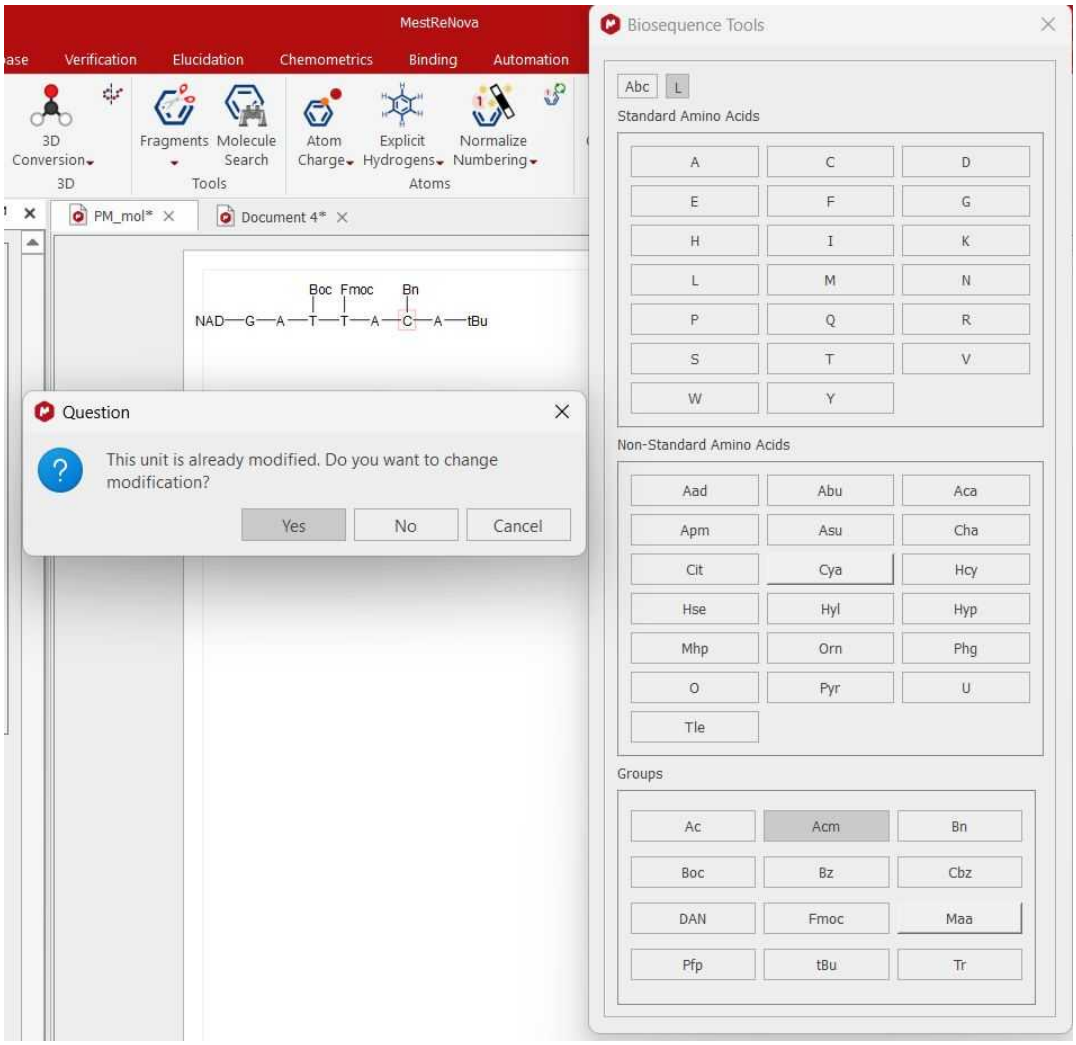


If there are several options to attach the group, you will get a dialog box to select the desired heteroatom:



When an AA already has a position with a protecting group, you can replace it for another one after having obtained the expected warning message:

© 2024 MESTRELAB RESEARCH



You can paste structures from the clipboard if the string contains 3-letters amino acids shortnames. The polymer string could be with or without delimeters (space, '-' or '.').

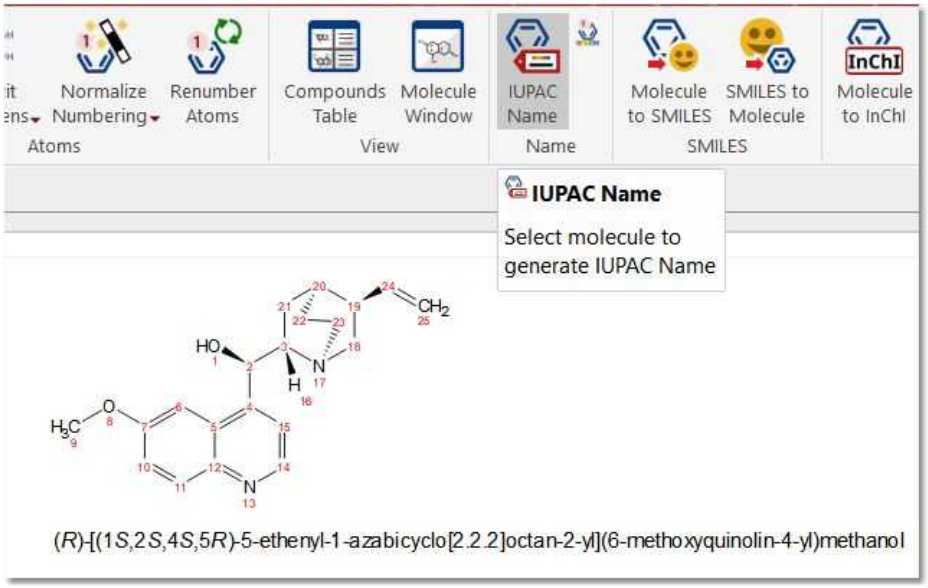
It also works when pasting from clipboard biopolymer strings with 1-letter amino acids shortnames. Example: ACDECCTRD. In this case, it only works when Biosequence Tools dialog is opened.

See also this video: https://youtu.be/W070ciW46so

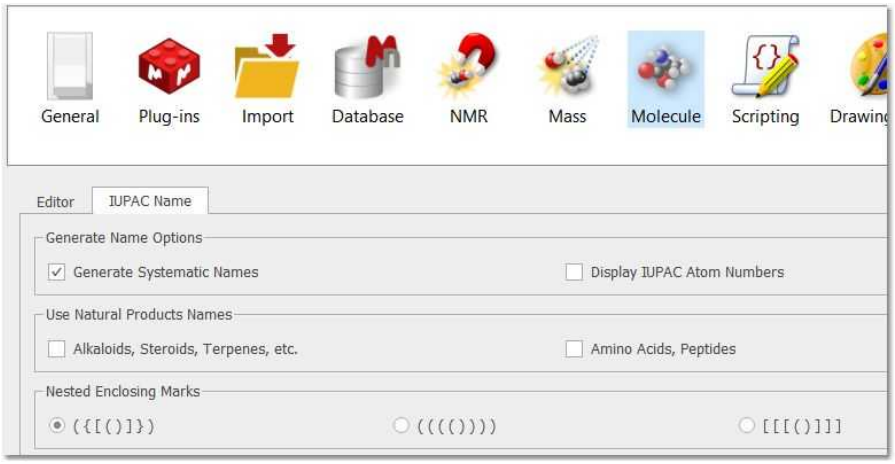
You can export your molecules as .mol, mol file V3000, .cdxml, .sdf or .jcamp by following the menu 'File/Save as' and selecting the desired format from the scroll down menu.

12.1 IUPAC Name

This button of the molecule ribbon will generate the IUPAC name from a given molecular structure. Please bear in mind that a separate license will be needed for this plugin.

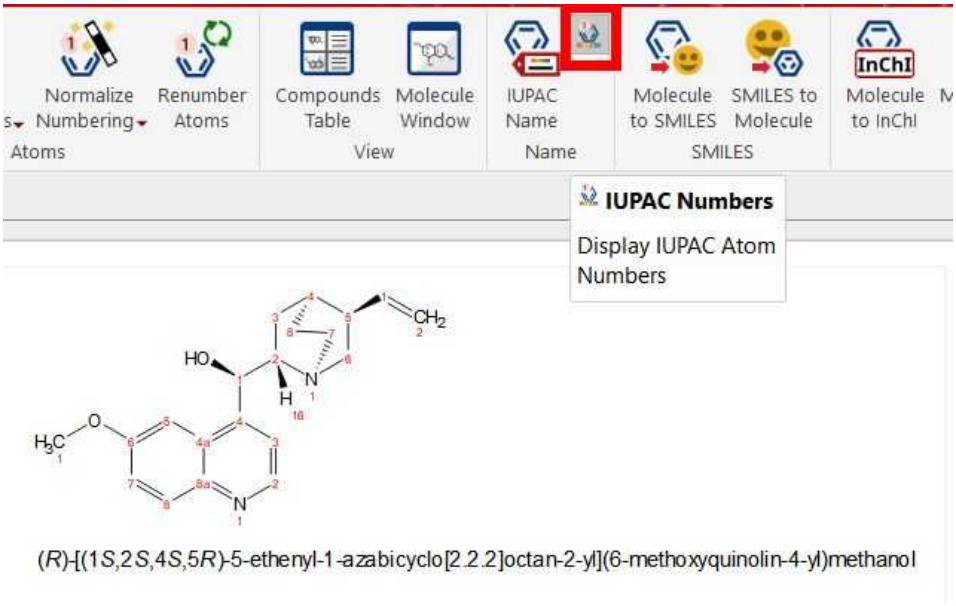


Some molecules have trivial names, like "1,3-dimethylbenzene" instead of "m-xylene". Follow the menu 'File/Preferences/Molecule/IUPAC NAME' and check the 'Generate Systematic Name'. When the box is unchecked, Mnova generates trivial names when it is possible.



If you are interested in getting IUPAC numbering, check the applicable box for that and generate the IUPAC name of your molecular structure. You will also find a button in the ribbon to display the IUPAC numbers, after having generated the IUPAC Name:

© 2024 MESTRELAB RESEARCH



When the "Use Natural Products Names' or/and 'Amino Acids, Peptides' boxes are checked, Mnova will generate IUPAC names for alkaloids, steroids, terpenes, amino acids, peptides, etc

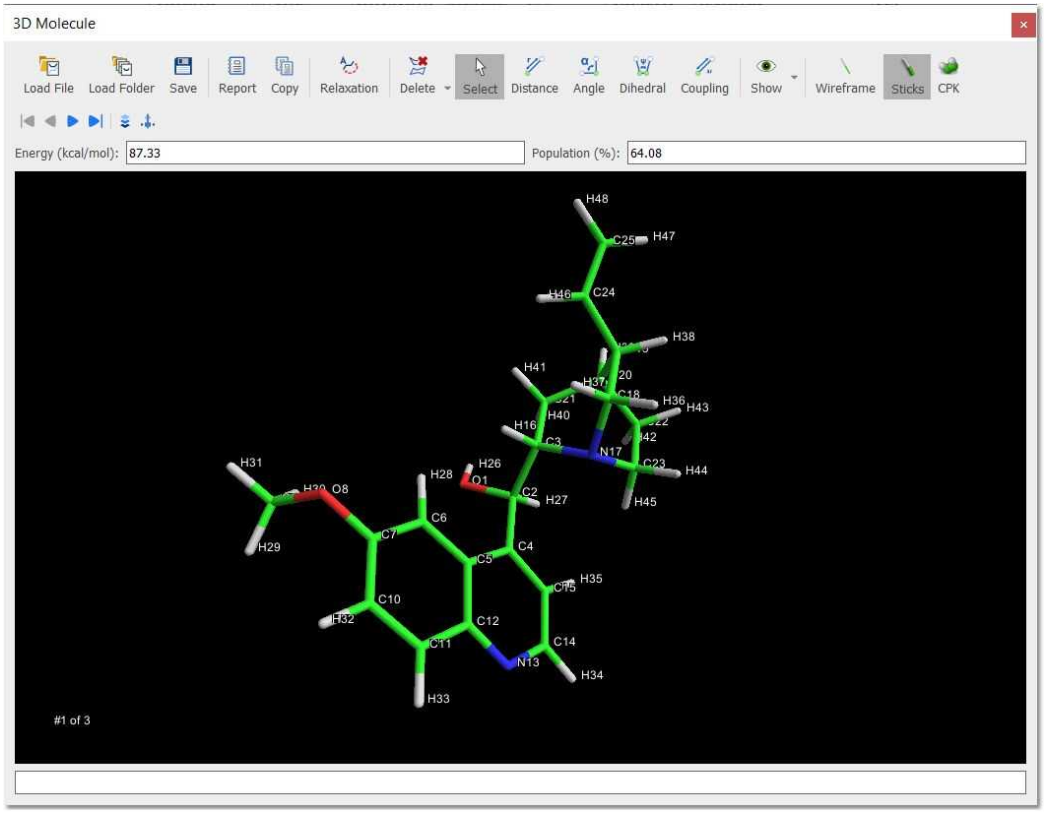
The "Nested Enclosing Marks" includes three options to choose the types of enclosing marks to generate the IUPAC name of a molecule. By default is selected the first option, which is the preferable in Preferred IUPAC Name indicated in the Blue Book 2013.

Further information can be found here:
https://resources.mestrelab.com/mnova-iupac-name-algorithm/

12.2 3D Molecule

You can activate the 3D molecule viewer by following the menu 'View/Tables/Molecule/3D Molecule':

© 2024 MESTRELAB RESEARCH



You will be able to navigate through the set of conformers by using the **'Navigator toolbar'** 

Molecular 3D structures can be managed by:

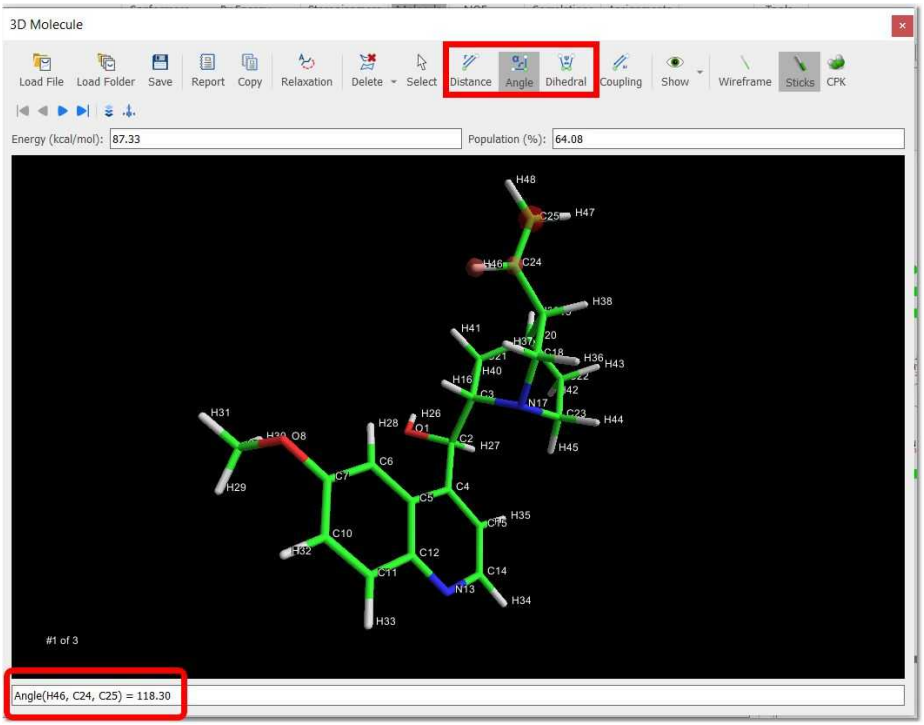
- Clicking on the right mouse button and dragging (or scrolling up or down the mouse wheel) to zoom in/out the molecule structure.

- Clicking on the left mouse button and dragging to apply a *TrackBall Rotation* (the user is able to control the movement of a ball which contains the molecule).

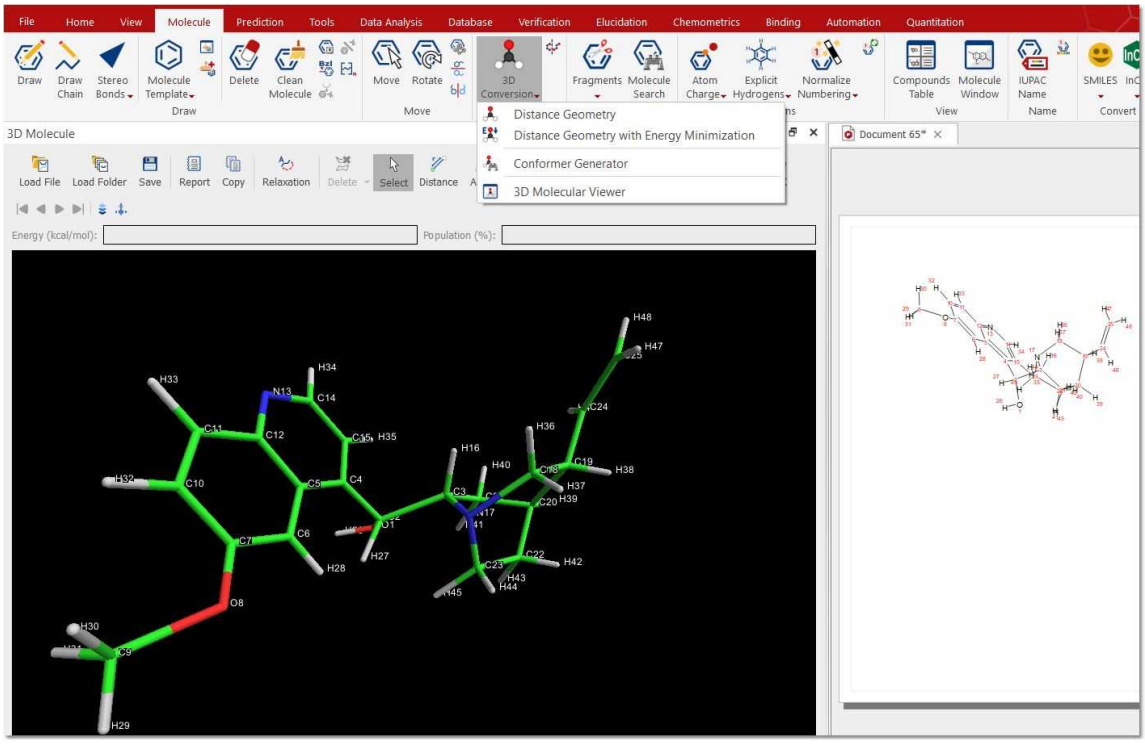
From this panel, the user will also be able to select the rendering mode (Wireframe, Sticks and Ball Sticks).

You will be able to measure bond distances, angles and dihedrals by just clicking on the corresponding buttons of the toolbar and next selecting the desired atoms to analyze.

© 2024 MESTRELAB RESEARCH

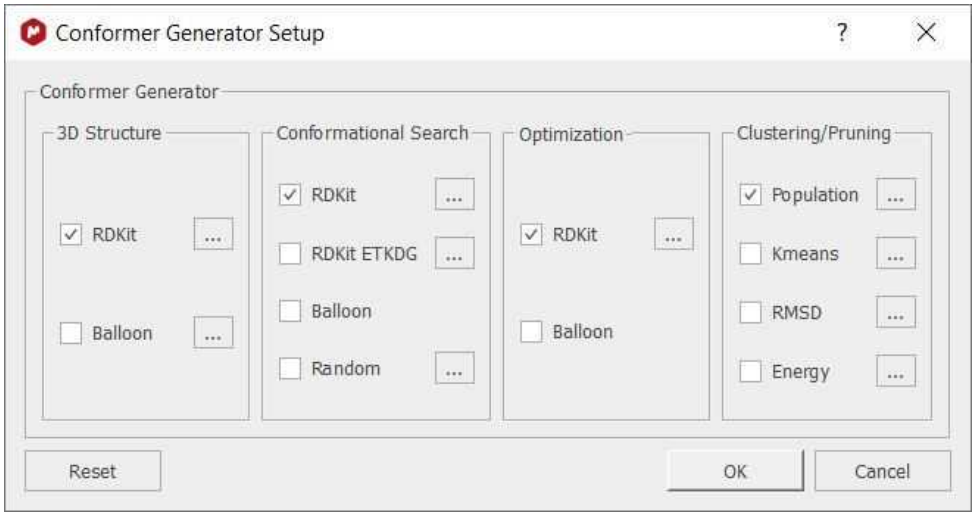


Click on the 3D conversion feature button to convert a 2D molecule in a 3D structure, calculating the energy for each conformer and their population:



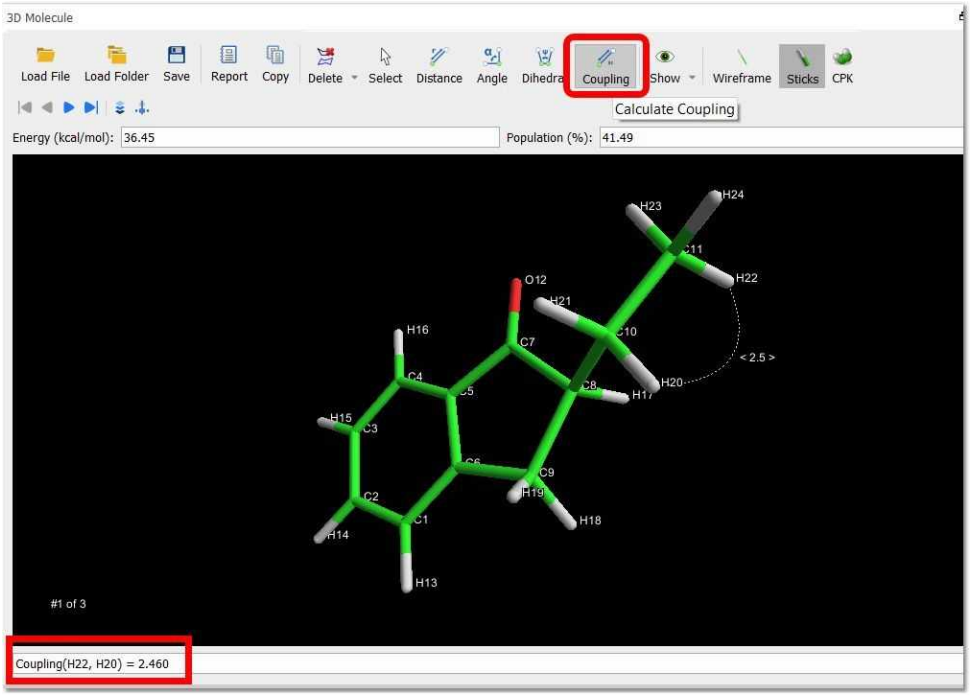
NOTE: If you are not getting the 3D molecule in the viewer, please change the OpenGL engine from the 'Preferences/Interface' dialog.

Clicking on the Conformer Generator option will allow you to select the algorithms for the calculation:



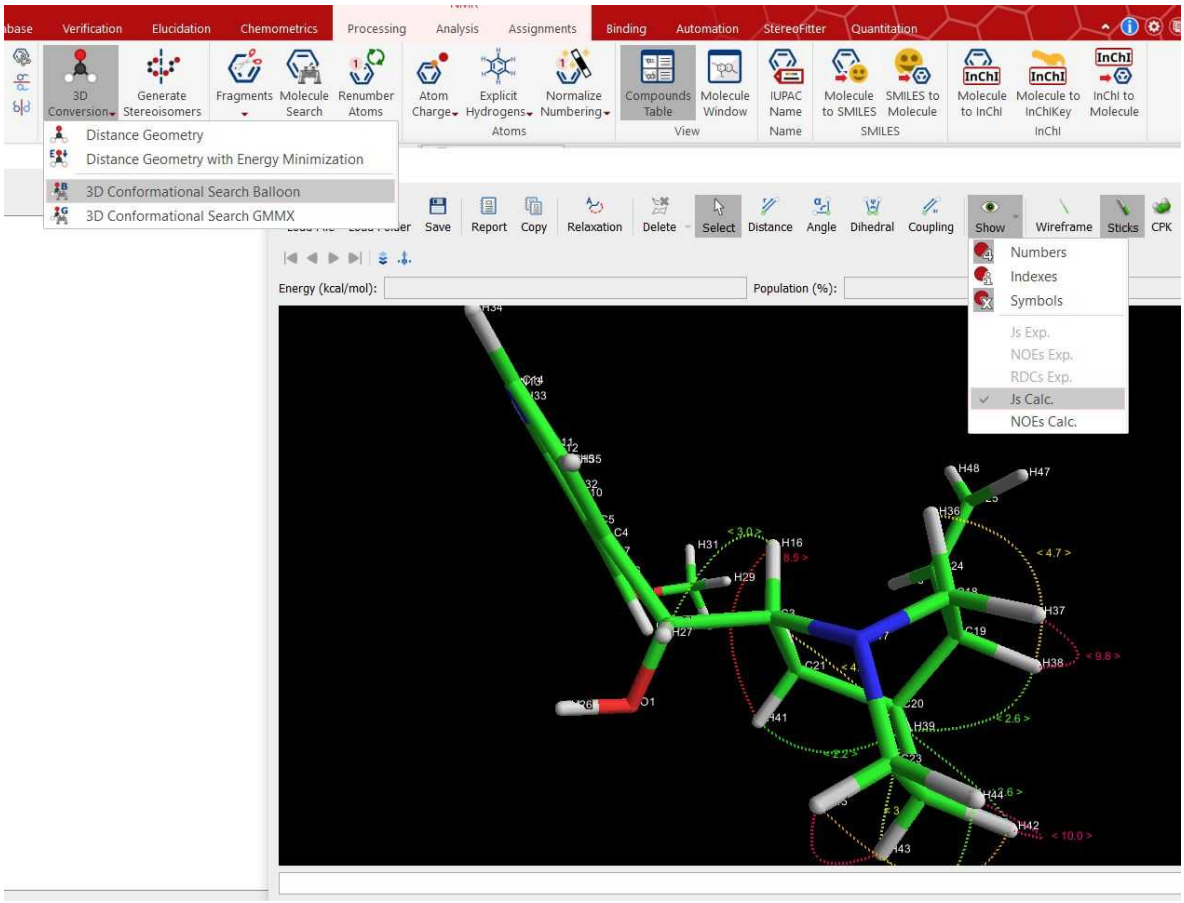
When loading a .mol file and using any of the available options, all possible conformers will be displayed in the 3D Molecule panel, including for each conformer: the Energy (kcal/mol) and Population (%).

You can also calculate 3J[HCCH] couplings in sp3 carbons after having applied a 'distance geometry calculation'. Next, click on the 'Coupling' button, and select the two atoms of interest:



You can also calculate 3J[HCCH] couplings in sp3 carbons by doing a 3D conformational search with Balloon or GMMX analysis, making sure that you display the calculated J's values:

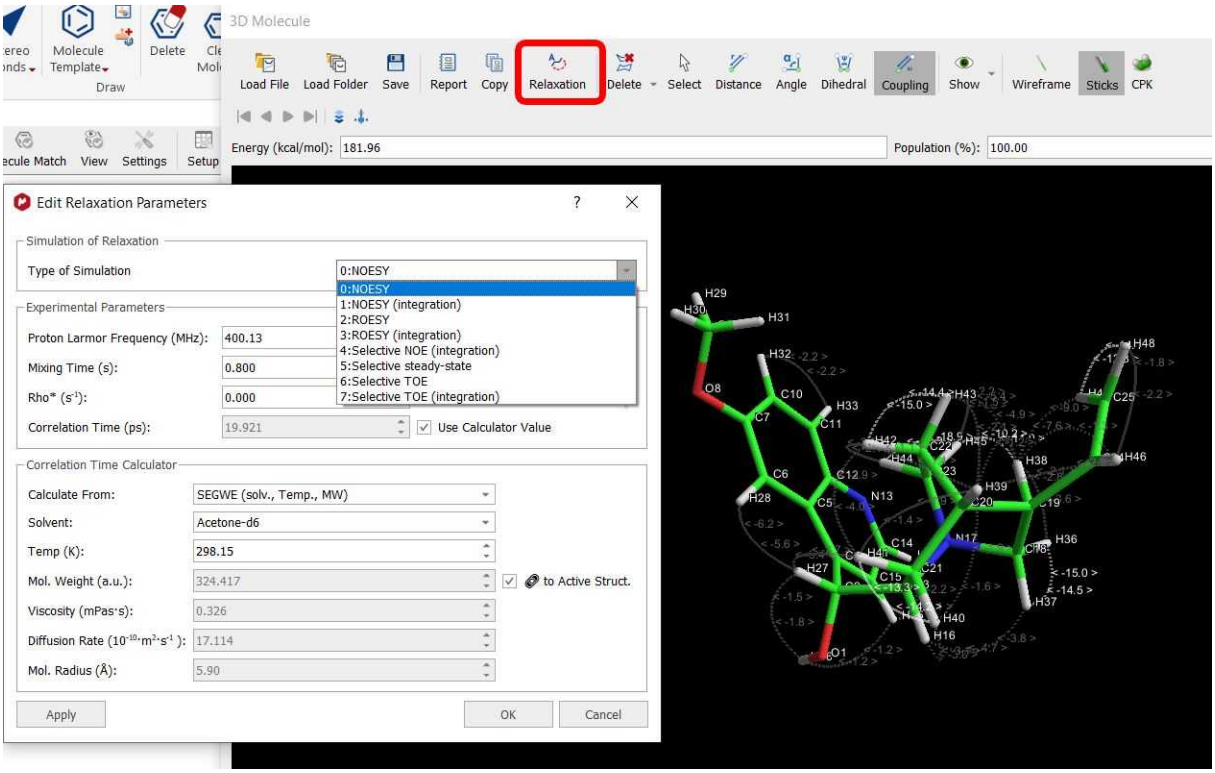
© 2024 MESTRELAB RESEARCH



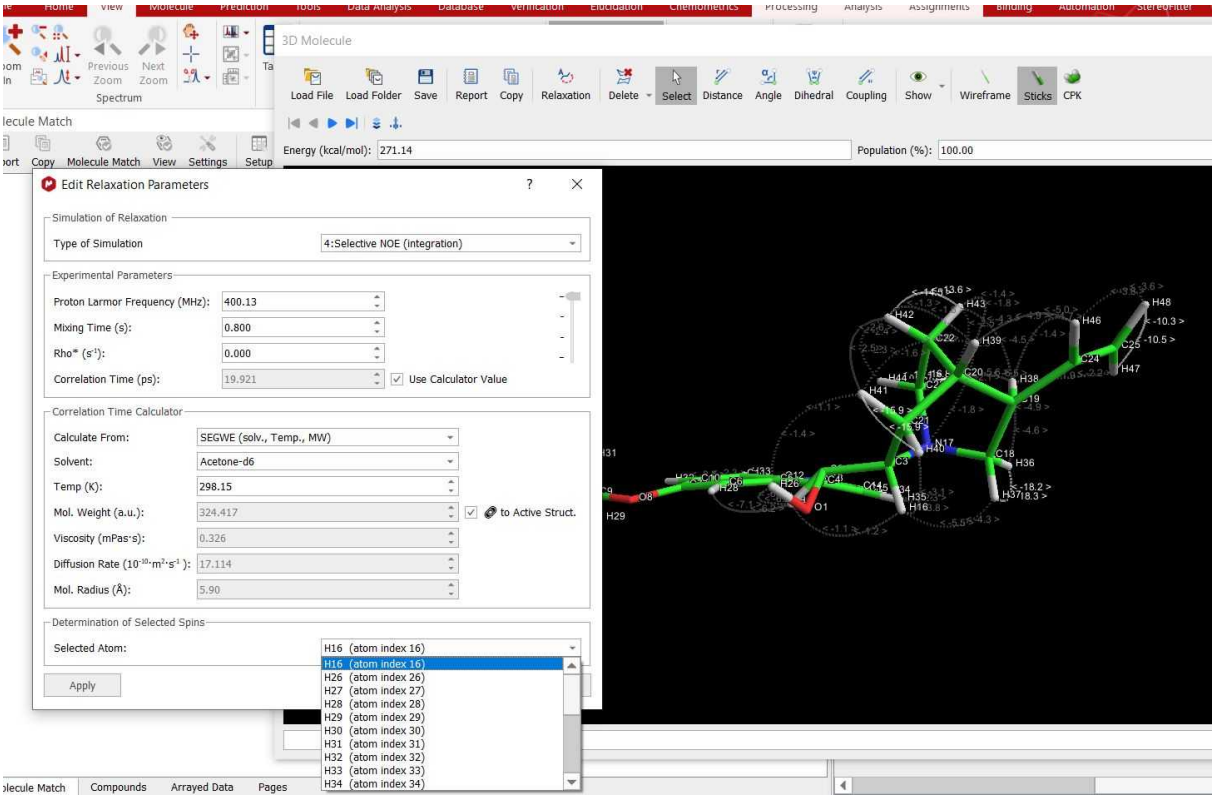
The J values and lines will be displayed with different colors (cyan to 0 Hz, green to 2 Hz, dark yellow to 4 Hz, orange to 6 Hz, red to10 Hz, magenta to 12 Hz, blue to 16 Hz and white to 20 Hz).

If your 3D molecule contains explicit protons, you can do relaxation analysis by clicking on the applicable button, which will display a new dialog to select the desired parameters:

© 2024 MESTRELAB RESEARCH



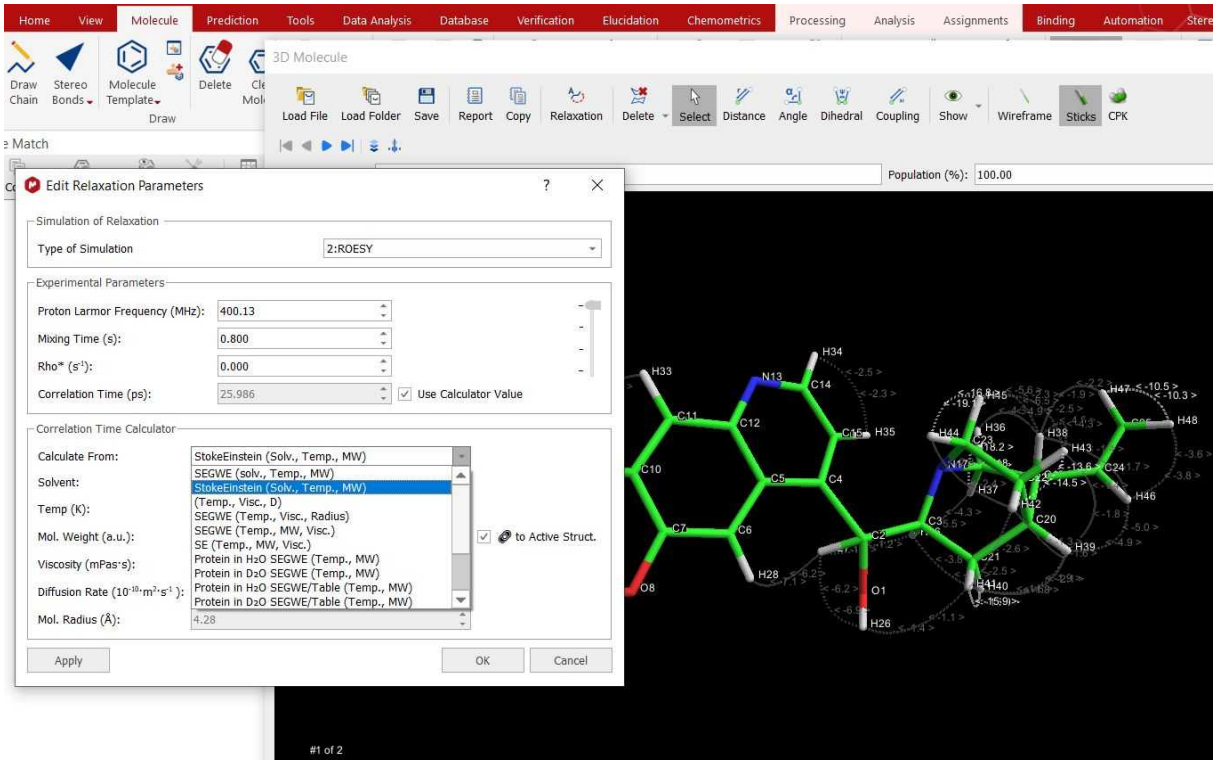
When choosing a “selective” simulation, an additional section named "Determination of selected spins" is shown at the bottom of the dialog:



© 2024 MESTRELAB RESEARCH

The correlation time value can be added manually or it can be calculated by using the “Correlation Time calculator”, which is displayed when the option "use calculator value" is checked.

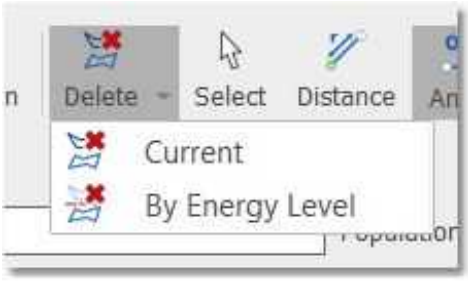
Once there you can select different methods, solvents, temperature, molecular weight, viscosity, Diffussion rate, molecular radius, etc...



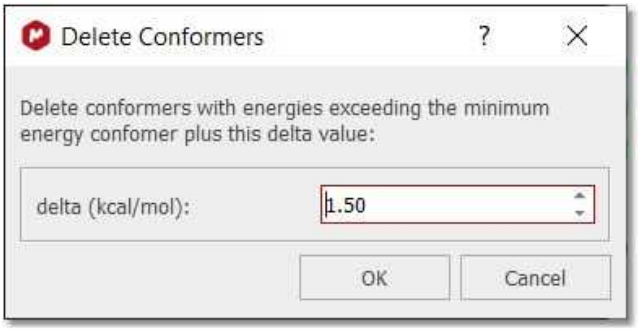
When a method is selected in which the molecular weight is an input parameter, there is an option named "to Active Struct" next to the MW field. When this option is unchecked, the Molecular weight is editable.

The negative NOEs values will appear as white labels, the positive will be red and the absolute values less than 10 will be (grey) darker.

You could delete the undesired conformers just by clicking on 'Delete current conformer' button:



You can also delete conformers by energy level:



The '3D Conformation Search GMMX' tool will need a valid license for the Stereofitter plugin.

© 2024 MESTRELAB RESEARCH

Part XIII

13 Prediction

The handling of molecular structures within **Mnova** also allows the user to carry out **spectral predictions**. Although the process described here could be applied to predictions by means of any commercially available or in-house prediction package, currently **Mnova** will carry out predictions by using the prediction application **NMRPredict**, by **Modgraph Consultants Ltd** and our own Mestrelab Predictor.

NMR Prediction in Mnova follows the concept of “unity creates strength”. The basic idea is to combine several predictors together to get a better predictive power. We have borrowed from the field of Machine Learning the term "**ensemble**" to define this new prediction procedure and we have written about it in this article, “Ensemble NMR Prediction” , where some results using 13C NMR data are given.

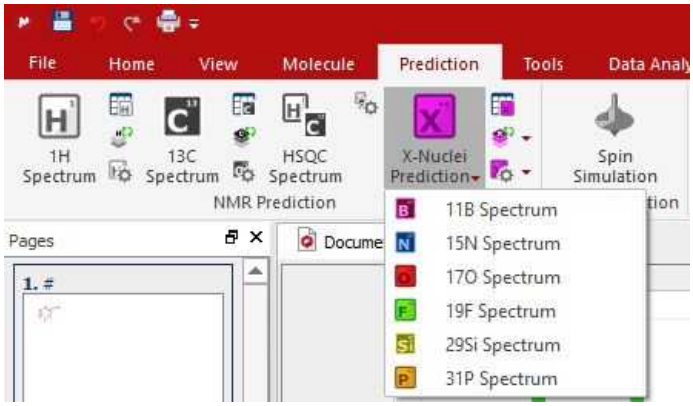
More information about Mestrelab Predictor can be found in this **paper**.



A few notes on NMR Prediction

- ✓ Our intention is for **Mnova** to support predictions with a range of prediction packages as wide as possible.
- ✓ Currently, predictions can be carried out by using **NMRPredict**, the prediction software by Modgraph Consultants Ltd or our Mestrelab Predictor. An evaluation version of NMRPredict can be downloaded from http://www.modgraph.co.uk
- ✓ **NMRPredict** comes into two different flavours:
  NMRPredict Desktop
  NMRPredict server-based
- ✓ Customers who have their own in-house prediction package are welcome to contact us to discuss a similar, seamless integration of their own prediction package. Please write to us at support@mestrelab.com.
- ✓ Please note that NMRPredict server is a different software package to Mnova and therefore, in order to carry out predictions via this package, a license of NMRPredict server must be held separately, once the NMRPredict evaluation has expired.

Mnova incorporates a fast simulation mode of ^{1}H, ^{13}C and X-nuclei (^{11}B, ^{15}N, ^{17}O, ^{19}F, ^{29}Si, ^{31}P) NMR spectra, called **NMRPredict.**



The reference used for the prediction are listed below:

^{1}H, ^{13}C and ^{29}Si (0.00 ppm, TMS); ^{11}B (0.00 ppm, $BF_3.Et_2O$); ^{15}N (0.00 ppm, NH_3); ^{17}O (0.00 ppm, Water); ^{19}F (0.00 ppm CF_3COOH); ^{31}P (0.00 ppm, H_3PO_4),

© 2024 MESTRELAB RESEARCH

You can change the references by editing the file named "CSEARCH.REF" stored in the Mnova installation folder:

C:\Program Files (x86)\Mestrelab Research S.L\MestReNova\NMRPredict\csearchho3abhijmab

The path to the file in Mac
is /Applications/MestReNova.app/Contents/MacOS/NMRPredict/csearchho3abfhijkmab/CSEARCH.REF

For example, The F19 is referenced CF3COOH. To change the reference, you will need to delete these lines (in the CSEARCH.REF file above):

```
# Reference F19 against CF3COOH
#
    4   -78.5                           CF3COOH
```

This application does not need to connect itself to a server for the spectra prediction. Besides, it makes uses of the fastest prediction algorithms (**Neural Network, Charge, Increments** and **Best**) implemented into **NMRPredict**.

Watch a miniclip on this functionality here

With the Mass plugin enabled, you could also predict MS spectra.

13.1 Mestrelab Predictor

Mnova comes with a new module for the fast and accurate prediction of 1H and 13C NMR spectra. It consists of a novel proprietary algorithm based on a *machine learning* algorithm (Mestrelab Predictor) which has been training with a large database of experimental assigned 1H and 13C chemical shifts as well as proton scalar coupling constants.

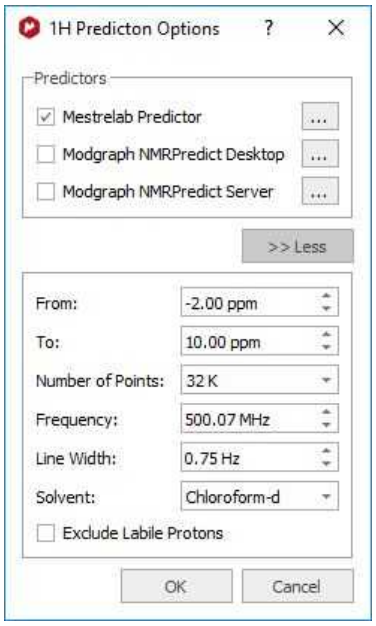
More information about Mestrelab Predictor can be found **here** and in this **paper**.

Running a Prediction

Once a molecule is available in Mnova, predicting a 1H or 13C spectrum can be done by simple selecting that option either from the *Predict* menu or by right clicking on the molecule and selecting the prediction from corresponding contextual menu.

First time a prediction is run, it can take a few seconds for the mathematical prediction model to be loaded in memory. This is done only once, so subsequent predictions will be extremely fast, although the model will be unloaded from memory if no predictions are run during five minutes.

Prediction options



From/To: Lower and upper limits for the chemical shift scale

Number of Points: Number of points used to build the predicted spectrum. Actually, Mnova predicts a time domain signal (FID) which is automatically Fourier Transformed to yield the final predicted frequency domain spectrum.

Line Width: Line width at half height of all peaks in the predicted spectrum.

Solvent: Prediction solvent.

13.2 Modgraph NMR Predict Desktop

Mnova integrates a fast simulation module of ^{1}H and ^{13}C-NMR spectra, called **Modgraph NMRPredict Desktop**.

See also 'How to predict a spectrum with NMRPredict Desktop?

NMRPredict Desktop is faster than **NMRPredict server-based**, because it does not need to log in to a server to predict a spectrum. Besides, it makes use of the fastest prediction algorithms (**Neural Network**, **Increments** and **Best**) implemented into **NMRPredict**. Other very significant advantages are that the software can be used without an internet connection, there are no access issues with firewalls, and no risk to confidential data being sent over the internet.

NMRPredict Desktop uses a Neural Network system for the prediction of ^{13}C-NMR spectra, and the NMR tables (**increments** methodology) of Professor Ernö Pretsch (ETH, Zürich) for a fast simulation mode of ^{1}H and **X-nuclei:** ^{11}B, ^{15}N, ^{17}O, ^{19}F, ^{29}Si, ^{31}P NMR spectra.

NMR Predict Desktop also includes the calculation of heteronuclear (HF, HP, CF and CP) couplings.

The **Best** algorithm is the combined approach that is capable of producing significantly improved proton NMR predictions for the data set upon which it has been tested down to below the 0.2 ppm target error.

You will find further information about this feature in:
http://www.modgraph.co.uk/best_proton_press_release.htm

© 2024 MESTRELAB RESEARCH

Please bear in mind that we are not giving any guarantees that the “Best” will always give prediction accuracy as low as 0.18 ppm. Statistically this is what we got against the 1.1 million shift values in the 90,000 data we used. This may or may not be the case with user data.

The **Neural Network algorithm** is much more general and error tolerant than the HOSE code approach (based on a reference spectra database) and is much more accurate at predicting shifts not found in the database.

The Neural Network prediction method in **NMRPredict Desktop** was developed during the mid 1990s in the group of Professor Robien at the University of Vienna. It was developed by V Purtuc and gave a very broad application range to cover general organic chemistry.

The basic design principles as defined in 1995 were:

- to be general enough and be able to handle problems from basic organic chemistry to complex natural products.
- to be able to handle "unusual" organic chemistry like organometallics.
- to be solvent specific during prediction.
- to be able to use stereochemical information not restricted by ring size, in the same way as could be done within the HOSE code technology.
- to make sure that only **inter**polation and not **extra**polation occured when making predictions.

The Neural Network implemented into **NMRPredict Desktop** has been thoroughly tested and proved to be both reliable and accurate. The 4,000,000 assigned chemical shift values of the available 345,000 reference spectra can be predicted with an average deviation between experimental versus calculated of below 2.00 ppm. This includes compounds which are not well handled by traditional prediction programs, such as ferrocenes and chromium complexes.



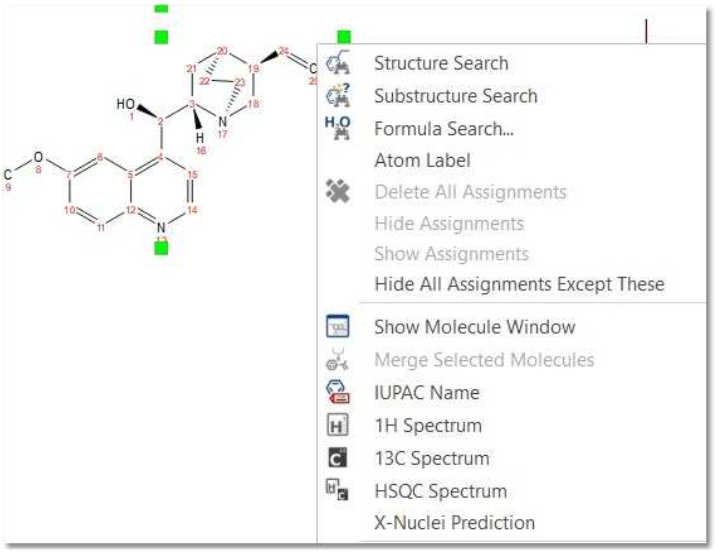
ATTENTION

Bear in mind that, in terms of stereochemistry, drawing a structure as a chair or as a boat means nothing to the .mol file and the computer. In those cases the user must draw all the corresponding bonds to show the stereochemistry of the molecule, i.e., you must explicitly draw up and down bonds using wedges.

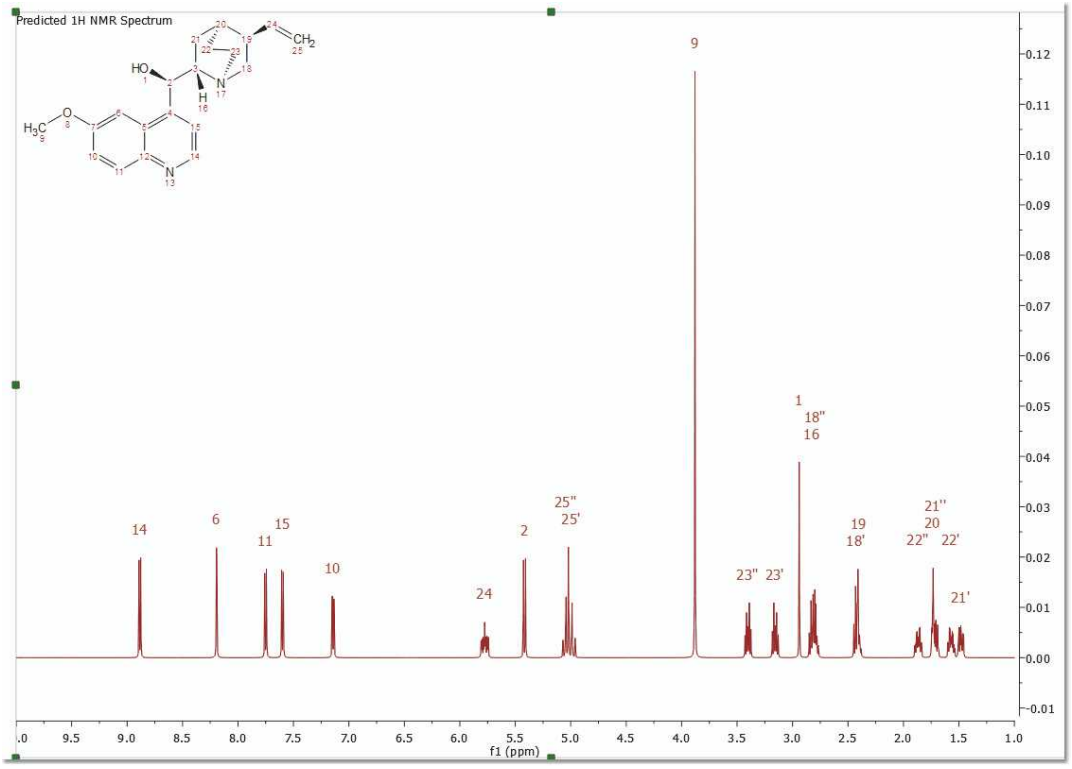
13.2.1 Running predictions

Carrying out of predictions in Mnova with **NMRPredict Desktop** is very easy. Just load a molecule into Mnova and right click on it to select the desired prediction (or follow the 'Prediction' menu):

© 2024 MESTRELAB RESEARCH



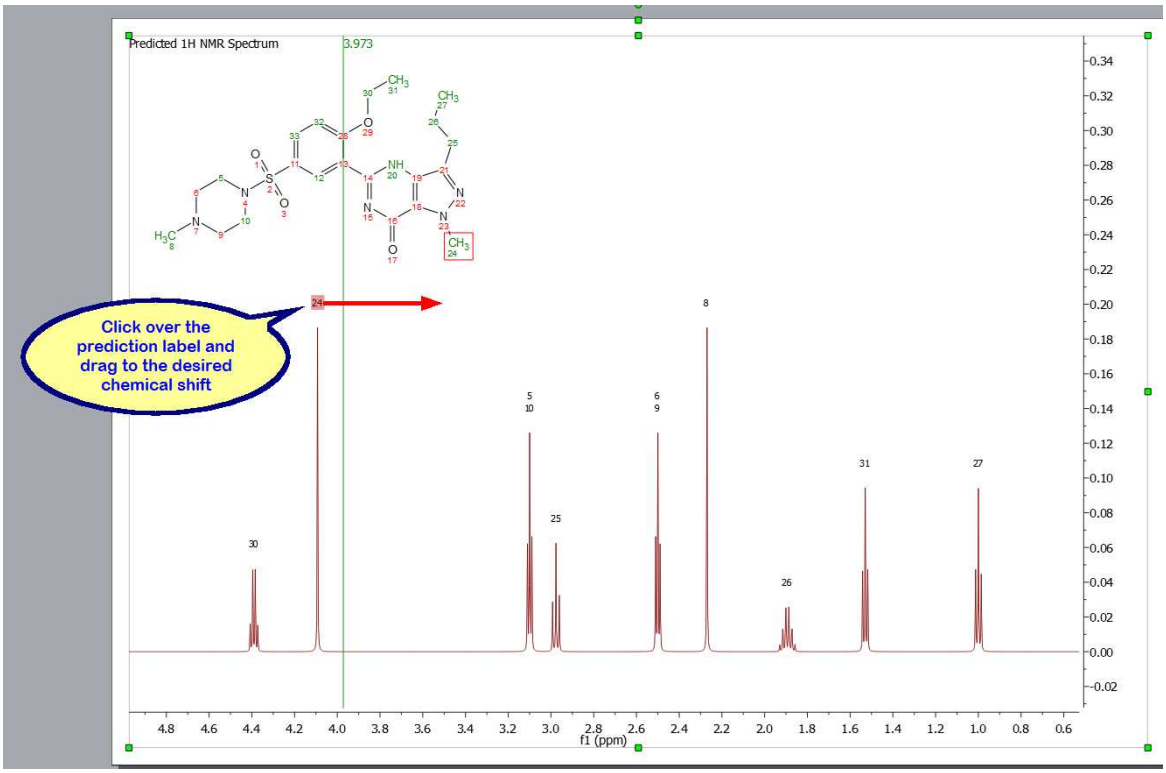
After that, you will obtain the desired predicted spectrum using a rigorous full quantum mechanics method taking into account any second order effect present in the predicted spin(s) systems(s):



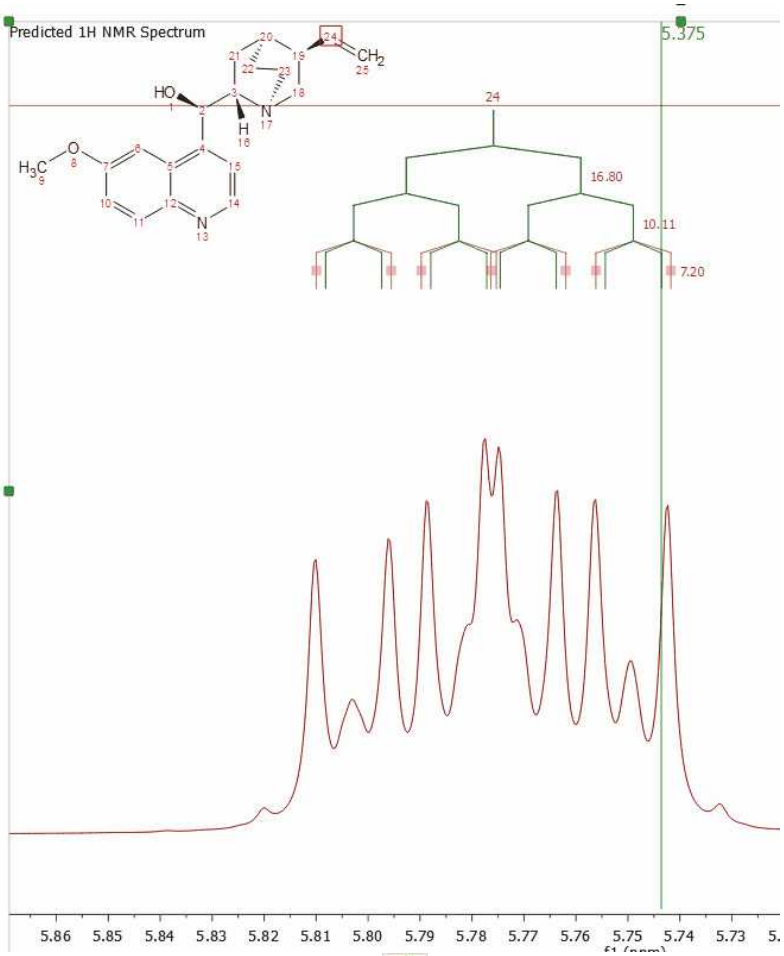
This predicted spectrum can be analyzed as a real one (e.g. it can be integrated, etc). The user can also visualize and edit the chemical shift and coupling constants values from the tables of the predicted spectrum by following the menu 'View/Table/1H Prediction'. Please bear in mind that the peak labels and the multiplet boxes will show the applicable atom numbers of the prediction.

Click&drag over the prediction label to graphically modify the chemical shift of the predicted signals:

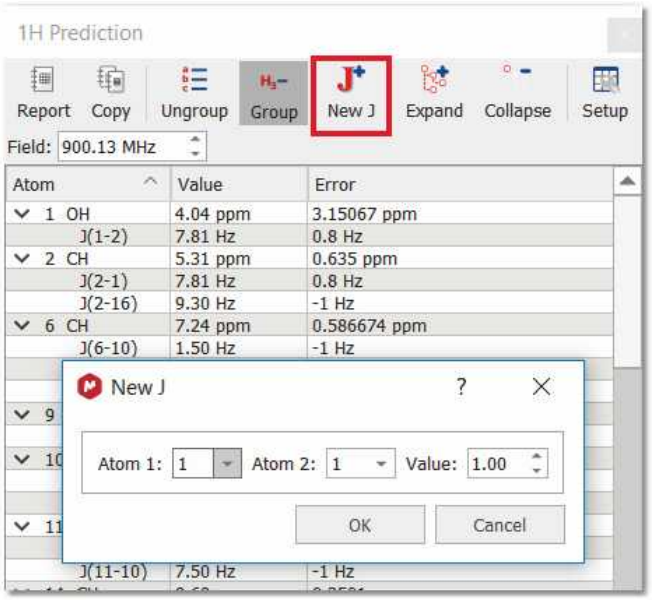
© 2024 MESTRELAB RESEARCH



You can change the predicted coupling constants by showing the multipet tree from the 'Prediction Properties' dialog box and clicking&dragging:



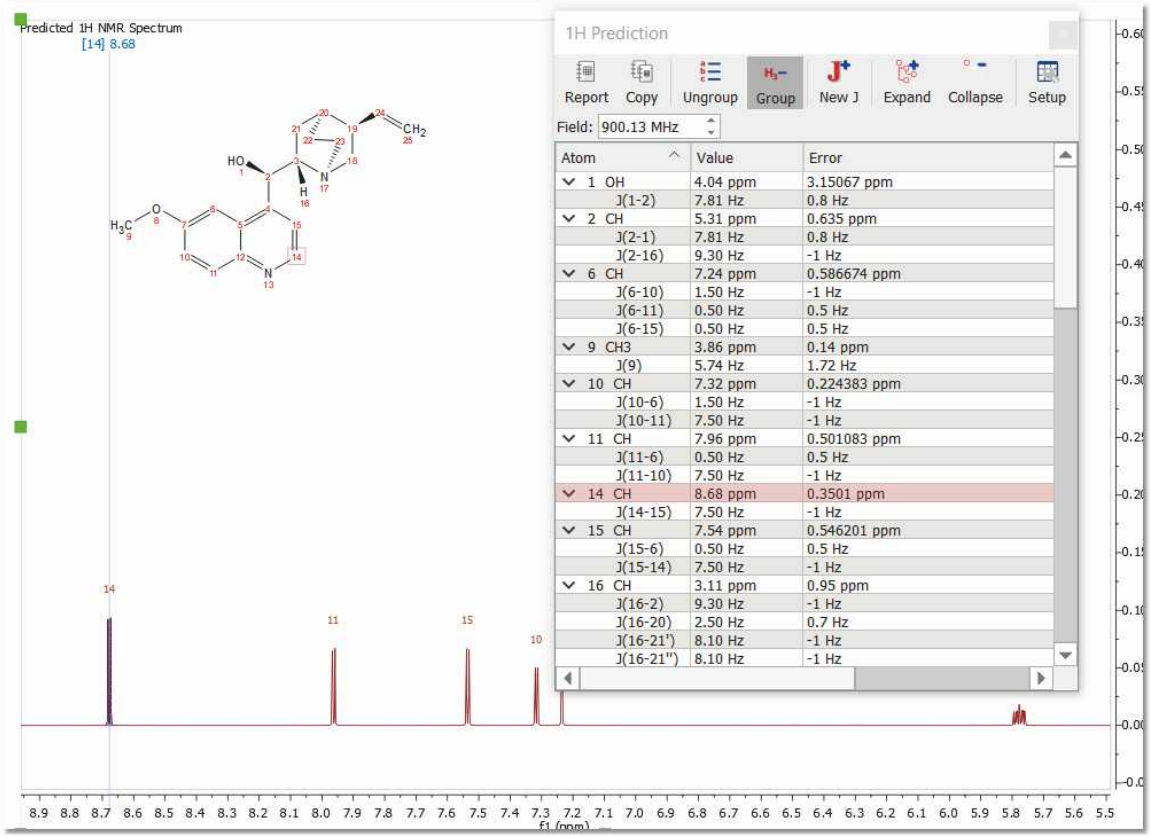
Click on the on the 'New J' button  to introduce new coupling constants:



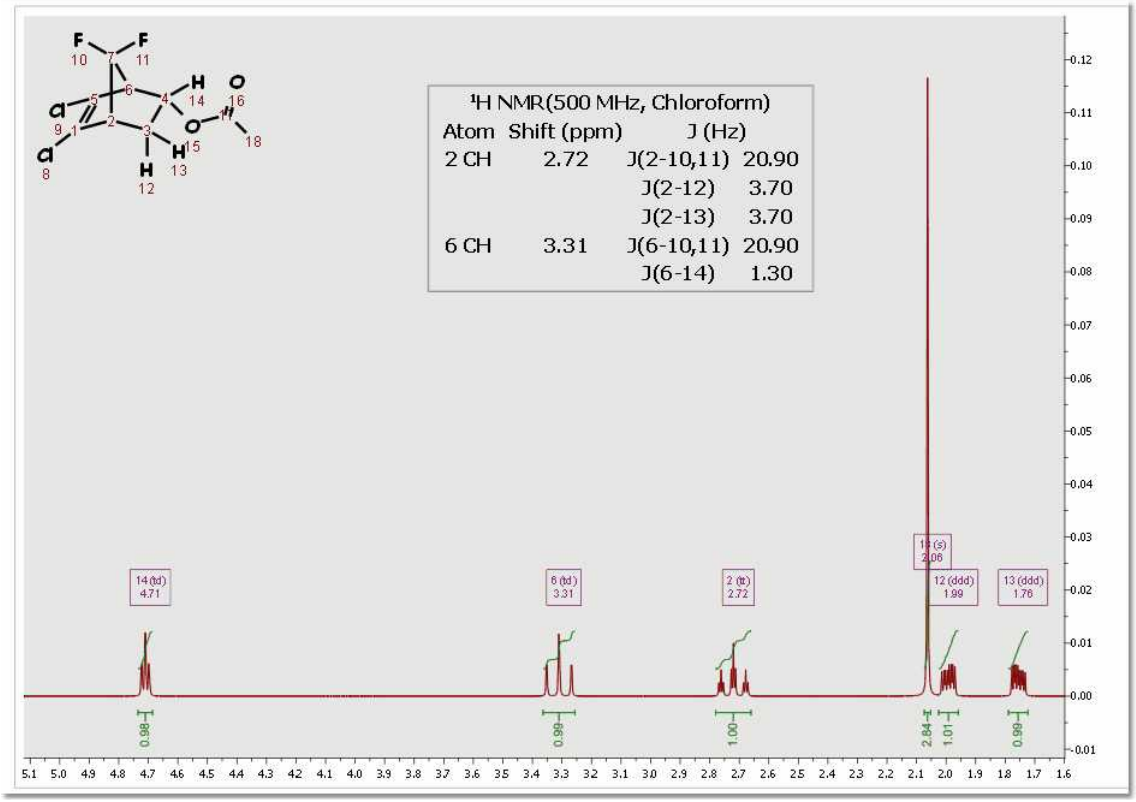
© 2024 MESTRELAB RESEARCH

After that, the 'New J' dialog box will be displayed and you will only need to select the atoms involved in the coupling and the value of the new coupling constant. Click on OK and the new coupling constant will be added to the spectrum and also to the prediction table.

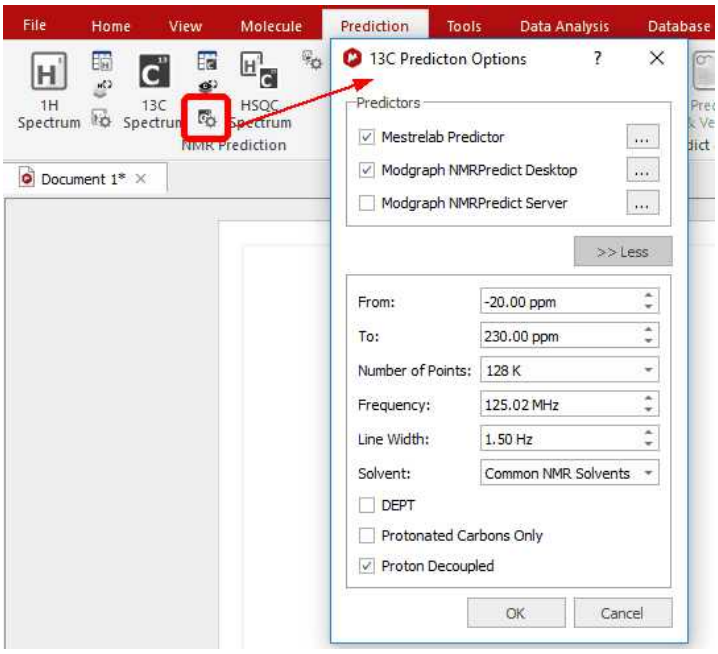
Finally, to know the "predicted assignment", just hover the mouse over the desired atom, (so that it becomes highlighted in a red box) and the corresponding signal in the spectrum will automatically be highlighted in blue; or vice versa (hovering the mouse over the peak will highlight the applicable atom on the molecular structure). You will also get highlights in the applicable row of the 'Prediction table':



Please bear in mind that NMR Predict Desktop also includes the calculation of heteronuclear (HF, HP, CF and CP) couplings:

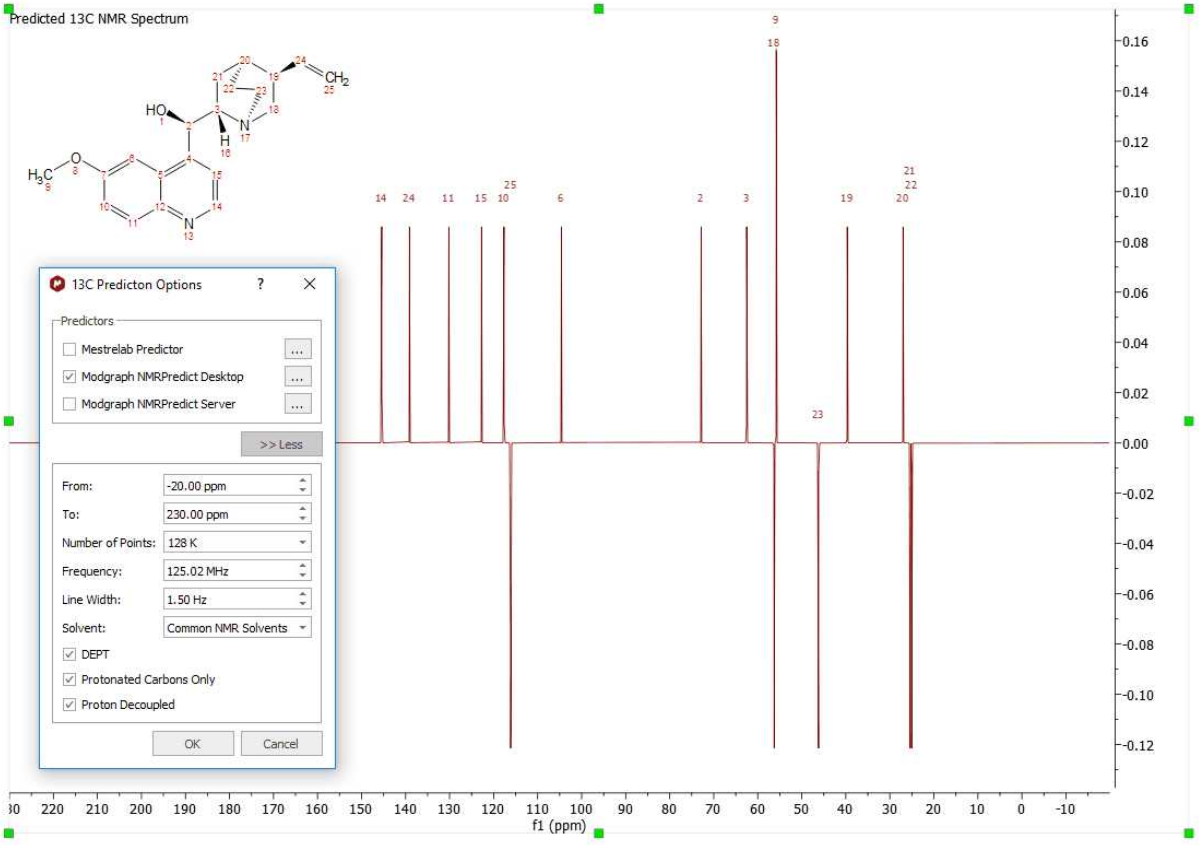


If you need the ^{13}C-NMR predicted spectrum, follow the same instructions but selecting the options for '13C' (be sure that you select the correct icon for 13C, red square in the figure below). As you can see in the '13C Predictor Option', you can select the limits, the number of points, the frequency and the line width of the spectrum. You can predict 'DEPT' (CH and CH3 up and CH2 and Quaternary carbons down) and spectra with only protonated carbons (DEPT 45) by checking the applicable boxes. If you want to get a DEPT 135, you must check both boxes.



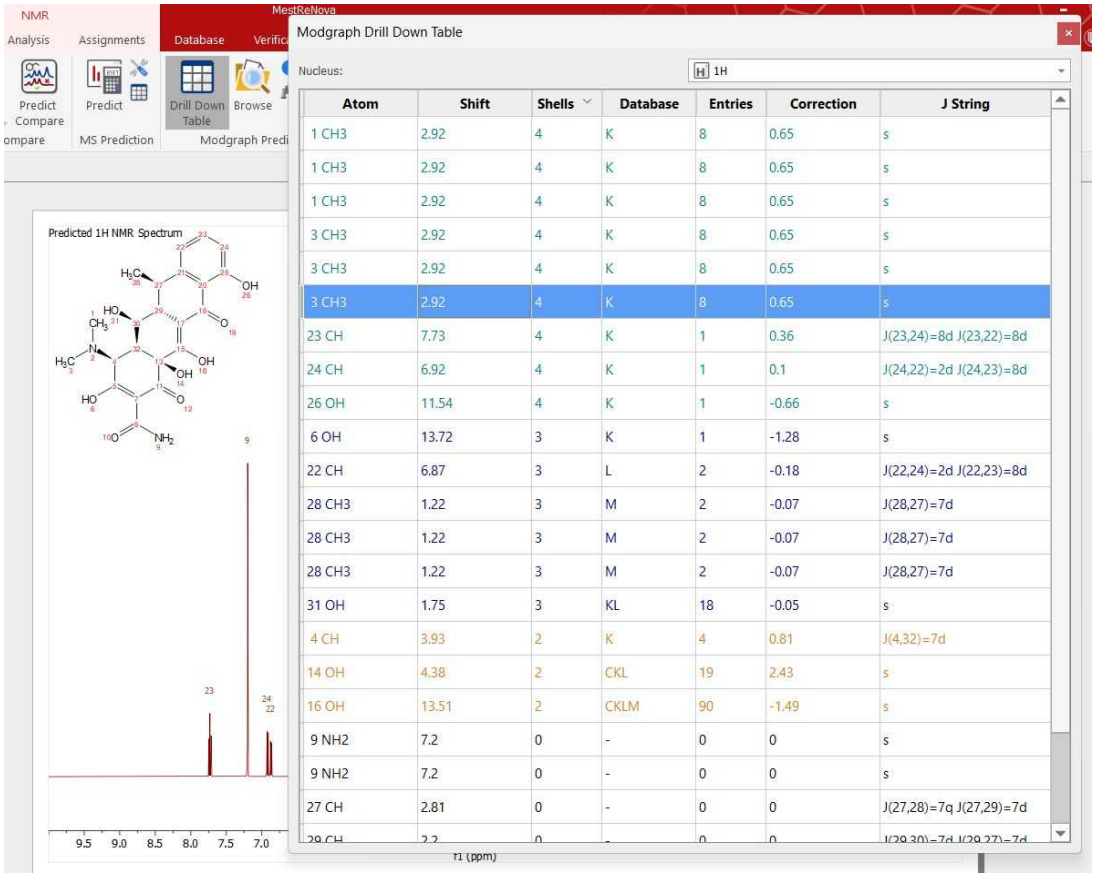
© 2024 MESTRELAB RESEARCH

Here you can see an example of a DEPT 135 prediction:



When running a prediction you can drill down into the system database predictions to check the number of shells and entries involved in the prediction. The details can be accessed through Modgraph Predictions table (by following the menu 'View/Tables/Modgraph NMR Predict/Modgraph Drill down Table') or by clicking on the 'Drill down Table' button from the 'Prediction' ribbon.

© 2024 MESTRELAB RESEARCH



Atom	Shift	Shells	Database	Entries	Correction	J String
1 CH3	2.92	4	K	8	0.65	s
1 CH3	2.92	4	K	8	0.65	s
1 CH3	2.92	4	K	8	0.65	s
3 CH3	2.92	4	K	8	0.65	s
3 CH3	2.92	4	K	8	0.65	s
3 CH3	2.92	4	K	8	0.65	s
23 CH	7.73	4	K	1	0.36	J(23,24)=8d J(23,22)=8d
24 CH	6.92	4	K	1	0.1	J(24,22)=2d J(24,23)=8d
26 OH	11.54	4	K	1	-0.66	s
6 OH	13.72	3	K	1	-1.28	s
22 CH	6.87	3	L	2	-0.18	J(22,24)=2d J(22,23)=8d
28 CH3	1.22	3	M	2	-0.07	J(28,27)=7d
28 CH3	1.22	3	M	2	-0.07	J(28,27)=7d
28 CH3	1.22	3	M	2	-0.07	J(28,27)=7d
31 OH	1.75	3	KL	18	-0.05	s
4 CH	3.93	2	K	4	0.81	J(4,32)=7d
14 OH	4.38	2	CKL	19	2.43	s
16 OH	13.51	2	CKLM	90	-1.49	s
9 NH2	7.2	0	-	0	0	s
9 NH2	7.2	0	-	0	0	s
27 CH	2.81	0	-	0	0	J(27,28)=7q J(27,29)=7d

The rows are colored according to the "Shells" number that determines the quality of the prediction:

0 shells -> black

1 shells -> red

2 shells -> orange

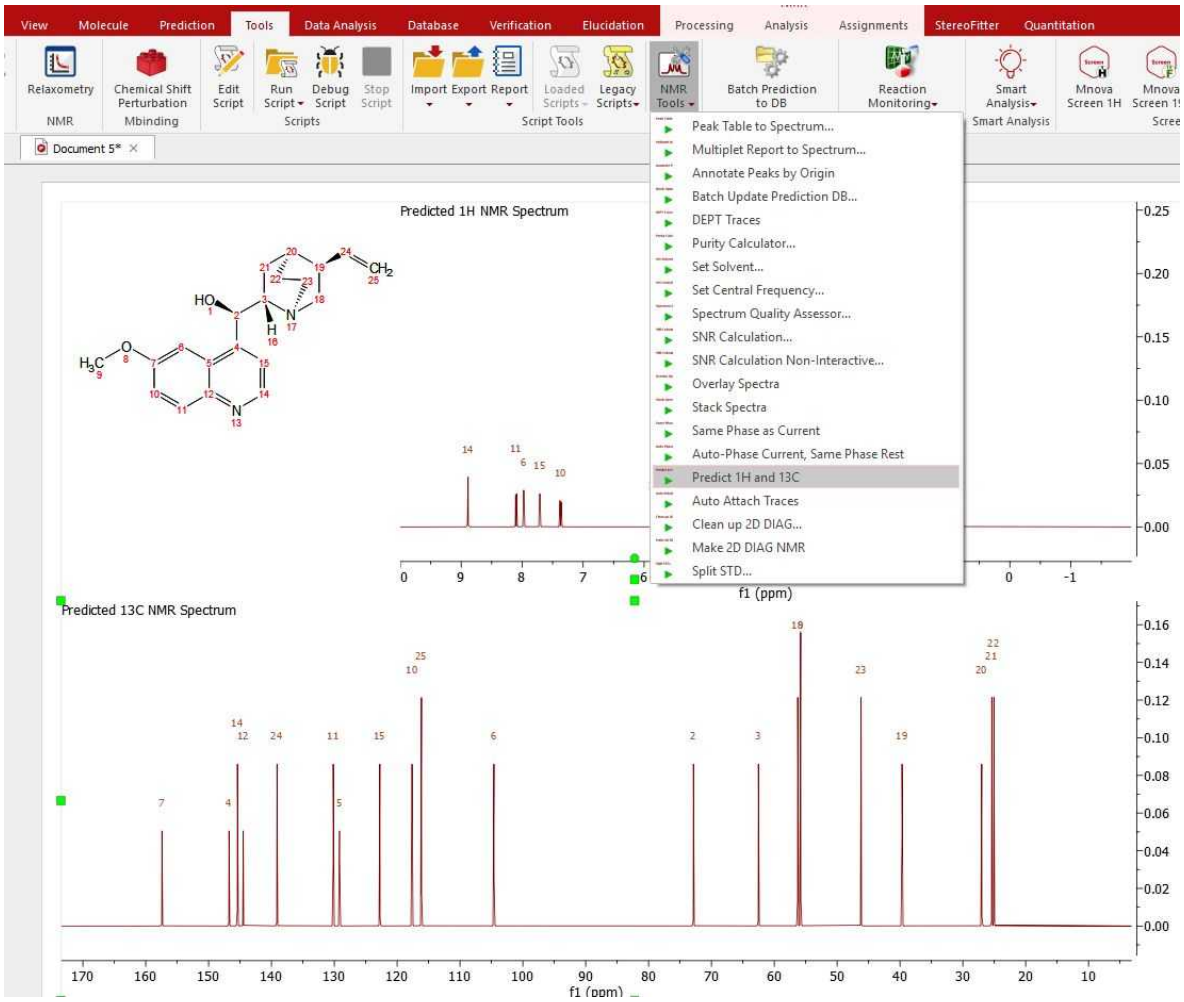
3 shells -> blue

4 shells -> cyan

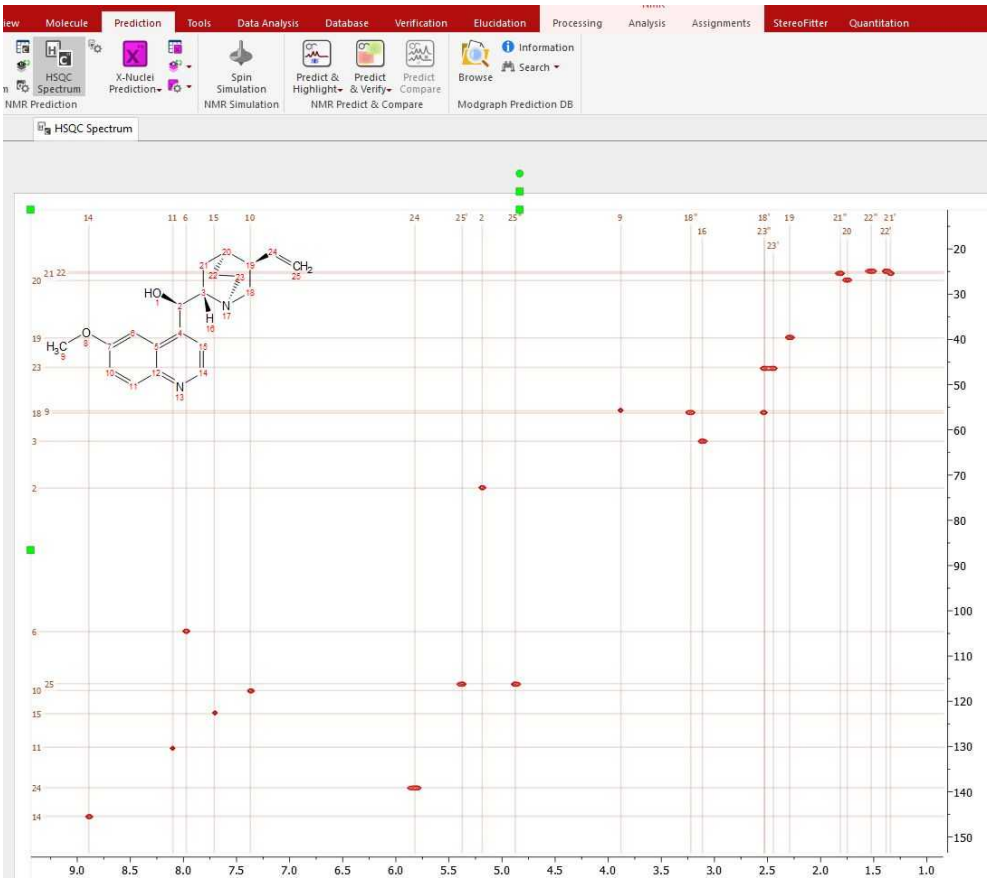
5 shells -> green

Follow the menu 'Tools/NMR Tools/Predict 1H and 13C' to get both predictions in the same page:

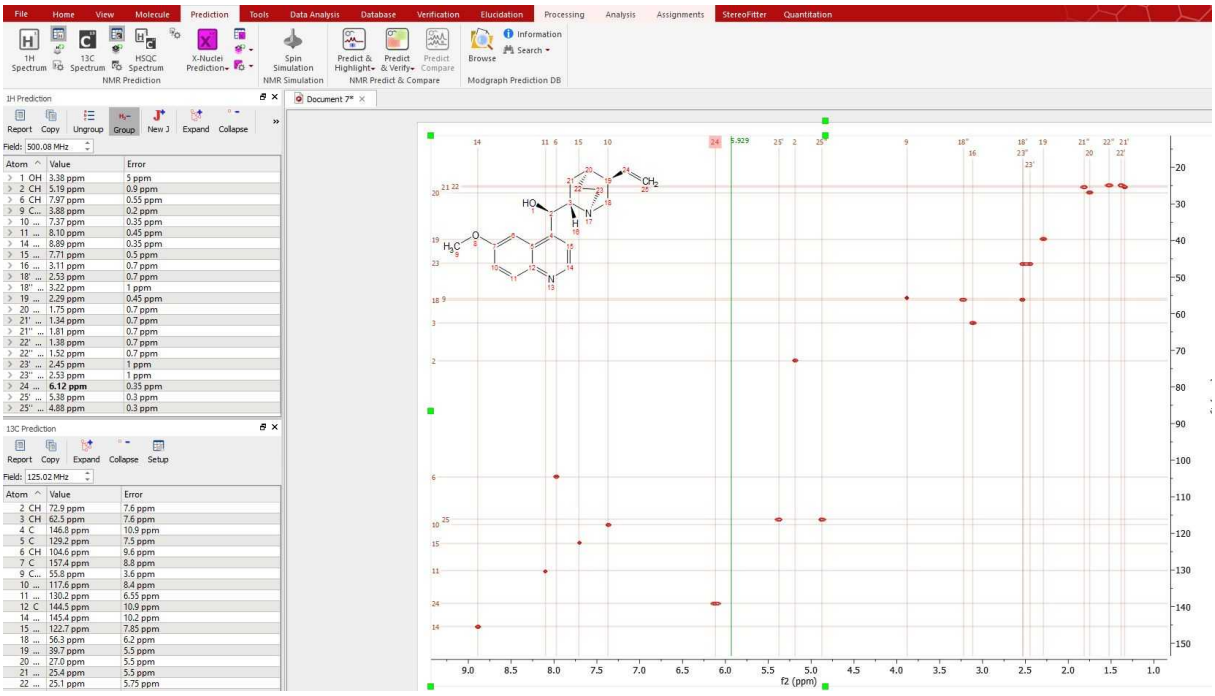
© 2024 MESTRELAB RESEARCH



Follow the menu 'Prediction/HSQC Spectra' to predict a HSQC spectra from a given molecule:

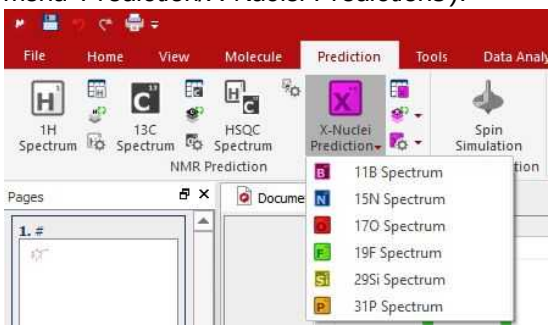


You can modify the predicted chemical shifts either graphically, by clicking&dragging any prediction label, or by typing the new values in the prediction tables:

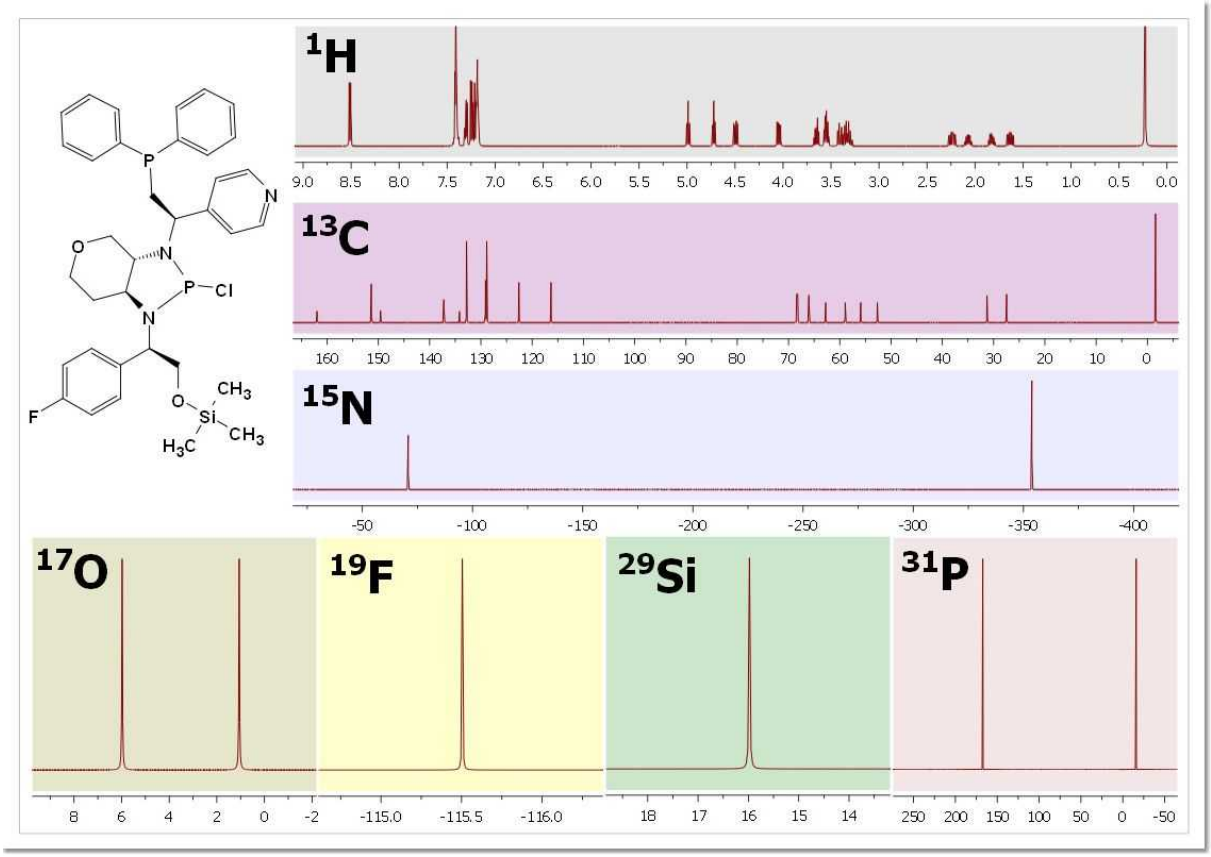


© 2024 MESTRELAB RESEARCH

The same procedure will be followed to run X-Nuclei predictions ^{11}B, ^{15}N, ^{17}O, ^{19}F, ^{29}Si, ^{31}P (under the menu 'Prediction/X-Nuclei Predictions'):



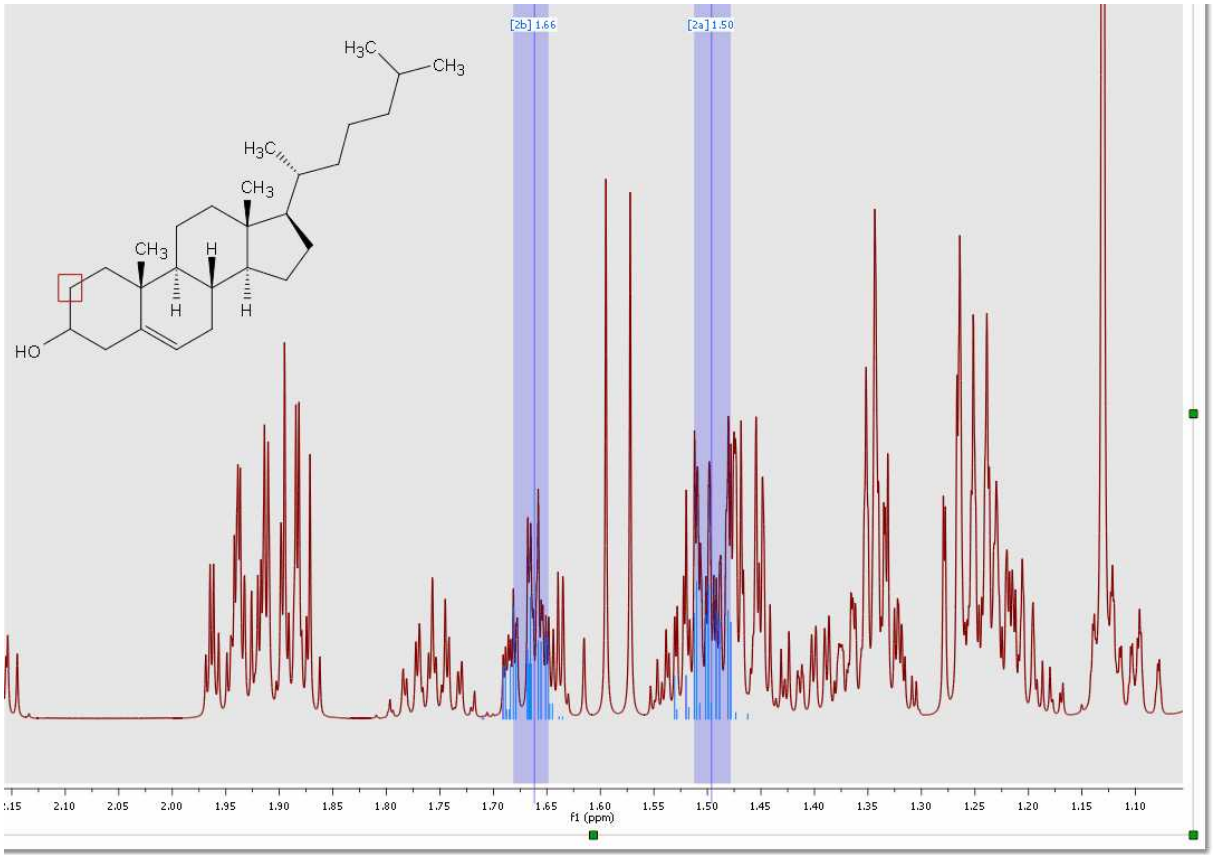
And here you can see an example:



ATTENTION

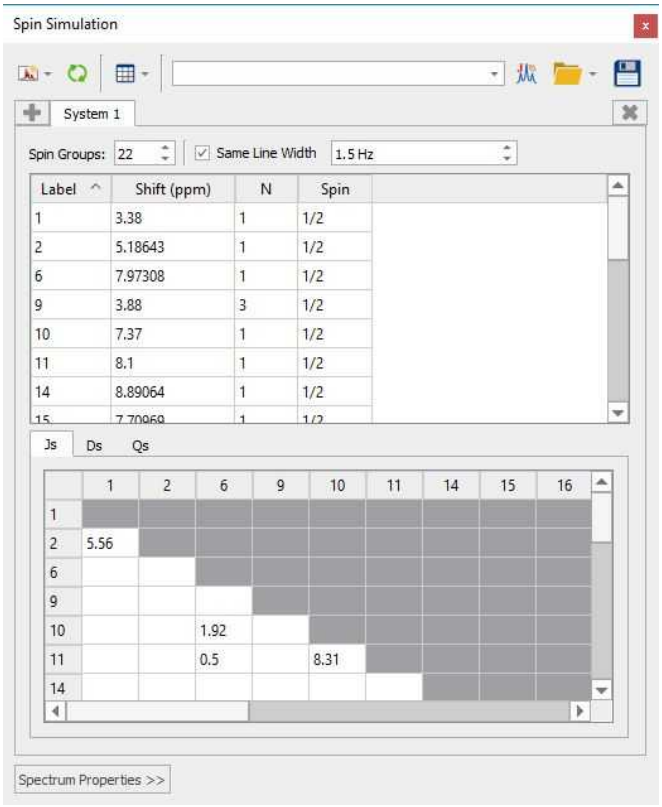
Take advantage of the full quantum mechanics simulations available within the Mnova GUI. Display spectra and simulate higher order effects and strong coupling for any size spin systems

You can see (in the picture below) the higher order effects observed in the 1H predicted spectrum of the cholesterol:



After having run an NMR prediction, the Spin Simulation panel will be automatically populated with the predicted values:

© 2024 MESTRELAB RESEARCH



Modifications done in the spin simulation panel will be reflected in the 1H Prediction table (an vice versa); those changes will be graphically updated in the applicable synthetic spectrum.

See also:
[How to predict a spectrum with NMRPredict Desktop?]

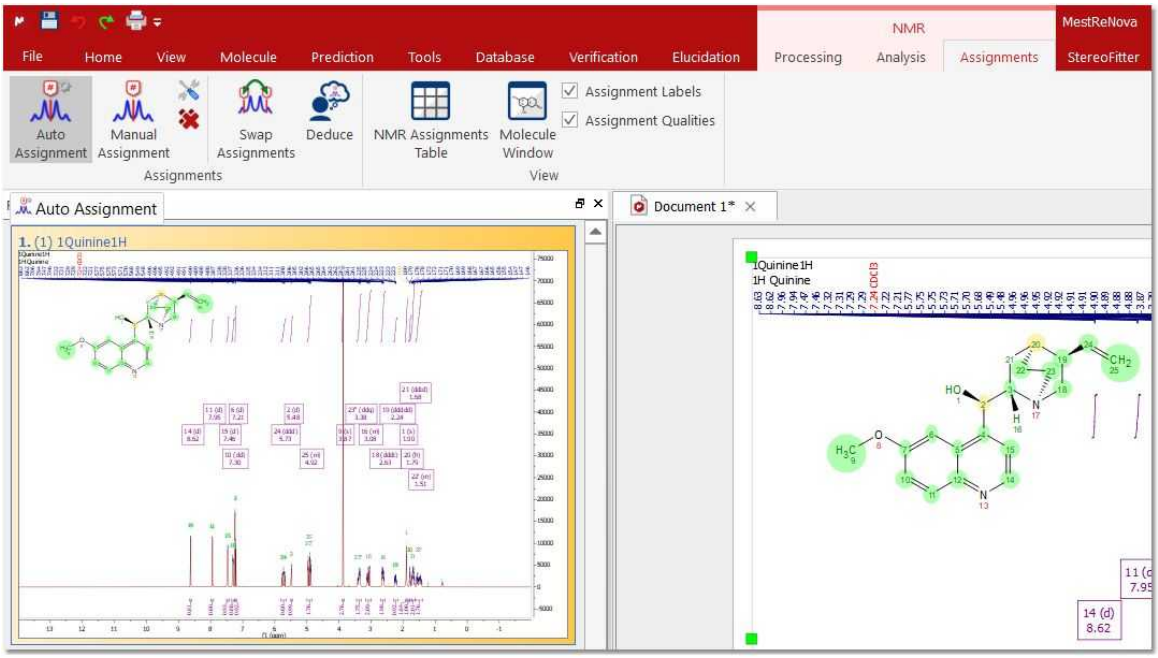
13.2.2 Automatic Assignments

(NMR plugin is needed)

Mnova automatically does atom to peak assignments to 1H, 13C and HSQC spectra.

Just load your NMR spectrum (1H, 13C and HSQC; alone or combined) with the applicable molecular structure and follow the menu 'Assignments/Auto Assignments':

© 2024 MESTRELAB RESEARCH



Mnova will automatically run a multiplet analysis and will fully assign your spectra with the applicable molecular structure. The multiplet boxes will be renamed with the corresponding atom number of the molecule and you will get an assignment label with the atom number over the applicable peak. In addition, hovering the mouse over the atom will highlight the applicable signal in the spectrum.

See also 'How to carry out assignments?'

13.2.3 Predict & Highlight

You will find the quinine datasets under the 'File/Help/Download Example datasets' menu. NMR plugin is needed.

This feature will calculate in the background a simulation of the spectrum of the molecular structure present in the spectral window, highlighting the expected chemical shifts when the user hover the mouse over a proton or a carbon. This tool will be very useful to help the user in the process of assigning of NMR spectra.

The user will be able to use this tool with 1D and HSQC NMR spectra just by pasting the applicable molecular structure (quinine, in this example) over the spectrum and following the menu 'Prediction/Predict & Highlight', as is shown in the picture below:

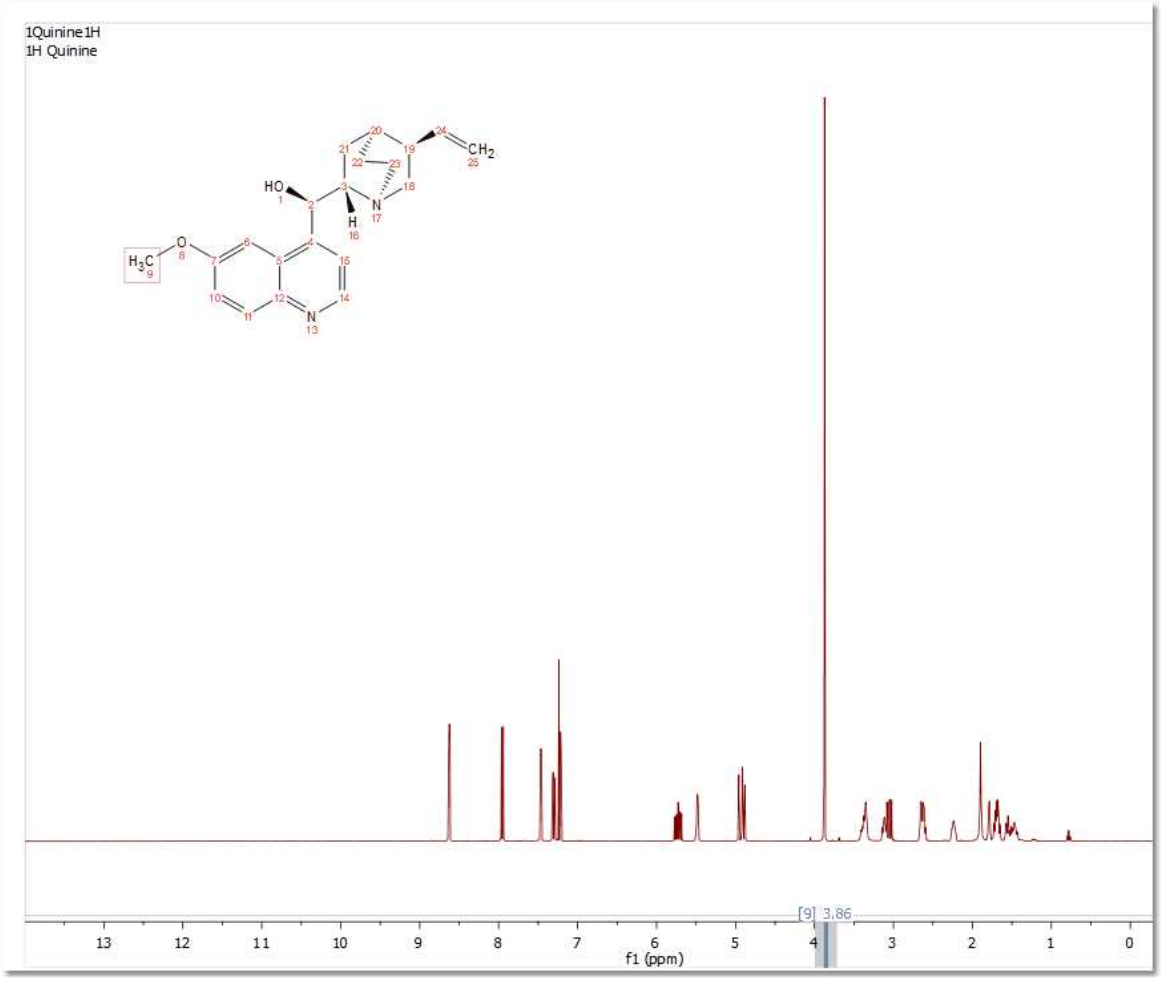


The algorithm of the simulation carried out in the background will be the selected in the 'Prediction/Prediction Nuclei Options' menu.

The same feature can be applied by selecting the molecule on the Compounds Table.

© 2024 MESTRELAB RESEARCH

After having applied this feature, the user will be able to notice that hovering the mouse over the atom will highlight the applicable simulated area in the spectrum. So the user will know that this signal will correspond with the proton of the carbon number 9 in the quinine.



The user will be able to apply the same feature with a ^{13}C or HSQC spectrum.

13.2.4 Predict & Compare

(NMR plugin is needed)

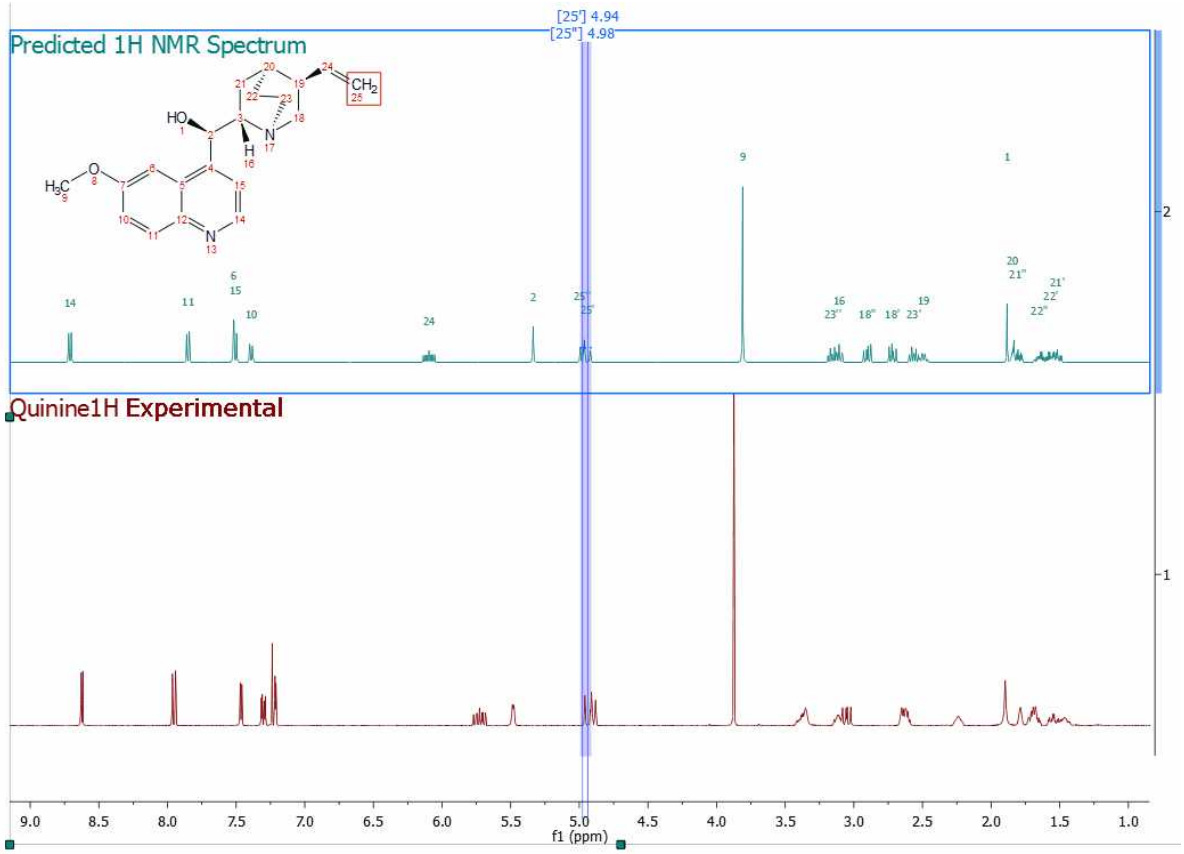
This feature will display in stacked mode, the experimental dataset and a simulation of the spectrum of the molecular structure present in the spectral window, highlighting the expected chemical shifts when the user hovers the mouse over a proton or a carbon. This tool can be used of assistance in the process of assigning ^{1}H and ^{13}C NMR spectra.

The user will be able to use this tool with ^{1}H and ^{13}C NMR spectra just by pasting the applicable molecular structure (quinine, in this example) over the spectrum and following the menu 'Prediction/Predict & Compare'. The same feature can be applied by selecting the molecule from the Compounds Table (without a need for having the molecule pasted on the relevant page, affording users the flexibility to run different predictions without having to generate many instances of the molecule on their report).

© 2024 MESTRELAB RESEARCH

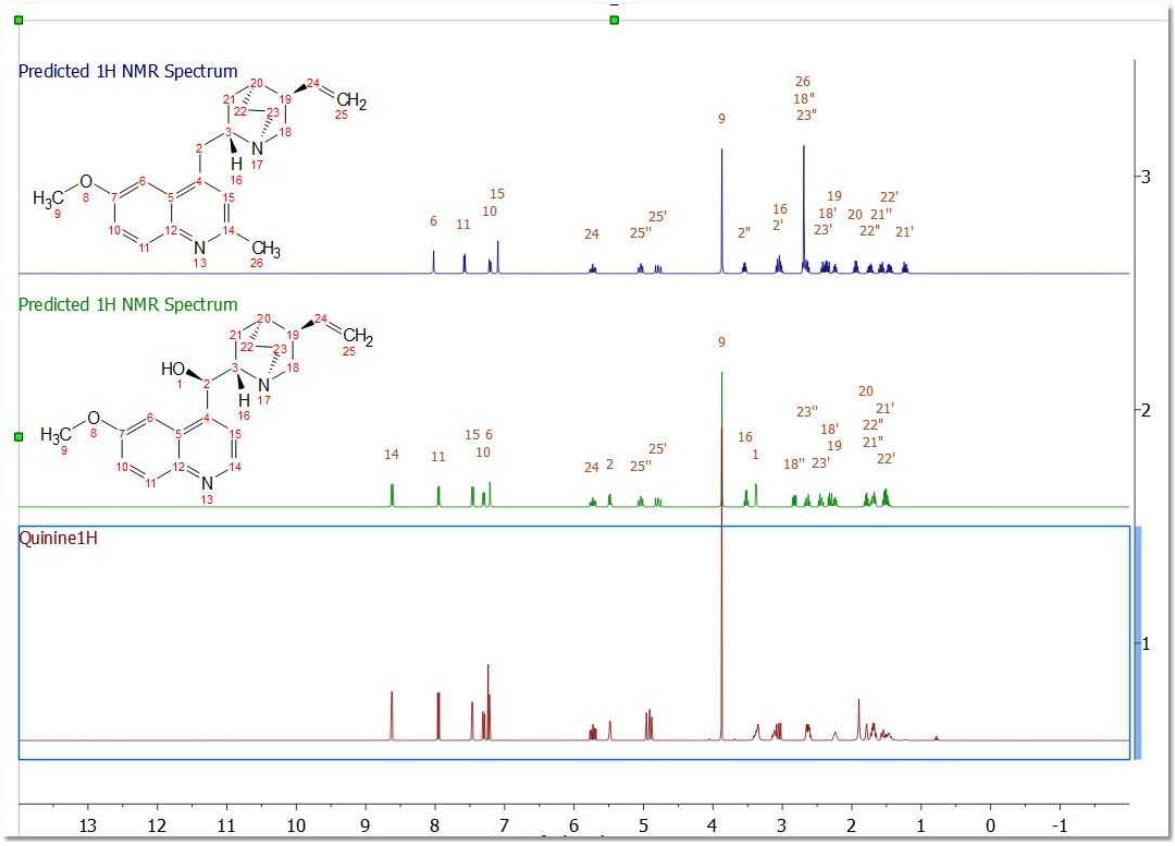
After the prediction is run, Mnova synthesizes the corresponding 1H-NMR spectrum using a rigorous full quantum mechanics method taking into account any second order effect present in the predicted spin(s) systems(s).

For example, in the figure below, you can see the results of running a prediction and comparing the resulting 1H spectrum with its experimental counterpart:



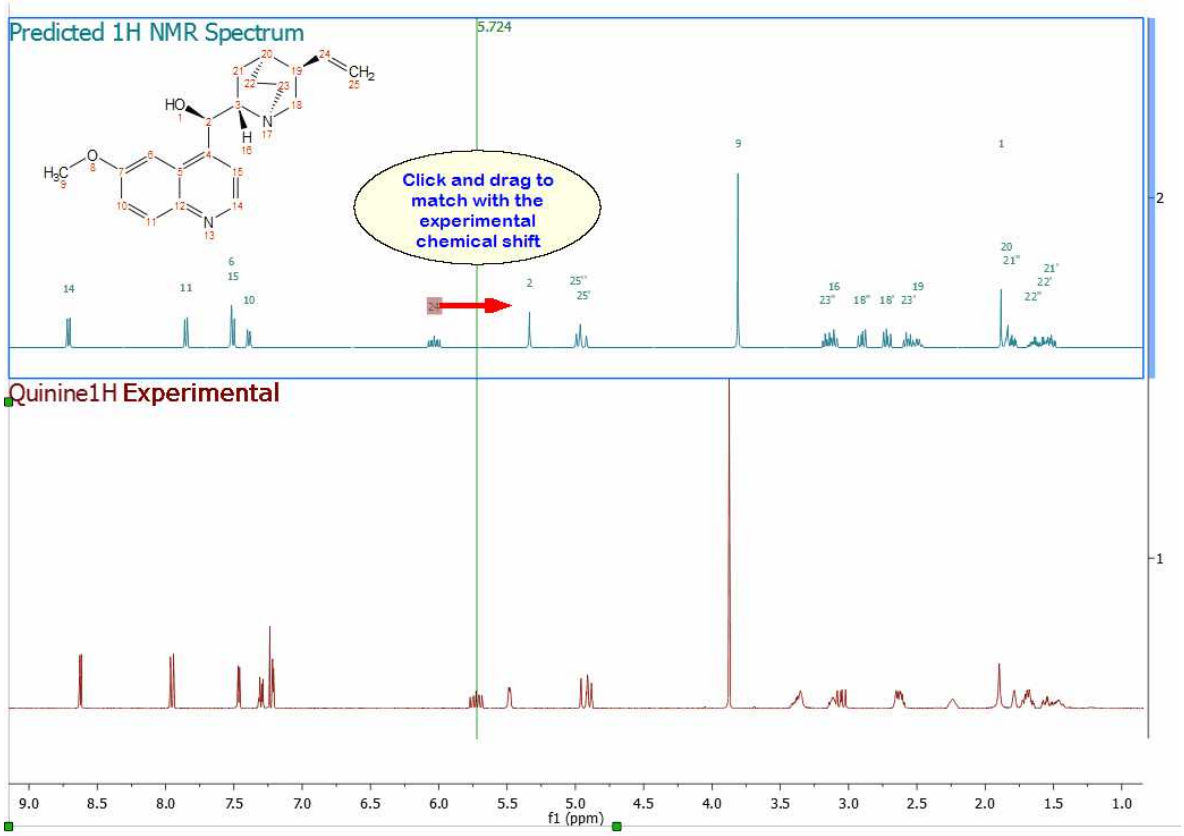
As you can see, you will get the predicted spectrum on top with the proton assignments labels. You can draw (or load) a second molecule over the 'Predict&Compare' result, in order to get a new prediction in the stack:

© 2024 MESTRELAB RESEARCH



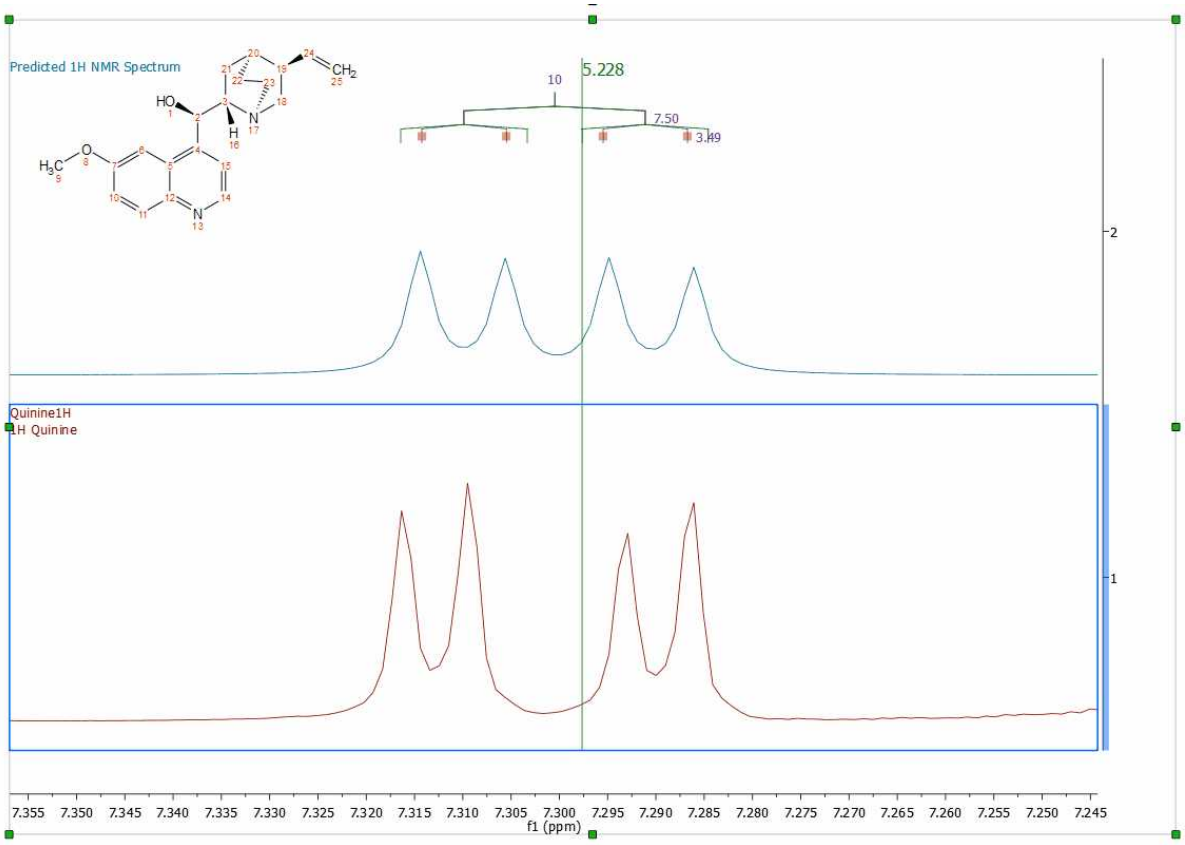
Hovering the mouse over the assignment labels and clicking&dragging will change the chemical shift in in real time:

© 2024 MESTRELAB RESEARCH

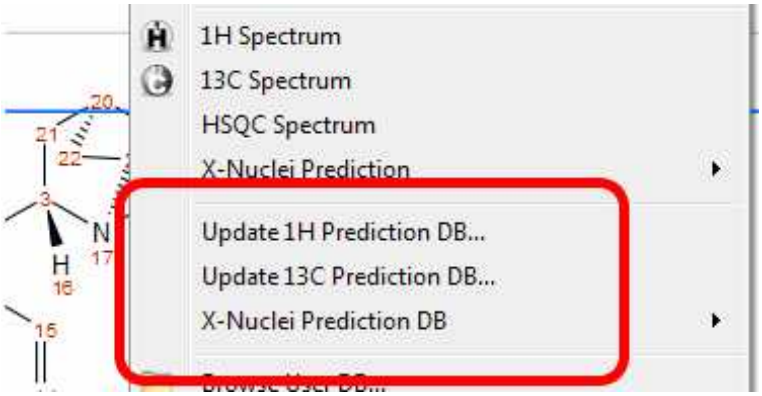


Even you can graphically modified the predited coupling constants (just by clicking&dragging) if you have selected the option to show the coupling tree in the Multiplet properties

© 2024 MESTRELAB RESEARCH

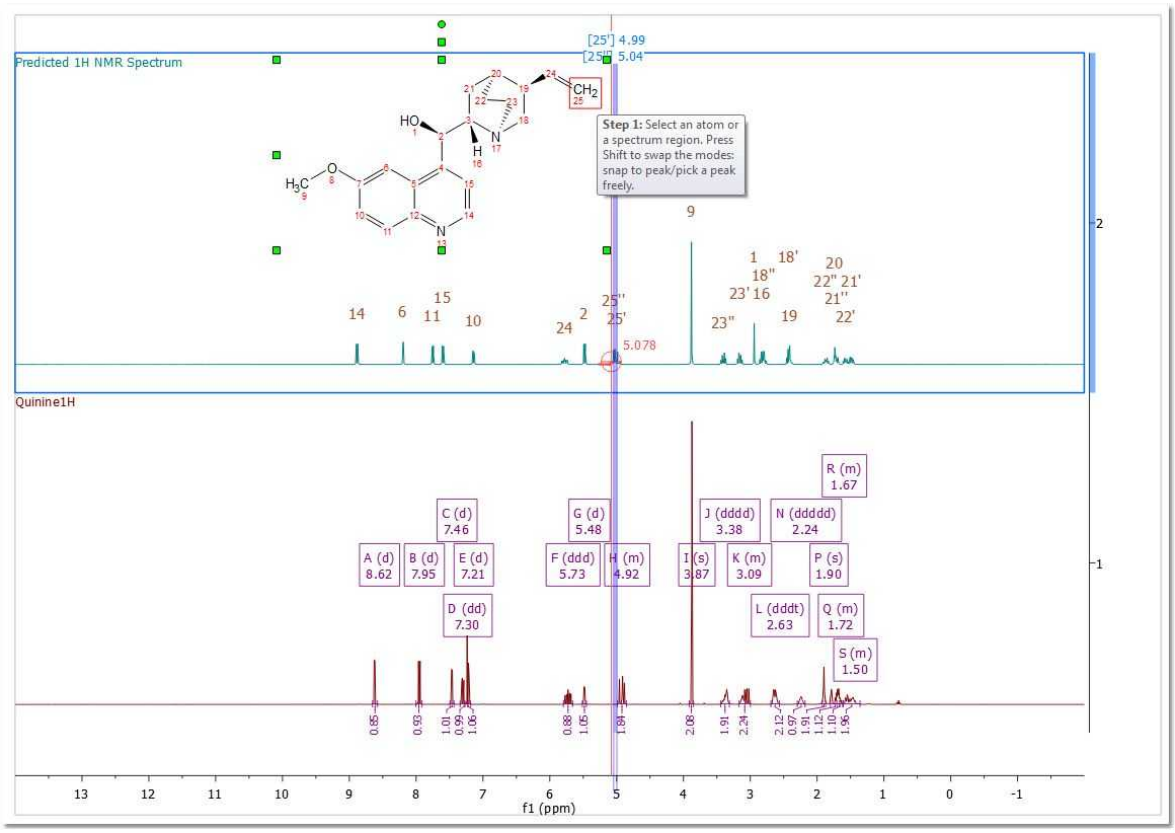


Once you have modified the prediction, you can update those changes to the prediction Database to enhance the prediction results of similar structures. To do it, just right click on the molecular structure and select 'Update Prediction DB':



If you load a 1H-NMR spectrum with a multiplet analysis and run Predict and Compare; you will get a stack plot with the multiplet boxes displayed in the experimental spectrum, so you can easily assign those multiplets to the molecular structure and finally update the 'Prediction DB' if you wish:

© 2024 MESTRELAB RESEARCH



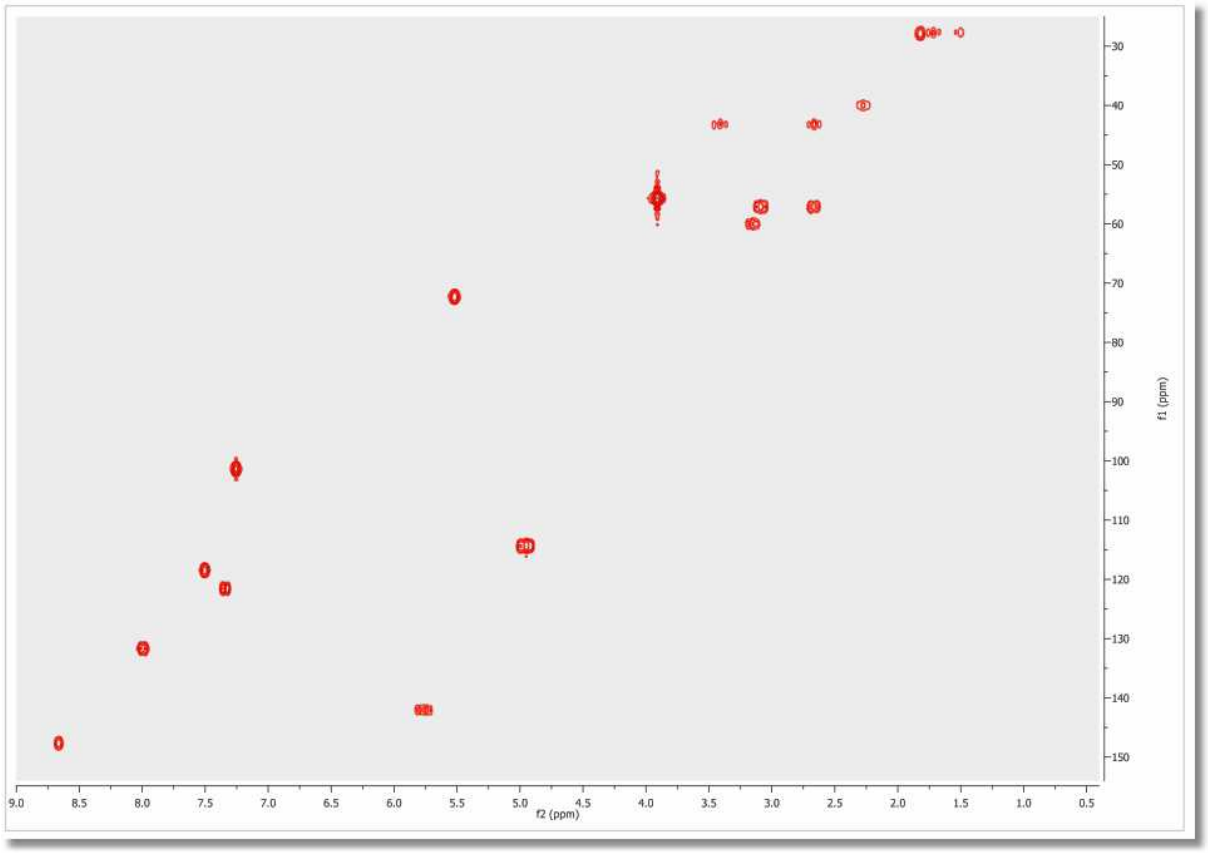
13.2.5 Predict & Verify

This chapter describes a new feature to validate molecular structures, which is based on the concurrent analysis of two simulated 1D (1H and 13C) NMR spectra and a real 2D NMR one-bond correlation spectrum, such as HMQC or HSQC (experiments which correlate the chemical shift of the proton with the chemical shift of the directly bonded carbon).

The procedure to apply a **Visual Verification** with Mnova is very easy, just load your HSQC or HMQC spectrum, and follow the menu: 'Prediction/Predict & verify/HSQC' with the applicable molecular structure pasted on the spectral window as is shown in the pictures below:

1st: Load the HSQC or the HMQC spectrum in Mnova:

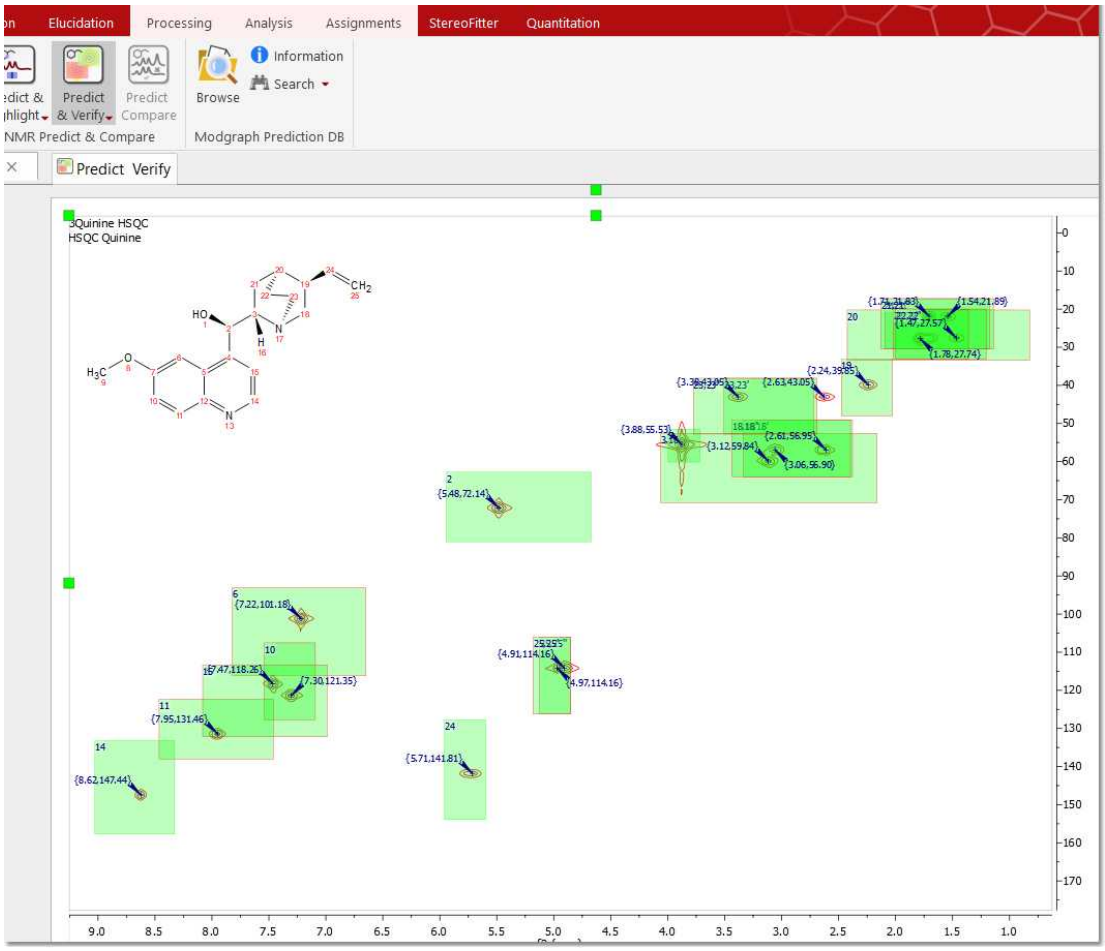
© 2024 MESTRELAB RESEARCH



2nd: Paste (or load) the applicable molecular structure. Bear in mind that molecular structures can be imported as a **.mol** file by simply using the 'Open' command exactly in the same way as this would be applied to a spectrum. Keep in mind that you are also able to copy & paste structures from **ChemDraw, ChemSketch** and **IsisDraw**.

3rd: Follow the menu: 'Prediction/Predict & Verify/HSQC' to obtain the corresponding analysis:

© 2024 MESTRELAB RESEARCH

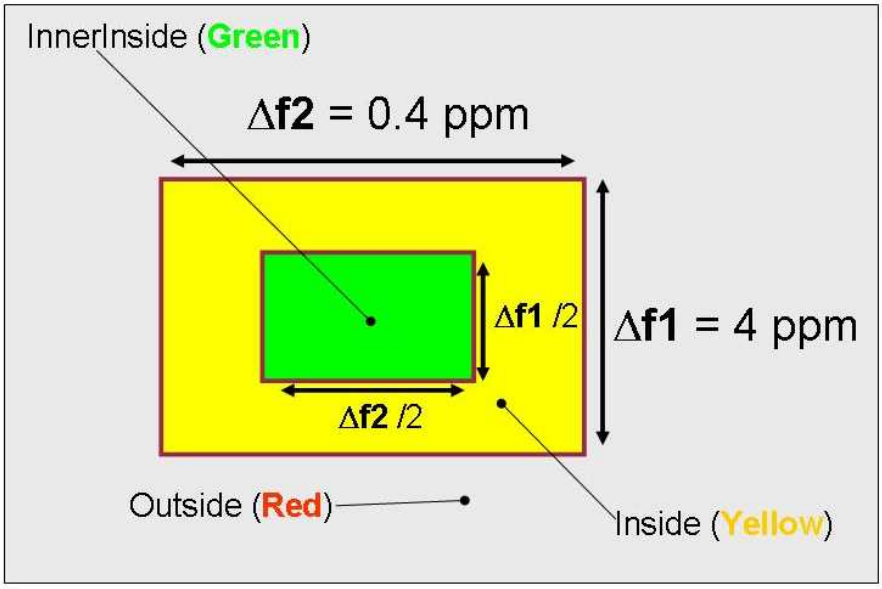


The same feature can be applied by selecting the molecule in the Compounds Table.

As you can see in the picture above, green, yellow and red rectangles will be obtained in the analysis.

A green rectangle will appear when the simulated value fits with the experimental one (the experimental value falls within a rectangular window, drawn around the simulated value, with dimensions of 0.2 ppm along the proton dimension and 2 ppm along the carbon dimension). A yellow rectangle will be displayed when an experimental peak falls within a window which is between 0.2 and 0.4 ppm along the proton dimension and 2 and 4 ppm along the carbon dimension. Finally, the red rectangles are displayed when no experimental peak is found within the latter rectangle, with the simulated value at its center and dimensions of 0.4 ppm along the proton dimension and 4 ppm along the carbon dimension.

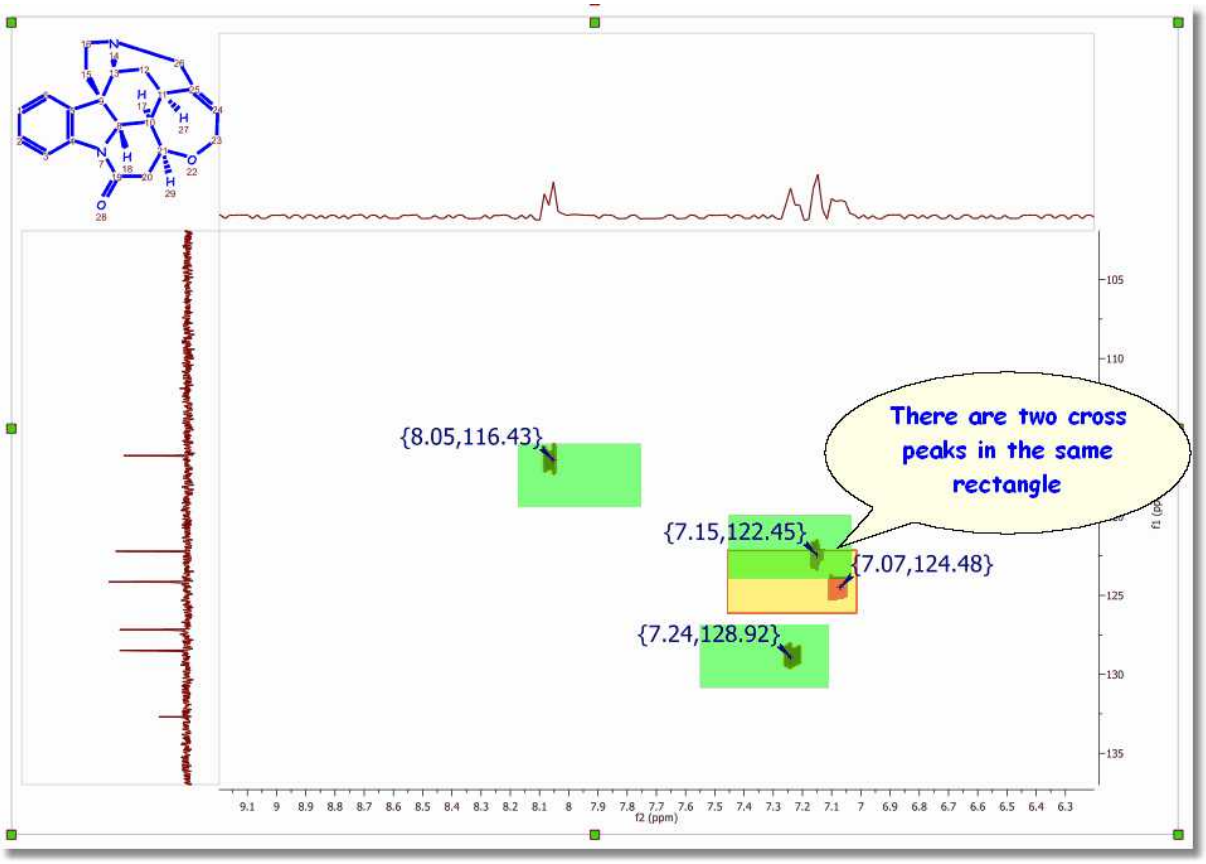
© 2024 MESTRELAB RESEARCH



For example, if we have a real cross peak in the HSQC spectrum at (1.00, 10.0) ppm and a simulated value of (1.05, 10.2) ppm, then we shall obtain a green rectangle, because there is a experimental peak within the rectangle with a predicted peak at its center and sides of 0.2 ppm along the proton and 2 ppm along the carbon dimensions, which we will call 'inner inside'. If we would have a simulated value of (1.25, 11.0) ppm, then we would get a yellow rectangle drawn around the simulated value, as the experimental peak values would fall outside the 'inner inside' but within the area, drawn around the predicted peak, with sides of 0.4 ppm along the proton dimension. In this case the carbon simulation would be fine, but we would get a yellow rectangle due to the deviation in the proton prediction. If the simulated value for this cross peak was (1.41, 15.0) ppm, then we would obtain a red rectangle.

Finally, we shall get a rectangle with a red border when more than one experimental cross peak falls within the green or yellow rectangles drawn around a predicted peak, as is shown in the picture below:

© 2024 MESTRELAB RESEARCH



The user also has the ability to display any combination of rectangles, such as only red rectangles or green and yellow rectangles, for example. These options are available from the 'Prediction/Predict & Verify' menu.

Bear in mind that the results reported by visual verification will depend on the prediction algorithm used in the spectral simulation.

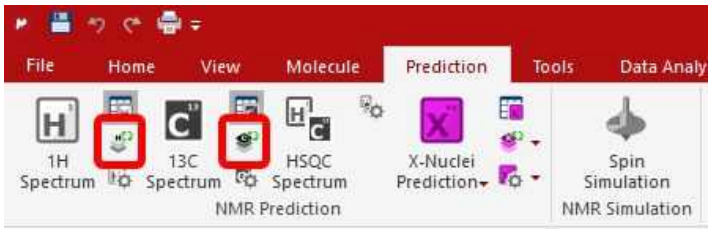
13.2.6 Prediction Database

This feature will allow the user to build databases with experimental assigned spectra which can be used to enhance the prediction results of similar structures.

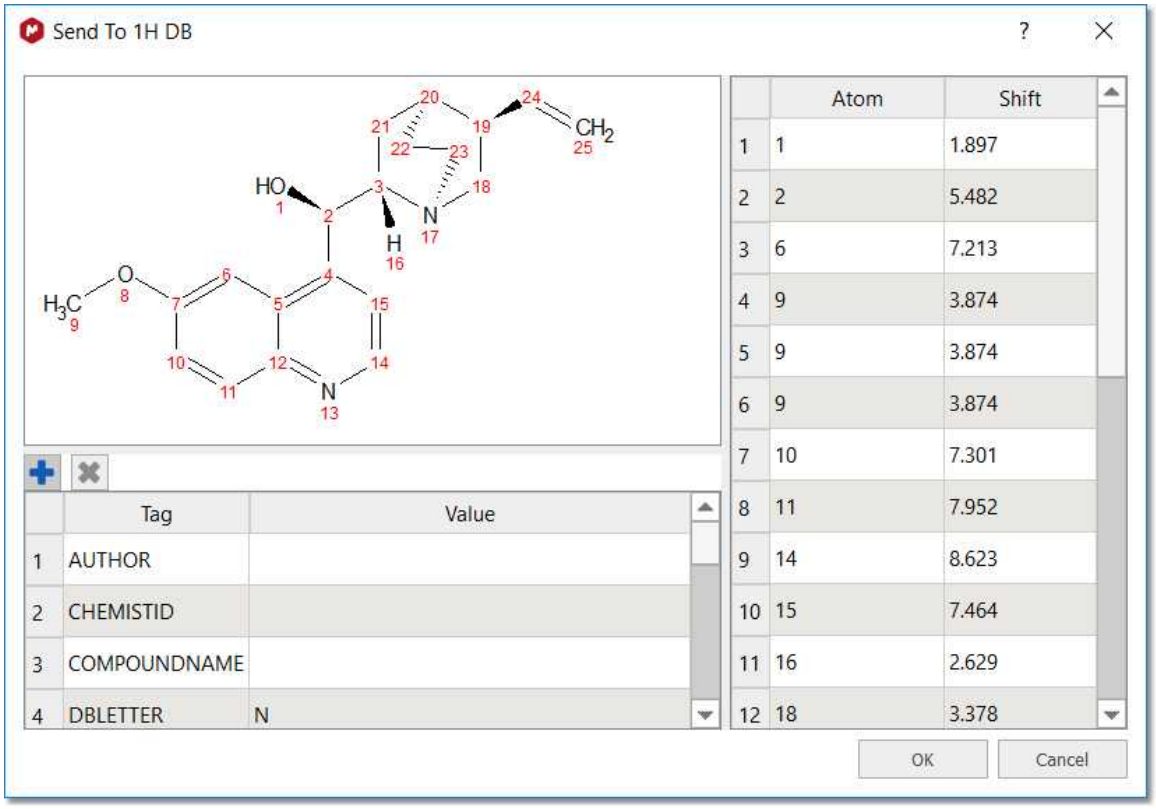
You can build ^{1}H, ^{13}C and X nuclei (^{11}B, ^{15}N, ^{17}O, ^{19}F, ^{29}Si, ^{31}P) NMR databases from any Mnova document containing a molecular structure with the resulting assignments.

Building the database is really easy, just open your NMR dataset with the molecular structure; make the assignments and follow the menu 'Prediction/Update 1H Prediction DB' (you can also update the prediction database from the Well Plates view of the DB plugin by right clicking on any of the plates, or even directly from a predicted spectrum).

© 2024 MESTRELAB RESEARCH

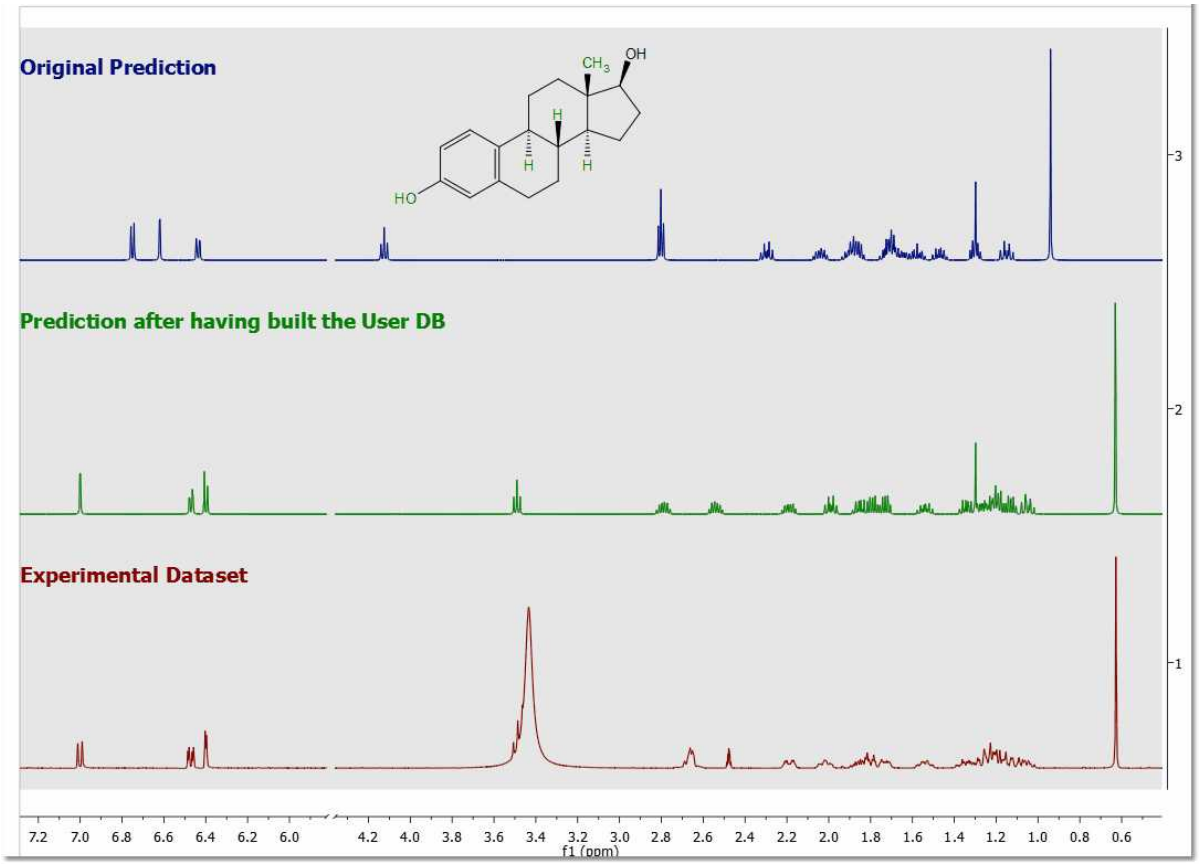


This will display a new dialog box, with information about the molecular structure and the assignments. From here, you can select the DB Letter to save your assignments (from 'N' to 'Z'; the database letter chosen represents the unit which can be excluded from any subsequent prediction or search. Thus, the database letter should be carefully selected on an organization wide basis).



Here we can see the effect of having updated the prediction database. The blue spectrum corresponds with the original dataset, the green one with the original prediction (prior to the update of the Database) and the red one corresponds with the resulting predicted spectrum after having updated the database with the assigned experimental dataset. As you can see, now the prediction is even more accurate!

© 2024 MESTRELAB RESEARCH

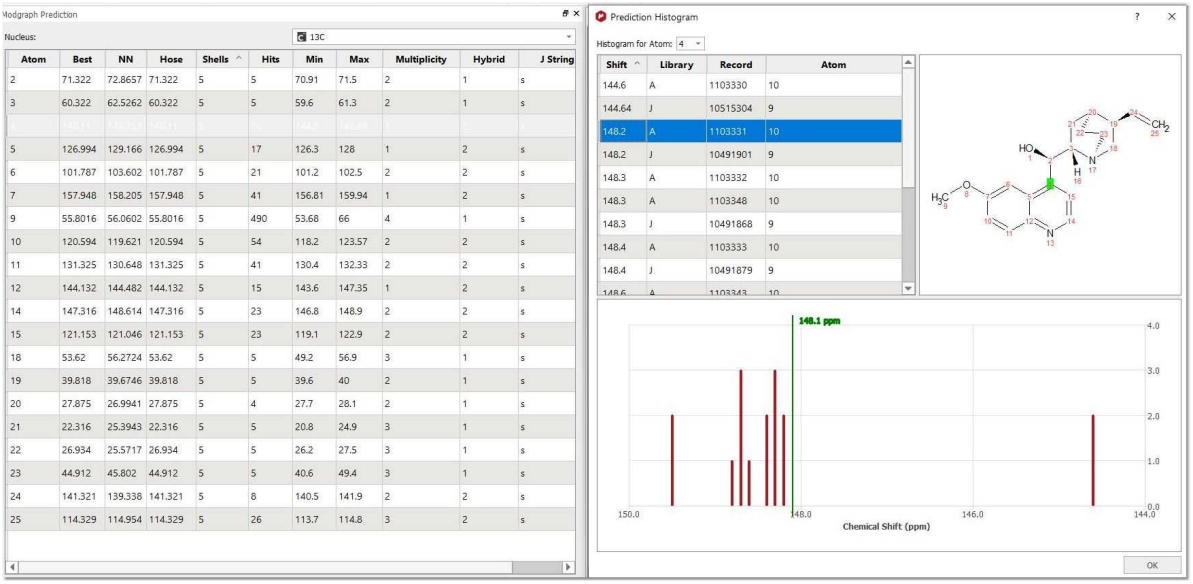


When running a 1H or a 13C prediction you can drill down into any of the 13C user database predictions (if you do not have licenses of NMRPredict Server, you will only see your own prediction Databases; from N to Z). The details can be accessed through Modgraph Predictions table (by following the menu 'View/Tables/Modgraph NMR Predict/Modgraph Drill down Table') or by clicking on the 'Drill down Table' button from the 'Prediction' ribbon.

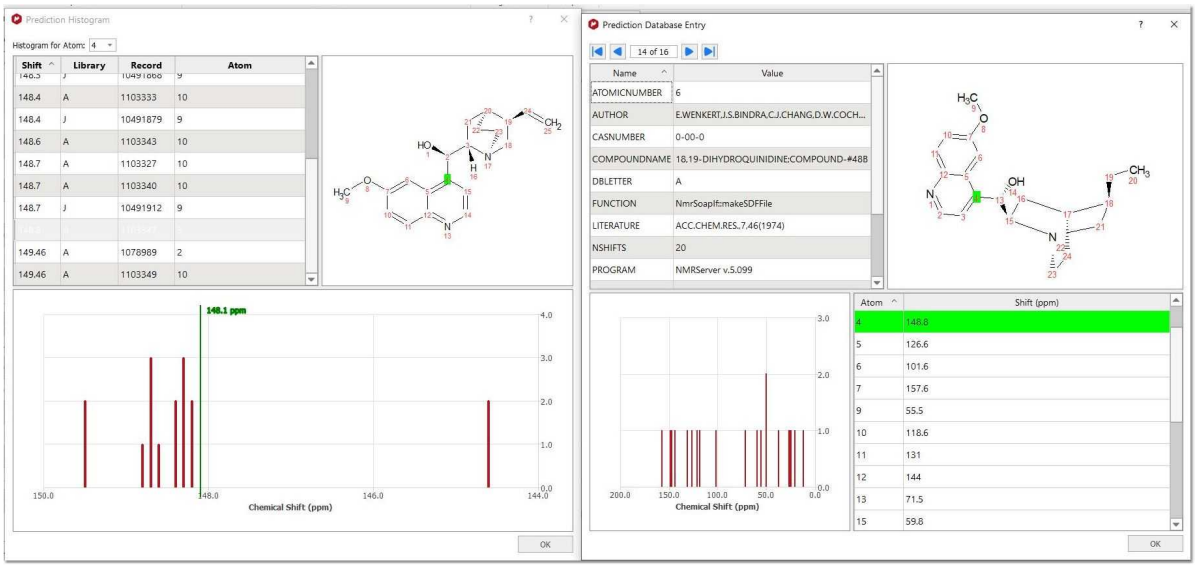


After having double clicked on a row, the whole list of shifts used by Modgraph predictor to generate the final predicted shift will be available.

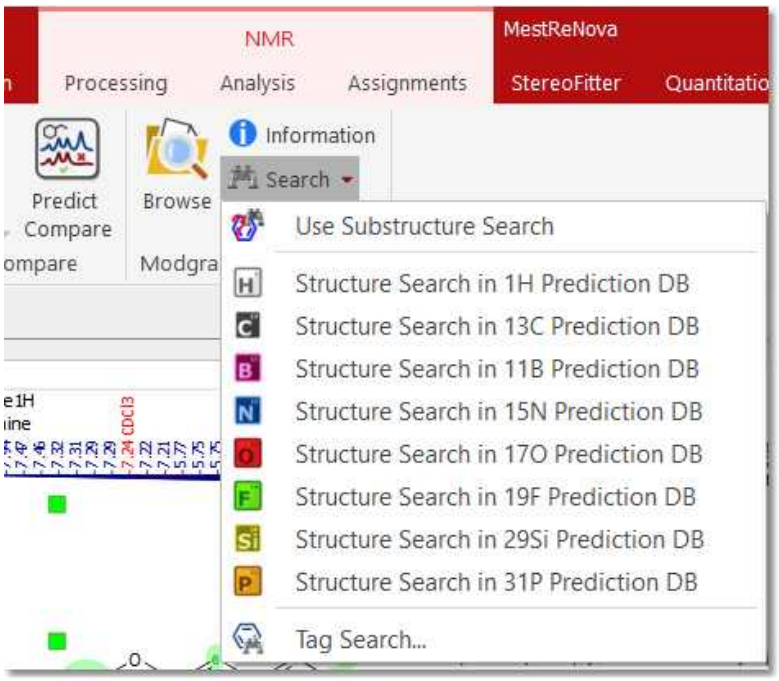
© 2024 MESTRELAB RESEARCH



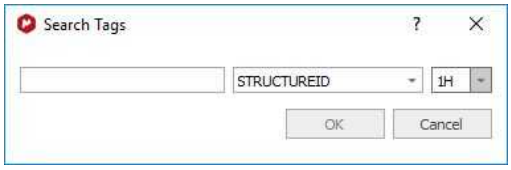
Then, you can access the details of each compound from the database, by clicking on any row in the listed shifts:



Once you have built your assignments database, load a molecular structure with assignments and select any structure search option under the 'Prediction' menu to search for structures and substructures in the DB:

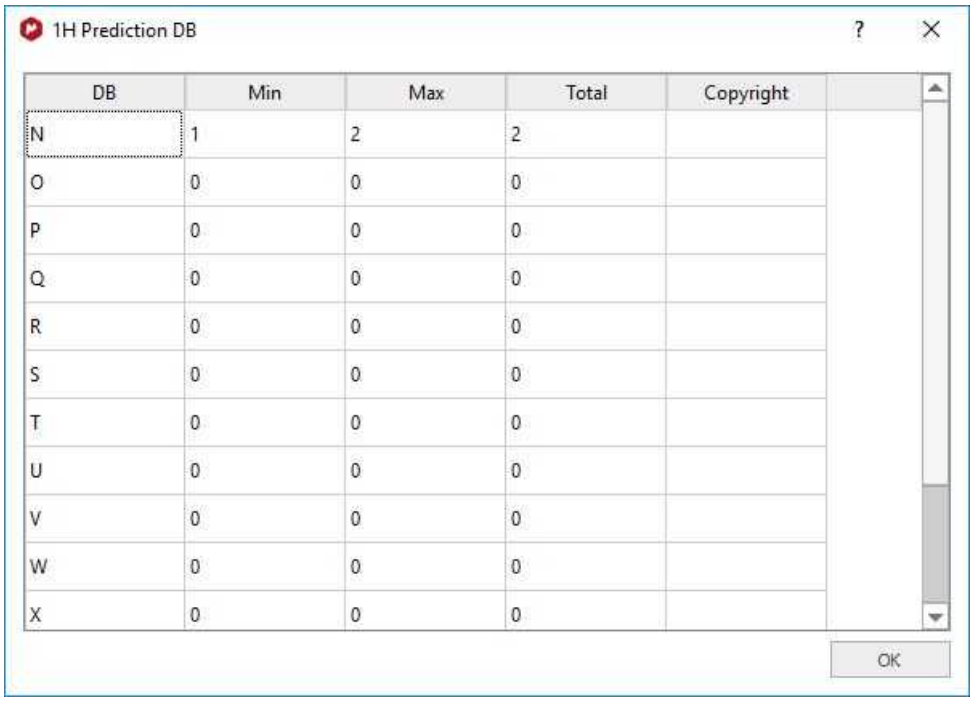


You can also Search for Tags:



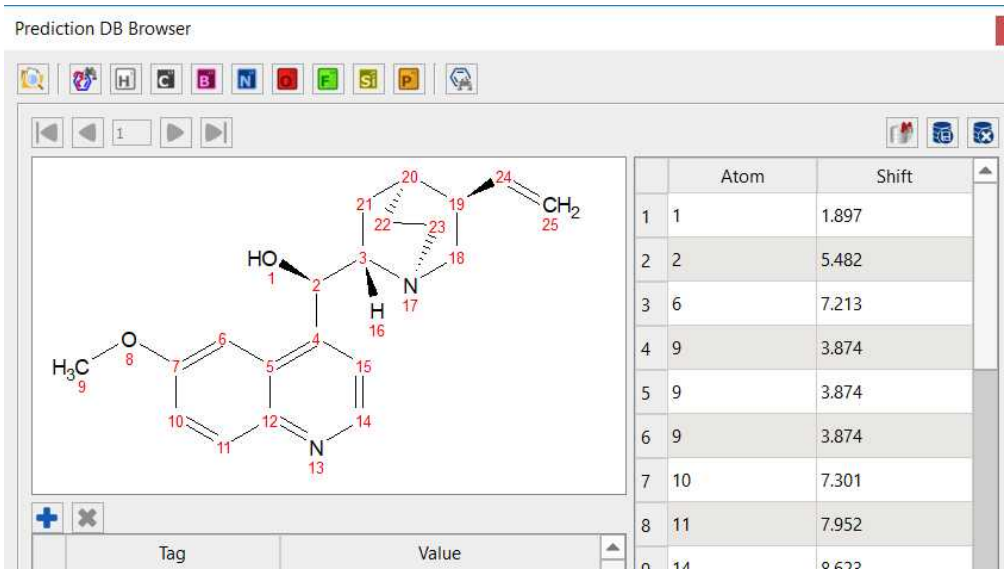
Selecting 'Prediction DB Info' will show you the number of records for each nucleus in each prediction DB:

© 2024 MESTRELAB RESEARCH



Min and Max values correspond to the number of entries in each database, where the deleted entries are also counted.

Follow the menu 'Prediction/Browse Prediction DB' or 'View/Panels/Prediction DB Browser' to navigate through the database:



From this browser, you will be able to navigate through the different compounds in the DB by using the 'blue arrows'. You can paste the assigned molecular structure into Mnova by clicking on this button 
You can edit any assignment by double clicking on the cell and typing the new value. Once you have changed the value, click on this icon  to update the changes in the DB. Click on this button  to remove any molecule from the DB.

© 2024 MESTRELAB RESEARCH

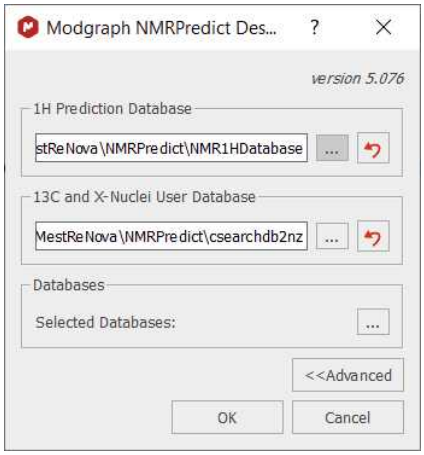
Select another DB letter (from 'N' to 'Z') by clicking on the 'Browse DB' button:



From the Prediction DB Browser you can also run substructures searches, by clicking on the resulting icon for each nuclei:



The location of the prediction database can be selected from the 'Prediction Nuclei Options' menu, after having clicked on the 'Predictor Properties' button.



Please make sure that you have rights to write in the folders above (if not, you will need to change the paths)

Prediction DBs are stored by default in the following locations (with writable permissions).

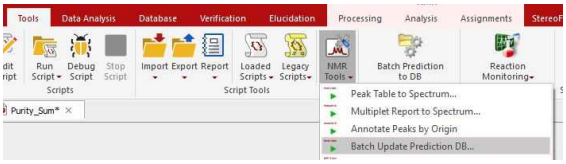
- 1H: C:\Users*Username*\AppData\Roaming\Mestrelab Research S.L. \MestReNova\NMRPredict\NMR1HDatabase

- 13C and X-nuclei: C:\Users*Username*\AppData\Roaming\Mestrelab Research S.L. \MestReNova\NMRPredict\csearchdb2nz

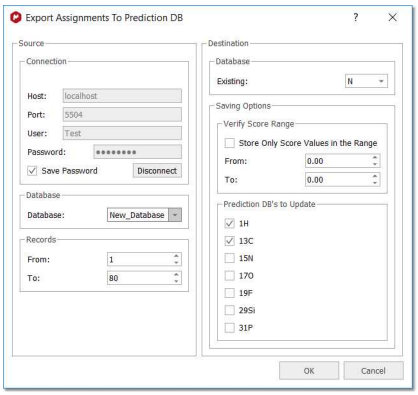
However, if you are getting a red path, please try to find the "*13C and X-nuclei folder*" called "**csearchdb2nz**". Then copy the "**csearchdb2nz**" folder to any other location where you have writing privileges (for example to your 'My documents' folder or directly to your 'desktop'). Finally select the path in the 'Prediction Databases Setup' dialog box and try to update the prediction.

If you have loaded a molecule in Mnova (or a SDF containing several molecules) with assignments, you can update the prediction Database by following the menu 'Tools//NMR Tools/Batch Update Prediction DB':

© 2024 MESTRELAB RESEARCH



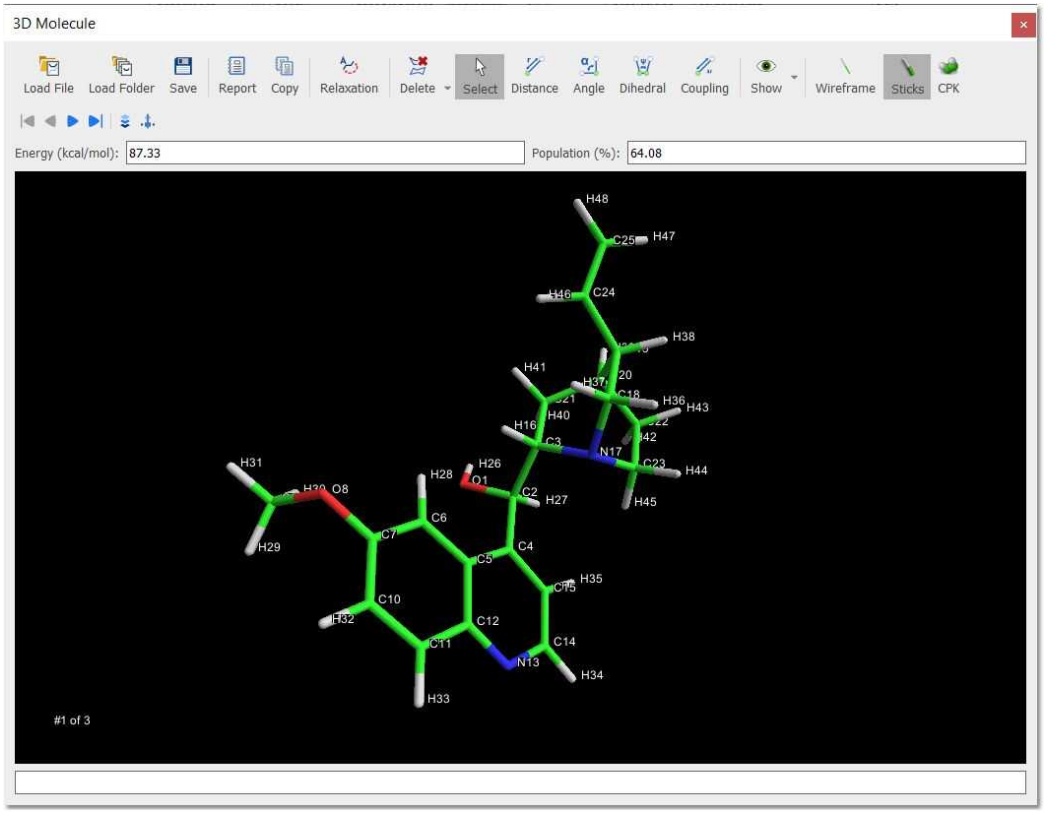
Follow the menu 'Tools/NMR Tools/Batch Update Prediction DB' to export the assignments that you have in your Mnova Database into the Prediction Database:



This script will be useful for example to import external prediction Databases into a Mnova Database and from here export the assignments to the Mnova prediction DB (in combination with other scripts as it is described in this chapter)

13.2.7 3D Molecule Viewer

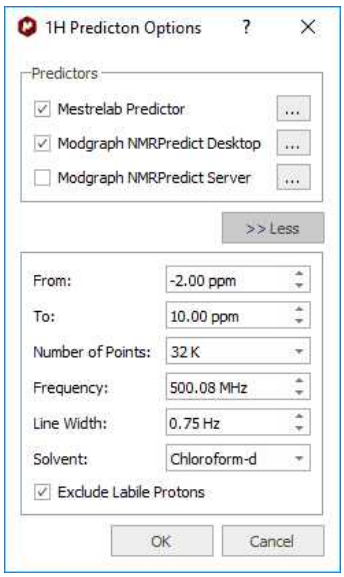
You can activate the 3D molecule viewer after having running a prediction which involves more than one conformer. In that case, just highlight the molecular structure and follow the menu 'View/Tables/3D Molecule':



For further information about this panel, please check this chapter.

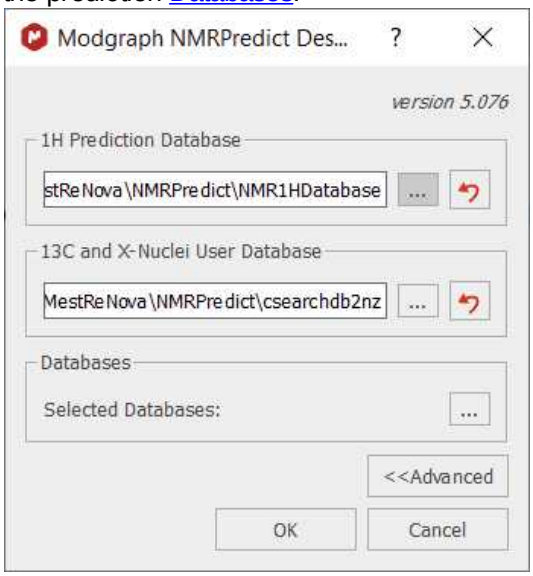
13.2.8 Prediction Options and Properties

Following the menu Prediction/Prediction Nuclei Options will display a dialog box from where you will be able to select the chemical shift limits of the spectral window, as well as other parameters, such as number of points, frequency, Line Width, solvent and minimum coupling constant value. If you are going to predict a ^{1}H-NMR spectrum, you can also exclude the 'Labile Protons' from this window



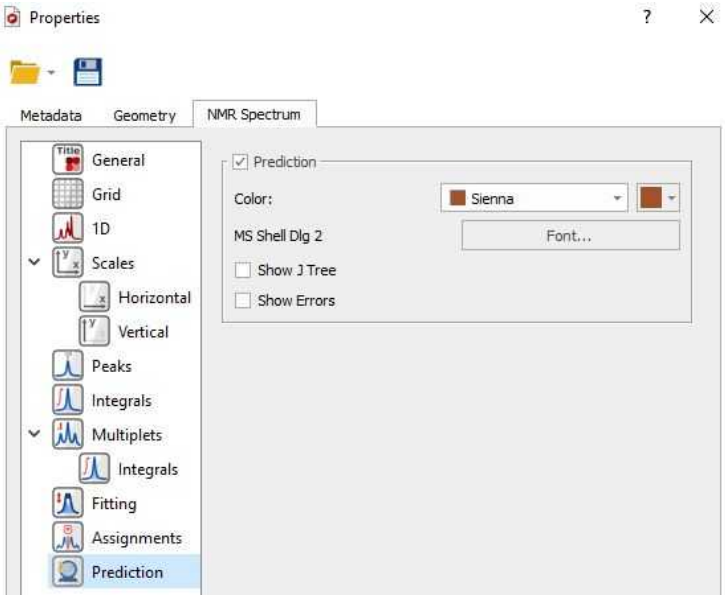
© 2024 MESTRELAB RESEARCH

Clicking on the 'Predictor Properties' button will display a dialog box to allow you to select the location of the prediction Databases:

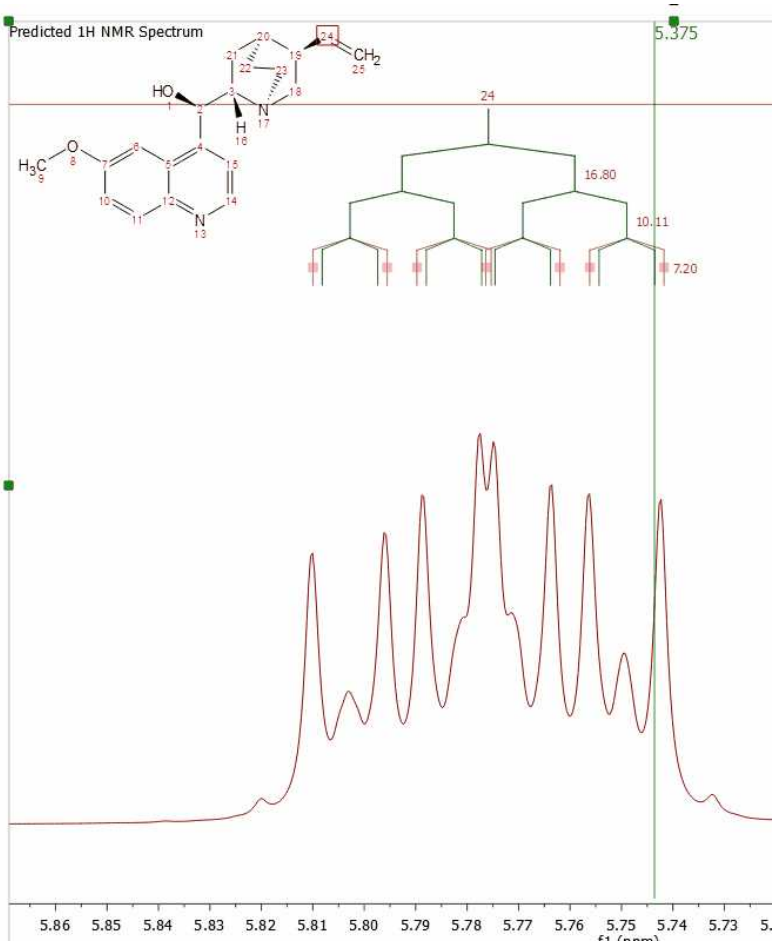


Properties

Double clicking on the predicted spectrum will display the 'Properties' dialog box:



Selecting 'Prediction' from the left tabbed menu will allow you to change the color and font of the prediction labels and also to display graphically the J Tree and the prediction errors:



13.3 NMRPredict Server-Based

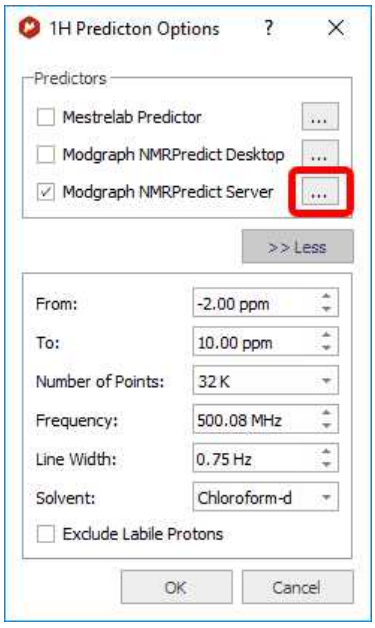
NMR Prediction: The handling of molecular structures within Mnova also allows the user to carry out **spectral predictions**. Although the process described here could be applied to predictions by means of any commercially available or in-house prediction package, currently Mnova will carry out predictions by using the prediction application **NMRPredict**, by Modgraph Consultants Ltd. Please note that this is a separate plugin which must be licensed in addition to Mnova.

© 2024 MESTRELAB RESEARCH

A few notes on NMR Prediction Server-based

- ✓ As opposed **to NMRPredict Desktop, NMRPredict server-based** is a **client-server** application, so to carry out the predictions you will have to log in to the server via a dialog box shown by Mnova. Log in details will be provided to you when downloading **NMRPredict**. This login will only have to be carried out the first time you run a prediction with Mnova. After that, the software should remember your login details and do the log in in the background.
- ✓ Because it is a client-server application and the data are being sent over the internet to the server, industrial testers may wish to have an in-house installation of NMRPredict for security reasons. If this is the case, just write to us with your request at nmrpredict@mestrelab.com.
- ✓ Customers who have their own in-house prediction package are welcome to contact us to discuss a similar, seamless integration of their own prediction package. Please write to us at support@mestrelab.com.
- ✓ Please note that NMRPredict is a different software package to Mnova and therefore, in order to carry out predictions via this package, a license of NMRPredict must be held separately, once the NMRPredict evaluation has expired.

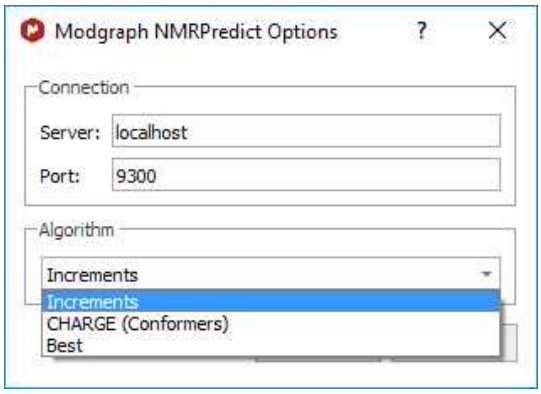
Carrying out of predictions in Mnova is very simple. With a molecular structure open in the active page of Mnova, just go to the 'Prediction' menu and select the applicable Prediction Options; then a dialog box will open (One for each Nuclei).



This open dialog allows the user to set a series of prediction parameters before carrying out the prediction: limits, number of points, frequency of the spectrometer, line width, solvent and the Predictor

methodology. To carry out a server-based NMR prediction the user has to select 'Modgraph NMRPredict Server' in the 'Predictor' edit box.

Clicking on the '...' (red square in the capture above), the user will be able to select the algorithm for the ^{1}H-NMR prediction (Increment or Charge):



The Increment algorithm (based in Ernö Pretsch´s NMR tables) is the same as NMRPredict Desktop uses to predict NMR spectra

The CHARGE program offers ^{1}H-NMR prediction based upon partial atomic charges and steric interactions. This program offers the **first** quantitative prediction of the proton chemical shifts of a variety of organic compounds.

The program works by first generating 3D conformers from a 2D structure through a choice of *force fields* and then predicting proton spectra for all the conformers. The program works by looking at functional groups which have parametrised by Professor Abraham.

To read more about the CHARGE theory, please visit the following link:

http://www.modgraph.co.uk/product_nmr_proton.htm

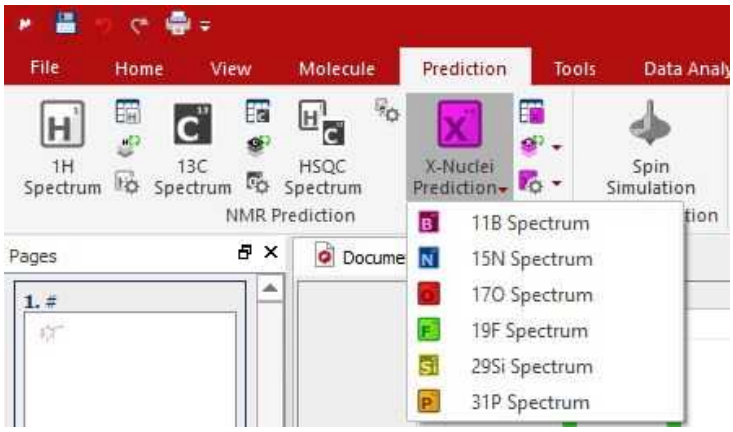


Please remember that:

NMRPredict is a **client-server** application, so to carry out the predictions you will have to log in to the server via a dialog box shown by Mnova. Log in details will be provided to you when downloading **NMRPredict**. This login will only have to be carried out the first time you run a prediction with Mnova. After that, the software should remember your login details and do the log in in the background.

Finally, just go to the 'Prediction' menu and select the desired prediction.

© 2024 MESTRELAB RESEARCH



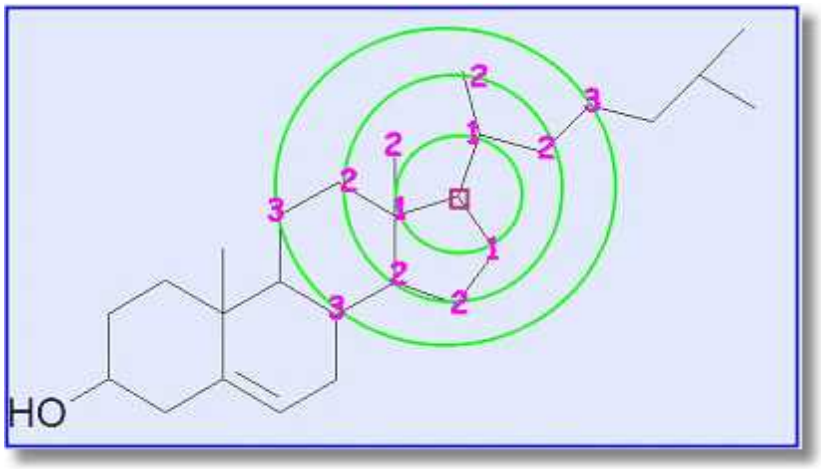
This will send a request to the server, which will run a prediction and return a set of values which Mnova will display as a spectrum on the screen. You will immediately notice that this will be an assigned spectrum, and therefore hovering of the mouse over the atoms will highlight the applicable peak on the spectrum and viceversa.



A few notes on the synthetization of NMR 1H spectra

✓ It is important to note that the simulation carried out by Mnova when displaying the predicted values as a spectrum **is not first order**. The software is carrying out the **full quantum-mechanics calculations** to arrive at the displayed spectrum. This is very computationally intensive and results in such simulation module having a limitation, for 1H spectra, which prevents it from simulating spin systems larger than 13 spins. However, we have developed a new algorithm to overcome this problem.

The prediction of the 13C-NMR is based on the HOSE code approach. The **HOSE** (**H**ierarchical **O**rganisation of **S**pherical **E**nvironments) code approach starts at the carbon atom whose shift is to be predicted, looks one bond away from the carbon and tries to find this same environment in its database. If it is successful it moves two bonds away and tries again and so on until it either comes across something not represented in the database or it reaches the boundary of the molecule.



© 2024 MESTRELAB RESEARCH

The HOSE code approach works very well for query structures which are well represented in the reference collection. If atoms can be predicted to three spheres or more (NMRPredict goes to a maximum of five spheres) the prediction can be considered to be very reliable.

However, if the query structure is not well represented in the database and the atom can only be predicted to one or two spheres the prediction cannot be relied upon at all. Also the HOSE code approach exactly reproduces the contents of the reference database – including every error within the reference database.

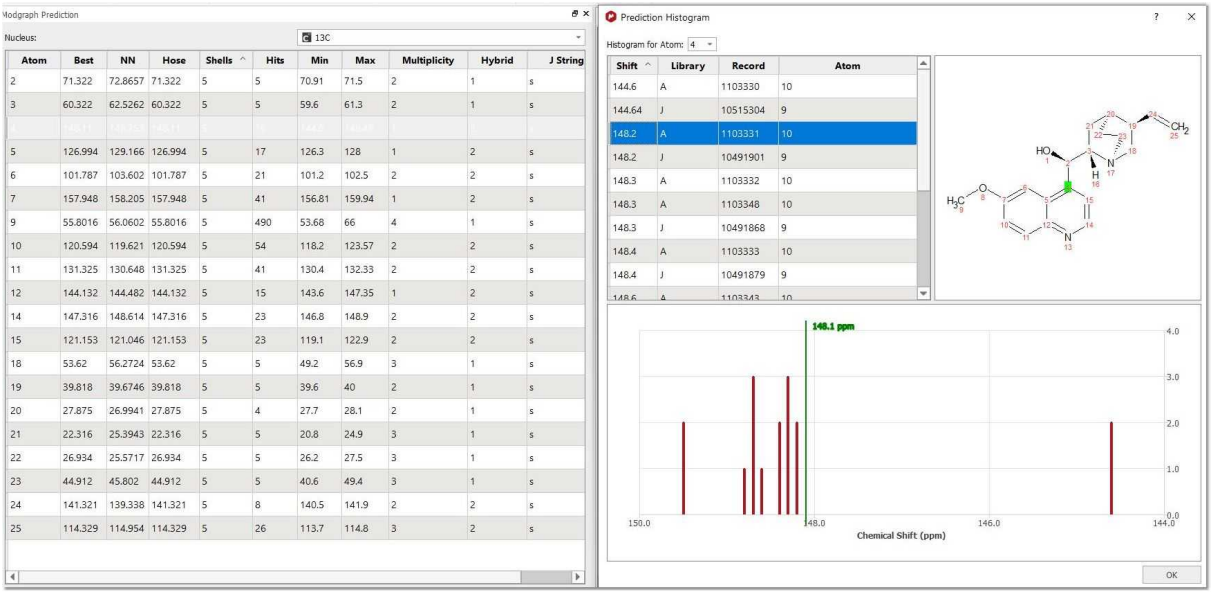
If atoms can only be predicted to one or two spheres the only solution traditionally was to add your own similar data and use that for prediction. NMRPredict also uses a unique Neural Network algorithm, which is much more general and error tolerant than the HOSE code approach and is much more accurate at predicting atoms it has not seen in its database.

13.3.1 Drill down to full 13C databases

A license for Modgraph NMRPredict Desktop Full is needed to access all 13C prediction libraries.

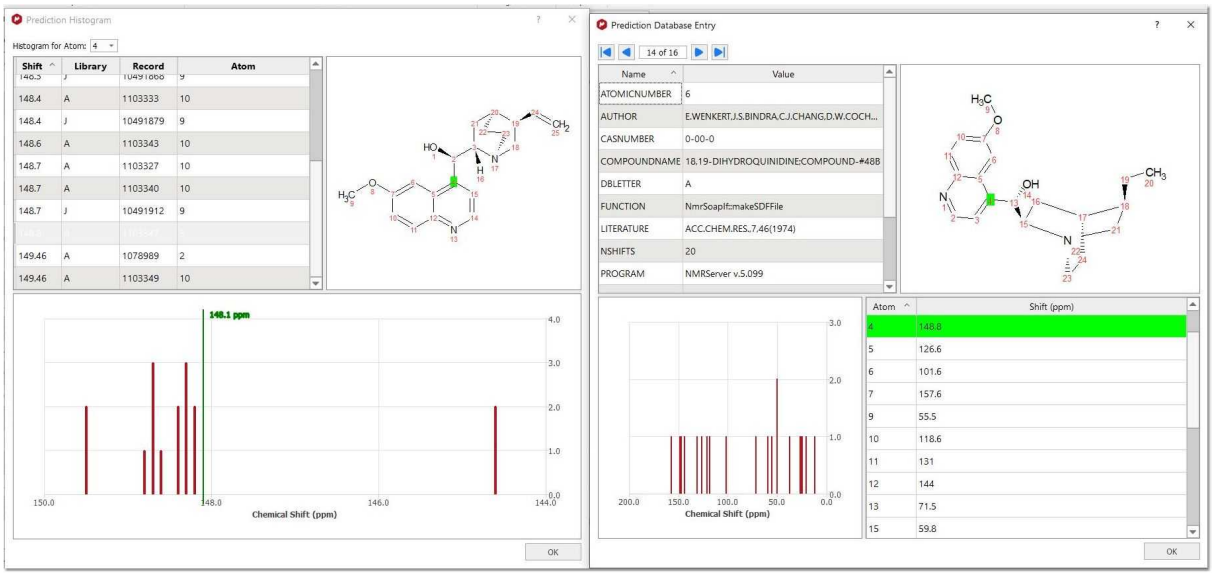
When running a 13C prediction you can drill down into any of the 13C database predictions (A-Z) used by Modgraph NMRPredict Desktop. The details can be accessed through Modgraph Predictions table.

After having double clicked on a row, the whole list of shifts used by Modgraph predictor to generate the final predicted shift will be available.



Then, you can access the details of each compound from the database, by clicking on any row in the listed shifts:

© 2024 MESTRELAB RESEARCH

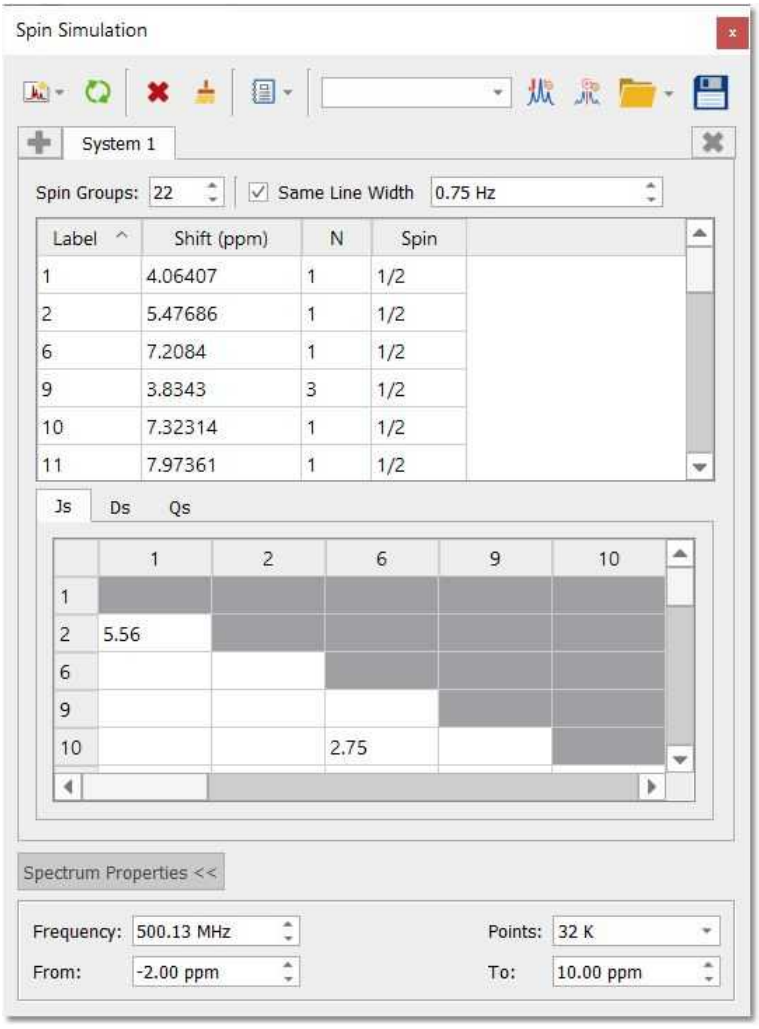


If no license is activated, these details of each compound from the databases can't be accessed.

13.4 Spin Simulation

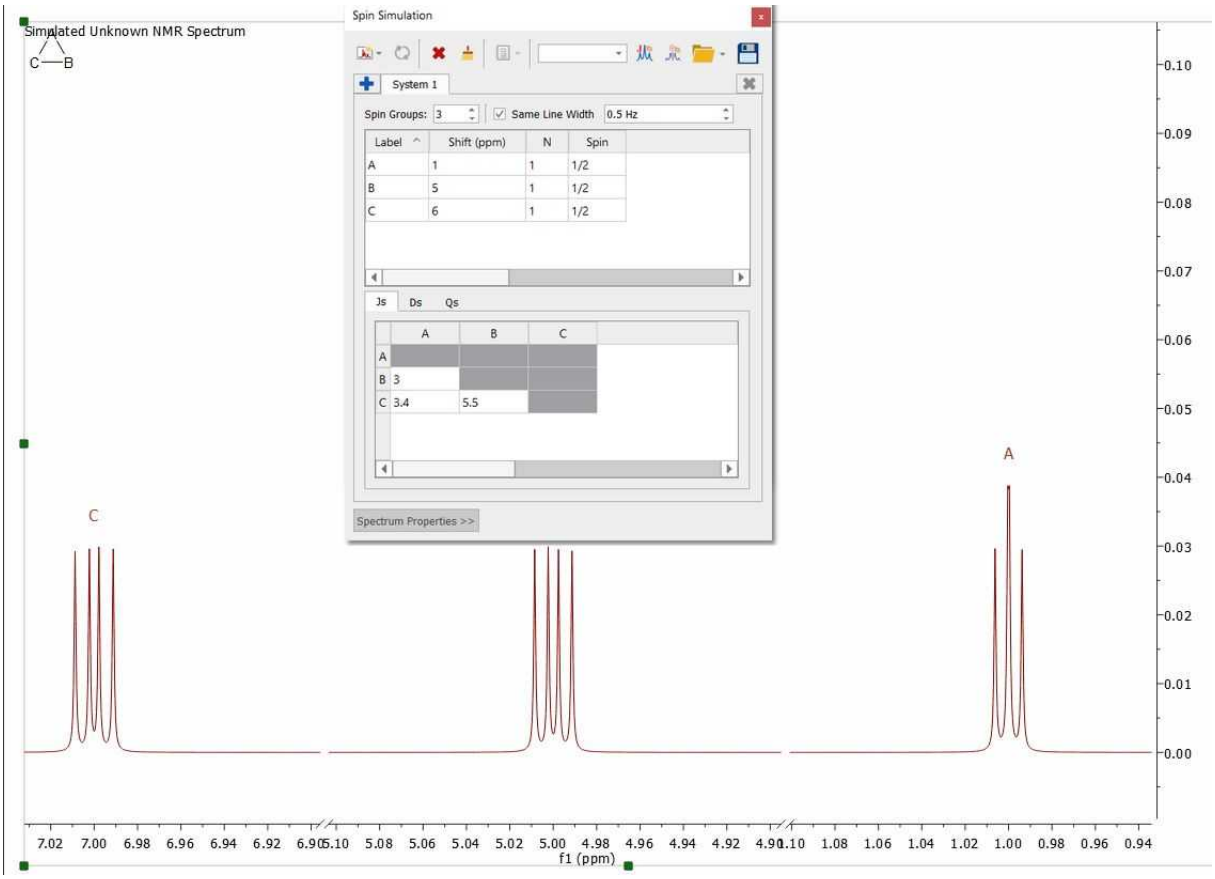
NOTE: The NMRpredict Desktop plugin must be installed (a license for this plugin is not needed).

This module of MestReNova is an efficient simulator for high resolution NMR spectra which can be used by following the menu 'View/Tables/Spin Simulation (or Prediction/Spin Simulation') which will display the applicable dialog box:



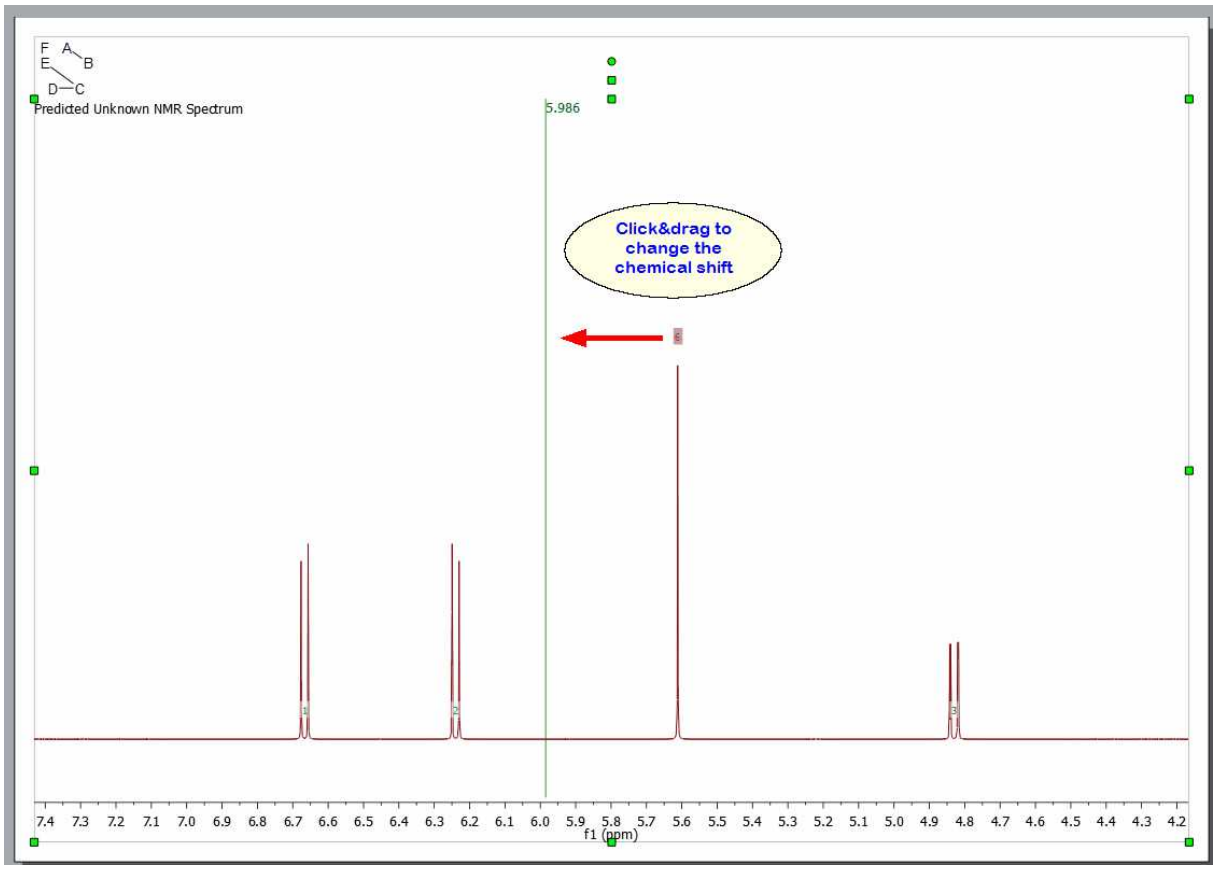
Once there, we can easily simulate a spectrum by entering the desired values. For example, 3 protons (3 spins Groups) called A, B and C, with chemical shifts 1, 5 and 7 ppm respectively. Their coupling constants were J(AB) = 3Hz, J(BC) = 5.5Hz and J(CA) = 3.4Hz. Finally, select the number of points and the spectral limits and click on the 'New Simulation' button to generate the corresponding spectrum:

© 2024 MESTRELAB RESEARCH



Once you have simulated the spectrum, you can modify any value in the table and obtain the new spectrum by clicking on the 'Recalculate' button . Click on the 'delete' button to remove a specific row from the table, or click on the 'clear all' button to start from the scratch. You can introduce 'Dipolar' or 'Quadrupolar' coupling constants and change the 'number of nuclei', 'Spin' and the 'Line Width' values by clicking and entering the desired values on the corresponding boxes. Finally, click on the 'Recalculate' button to update the changes in the spectrum.

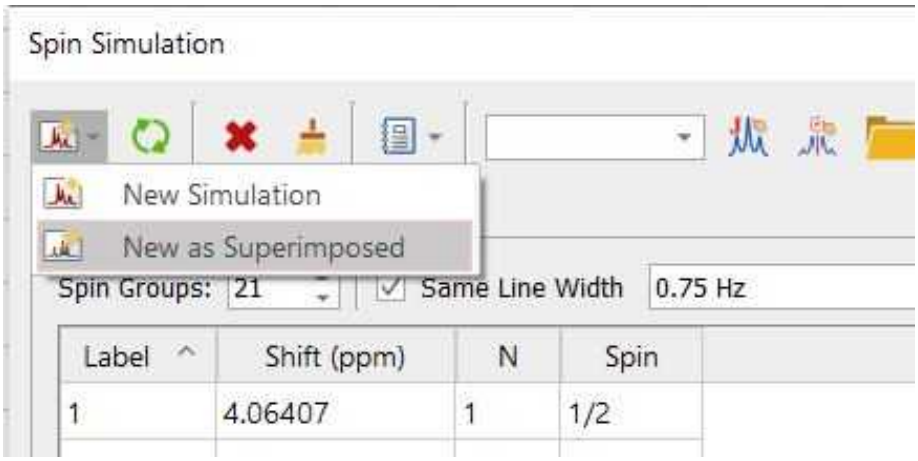
Click&drag the assignment label to change the chemical shift of any signal of the spin-simulated spectrum:



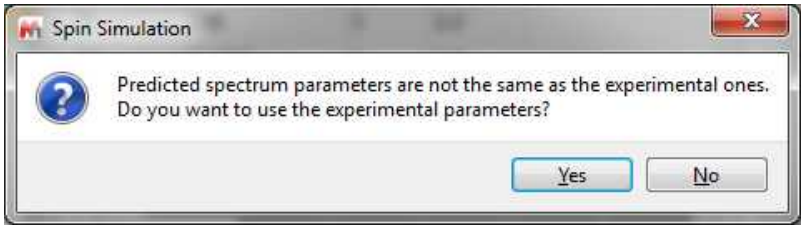
Traditionally, Spin Simulation algorithm were limited to 11-12 magnetic unequivalent coupled particles. However, this is completely insufficient to tackle the 'small molecules' of today. Consequently, simulation nowadays calls for brand new approximate algorithms involving, for example, fragmentation techniques.
Mnova SpinSimulation toolkit includes such sophisticated fragmentation algorithm which makes it possible the simulation of any spin system regardless of its size.

Select 'New as Superimposed' over an experimental spectrum to stack the synthetic spectrum with an experimental one:

© 2024 MESTRELAB RESEARCH

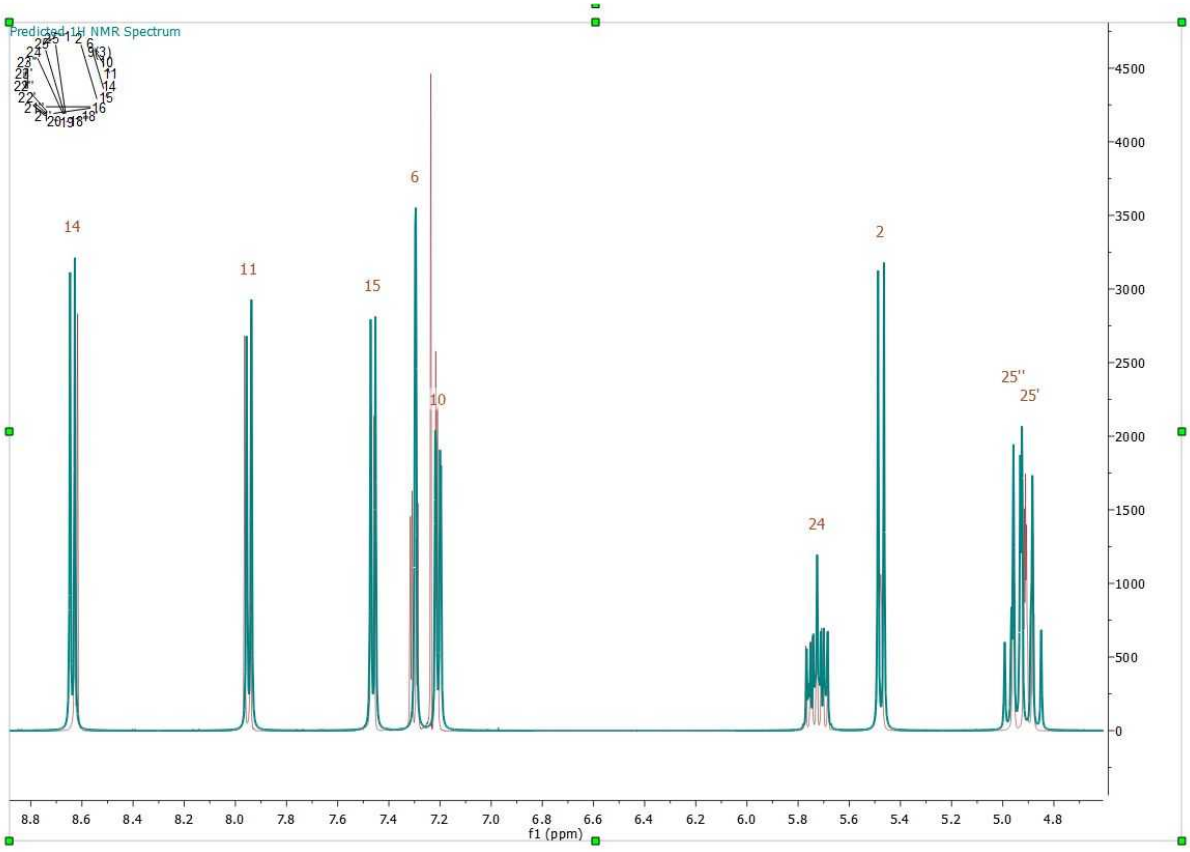


If parameters of experimental spectrum and simulated spectrum are not the same, when you try to generate the calculated spectrum as superimposed a message will be displayed:



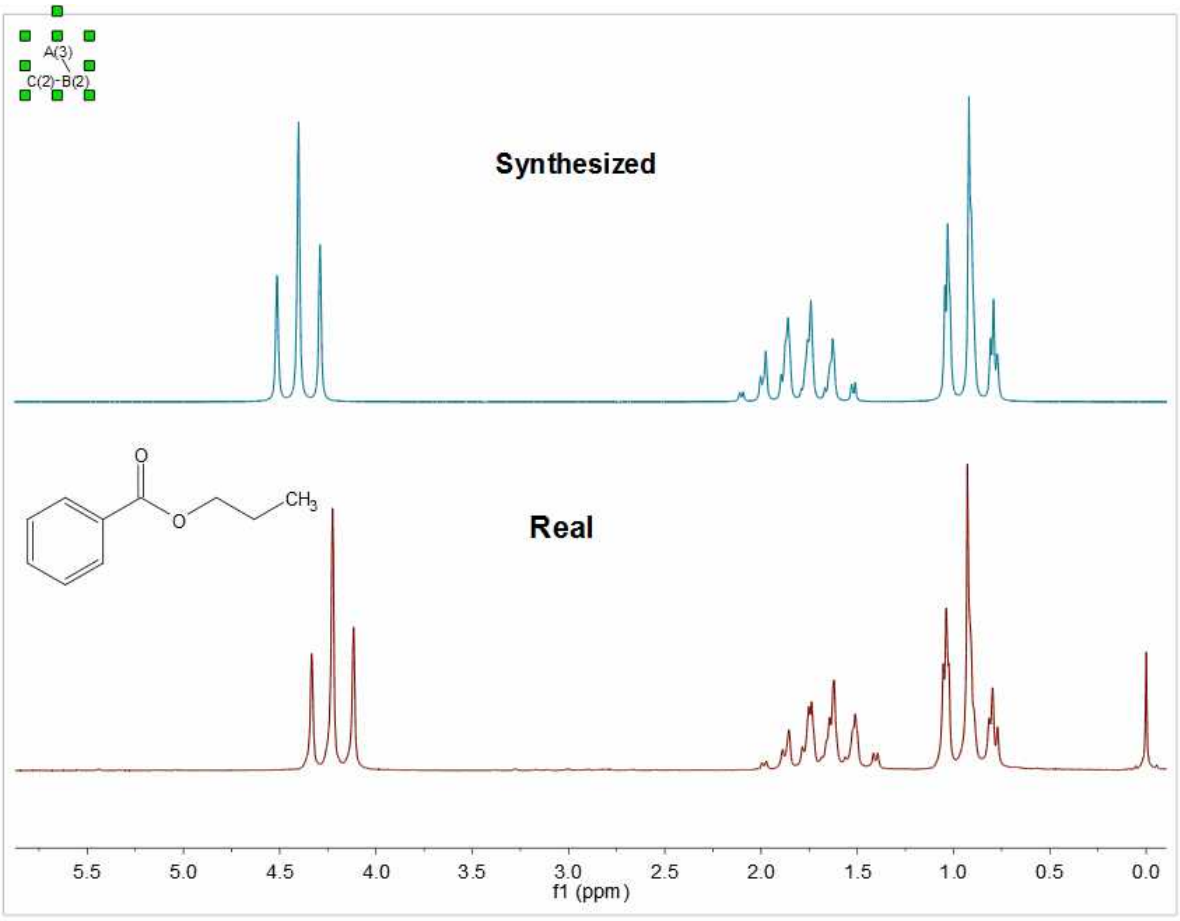
Clicking on Yes, the Spectrum Properties parameters will be modified to match with the experimental ones.

© 2024 MESTRELAB RESEARCH

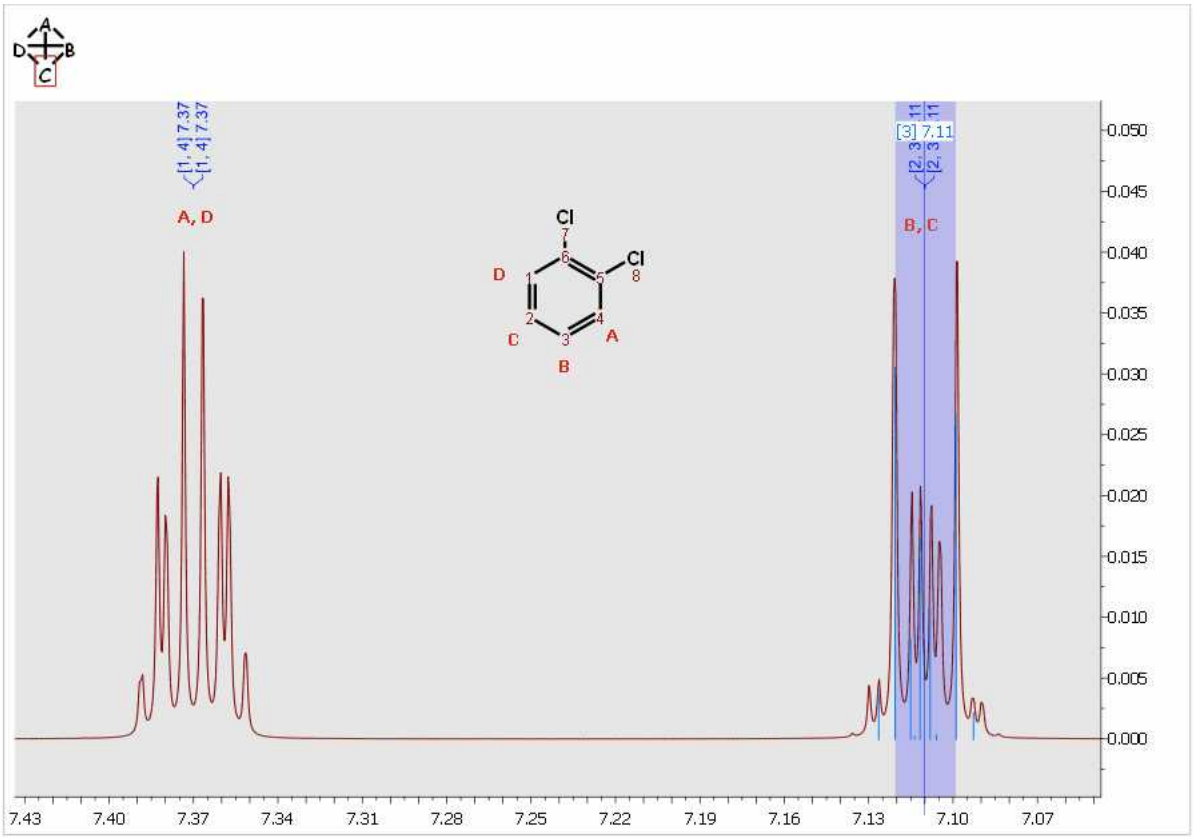


© 2024 MESTRELAB RESEARCH

Here you can see another example comparing an experimental dataset with the simulated one:



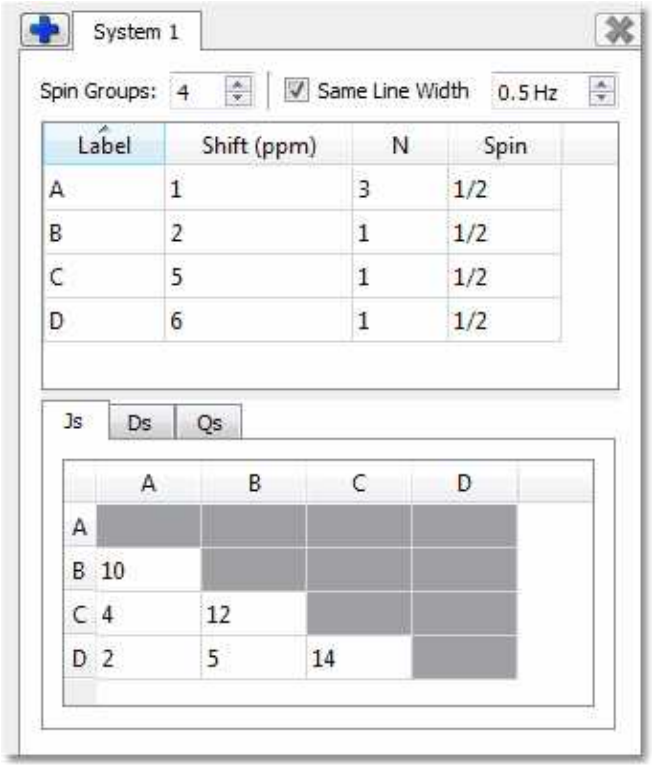
You can see below the simulated spectrum of the 'orto-dichlorobencene'; which is a AA'BB' system:



How can I simulate an A3BXY system?

Here you can see an example of an A3BXY system:

© 2024 MESTRELAB RESEARCH



To get the table above just follow this procedure:

1. Firstly, create a system with 4 spin groups. Please bear in mind that A3 is a unique spin group with 3 magnetic equivalent spins, so you will need to type N=3 in the label A..

2. Then, type the desired values for the other nuclei (B, C, D) with N = 1 for all of them (enter also the desired chemical shift value). Please bear in mind that Mnova does not need to know if the coupling is strong or weak, because Mnova makes the quantic calculations by using a rigorous method taking into account the chemical shift and the coupling constants

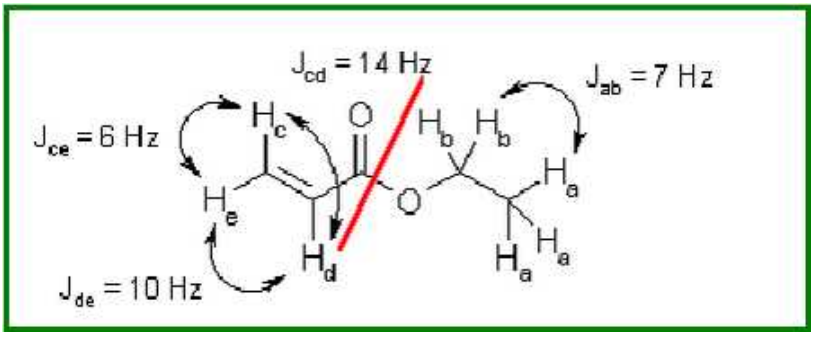
3. Once you have entered all the values and selected the Larmor Frequency and number of digital points, Linewidth for each nuclei, etc..., just click on the 'New Simulation' button to get the synthesized spectrum.

How can I add new systems?

Click on the 'Add System' button to add several systems. A new system will be a fragment of the molecule that can be simulated separately since its protons do not interact with the rest. In other words, a set of nuclei that are related through their coupling constants. Once you have added a new system, you can change the population of the system by using the 'Population' edit box.

As an example, consider the case of a small molecule, ethyl acrylate CH2CHCOOC2H5

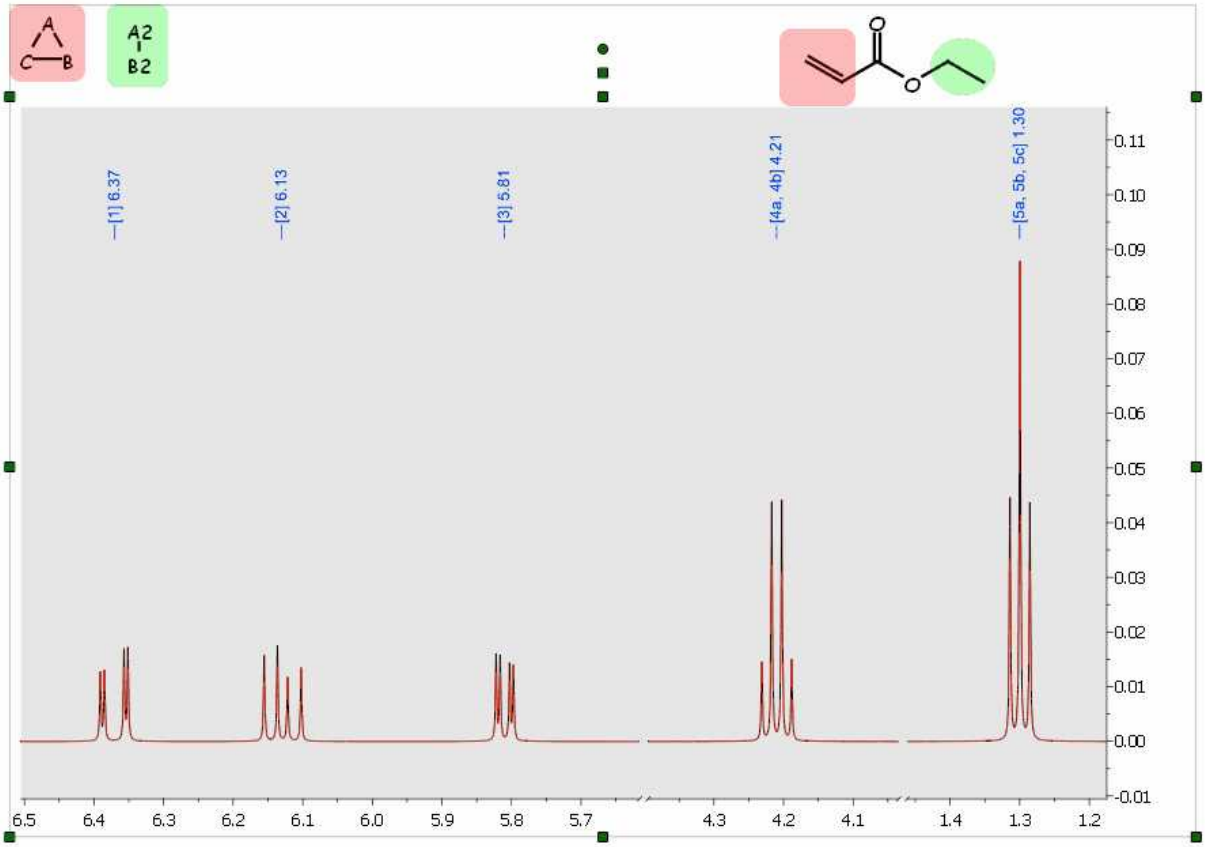
© 2024 MESTRELAB RESEARCH



As you can see, a standard simulation of the 1H-NMR spectrum of this molecule would require eight frequencies to be introduced, one for each proton. Moreover, you should introduce nine coupling constants as well. However, even a brief study of this spin system suggests us a more elegant solution.

Why not treat this spin system as two 'subsystems'? One 'subsystem', corresponding to the ethyl group, is composed of five protons with only two distinctive frequencies labeled a (the methyl group) and b (the methylene group), and only one coupling constant, Jab. Other 'subsystem' is composed of the three remaining protons (those of the vinyl group), with three frequencies, c, d and e, and three coupling constants, Jcd, Jce and Jde. This decomposition of the spin system in two subsystems is possible because they are not connected in any way. The decomposition of a spin system into subsystems can be carried out in many molecules, and is, for example, the conceptual basis of the NMR spectroscopic data tables.

You can see below, the synthesized spectrum of the ethyl acrylate; after having created two different systems; system A (vinyl protons) in red, and system B (ethyl group) in green:



© 2024 MESTRELAB RESEARCH

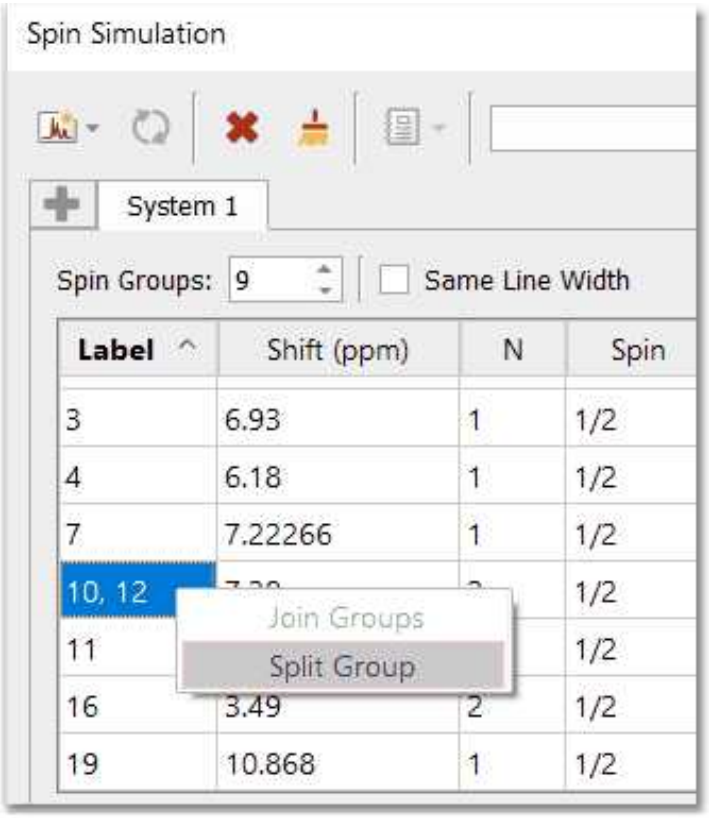
This decomposition method represents a great advance in terms of calculation speed, because the complexity of the calculation grows exponentially with the number of spins (nuclei) considered. However, we can perform even faster calculations and achieve greater flexibility going one step further.

Why not introduce only five frequencies, and tell the program how many protons correspond to each frequency? The introduction of magnetic equivalence allows the calculation to be performed using five spin groups, labeled from a to e, with their corresponding frequencies. The two first spin groups, a and b, have three and two protons respectively, while the other three spin groups have only one proton each. Of course, we need then only four coupling constants, Jab, Jcd, Jce and Jde. This process can be carried out in many molecules.

13.4.1 Spin Simulation panel

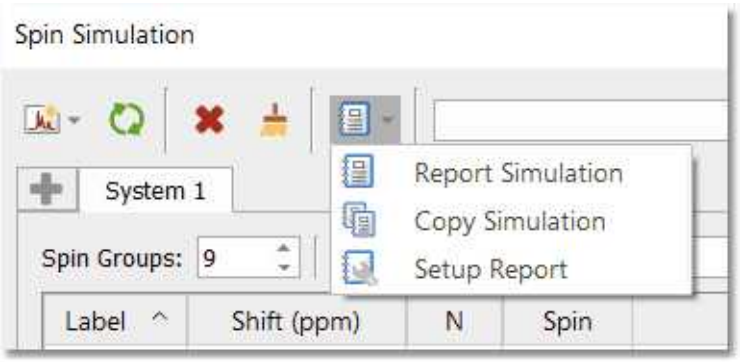
Click on the applicable buttons of the panel  to 'Load/Save' the spin systems (in XML format).

You can sort by chemical shift, atom number, etc... by clicking on the applicable header (of the Spin Simulation panel). Right click on the applicable label to join/split magnetically equivalent multiplets:



Check the 'Same Line Width' box to change all the 'line width' values at once.

You can report or copy to clipboard the Spin Simulation table:



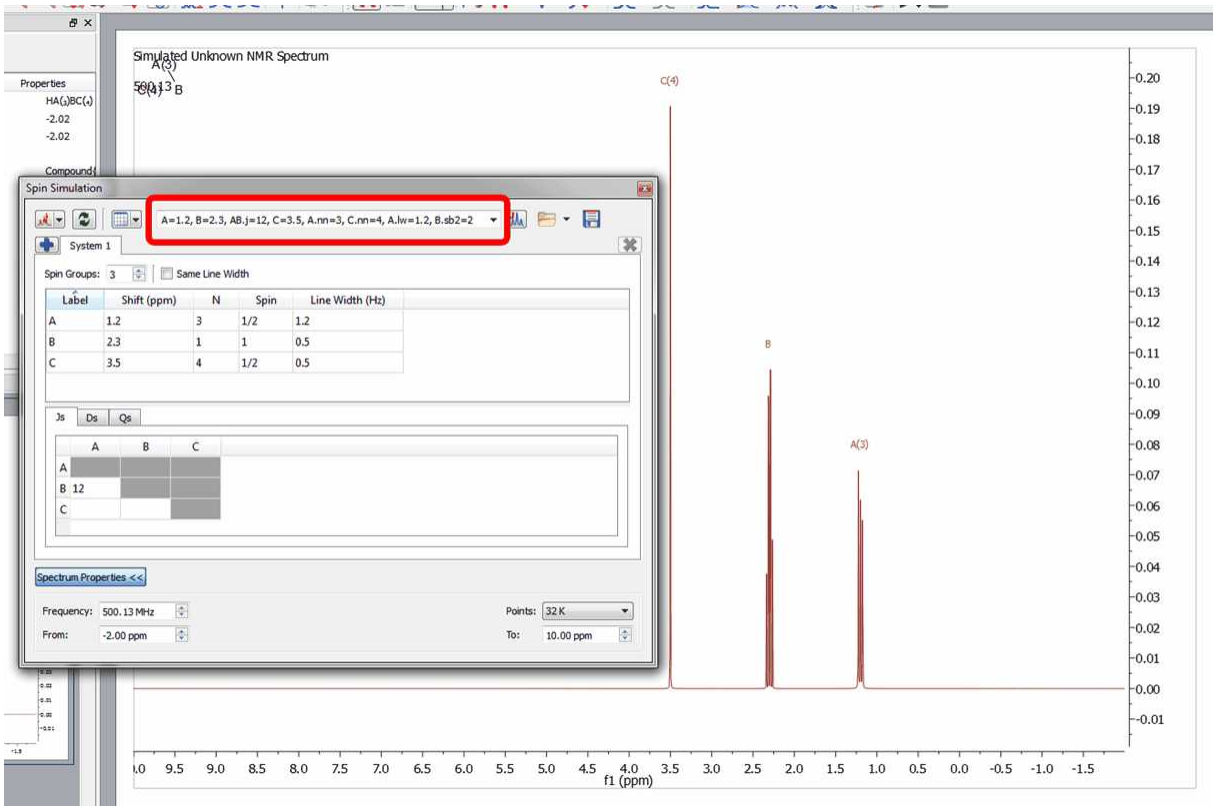
If you are using an experimental spectrum with multiplet analysis and/or assignments, you can use these buttons to fill the spin simulation table:



Command line for spin simulation

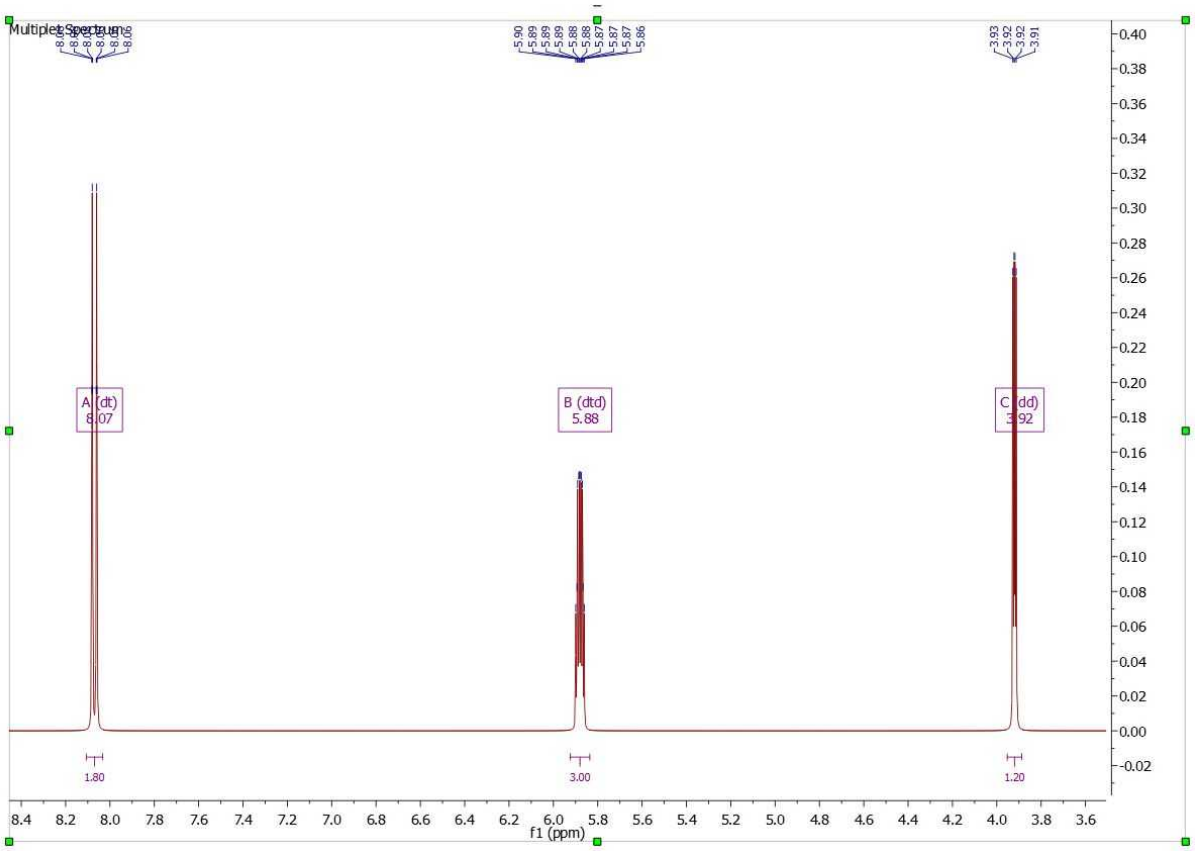
There is command line in the spin simulation panel to manually enter the values of interest. For example. when typing "A=1.2, B=2.3, AB.j=12, C=3.5, A.nn=3, C.nn=4, A.lw=1.2, B.sb2=2" the table in Spin Simulation panel will be filled as expected:

© 2024 MESTRELAB RESEARCH



Simulating a spectrum by importing a XML file

Here you can see an example. Loading the XML file into Mnova, will generate the spectrum below:

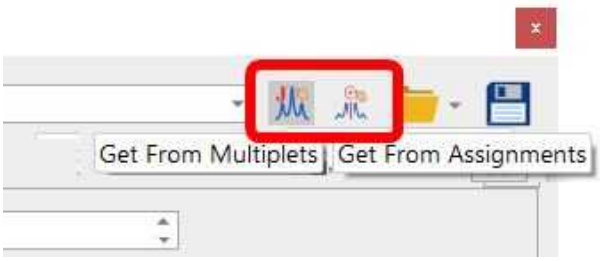


Simulation as .xml can also be imported from the command line as usual:

```
"C:\Program Files\Mestrelab Research S.L\MestReNova\MestReNova.exe" "C:\temp\Spin_simulation.xml"
```

Simulating a spectrum from the Multiplet Analysis or Assignments

The table of multiplets or assignments can be used for the simulation of the synthetic spectrum. To do it just run a multiplet analysis on a experimental spectrum (make the necessary corrections if it is needed) and open the 'Spin Simulation' panel to click on the 'Get From Multiplets' button to populate the table with chemical shift and coupling constants values. If your molecular structure contains assignments, you can use the "Get from Assignments' button.



Finally click on the 'New Simulation' button (or 'New as superimposed' if you want to get the experimental and the synthetic spectra overlaid):

See also "Importing spectral data from literature to synthesize a spectrum"

© 2024 MESTRELAB RESEARCH

Part

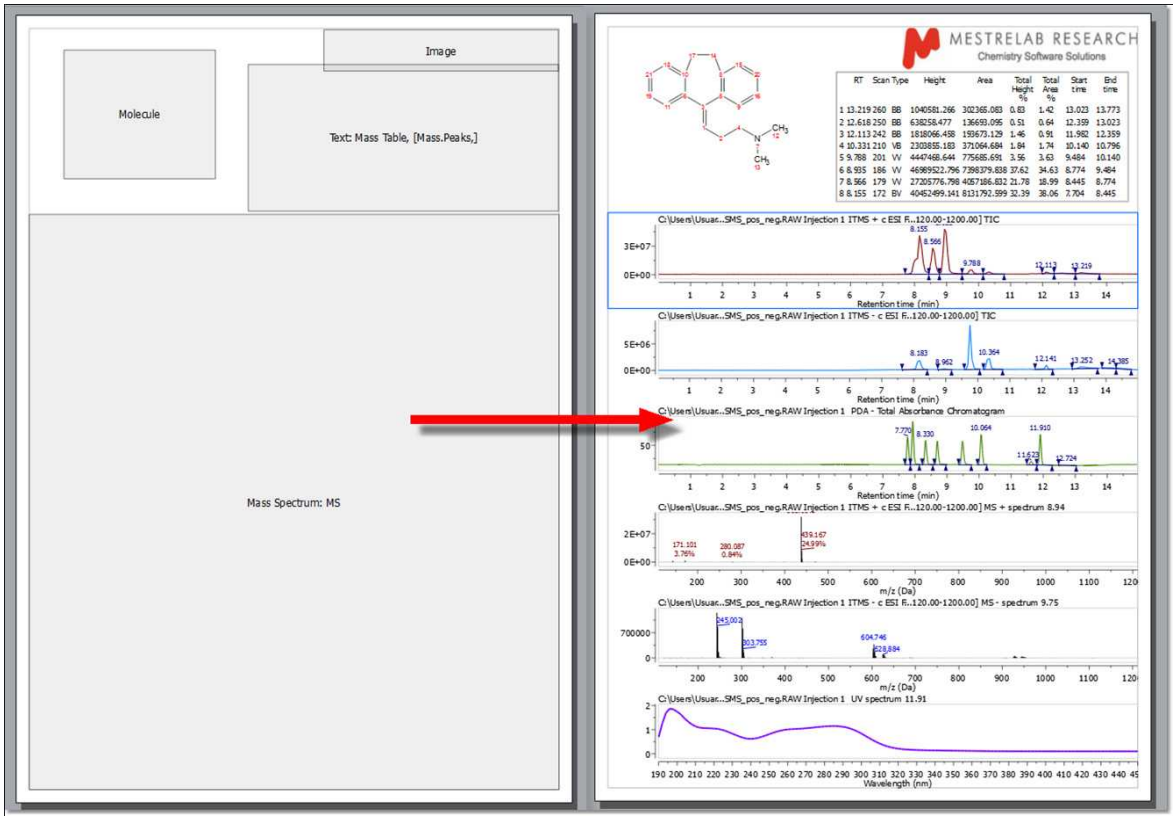


14 MSChrom

Mnova incorporates a plugin to process LC/GC chromatograms and mass spectra. The concept behind the multiplatform, multipage Graphical User Interface of Mnova is to allow the average chemist easy access to high quality processing and analysis of analytical data on the desktop computer and OS of choice. The MSChrom plugin gives the chemist the ability to work with LC/GC/MS data in combination with NMR data, within a single document.

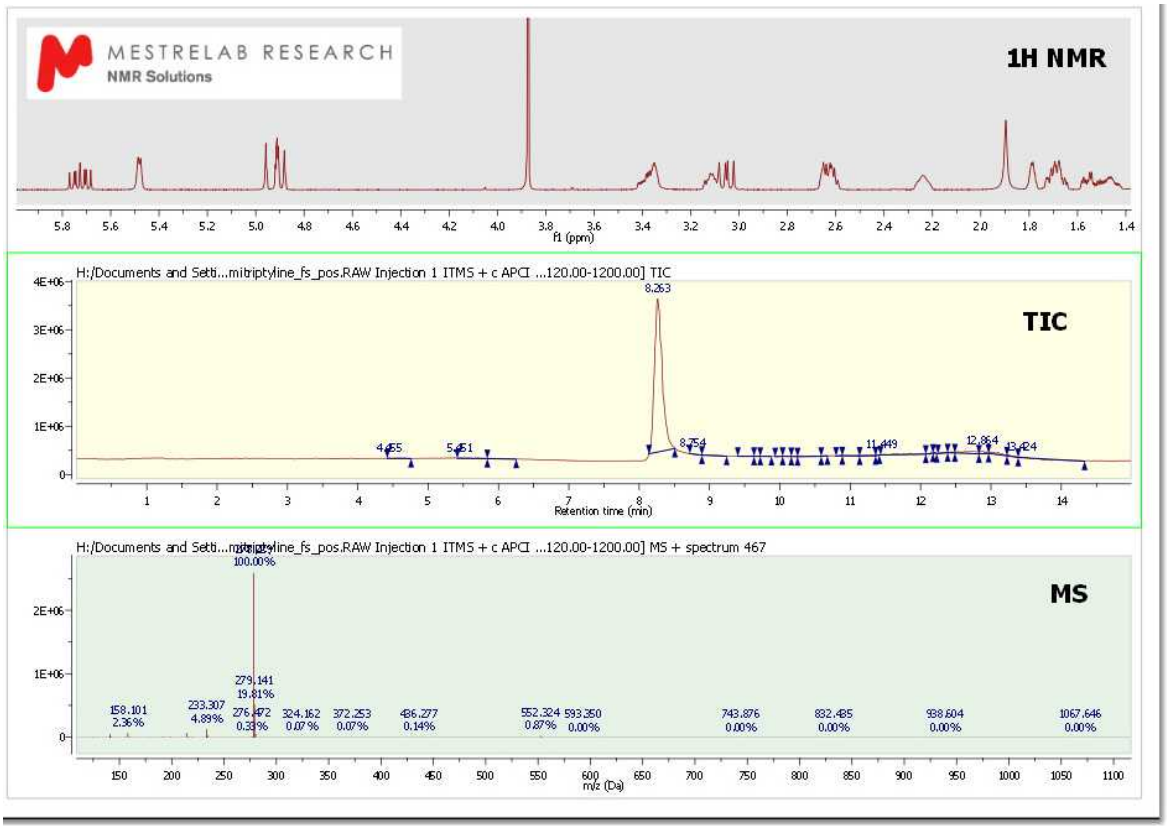
The **Mnova GUI gives you much more**. Benefit from day one of many other Mnova capabilities, such as scripting, reporting templates, graphical annotations, multiple exporting formats, including PDF, OLE integration with MS Office and many, many more.

In the picture below you can see an example of a layout template used for the MS plugin:



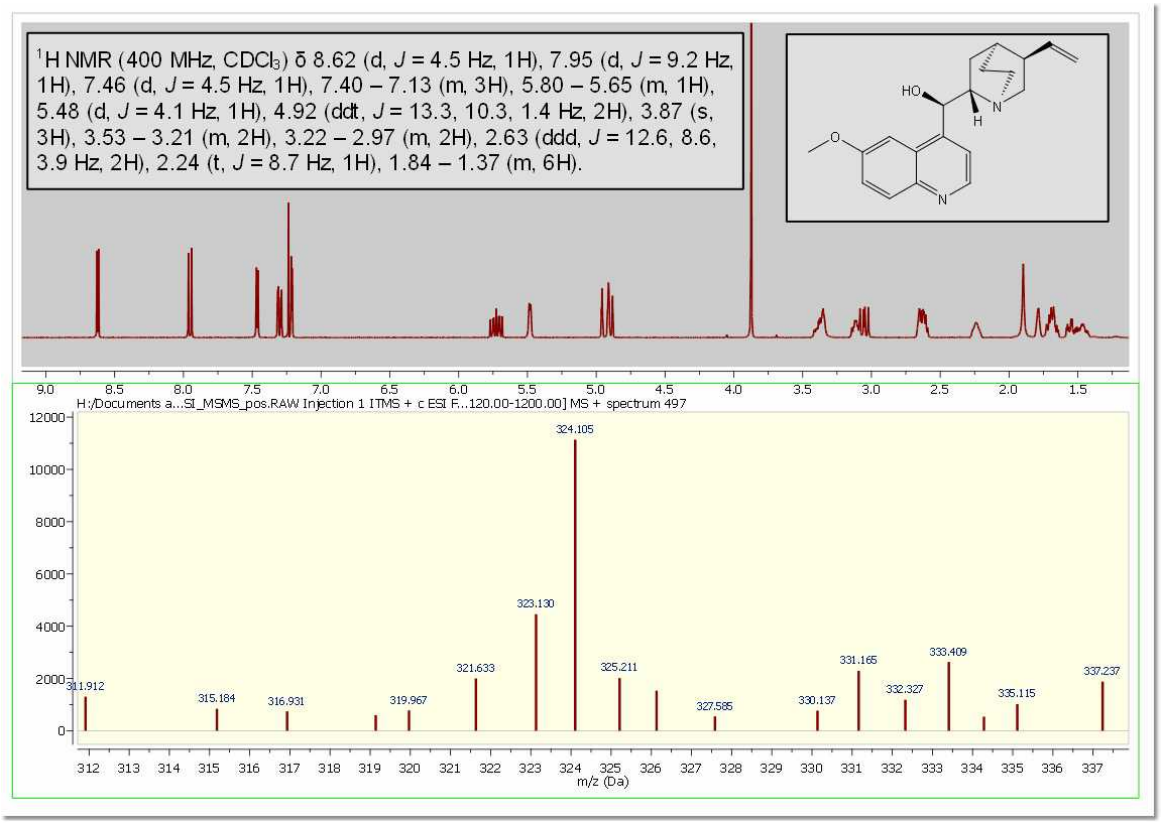
Mnova is an extremely versatile reporting tool, which will allow users to report even NMR and LC/GC/MS data together in the same document (or page):

© 2024 MESTRELAB RESEARCH



Here you can see another example with a MS and a 1H-NMR spectrum, containing a molecule structure and multiplet report:

© 2024 MESTRELAB RESEARCH



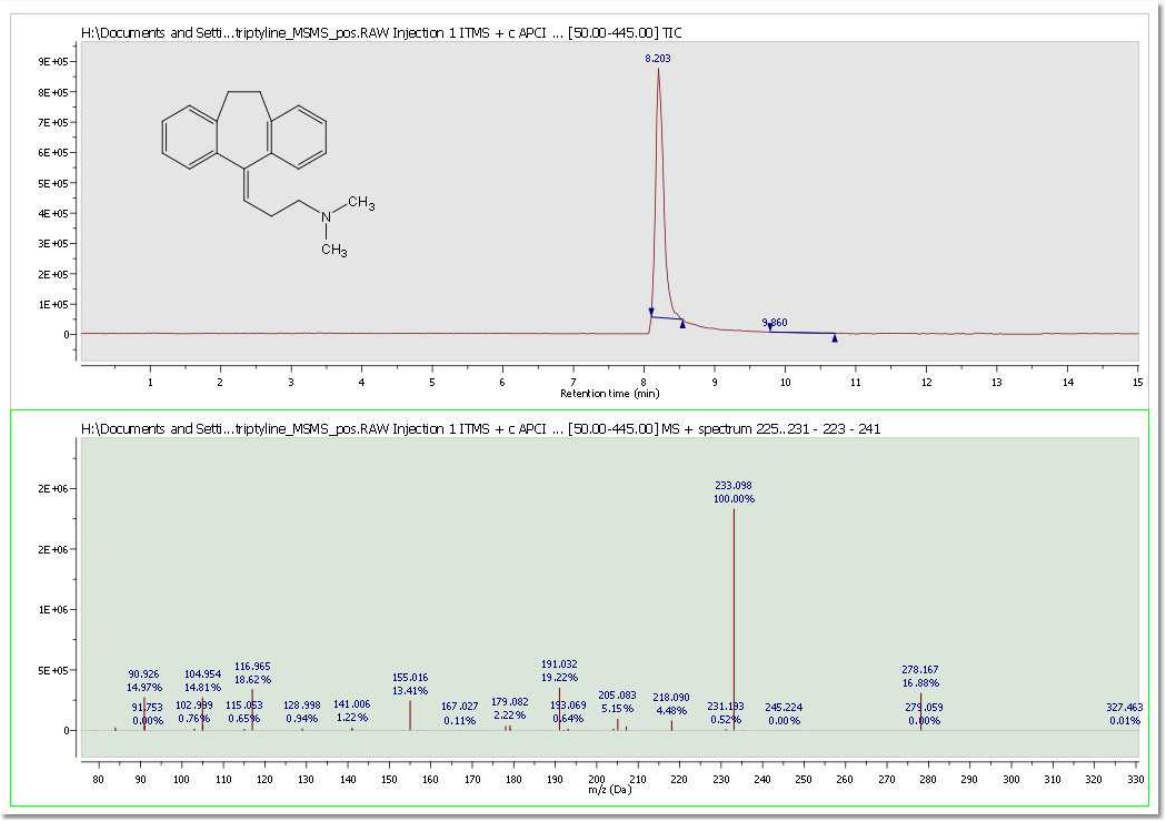
Following the Mnova concept, seamlessly interact with and handle data from different vendors. HP Agilent (ChemStation, Ion trap, MassHunter), Thermo Scientific–Xcalibur, Waters MassLynx, Waters UNIFI, Bruker (Compass, XMass), JEOL MSQ1000, JSON, Accu-ToF 4G LC-Plus, SCIEX Analyst, Sciex Data Explorer, Shimadzu, NetCDF ANDI-MS, Varian Saturn, mzData. mzML and mzXML. It provides a graphical user interface (GUI) for interaction with and display of dataset contents and tools and algorithms for basic processing of chromatograms and mass spectra.

Please bear in mind that Mnova MSChrom under Mac and Linux only supports:

- Agilent – ChemStation, Ion Trap
- Bruker Compass
- JEOL – MSQ1000
- MS CSV File
- Waters OpenLynx Report
- Waters – MassLynx (Mac only)
- mzData and mzXML

Here you can find the list of supported MS formats by OS:
https://resources.mestrelab.com/ms-supported-formats/

© 2024 MESTRELAB RESEARCH



The target user of the MSChrom plug-in is one whose goals are the elucidation and confirmation of small molecule structures.

The Mnova framework will allow the user to combine the results of multiple analyses into a consolidated report of results.

You will find below a glossary of the main terms relating to the MS plugin:

- **Adduct:** Ion formed by the interaction of an ion with one or more atoms or molecules to form an ion containing all the constituent atoms of the precursor ion as well as the additional atoms from the associated atoms or molecules.
- **Accurate Mass:** Experimentally determined mass of an ion that is used to determine an elemental formula. Typically, a mass accuracy of 5 ppm or better, at relatively low m/z values, is necessary to compute a correct elemental formula. See the section on elemental composition later in this document.
- **Base Peak**: The most intense peak of the mass spectrum which is assigned a relative abundance value of 100 percent. The lesser peaks are reported as a fraction of the base peak abundance.
- **Chemical ionization (CI):** Formation of an ion in the gas phase by the reaction of a neutral species with an ion. The process may involve transfer of an electron, a proton or other charged species between the reactants. Note: When a positive ion results from chemical ionization the term may be used without qualification. When a negative ion results the term negative ion chemical ionization should be used.
- **Electron Ionization (EI):** Ionization of an atom or molecule by electrons that are typically accelerated to energies between 10 and 150 eV in order to remove one or more electrons from the molecule. The term electron impact is deprecated.

© 2024 MESTRELAB RESEARCH

- **Electrospray Ionization (ESI):** A process in which ionized species in the gas phase are produced from a solution via highly charged fine droplets, by means of spraying the solution from a narrow-bore needle tip at atmospheric pressure in the presence of a high electric field (1,000 to 10,000 V potential).

- **Fast Atom Bombardment (FAB):** A method in which ions are produced in a mass spectrometer from non-volatile or thermally fragile organic molecules by bombarding the compound in the condensed phase with energy-rich neutral particles.

- **Isotope Cluster:** Set of mass peaks related to ions with the same chemical formula but containing different isotopes; e.g. the 16 and 17 Da peaks in a CH4 sample arising from 12CH4+ and 13CH4+ ions.

- **Mass Spectrometry/Mass Spectrometry (MS/MS):** The acquisition and study of the spectra of the electrically charged products or precursors of m/z selected ion or ions, or of precursor ions of a selected neutral mass loss. MS/MS can be accomplished using beam instruments incorporating more than one analyzer (tandem mass spectrometry in space) or in trap instruments (tandem mass spectrometry in time).

- **Mass Spectrum:** A plot of the relative abundances of ions as a function of their m/z (mass to charge ratio) values, representing data produced by a mass spectrometer.

- **Mass Resolution:** In a mass spectrum, the smallest mass difference (Δm) between two equal magnitude mass peaks such that the valley between them is a specified fraction of the peak height. Typically, a 10% valley is specified, or 50%, which is known as full width at half-maximum height (FWHM).

- **Mass Resolving Power:** In a mass spectrum, the observed mass divided by the difference between two masses that can be separated: m/Δm.

- **Mass Chromatogram:** A chromatogram obtained from the plot of the intensity of a single m/z value or of a range of m/z values for each mass spectra in a dataset versus time.

- **Molecular Isotope Cluster Chromatogram (MICC)**: The MICC is the sum of the mass chromatograms of each m/z value of an isotope cluster.

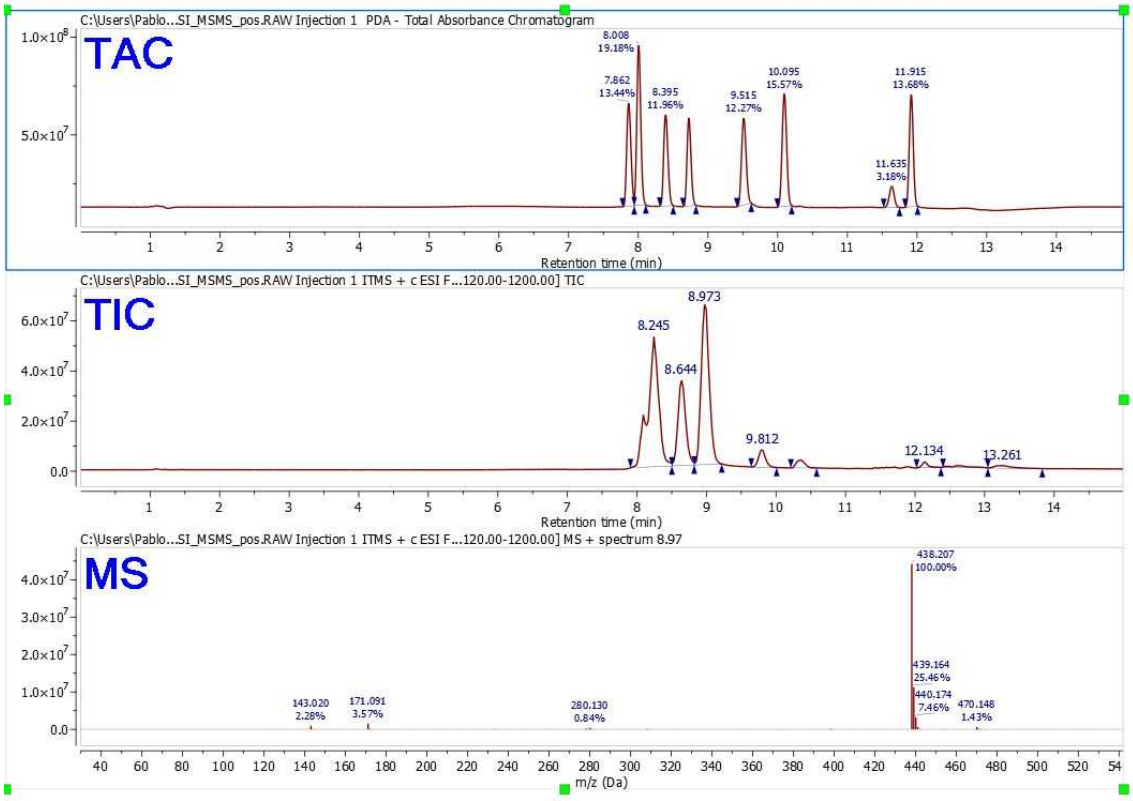
- **Total Ion Chromatogram (TIC):** The chromatogram obtained from the plot of the sum of intensities for each mass spectrum in a dataset versus time.

14.1 First Steps with the MSChrom Plugin

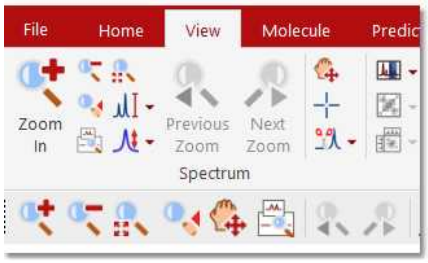
It is very easy to obtain your first results with the MSChrom plugin of Mnova:

1. Drag&drop the raw folder to Mnova in order to display the TAC (at the top of the window), the TIC (in the center) and the MS spectrum of the highest signal (this default can be changed from the 'Preferences/Mass/Setup')

© 2024 MESTRELAB RESEARCH

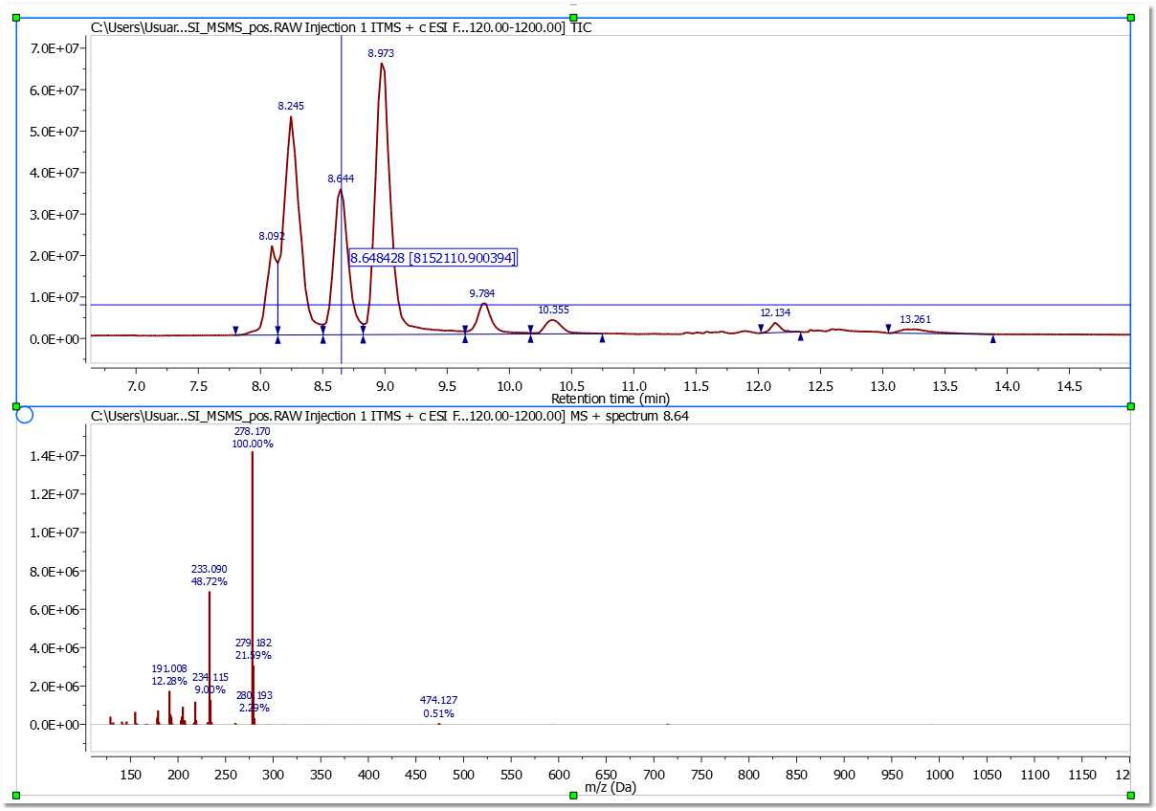


2. You can expand any scale (in either the TIC or MS) by using the 'zoom in' feature, which can be accessed by just pressing the 'Z' key or by using the corresponding icon of the toolbar .



3. You can select any other mass spectrum for a particular time point with the crosshair feature (press ESC key to keep the desired MS plot). For example in this case, we have selected the spectrum number 467 at a 'Retention time' of 8.26 min:

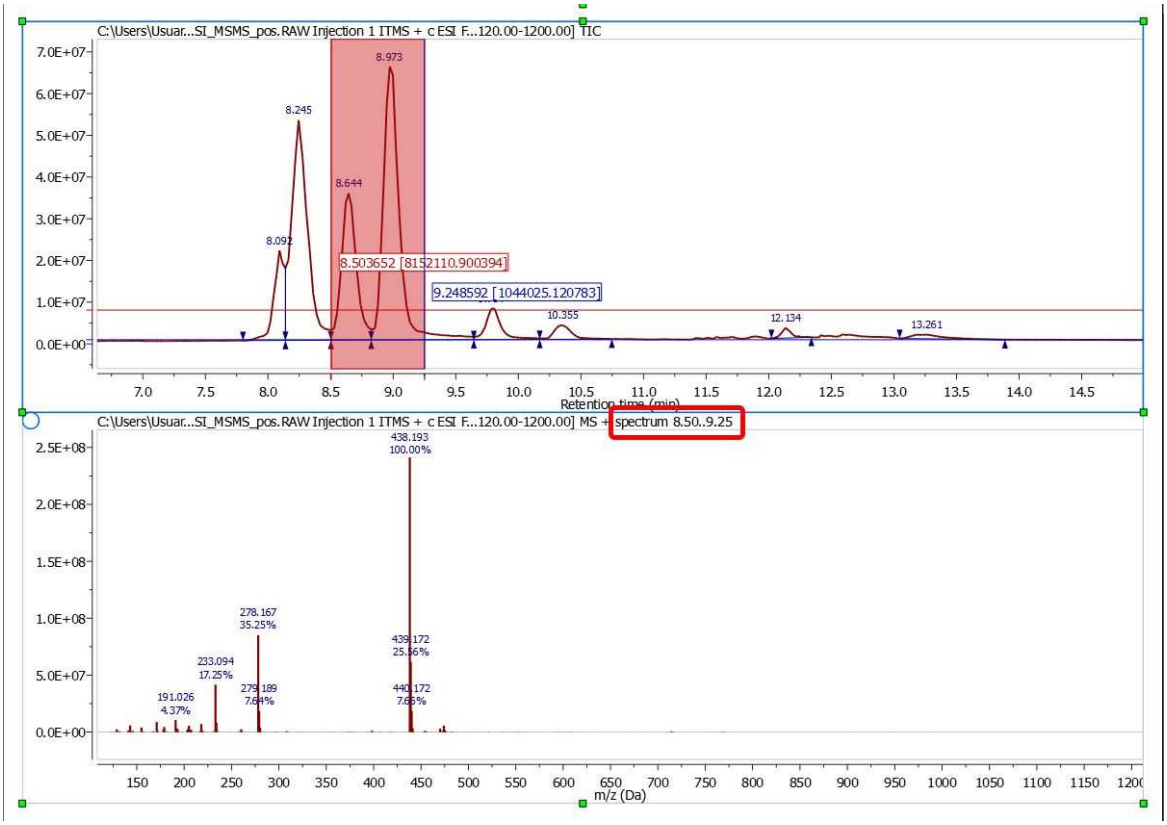
© 2024 MESTRELAB RESEARCH



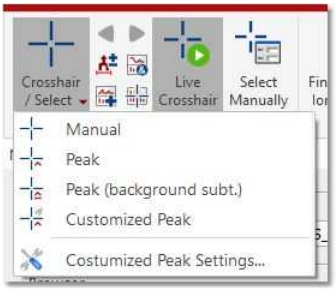
NOTE: The "Live Mass Spectrum Preview" preference will allow you to show the extracted mass spectrum *on the fly* when you hover the crosshair over the chromatogram (or trace).

4. If you need to average the mass spectra over a certain time range (CoAdd), just select the crosshair, hold down the left mouse button and drag over the appropriate time span in the TIC. A label at the top of the MS will inform you about the spectrum ranges (red rectangle in the picture below):

© 2024 MESTRELAB RESEARCH



If you want to 'CoAdd' the whole peak, just follow the menu 'Mass Analysis/Crosshair/Peak' and finally select the desired peak in the TIC.

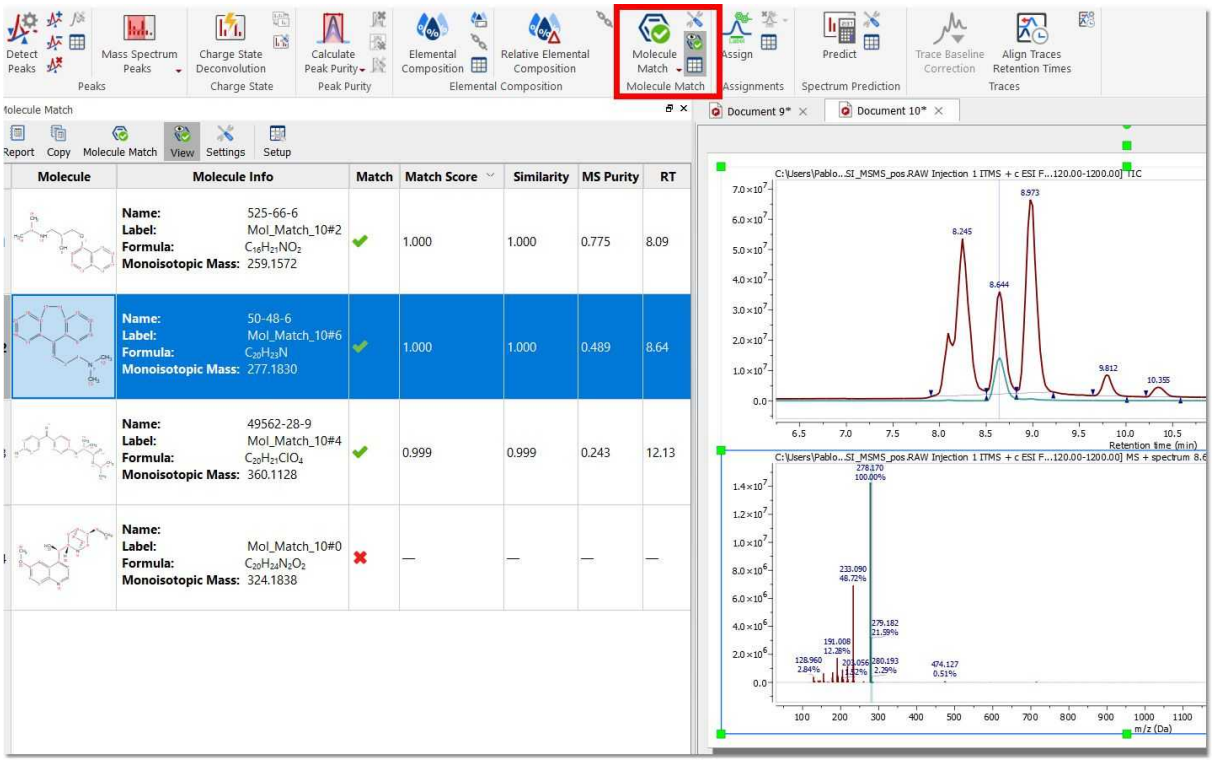


To generate a 'CoAdd' of the whole peak without the tail-peaks just select the mode 'Peak (Background Subt.)' under the 'Mass Analysis/Crosshair' scroll down menu.

5. You can add another MS spectrum to the page, just by clicking on the 'Append' button . Next, select the desired time span in the TIC in order to obtain the corresponding MS spectrum.

6. The 'Molecule Match' is very useful to see if a mass spectrum and a chromatogram match with one of the molecular structures of the document. With the TIC and the mass spectrum displayed on the screen and some .mol or .sdf files opened in the .mnova document, click on the 'Calculate Molecule Match' button of the toolbar . The 'Molecule Match' table will be displayed to see the results. This table will contain information about the 'Retention Time', Scan (number of spectrum), Match Score...

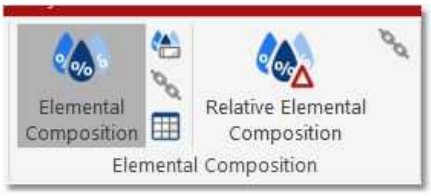
© 2024 MESTRELAB RESEARCH



Clicking on any molecule will display the MICC (Molecular Isotope Cluster Chromatogram), overlaid with the TIC.

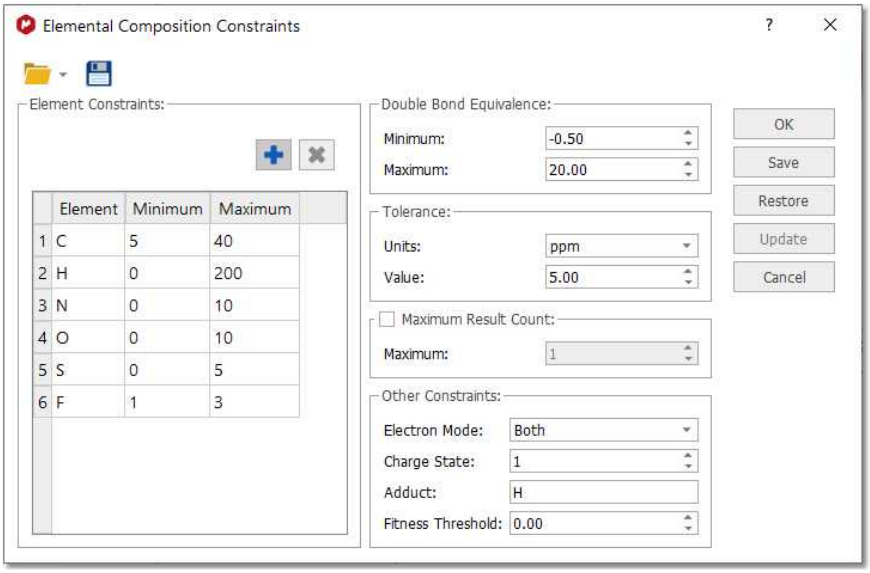
In addition, Mnova shows you in the chromatogram the retention time of the match (with a blue vertical line) and displays the corresponding MS spectrum overlaid with the theoretical one (in green at a M/Z around 278, in this case).

7. If you are using high resolution MS, it is possible to do an 'Elemental Composition' analysis of any peak of the Mass spectrum. First of all, follow the menu 'Mass Analysis/Elemental Composition/Constraints':



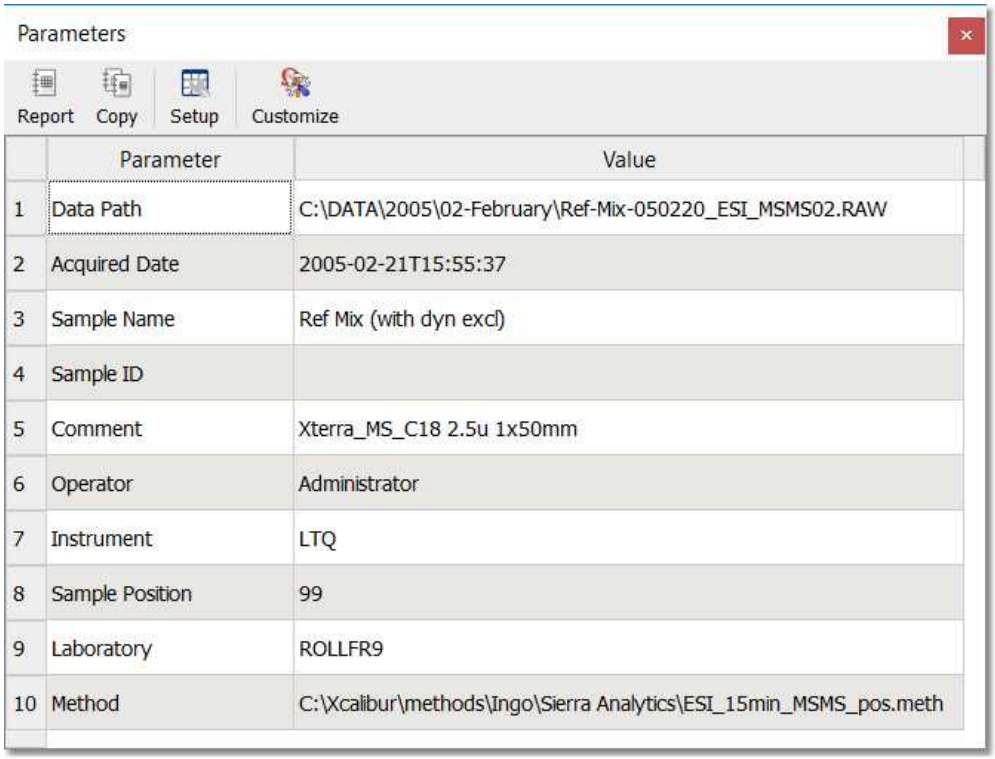
This will open the 'Elemental Composition Constraints' dialog box:

© 2024 MESTRELAB RESEARCH



Click on this icon  to add more 'Element Constraints'. Once you have finished with the constraints, click on the 'Elemental Composition' icon and select with the crosshair the desired peak of the Mass spectrum. A table with the results will be displayed (you can find this table under the 'View/Tables' menu). All the results obtained will satisfy the composition, DBE, and tolerance constraints, and will be ranked in decreasing order of fitness. Please bear in mind that a fitness value of 1 will be the perfect match.

8. Follow the menu 'View/Tables/Parameters' to display and report the 'Parameters table' :



	Parameter	Value
1	Data Path	C:\DATA\2005\02-February\Ref-Mix-050220_ESI_MSMS02.RAW
2	Acquired Date	2005-02-21T15:55:37
3	Sample Name	Ref Mix (with dyn excl)
4	Sample ID	
5	Comment	Xterra_MS_C18 2.5u 1x50mm
6	Operator	Administrator
7	Instrument	LTQ
8	Sample Position	99
9	Laboratory	ROLLFR9
10	Method	C:\Xcalibur\methods\Ingo\Sierra Analytics\ESI_15min_MSMS_pos.meth

© 2024 MESTRELAB RESEARCH

Finally, the user will be able to save , print  or export to PDF , the document by clicking on the corresponding icon in the toolbar (or under the 'File' menu). You can also export as JSON format by following the menu 'File/Saves As'

14.2 Working with the MSChrom plugin

Mnova MSChrom allows the user to navigate the TIC and display simultaneously any number of MS traces of interest, corresponding to specific points or to ranges in the TIC. All Mnova graphical functionalities (zooming, panning, create expansions, fit to height, increase/decrease intensities, etc) are available on all active spectra on screen. Spectra can be visualized in different modes: Standard, Peak spectrum, Background Subtracted Spectrum. Molecular structures (MOL files, SD files and copy/paste from ChemDraw, IsisDraw and ChemSketch) are also supported.

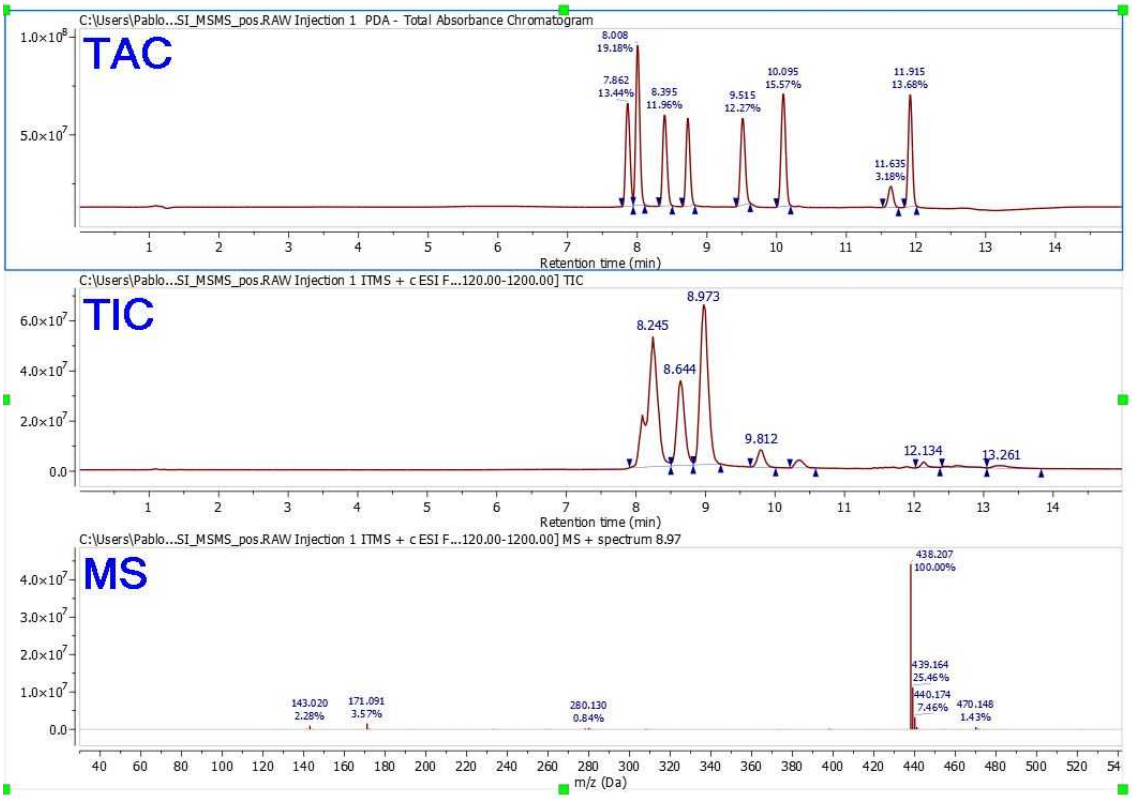
The Mass plugin of Mnova supports the following formats:

- AB SCIEX Analyst
- AB SCIEX Data Explorer (after having run DE software with admin privileges)
- Advion
- Agilent – ChemStation, MassHunter, Ion Trap
- Bruker Compass
- JEOL – MSQ1000, FastFlight, Accu-ToF 4G LC-Plus
- JSON
- MS CSV File
- NetCDF ANDI-MS
- Shimadzu LCMS, GCMS
- ThermoScientific Xcalibur
- Varian Saturn
- Waters MassLynx
- Waters OpenLynx Report
- Waters UNIFI
- mzXML, mzML and mzData (*.mzXML, *.mzML, *.mzData, *.xml)
- PerkinElmer Files

Here you can find the list of supported MS formats by OS:
https://resources.mestrelab.com/ms-supported-formats/

In order to load any sample in Mnova just Drag&drop the corresponding file to Mnova to display the TAC (at the top of the window), the TIC (in the center) and the MS spectrum of the highest signal (this default can be changed in the 'Preferences/Mass/Setup'). You can also use the ***'File/Open'*** menu, the keyboard shortcut **<Ctrl+O>** (Cmd+O in Mac) or click on the **'Open'** icon on the toolbar.

© 2024 MESTRELAB RESEARCH



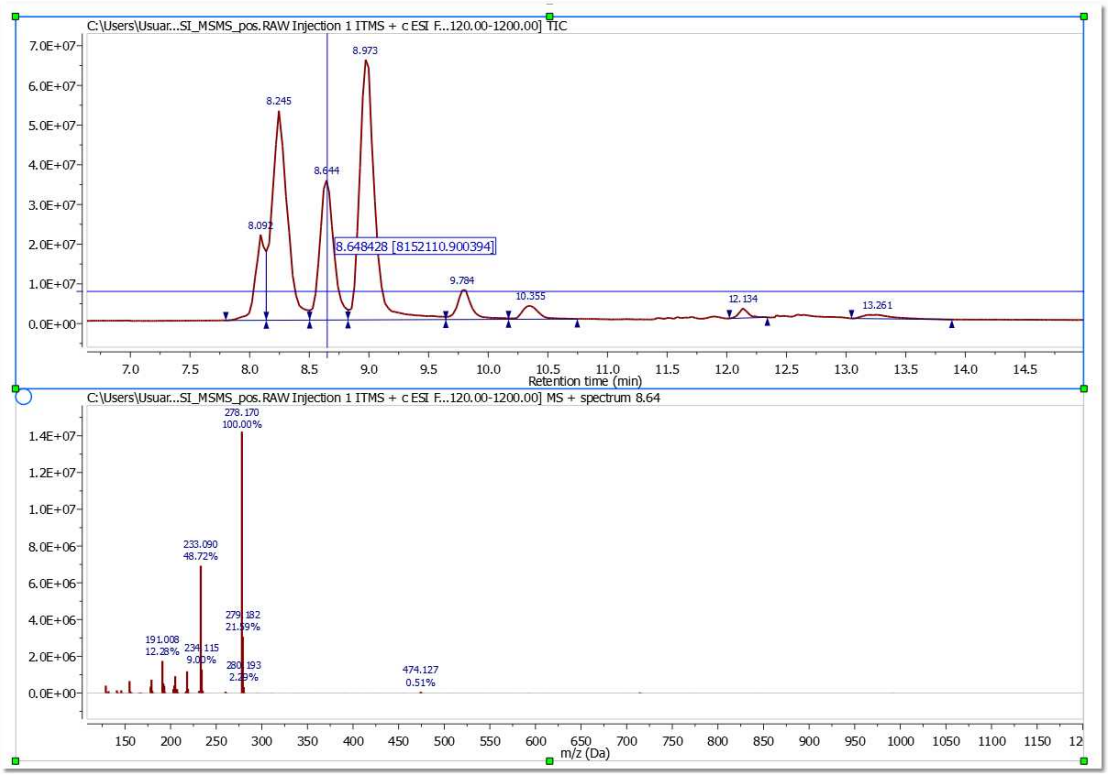
Double clicking on the spectral window will display the properties dialog box. From here you can modify the Grid, Axes, Line, Peaks and Title properties of the TIC and MS spectrum by selecting the corresponding tab in the dialog box. For further information about the Properties, please click here.
You can expand any scale (in either the TIC or MS) by using the 'zoom in' feature, which can be accessed by just pressing the 'Z' key or by using the corresponding icon of the toolbar . Please note that the intensity of the expanded region will be automatically fitted. However if you are not happy with it, you can change it by just clicking on the 'Increase or Decrease Intensity' icons: . Or by using the '+' or '-' keys.

You can select multiple chromatograms of the same dataset to zoom in/out simultaneously. The chromatograms can be selected as in a stack of NMR spectra:

- To select a single spectrum, left click on the spectrum while keeping pressed the Alt key
- To deselect a spectrum: left click + Ctrl/Cmd + Alt
- In order to select multiple spectra, follow the same operation as above while keeping pressed Alt and/or Shift keys.

You can select any other mass spectrum for a particular time point with the crosshair feature  (press ESC key to keep the desired MS plot). For example in this case, we have selected the spectrum number 467 at a 'Retention time' of 8.26 min:

© 2024 MESTRELAB RESEARCH

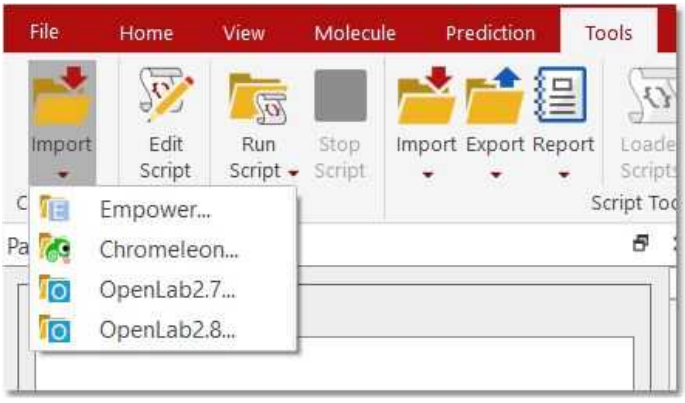


NOTE: The "Live Mass Spectrum Preview" preference will allow you to show the extracted mass spectrum on the fly when you hover the crosshair over the chromatogram (or trace).

14.2.1 Importing Empower, Chromeleon and OpenLab2 formats

Mnova can support Waters Empower (3.6), ThermoScientific Chromeleon (7.3) and Agilent OpenLab (2.7 and 2.8) formats if the vendor software is installed in the same computer. (It will not work if you are only using a client version of the vendor software).

To work with these datasets, just follow the 'Tools/Import CDS tools' ribbon:



Empower

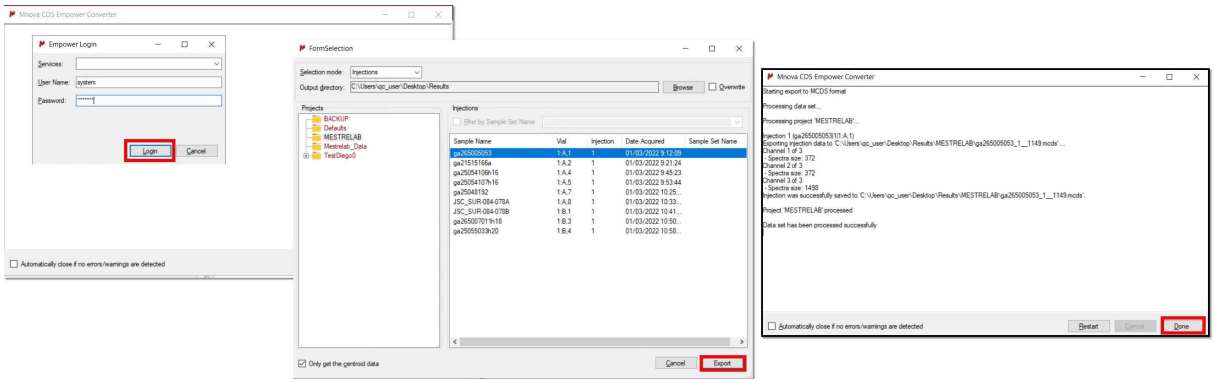
Selecting the Empower option will display several dialogues to convert your datasets. After having logged with Empower's credentials, you will have access to the 'Form Selection' dialog. From here, you can select the injections (or folders) from the desired project. Do not forget to select the output directory

© 2024 MESTRELAB RESEARCH

and click on the Export button to do the conversion and generate the .mcds files (which can be imported in Mnova as usual).

- An option "Overwrite" is available to indicate if the contents of the current output directory can be overwritten. With the option enabled, no warnings were obtained when importing for a second time the same injections in the same output directory; with the option disabled, a warning was obtained when the conflict was detected and no MCDS was generated.

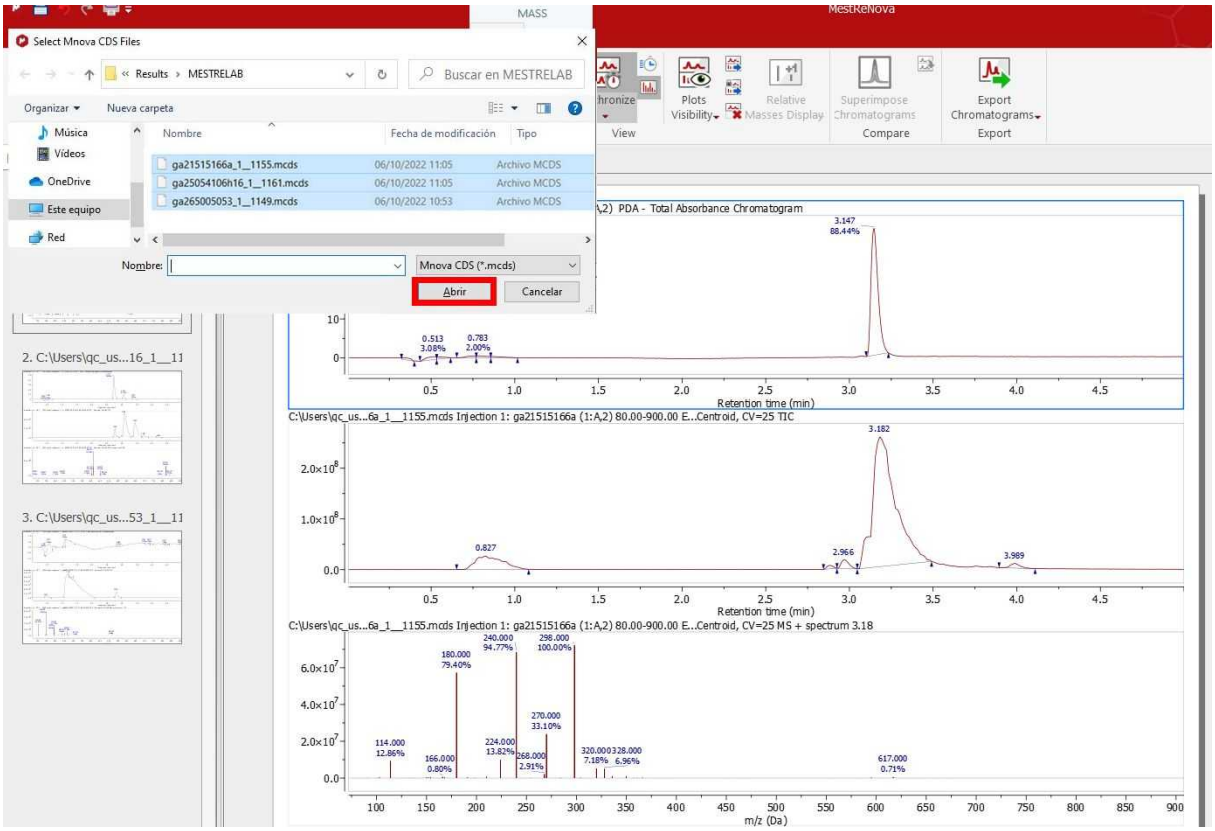
- An option "only get the centroid data" is available to switch between centroid and profile data.



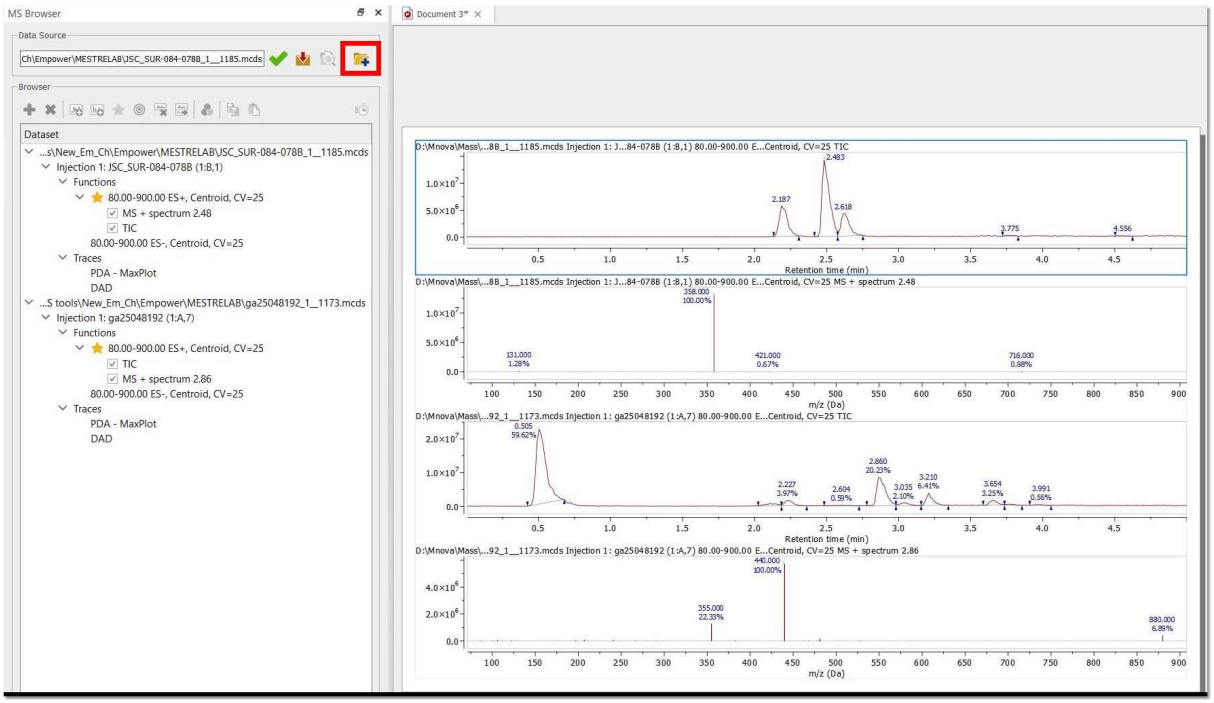
After having clicked on the 'Done' button, the injection will appear loaded in Mnova. In this window, you will find a checkbox to close the dialog in case of a clean output (i.e., no warnings/errors). If this option is unchecked or there is at least one warning/error in the conversion process, the dialog waits for the user action. If it is checked and there are no errors/warnings, the dialog closes automatically at the end of the conversion. At the end of the process, if the dialog is not closed automatically, it is possible to click the "Restart" button to select other injection/folder to be imported.

If you selected several injections, you will get a dialog to select which ones you want to import into Mnova:

© 2024 MESTRELAB RESEARCH



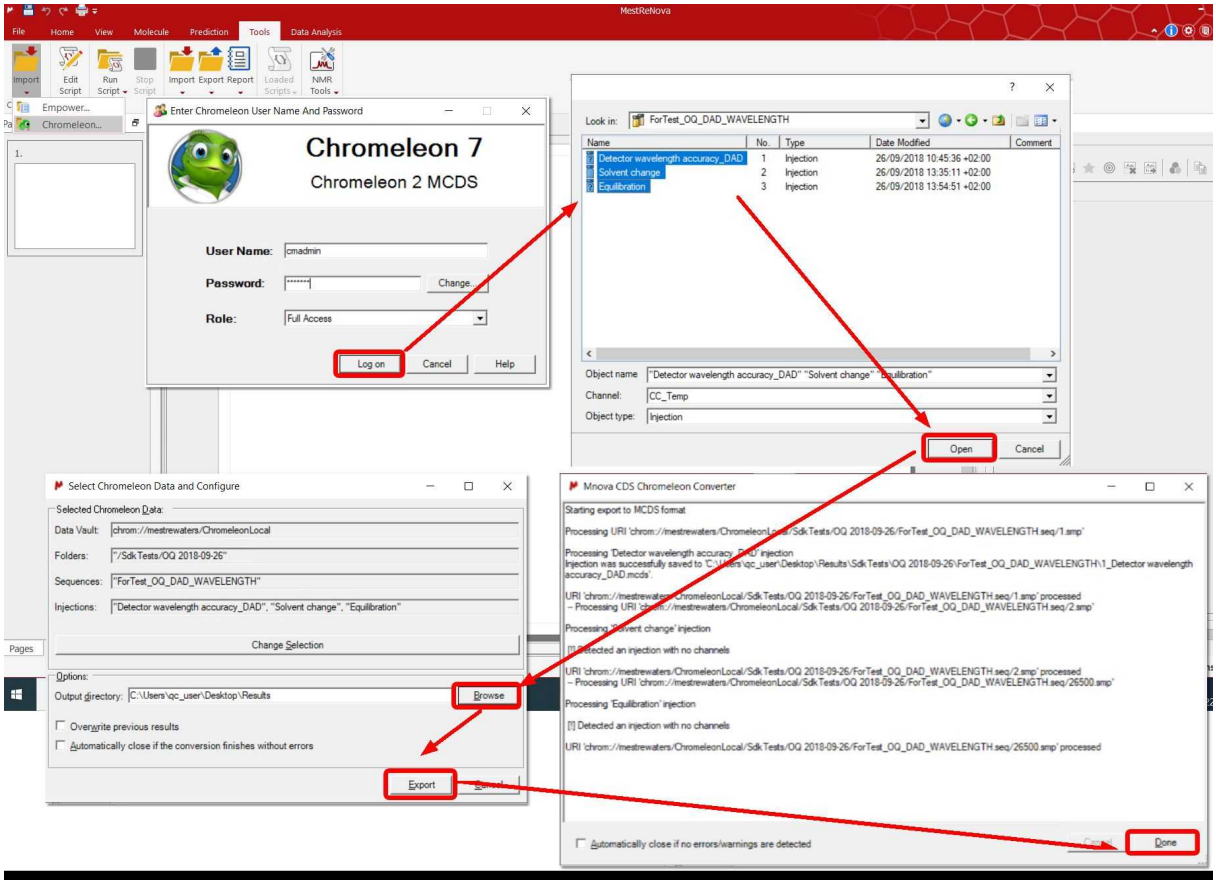
The injections can be loaded separately (one per page), or several injections can be loaded in the current MS data by clicking on the "Add MS dataset" button from the MS Browser:



Chromeleon

© 2024 MESTRELAB RESEARCH

With Chromeleon, the workflow will be pretty similar. After having logged, you would need to select the injections (sequences or folders), make sure that you choose an 'Output' folder for the results and click on the 'Export' button to start the conversion.

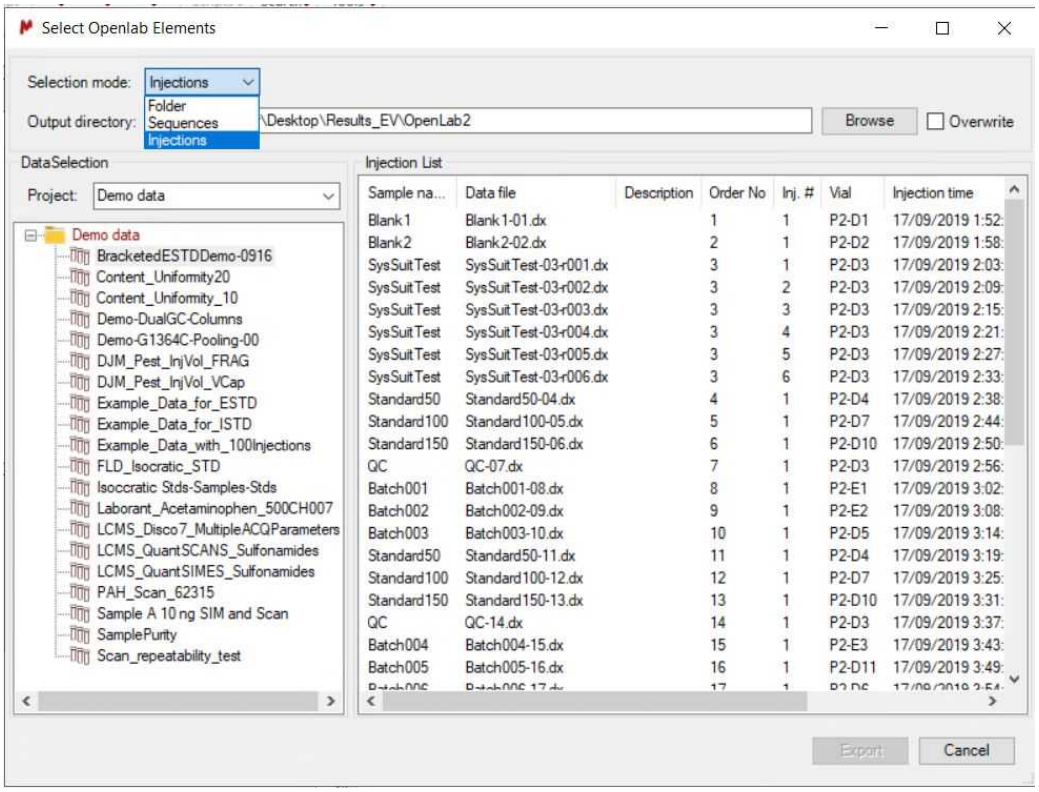


Once the conversion has finished, click on the 'Done' button to get your result in Mnova.

OpenLab2

When clicking Import/OpenLab2.7 (or 2.8) a dialog appears for OpenLab Login. Once you have entered your credentials, a new box will be displayed to allow you to browse the OpenLab data to select either Injections, Sequences (groups of injections) or Folders, via Selection Mode.

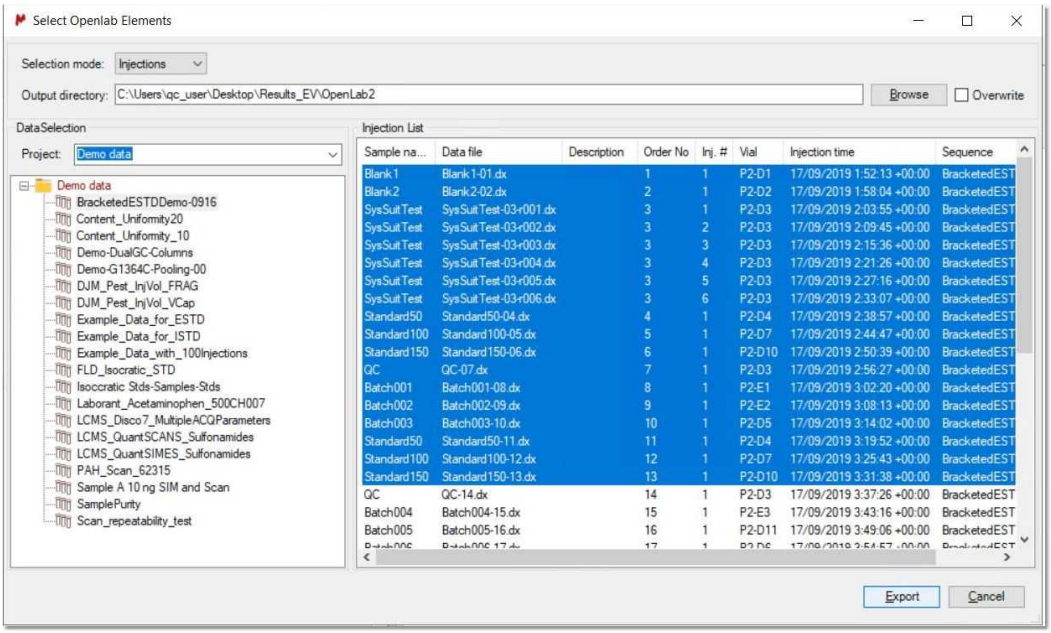
© 2024 MESTRELAB RESEARCH



This dialog allows you:
- to select either Injections/sequences/Folders, via Selection mode.
- define the output directory where all the .mcds file will be stored. The converted injections maintain the original directory structure. Only after selecting an output directory and selecting at least a folder, a sequence or an injection, the Export button is enabled.
- An option "Overwrite" is available (uncheked by default) to indicate if the contents of the current output directory can be overwritten. With the option enabled, no warnings were obtained when importing for a second time the same injections in the same output directory; with the option disabled, a warning was obtained when the conflict was detected and no MCDS was generated.

With the injections, you will be entitled to do multiple selection (no available for sequences or folders).
Once you have selected the desired injections, click on the Export button to launch the MCD conversion:

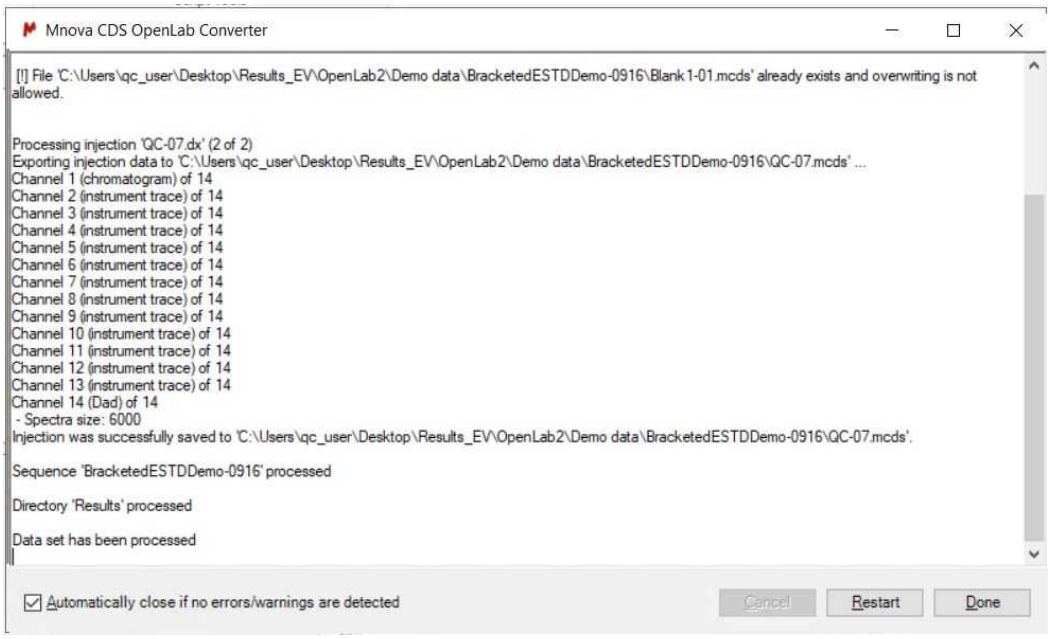
© 2024 MESTRELAB RESEARCH



During the process, a checkbox option is available to close this dialog in case of a clean output (i.e., no warnings/errors). If this option is unckeded or there is at least one warning/error in the conversion process, the dialog waits for the user action. If it is checked and there are no errors/warnings, the dialog closes automatically at the end of the conversion.

At the end of the process, if the dialog is not closed automatically, it is possible to click the "Restart" button to select other injection/folder to be imported.

NOTE: While the MCDS Empower Converter is open, Mnova can't work because it is waiting for the converter (it shows as if Mnova is not responding).



Once it has finished; click on the 'Done' button and Mnova wil be again accessible. A new dialog will be displayed to load one or several mcds files, mimiching the folder system of the original location. In case

that only one injection is imported, the dialog will not show up and the injection will be loaded automatically in Mnova.

Note. if you has problems with your OpenLab converter and you are not extracting the MS data, you may need to add an additional configuration step:

In the "openlab2mcds" directory of Mnova (by default, "C:\Program Files\Mestrelab Research S.L\MestReNova"), run **as administrator** the batch script "configure_openlab2mcds.bat" in order to correctly configure the converter with the Agilent dependencies. If OpenLab2 is installed at the default path ("C:\Program Files (x86)\Agilent Technologies"), a double click should be enough. If not, the script must be run in a CMD and the Agilent installation path must be passed as a command line argument.

14.2.2 Mass Tools

The Mass Tools ribbon menu contains these useful features:

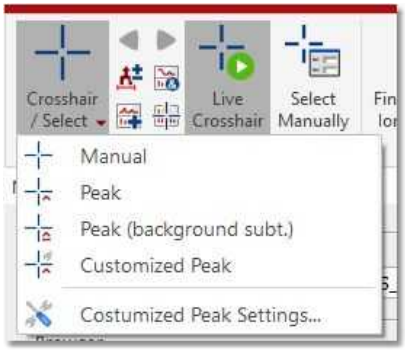


- **Spectrum Selection Mode**
- **Find Fragment Ion Spectrum**
- **New Mass chromatogram**
- **MS Browser**
- **Plots Visibility**
- **Relative Masses Display**
- **Superimpose Chromatogram**
- **Export and Stack chromatograms**

Let´s take look at them one by one.

14.2.2.1 Spectrum Selection Mode

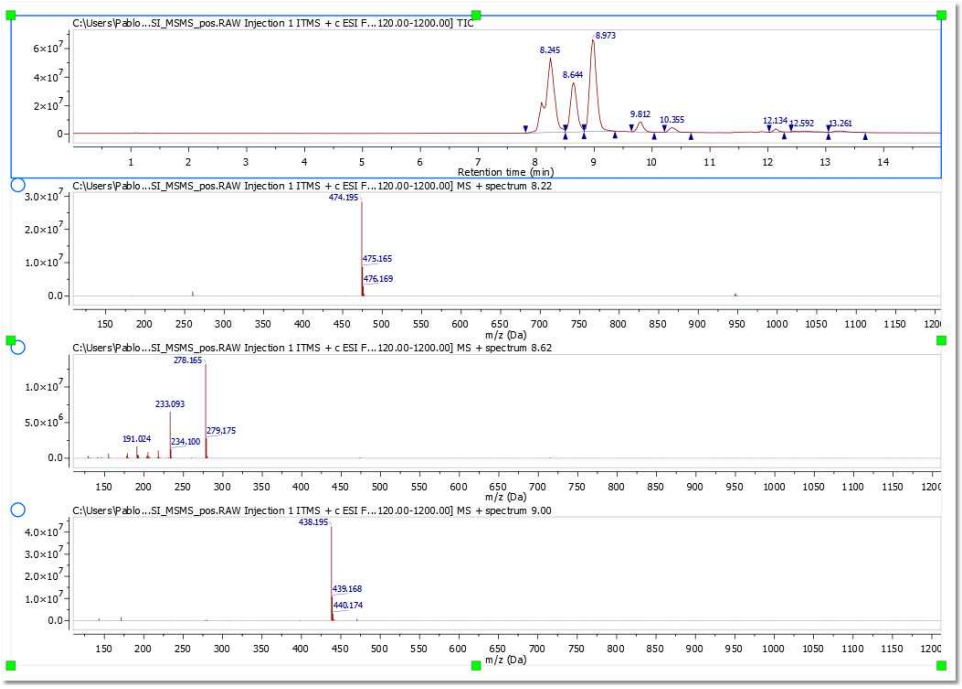
This feature will allow you to add additional MS spectra to your document and also to generate 'CoAdds' and subtractions.



Append:  it will allow you to add additional MS spectra to the main window just by selecting 'Append' in the corresponding menu and clicking with the crosshair on the corresponding signal of the TIC. If the 'Append' option is not selected, the current MS spectrum will change after having clicked on the corresponding signal of the TIC.

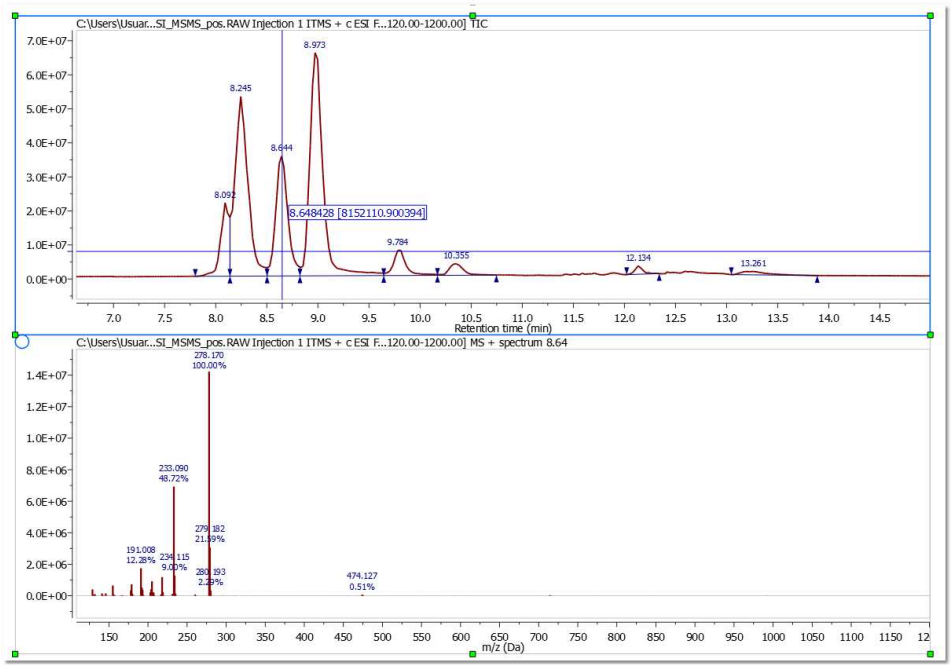
© 2024 MESTRELAB RESEARCH

In this case we have added the MS spectrum 738 which appears at 12.90 min.

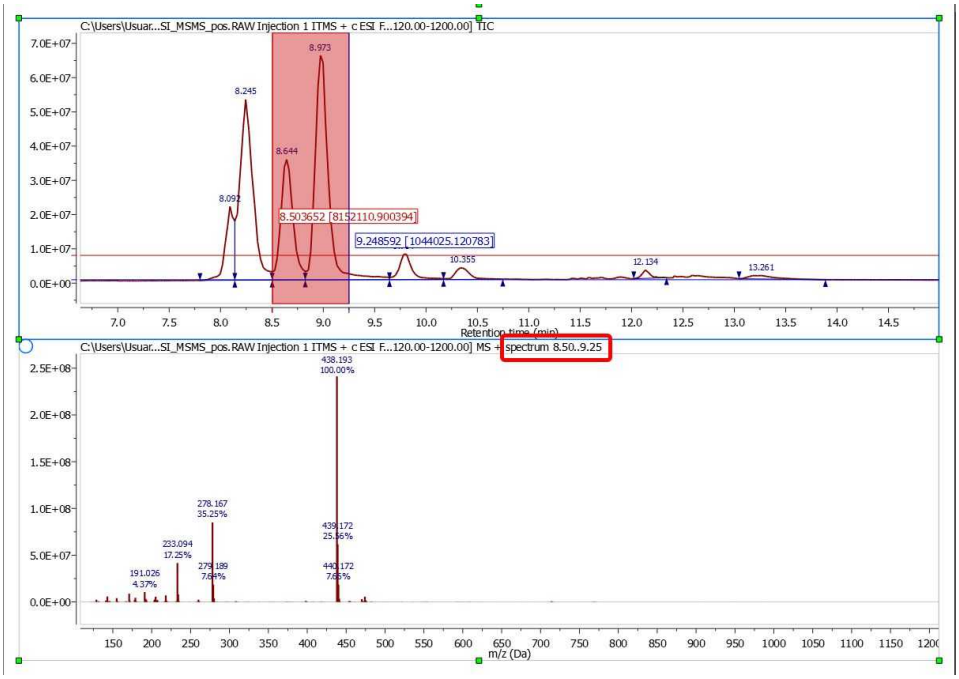


You can combine the 'Append' option with the different modes of the crosshair (Manual, Peak, Peak (Background Subt) and Customized Peak):

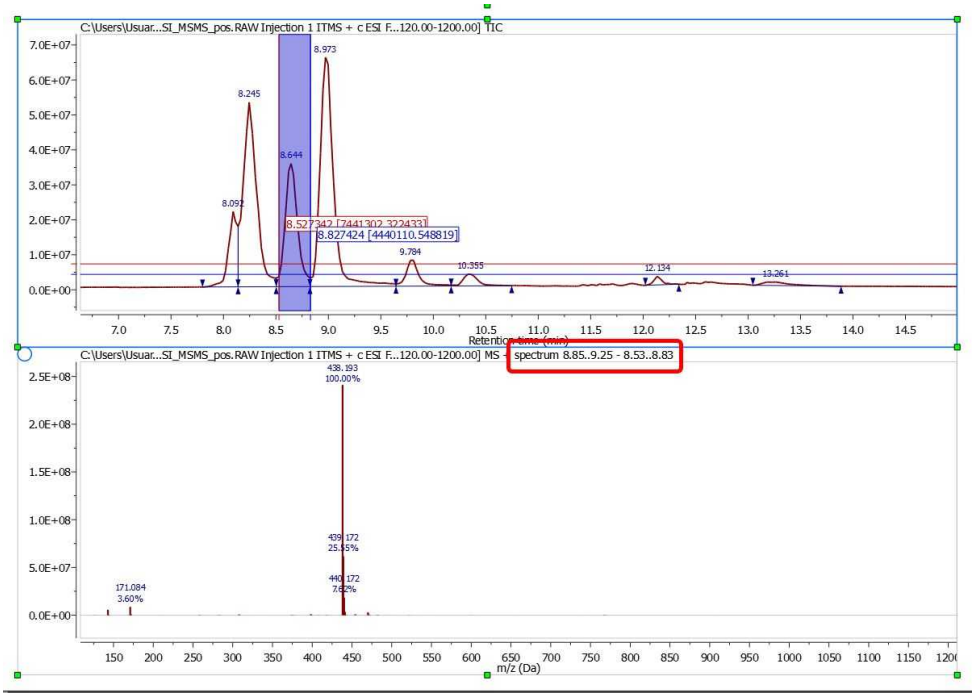
Manual: This is the default mode of the crosshair. In this mode, you can select any other mass spectrum for a particular time point with the crosshair feature  just by clicking on the corresponding signal of the TIC. For example in this case, we have selected the spectrum at a 'Retention time' of 8.64 min:



If you need to average the mass spectra over a certain time range (CoAdd), just hold down the left mouse button (after having selected the crosshair) and drag over the appropriate time span in the TIC. A label at the top of the MS will inform you about the spectrum ranges (red rectangle in the picture below):

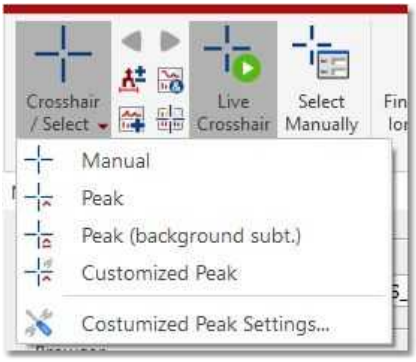


After having done the 'CoAdd' you can do a 'subtraction' by just selecting the 'Crosshair' feature, pressing the 'SHIFT' key and holding down the left mouse button and dragging over the desired time span in the TIC.



© 2024 MESTRELAB RESEARCH

Peak: Selecting this mode and clicking with the crosshair on the corresponding signal of the TIC, will generate a 'CoAdd' of the midle part of the peak. The spectra from the middle half are used because they're the highest intensity and s/n. Spectra at the peak edges are then the opposite, lower s/n and more likely to contain background. Often when a peak elutes, background is actually suppressed, especially with higher concentration components. This approach is a common technique.

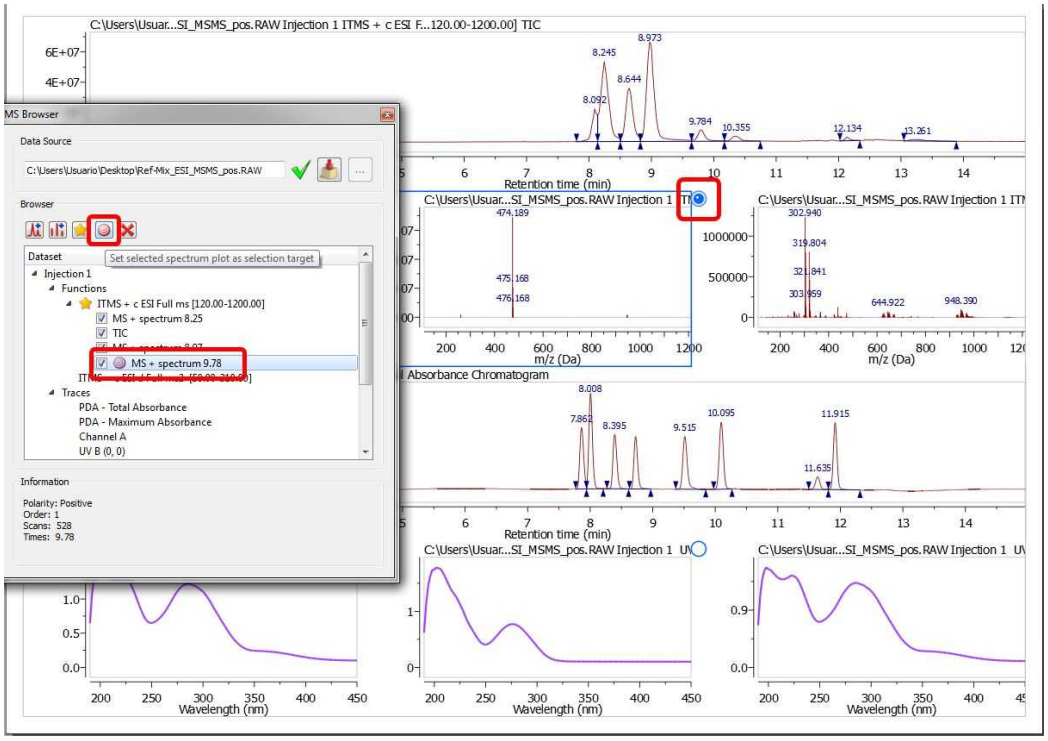


Peak (Background subt.):

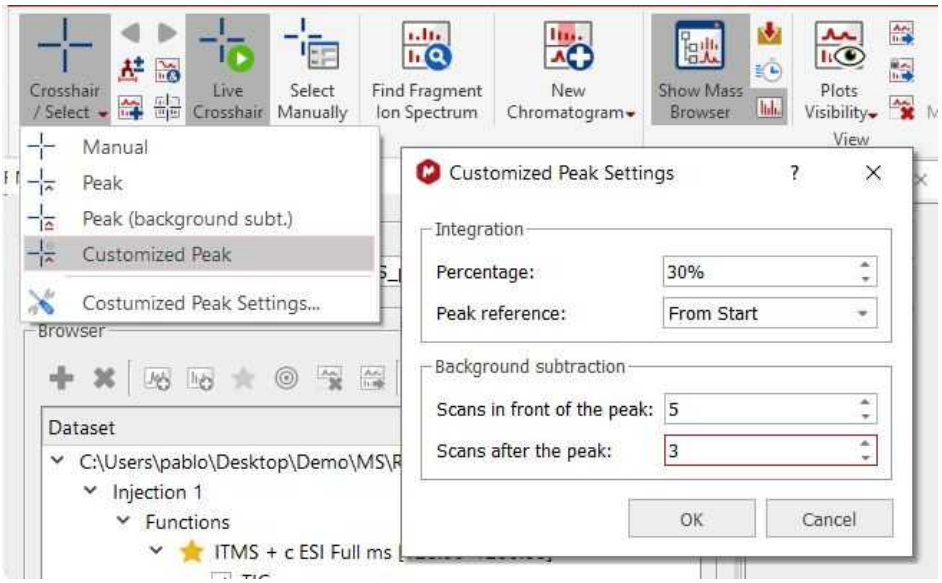
To generate a 'CoAdd' of the whole peak without the tail-peaks just select the mode 'Peak (Background Subt.)' under the 'Mass Analysis/Spectrum Selection Mode' menu and click with the crosshair on the desired signal of the TIC. You can do a 'subtraction' of any other chromatogram region just by selecting the 'Crosshair' feature, pressing the 'SHIFT' key and holding down the left mouse button and dragging over the desired time span in the TIC.

When you are in the spectrum selection mode (with the append mode disabled), you can choose graphically (by checking the blue circle) which of the plots must be replaced when you select a spectrum in a chromatogram. The selection target can be also done from the MS Browser, just by selecting the applicable MS and clicking on the 'selection' button .

© 2024 MESTRELAB RESEARCH



Customized Peak: This mode selects a peak of a chromatogram, integrates a percentage of the MS scans of the peak and automatically subtracts some scans before/after this peak. The implementation ensures that the integration has at least one MS scan.



If the selection is made with a point, this crosshair mode checks that the selected retention time point is within the peak limits. If it is not, the mode does nothing. Otherwise, the integration/subtraction is performed with the selected peak.

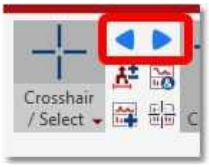
© 2024 MESTRELAB RESEARCH

If the selection is made with a range, this crosshair mode checks that the selected retention time range has any peaks. If it does not, the mode does nothing. Otherwise, the mode selects the most intense peak in the range (highest height) and performs the corresponding calculations.

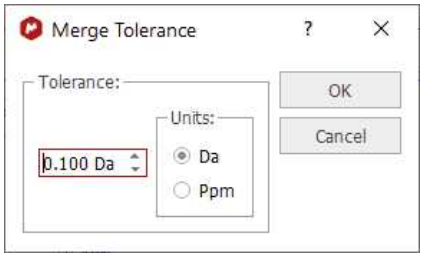
This mode can be configured from the the Customized Peak Settings dialog. From here, you can modify the percentage of peak integration (from 1% to 100%) and the peak reference (From Start (the first MS scans), Near Top (the MS scans nearest to the top of the peak) and To End (the last MS scans).

Also, the user can indicate the number of scans before/after the peak to be subtracted (zero or a positive integer). These settings are saved to the Mnova configuration (Registry Editor in Windows), so they are independent of the Mnova document.

You can move forward/backward scan by scan by clicking the buttons "Previous Scan" or "Next Scan" in the ribbon menu, or by using the shortcuts Alt+Left or Alt+Right arrow:



Set Merge Tolerance: From this dialog box, you can select the tolerance (in Da or ppm) of the coadded mass spectra.

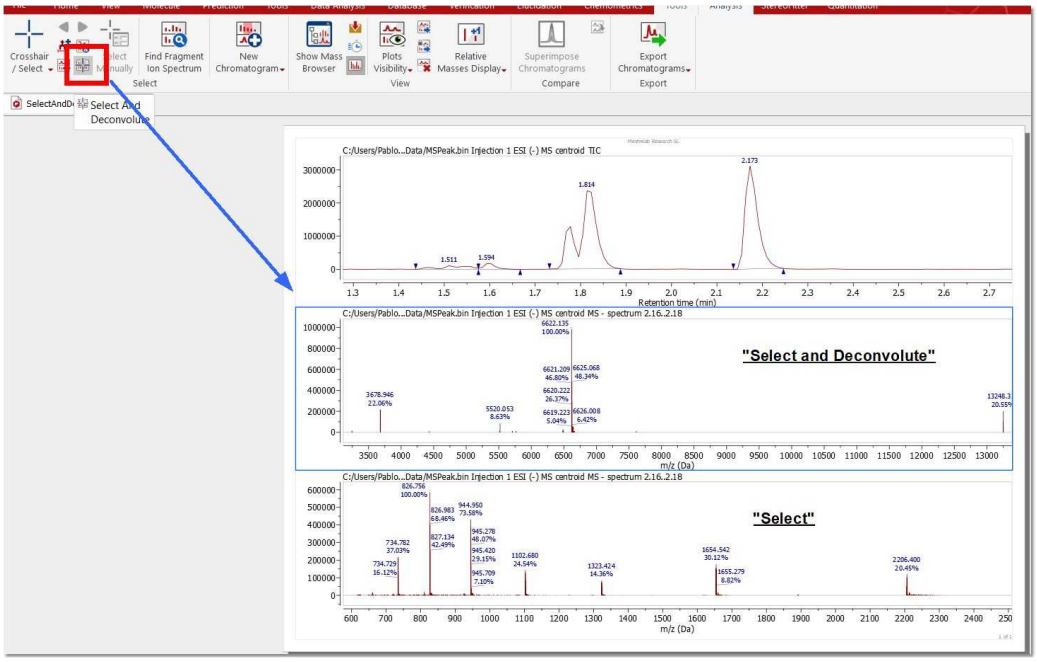


Merged tolerance is a m/z tolerance used any time mass spectra are summed or subtracted. The tolerance is applied to m/z values. Thus a merge tolerance of 0.1 Da means that if two spectra being summed contain, respectively, m/z 100.100 and m/z 100.150, the two mass peaks with be logically summed, since they're within 0.1 Da. The resultant intensity is the simple sum; the resultant m/z value will be the intensity-weighted average of 100.100 and 100.150.

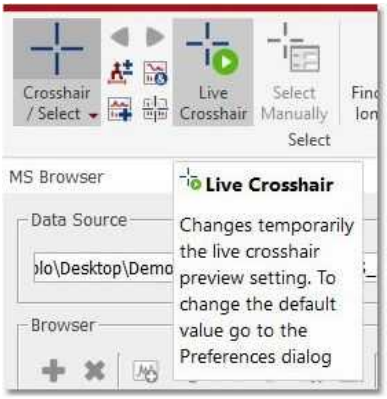
Select MS/UV together: When having this button pressed, if you select a position or a range in either the TIC or DAD trace, the MS spectrum and the UV spectra will be extracted at the same time.

Select and Deconvolute: If this mode is enabled (in the crosshair mode) the extracted spectrum gets also deconvoluted in the same step.

© 2024 MESTRELAB RESEARCH



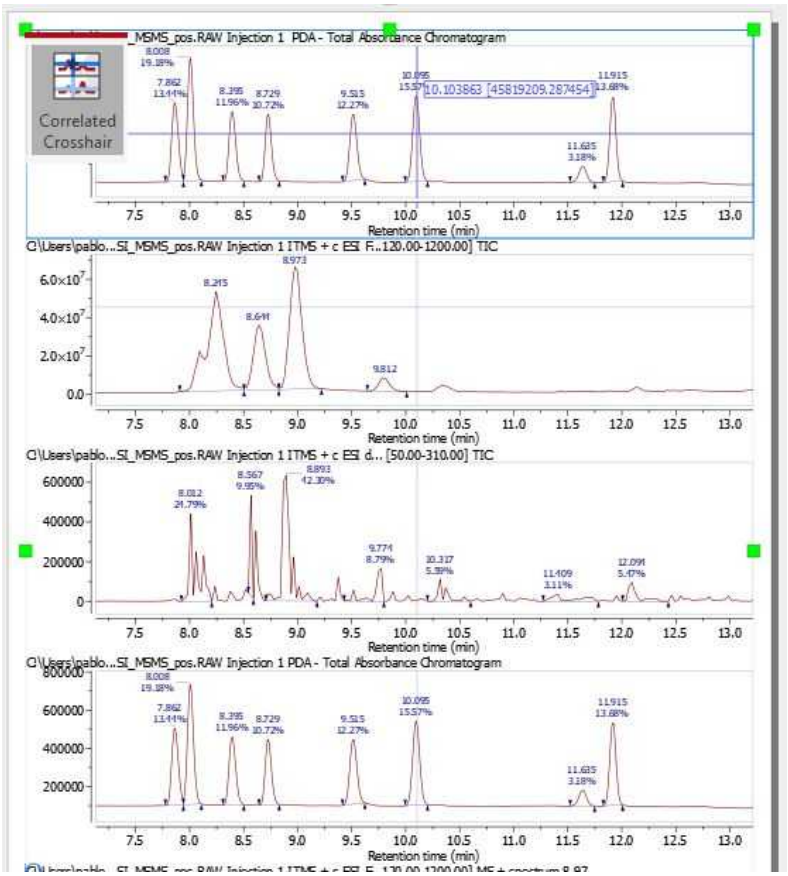
Live Crosshair: it will temporarily activate the 'Live view' mode which will display the applicable MS spectrum when hovering the 'crosshair' across the chromatogram.



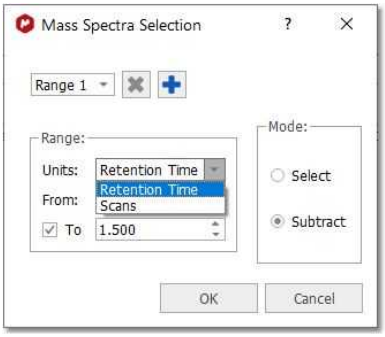
The 'Live Mass Spectrum Preview' option is also available under the 'File/Preferences/Mass/General' dialog box.

The **'Correlated Crosshair'** mode will display the crosshair as usual in the active plot and also as a semi-transparent crosshair in the none-active plots of the same type (chromatograms, mass spectra or UV spectra):

© 2024 MESTRELAB RESEARCH



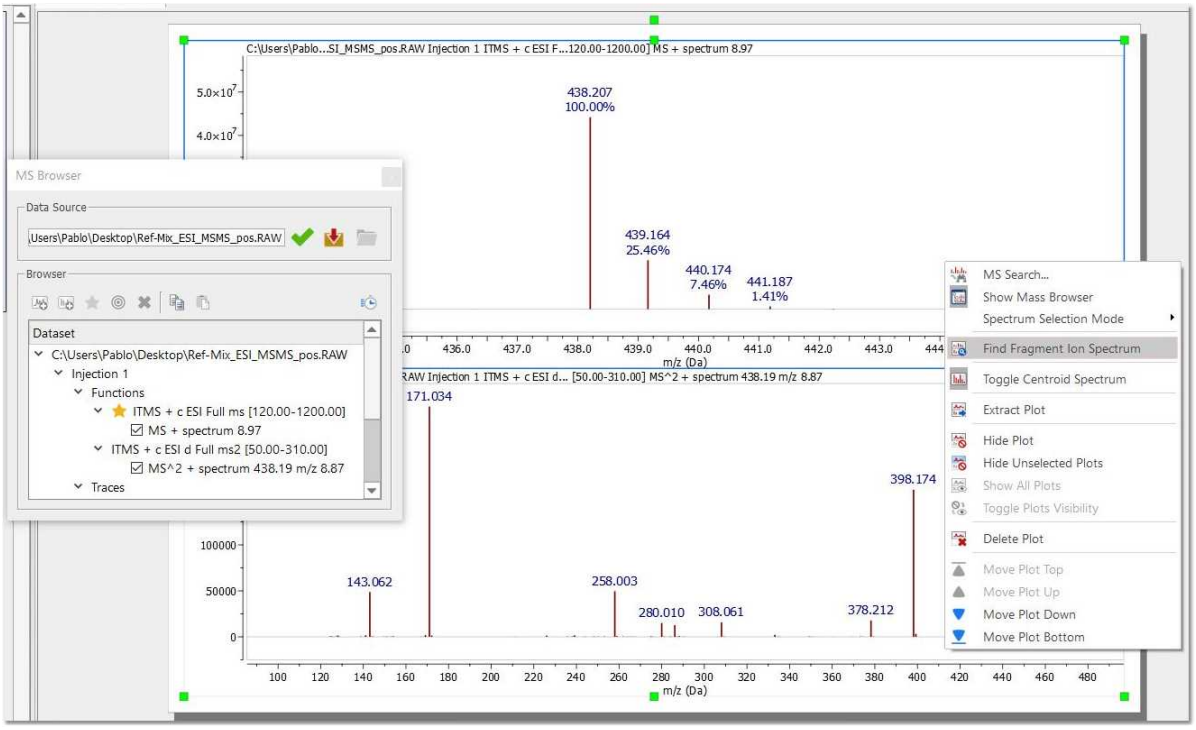
Select Manually: That will allow you to get (or subtract) the MS (and UV) spectrum at the desired retention time (or scan) range.



In the subtract mode, you can select multiple spectral ranges from the applicable scroll down menu.

Find Fragment ion spectrum: to easily display the applicable MS/MS spectrum from a MS dataset of the precursor, by matching the highest mass peak at around the same RT value. The highest mass peak in the MS spectrum depends on the displayed range, so if you make a zoom, the m/z value will be taken from the highest displayed peak.

© 2024 MESTRELAB RESEARCH



14.2.2.2 New Mass chromatogram

It is very useful to extract a MS spectrum by following the menu 'Mass Tools/New Chromatogram' or by clicking on the corresponding icon of the toolbar:



You can extract the MS chromatogram **graphically** or **manually**. To extract the MS graphically, just select the option and click on the MS (or click and drag if you want to select a range).

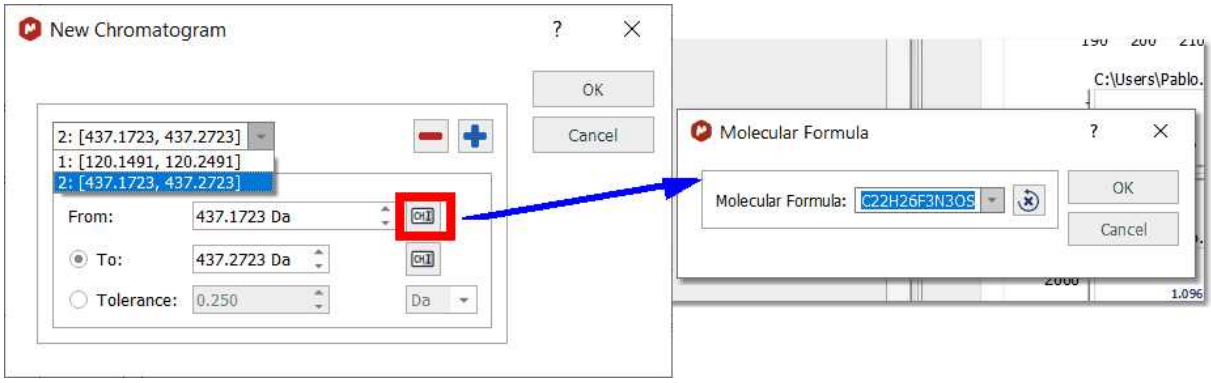


Holding down the 'SHIFT key' will activate the *'sticky mode'* which will move the cursor to the nearest peak

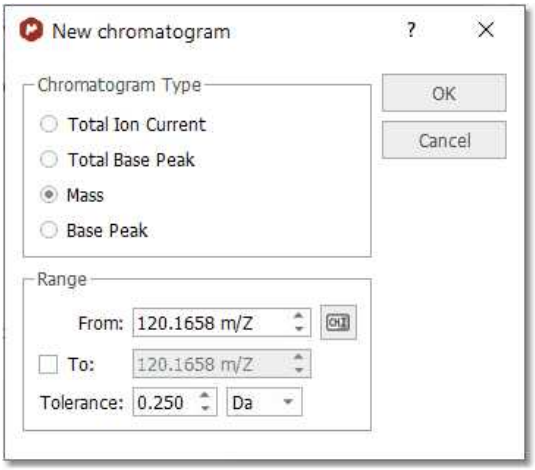
© 2024 MESTRELAB RESEARCH



You can also select the range (or ranges, by clicking on the blue plus button) of the new chromatogram **manually** (and by using a molecular formula, so that the m/z value is calculated automatically to extract the corresponding new mass chromatogram):

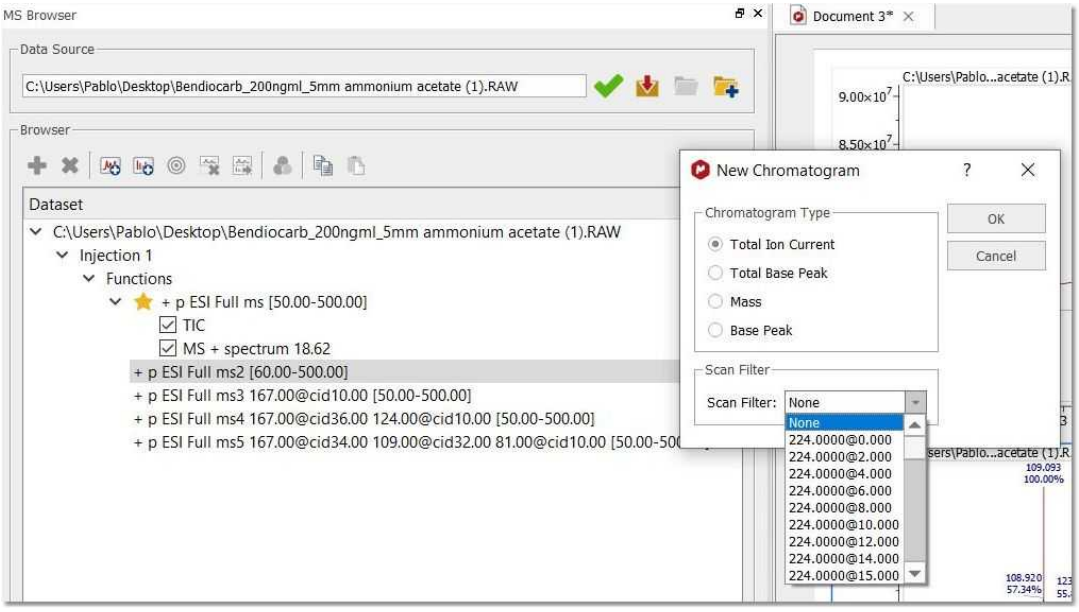


This also can be done from the MS Browser ('New Chromatogram/Mass'):



© 2024 MESTRELAB RESEARCH

You could also create a new chromatogram from a MS/MS function. In that case, if the provider supports it (such as Xcalibur from Thermoscientific), a *scan filter* dropdown will be displayed; showing the different precursors and collision energies. After having selected the desired filter, you will obtain the applicable chromatogram:

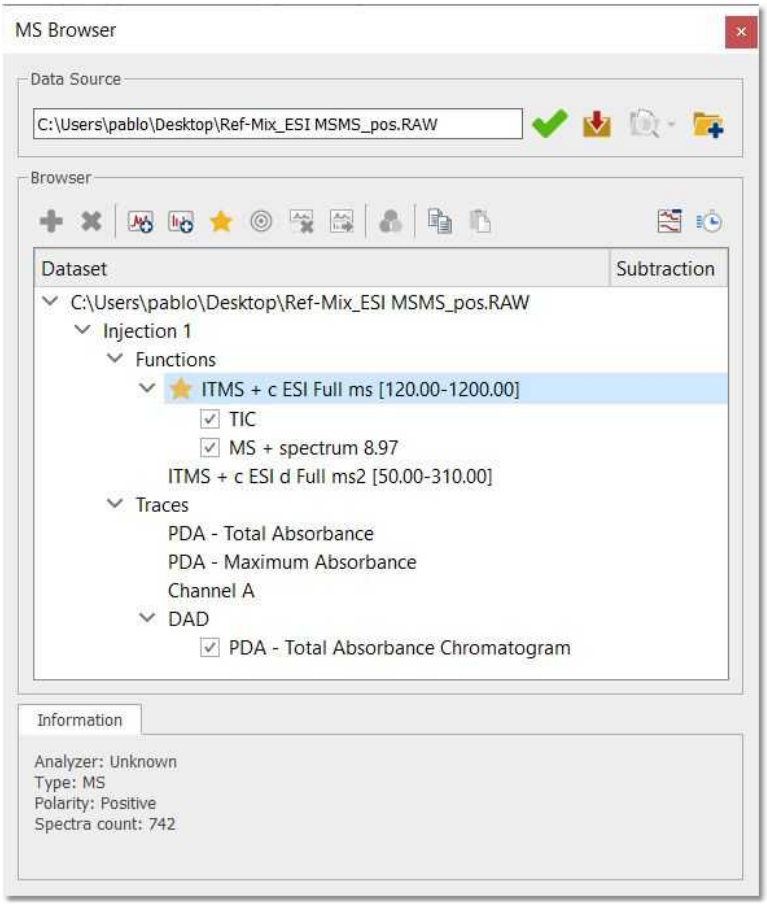


If you are interested in a Base Peak Chromatogram (BPC) for a selected mass range, just select the 'Base Peak' radio button and choose the desired range.

14.2.2.3 MS Browser

This panel will be displayed by default when you load a dataset. If it is hidden, you can show it by clicking on the corresponding icon  (or by following the menu 'View/Tables/Mass/MS Browser' or 'Mass Tools/Show MS Browser'). It is very useful and we recommend to dock it to the page navigator. From this panel you can easily open additional chromatograms or spectra at the same item:

© 2024 MESTRELAB RESEARCH



This dialog box will contain information about the 'Data Source'. If the current spectrum contains the original spectrum file, the 'Data Source' will show a green tick mark. If not, it will show a red cross (and the spectrum will have a red frame); which indicates that you will not be able to load any other part of the chromatogram and you will only be allowed to work with the spectrum that you have on the screen.

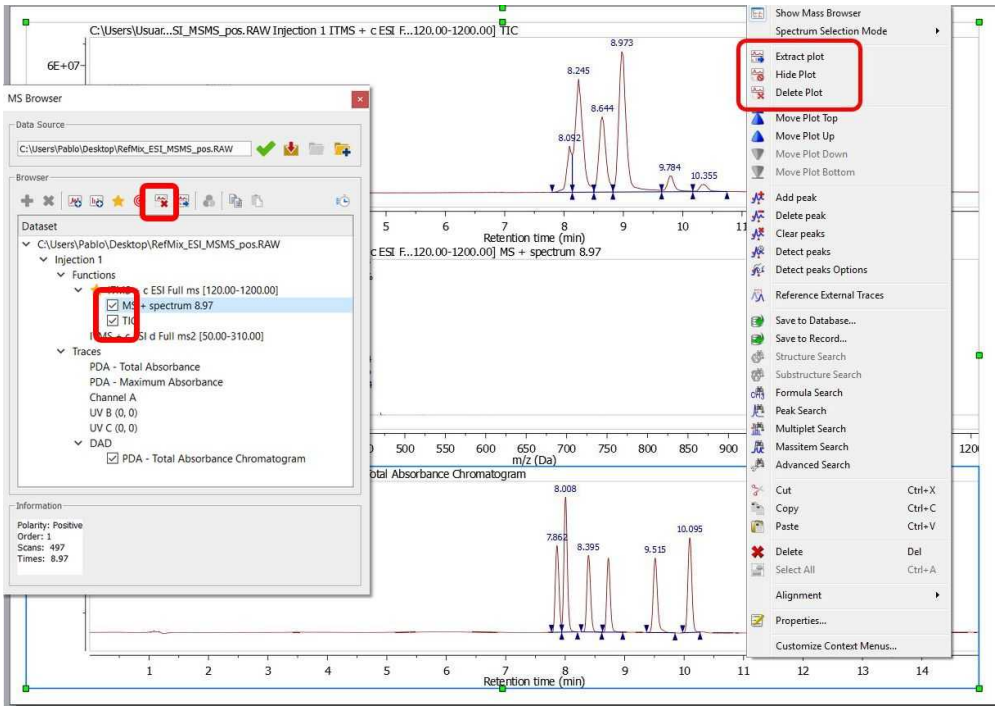
© 2024 MESTRELAB RESEARCH

Attention:

Please bear in mind that when you save a Mnova document with a TIC and a Mass spectrum, by default the raw file is not stored in the document (to avoid the generation of huge files), so you will need to keep the original raw file on your computer if you plan to continue working with the experiment in the future.
If you want to store the raw file on the Mnova document, click on the 'Fetch Full Dataset' button of the MS Browser.
Please make sure that if you change the path of the raw file (after having saved a Mnova document); you will need to load it by clicking on the 'Set Dataset File' button (of the MS Browser table) if you want to work with the experiment. Be careful in this situation, because Mnova will not be able to identify if you load an erroneous raw file (instead of the correct one).

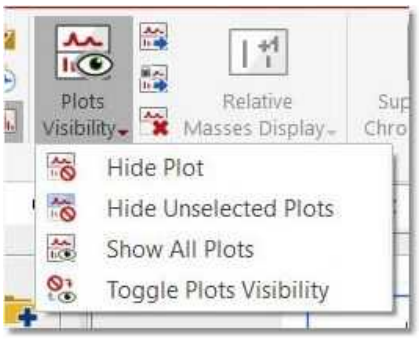
You can hide any chromatogram or MS spectrum by checking the corresponding check box in the 'MS Browser' or by selecting 'Hide Plot' in the context menu of the spectrum (which can be accessed by right clicking on the spectral window).

If you want to delete the plot from the Browser, just select it and click on the red cross button. The same result will be obtained by selecting 'Delete Plot' in the context menu. You can also hide and extract to any page the selected plot:

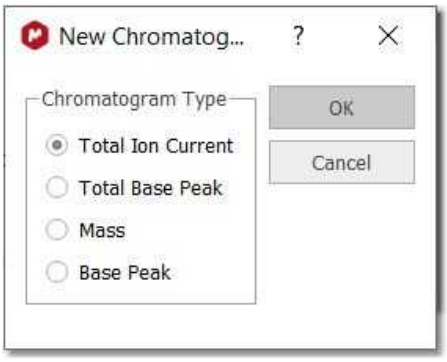


You can also use the Plots Visibility ribbon to hide/delete/extract plots:

© 2024 MESTRELAB RESEARCH



Double clicking on any function from the MS browser, will display the applicable dialog box, which will allow you to open the TIC, Base Peak or the Mass spectrum. The same result will be obtained by highlighting the applicable function and clicking on the 'New Chromatogram' icon:



- **TIC** (Total Ion Current): The sum of all the separate ion currents carried by the different ions contributing to a complete mass spectrum or in a specified m/z range of a mass spectrum (this is sometimes called the reconstructed ion current).

- **Base Peak**: The peak in a mass spectrum corresponding to the separated ion beam which has the greatest intensity which is assigned a relative intensity value of 100. The lesser peaks are reported as a percentage of it.

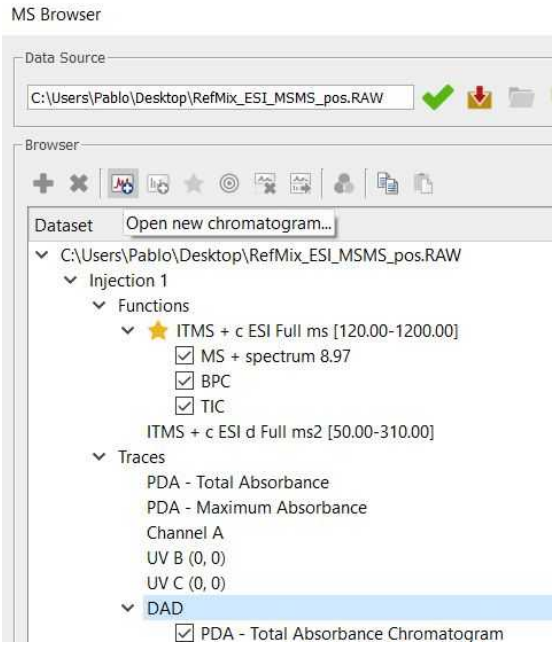
- **Mass**: clicking on this option will display the below dialog box which will allow you to select the range of the chromatogram between two values of m/z. You will obtain the TIC corresponding to the selected m/z range.

You will obtain the same result by selecting 'Manually' on the scroll down menu of the 'New Chromatogram' button of the toolbar:

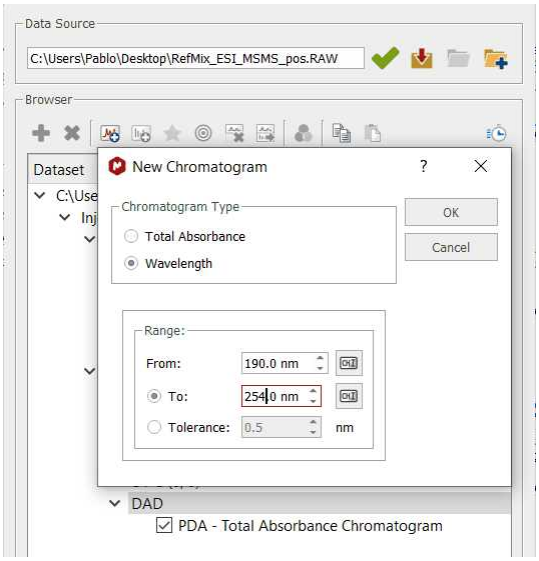


You can display any trace (i.e PDA or UV chromatograms) by double clicking on it or by selecting it on the MS Browser and then clicking on the 'Open new Chromatogram' icon.

© 2024 MESTRELAB RESEARCH



If your dataset contains UV traces (DAD) and it is in Masshunter, Bruker Compass, Shimadzu v5, Xcalibur or MassLynx format; you can extract them or select a range in the wavelength; by selecting the applicable option after having clicked on the 'Open new Chromatogram' icon

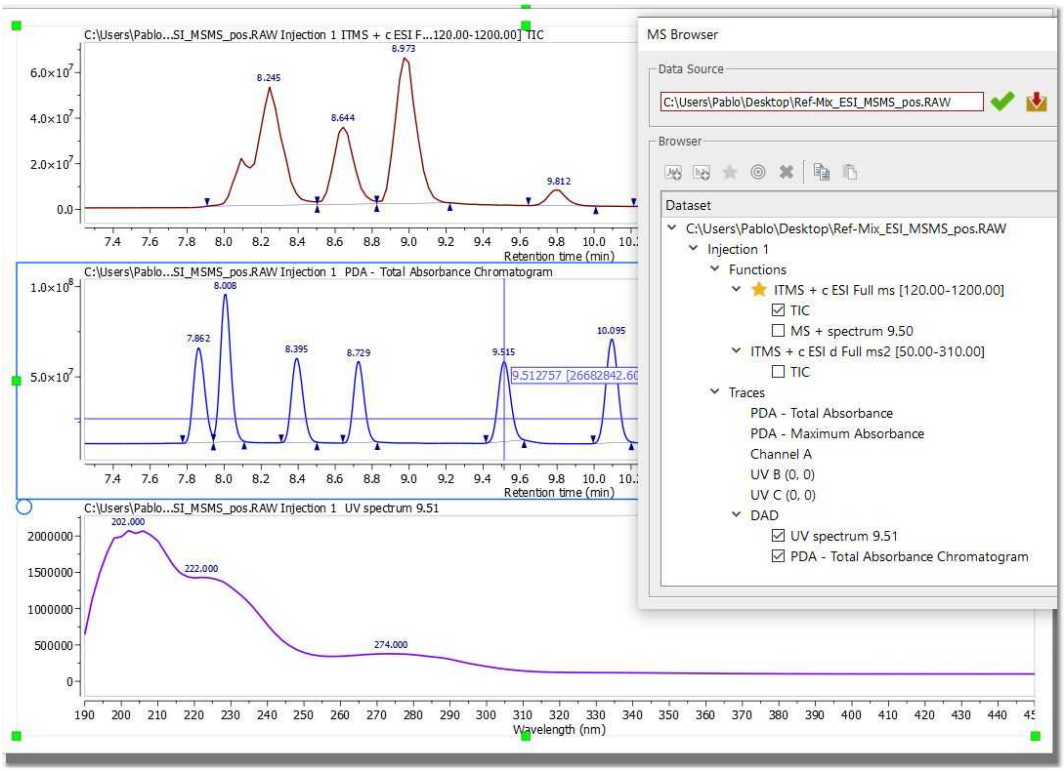


From the PDA-Total Absorbance Chromatogram, you will be able to extract the complete **UV spectrum.**

To select a UV spectra: the dataset must have the full UV spectra set (and not just a single or a set of UV chromatograms, ie, MS browser panel must show a sub-item called DAD under the Traces item).

Using the crosshair mode and **clicking** on a UV chromatogram while **holding Alt** pressed, will display the UV spectrum for the clicked retention time.

© 2024 MESTRELAB RESEARCH



You can display the UV together with the MS by selecting the applicable feature from the ribbon:



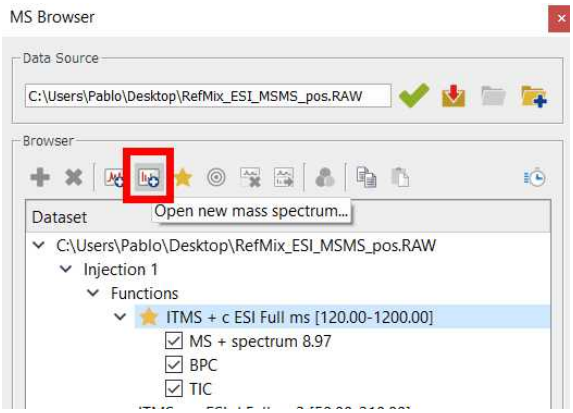
You can also Co-add UV chromatograms by clicking and dragging the crosshair to select a range on DAD chromatograms as you can do with the TICs. Please bear in mind that Mnova MSChrom only supports UV spectra from Masshunter, Bruker Compass, Shimadzu v5, Xcalibur or MassLynx formats.

The new button opens a new dialog that list all functions displayed in MS Browser that are compatible with the selected function: same spectrum number, same polarity,... In short, the same Information that is displayed at the bottom of the MS Browser (Note that "Information" is now a tab, not a group). This button is only enable when a MS function is selected and there is more than one function loaded in the MS Browser.

Note that the background function must be in the same MS item as the foreground function.

Highlighting any function in the MS Browser and clicking on the 'New Spectrum' icon will display the corresponding dialog box which will allow you to open the desired spectrum by just typing the number on the edit box:

© 2024 MESTRELAB RESEARCH

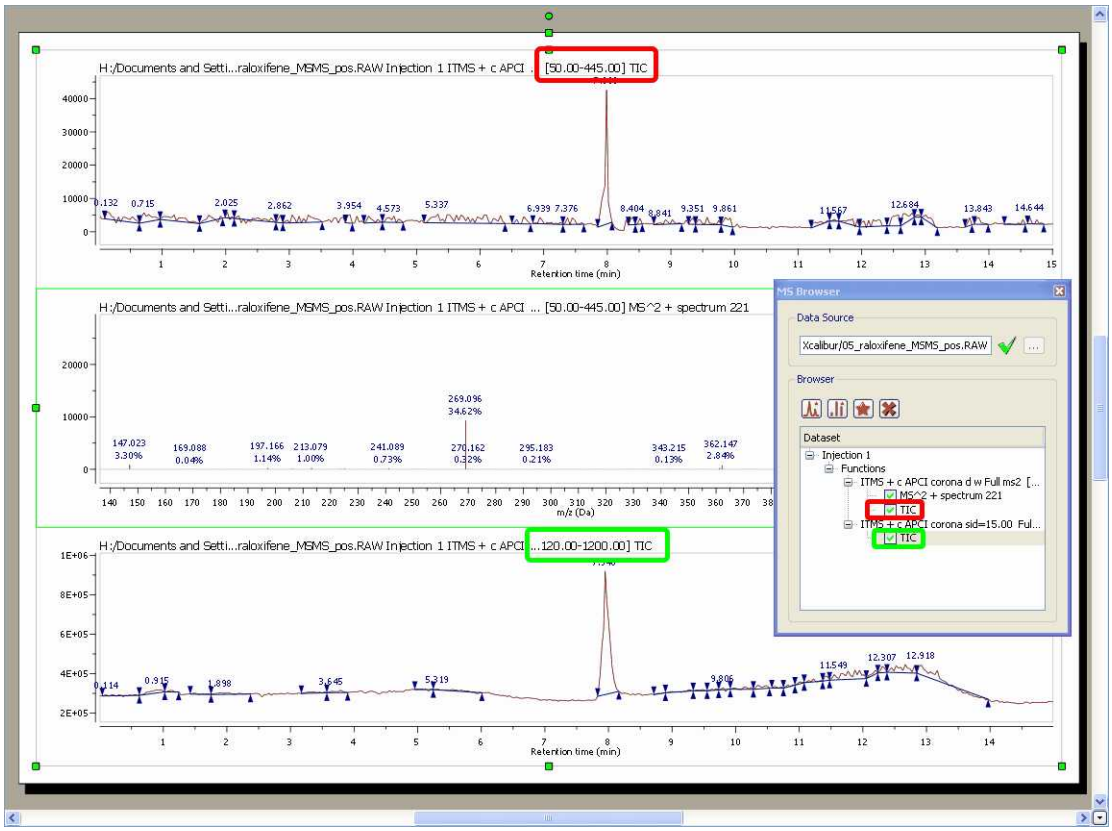


The below picture will show you a TIC (red), a Mass spectrum (number 467), the Base Peak (green) and the chromatogram between m/z=150.23-320.23 (pink):



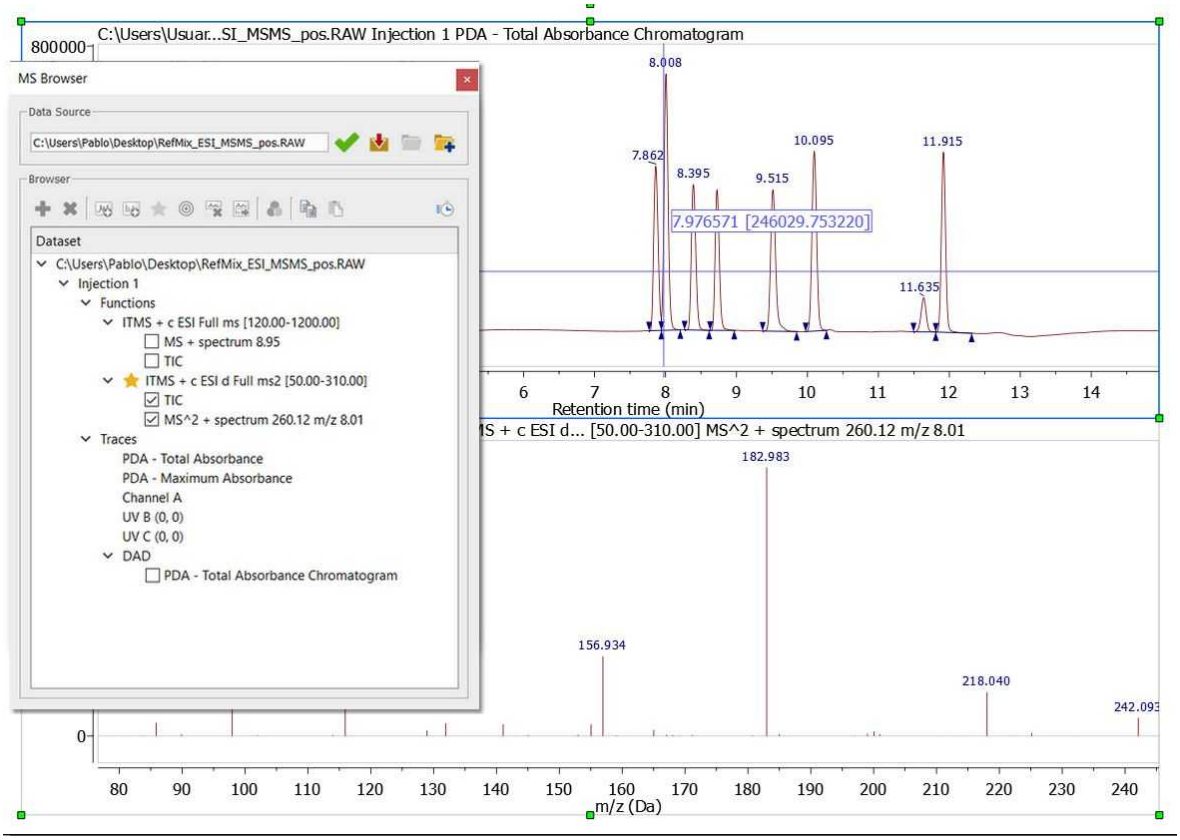
In the example below, you can see two TICs of two different functions:

© 2024 MESTRELAB RESEARCH

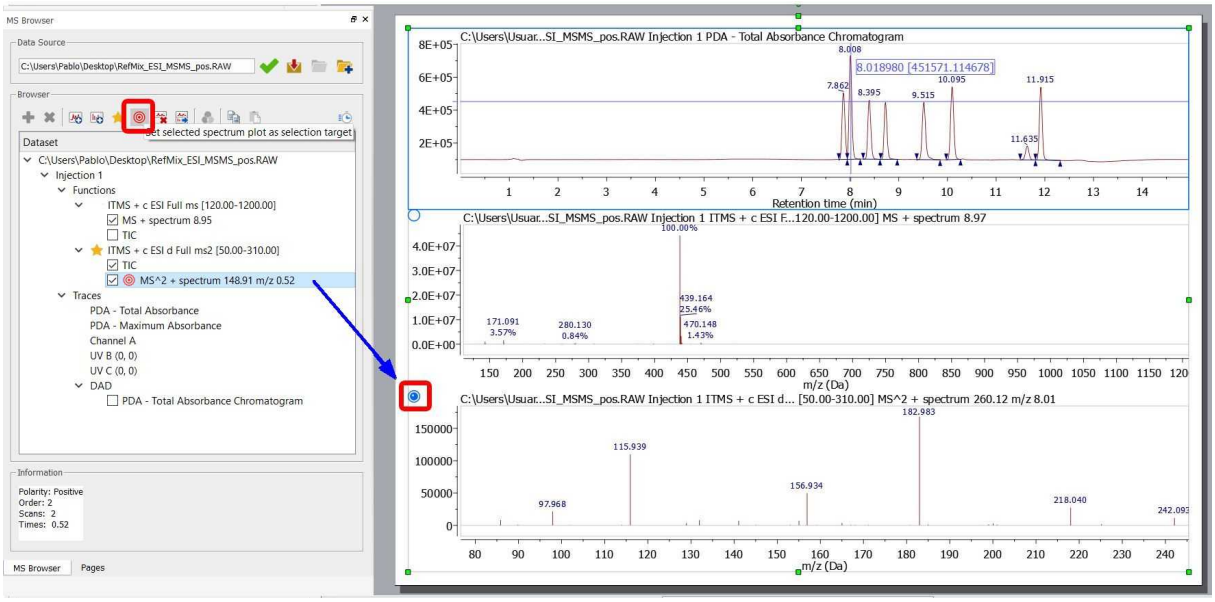


Selecting a function on the MS Browser window and clicking on this button , will set the selected function as active. This option will allow you to work with the traces chromatogram of different functions. It will be very useful for example if you have a PDA-Total Absorbance Chromatogram and you want to display the MS of one of the functions only:

© 2024 MESTRELAB RESEARCH

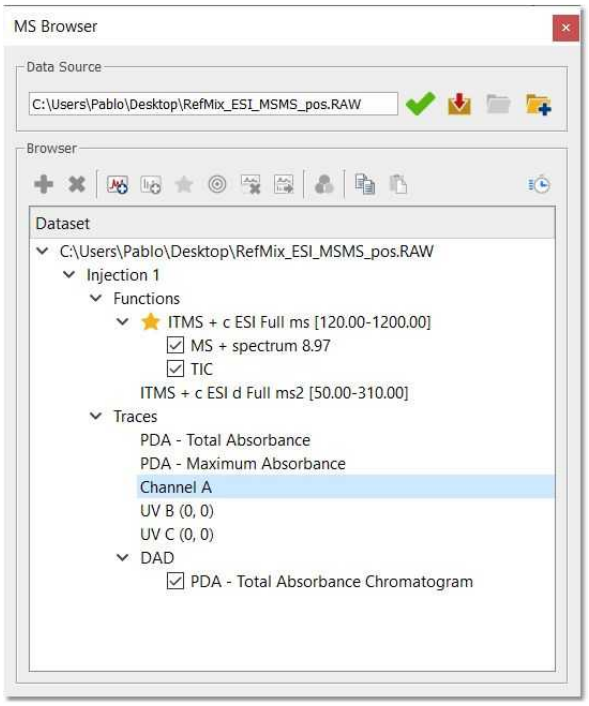


If you have two or more spectra opened in the document, you can use the feature to 'Select the spectrum plot as selection target'  in order to change that plot when you decide to display any other MS (after having clicked on a chromatogram under the crosshair mode). You will obtain the same result by clicking on the blue radio button  of the MS spectrum:



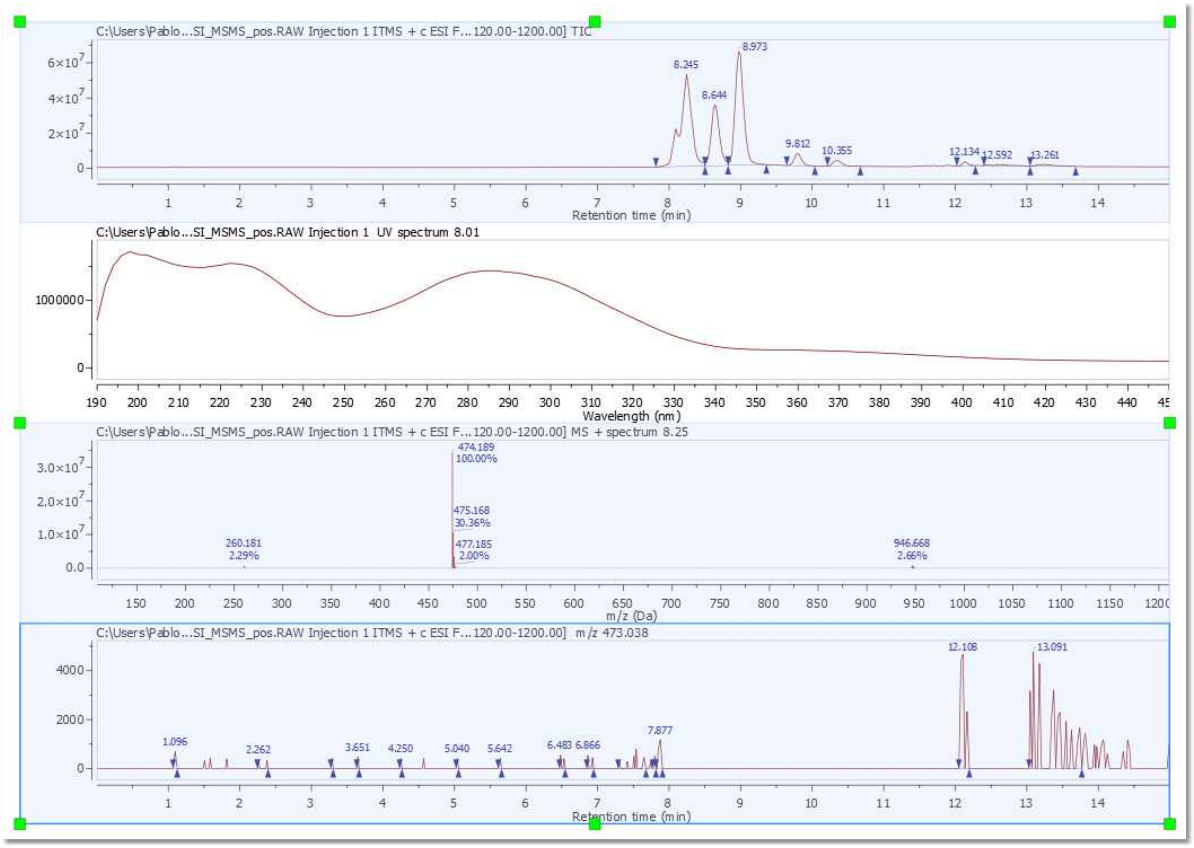
© 2024 MESTRELAB RESEARCH

Please bear in mind that different mass spectrometer acquisition data systems do different things with the names of traces. When we import traces, we do our best to retrieve sensible names, but they are not always present (or appear as 'Channel A' for example). We hope that the chemist who acquired the data will usually know what "Channel A" actually is.



You can select several chromatograms or spectra in the same page, for example to 'zoom in' or extract them to a new page at the same time. To do it, just hold down the 'Ctrl+Alt' ('Cmd+Alt' in Mac) key and click. Ctrl+SHIFT+Alt will select all chromatograms. To release the selection, press 'Ctrl+Alt' (Cmd+Alt in Mac) and click on the undesired item.

© 2024 MESTRELAB RESEARCH



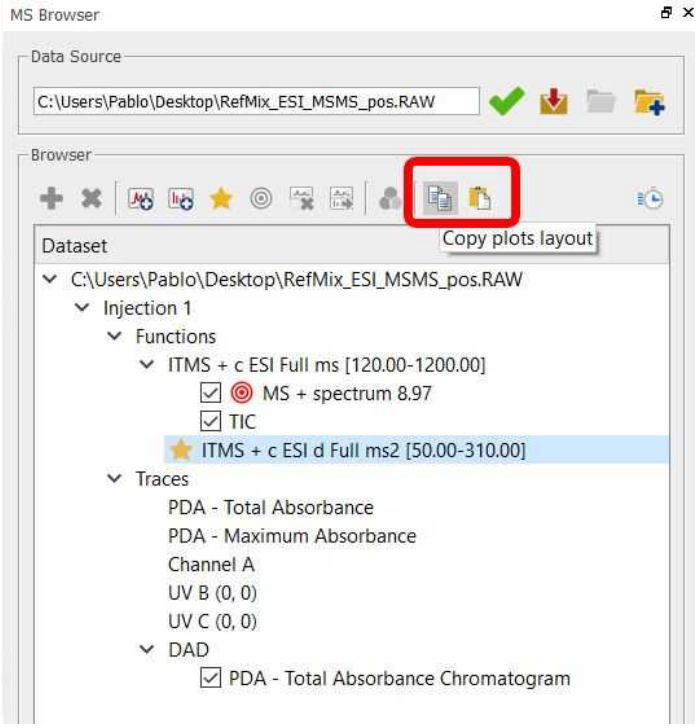


Shortcuts to select MS objects

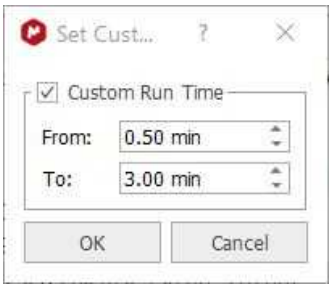
- Alt+click: select/deselect a single object.
- Ctrl+Alt+click: add/remove object from/to selection.
- Ctrl+Alt+Shift+click: select multiple MS objects in between last selection.

From the MS browser panel, you can copy the display configuration and paste it to a second dataset:

© 2024 MESTRELAB RESEARCH

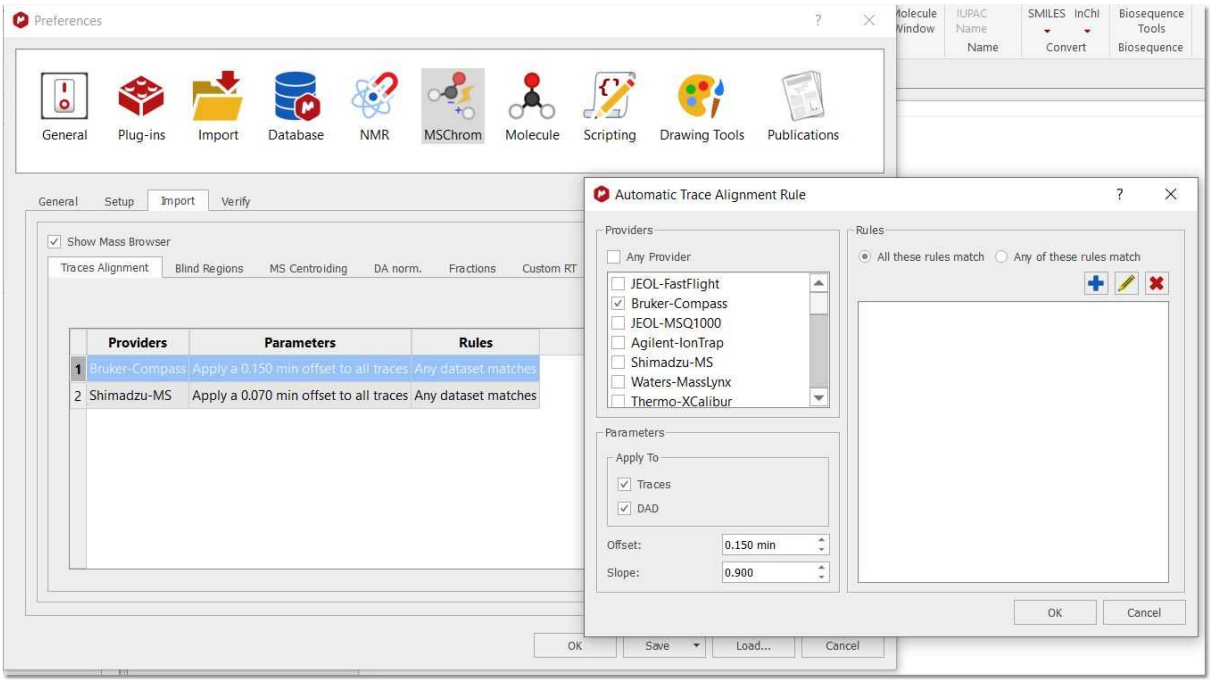


Use the this button  (from the ribbon or from the MS Browser panel) to specify a custom run time for a mass item, so all chromatograms will use that run time range as the full zoom out scale.

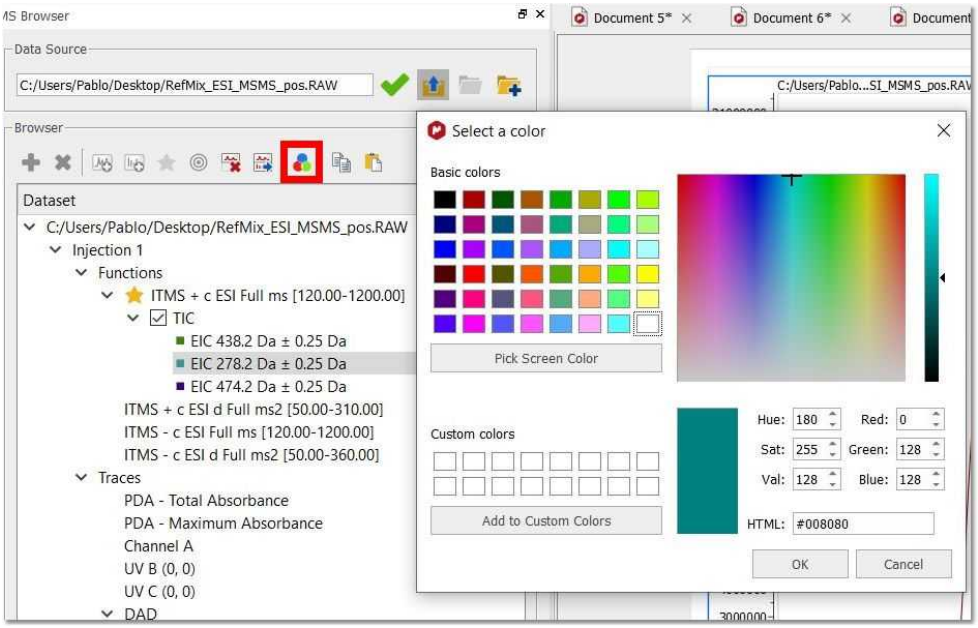


In addition,from the 'Preferences', you can define rules to apply custom run time ranges automatically when importing a mass dataset:

© 2024 MESTRELAB RESEARCH



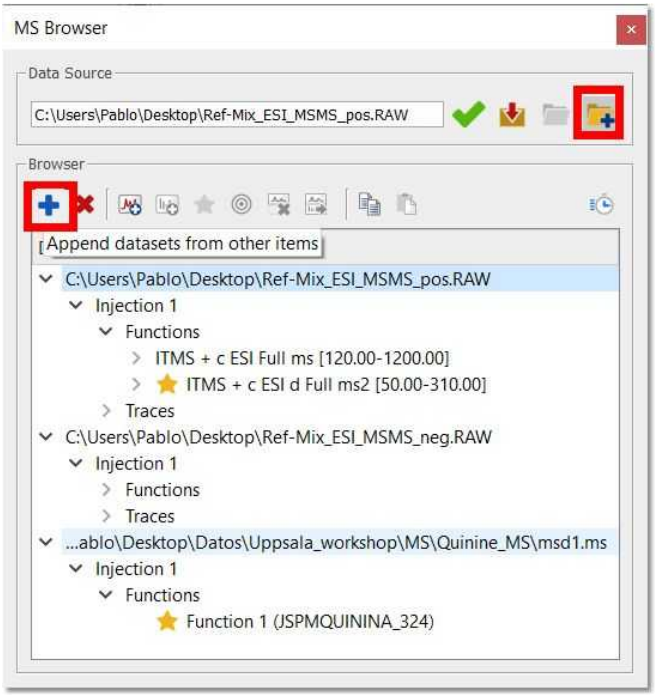
If you are working with superimposed chromatograms, you can change the colors from the MS Browser:



You can open several dataset files into the same mnova document. There are two ways to do it from the Mass Browser:

1. Click on the icon with a plus sign over a folder, at the right of the current dataset location, to choose another dataset to be added to the item
2. Click on the plus icon to chose a dataset from another mass item already in the document. There is also a minus icon to remove a dataset and all the plots belonging to it.

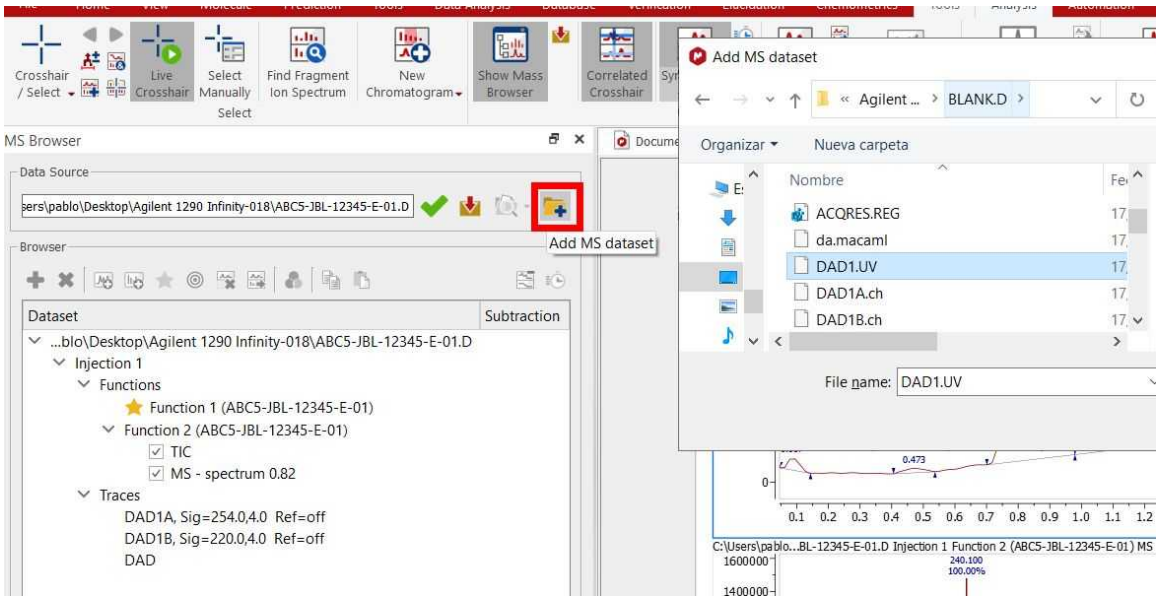
© 2024 MESTRELAB RESEARCH



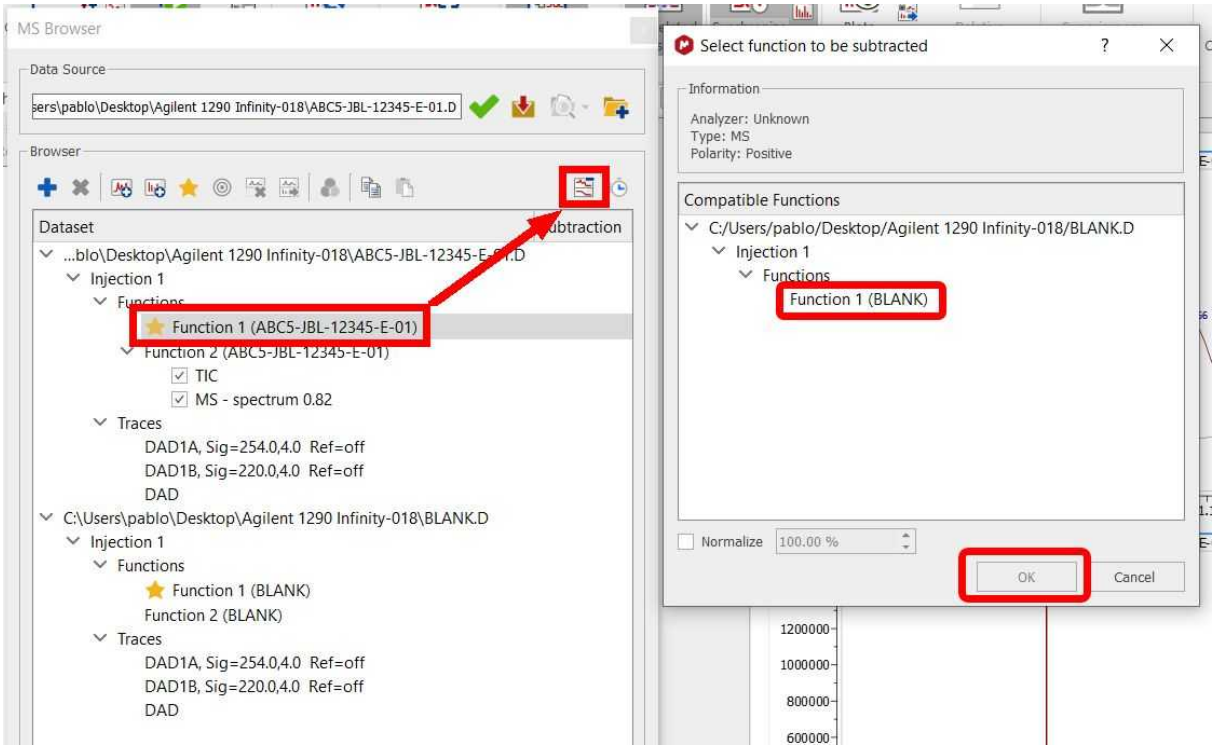
14.2.2.3.1 Chromatograms Subtraction

This feature is intended to subtract the background of your datasets or to apply a chromatogram or UV subtraction. See also these scripts.

First of all, you will need to load your dataset, which will display the main functions in the MS browser. Next click on the 'Add MS Dataset' button to select the file of your blank function (if the dataset contains the foreground and blank functions, you can skip this step):



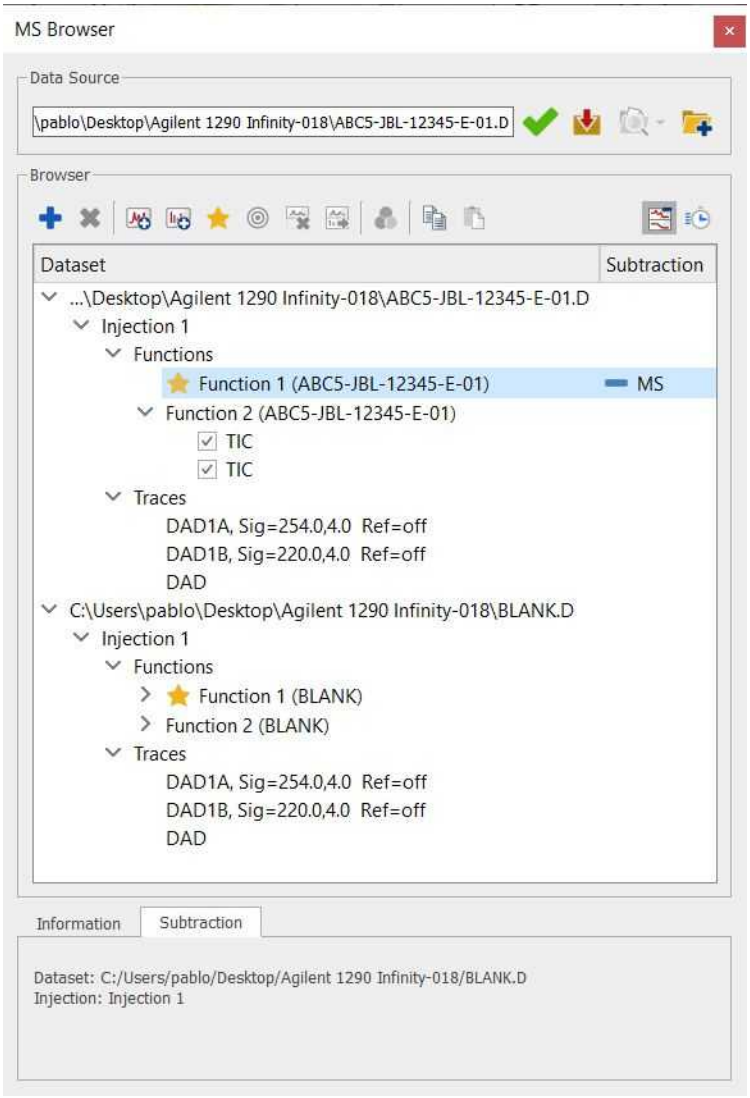
Next, select the main function and click on the 'MS Subtraction' button to select the function to be subtracted (blank):



Click on OK to get the result. If you are interested in applying any normalization, feel free to check the applicable box (and enter the desired value) before having clicked on the OK button.

The MS Browser will display a line under the 'Subtraction' column, to display that a subtraction was applied:

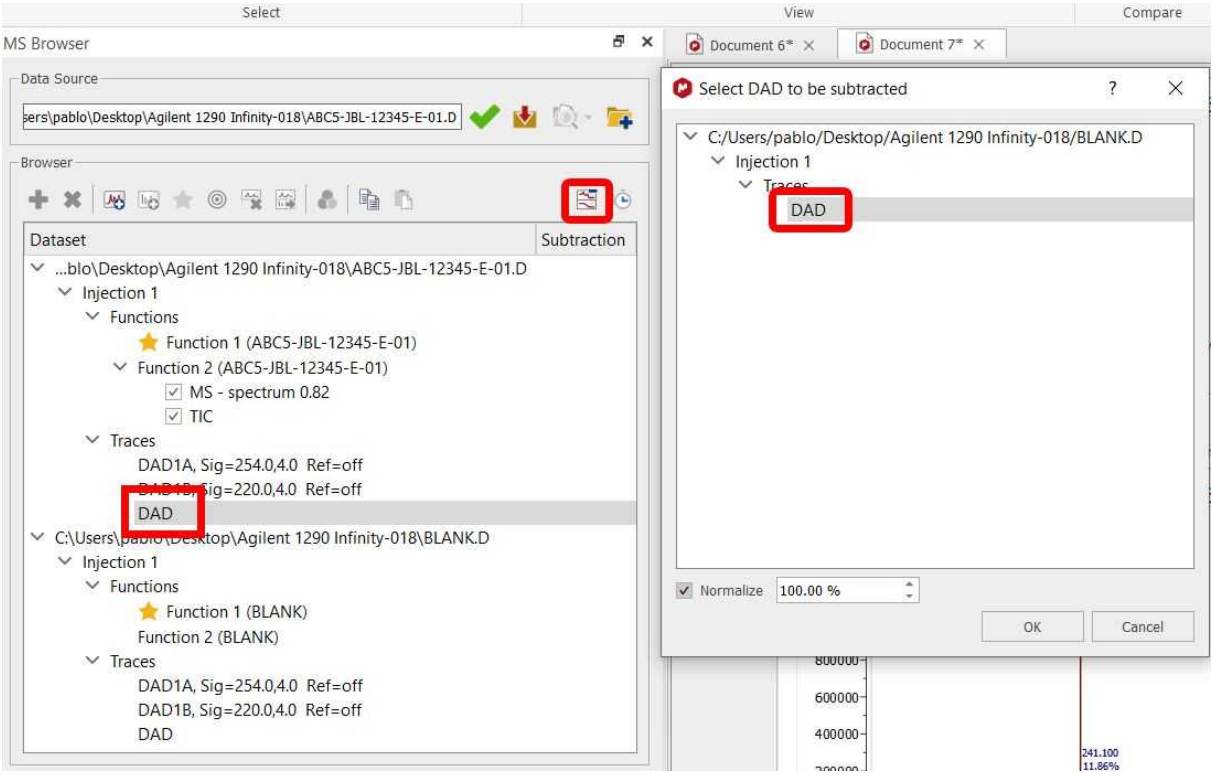
© 2024 MESTRELAB RESEARCH



You can remove the subtraction, just by selecting the function and clicking again on the MS subtraction button.

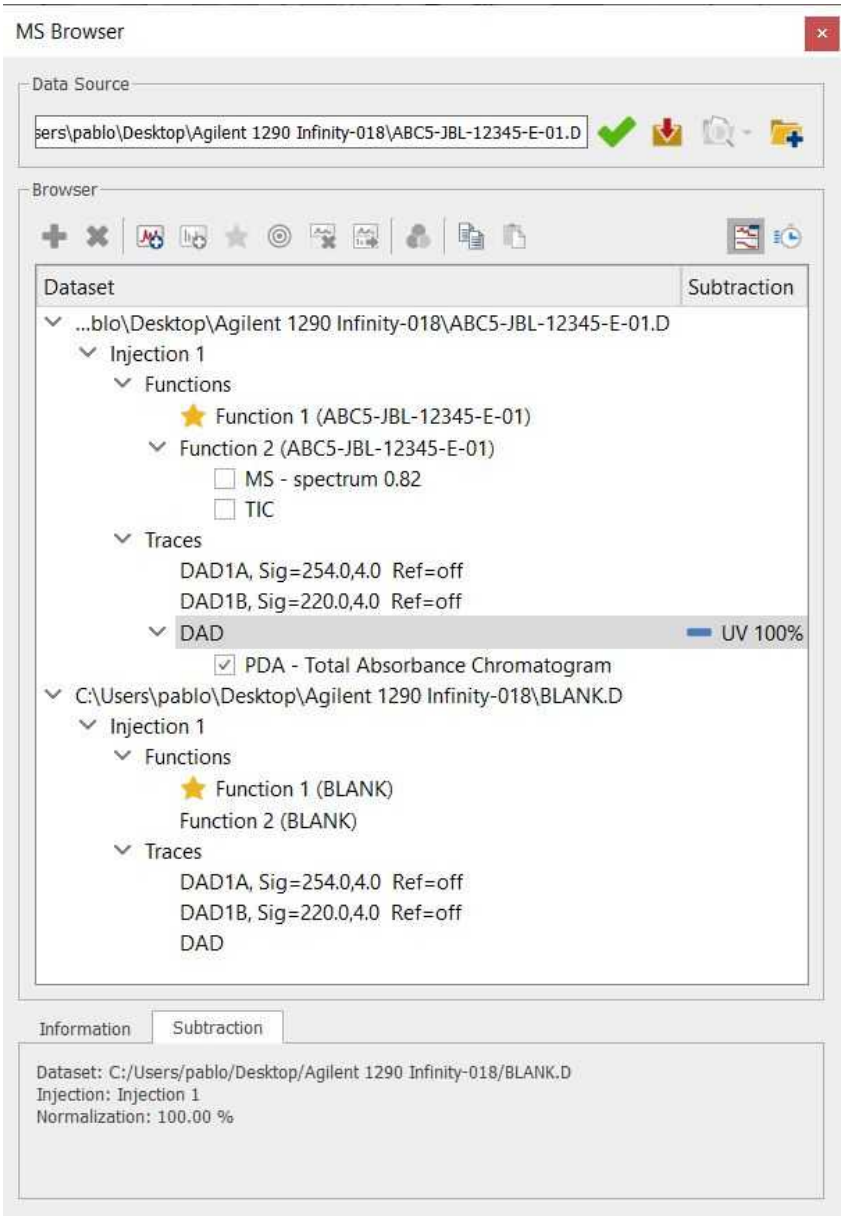
In the case that you are working with UV chromatograms, the workflow will be the same. In this case, you will need to select the DAD (or PDA) as the main function and subtract the applicable blank:

© 2024 MESTRELAB RESEARCH

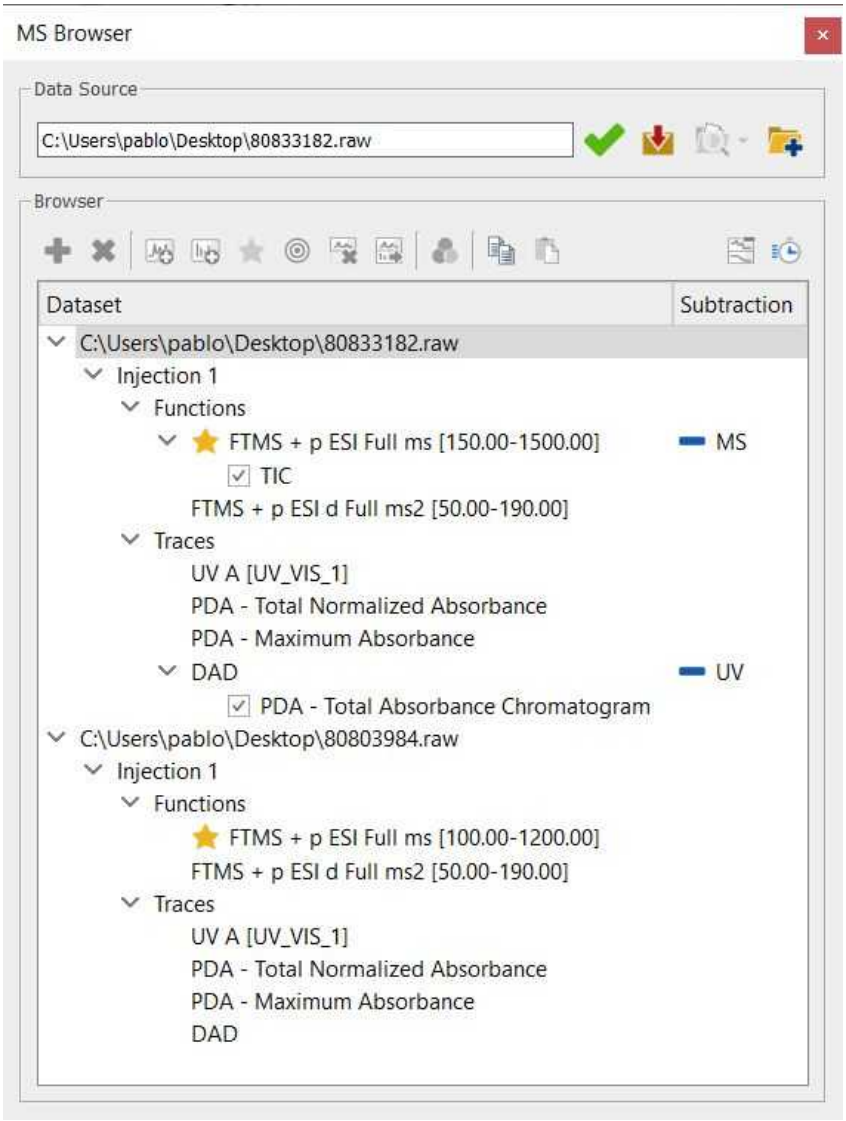


After having clicked on the OK button, you will get the expected result, with the applicable information under the 'Subtraction' column:

© 2024 MESTRELAB RESEARCH



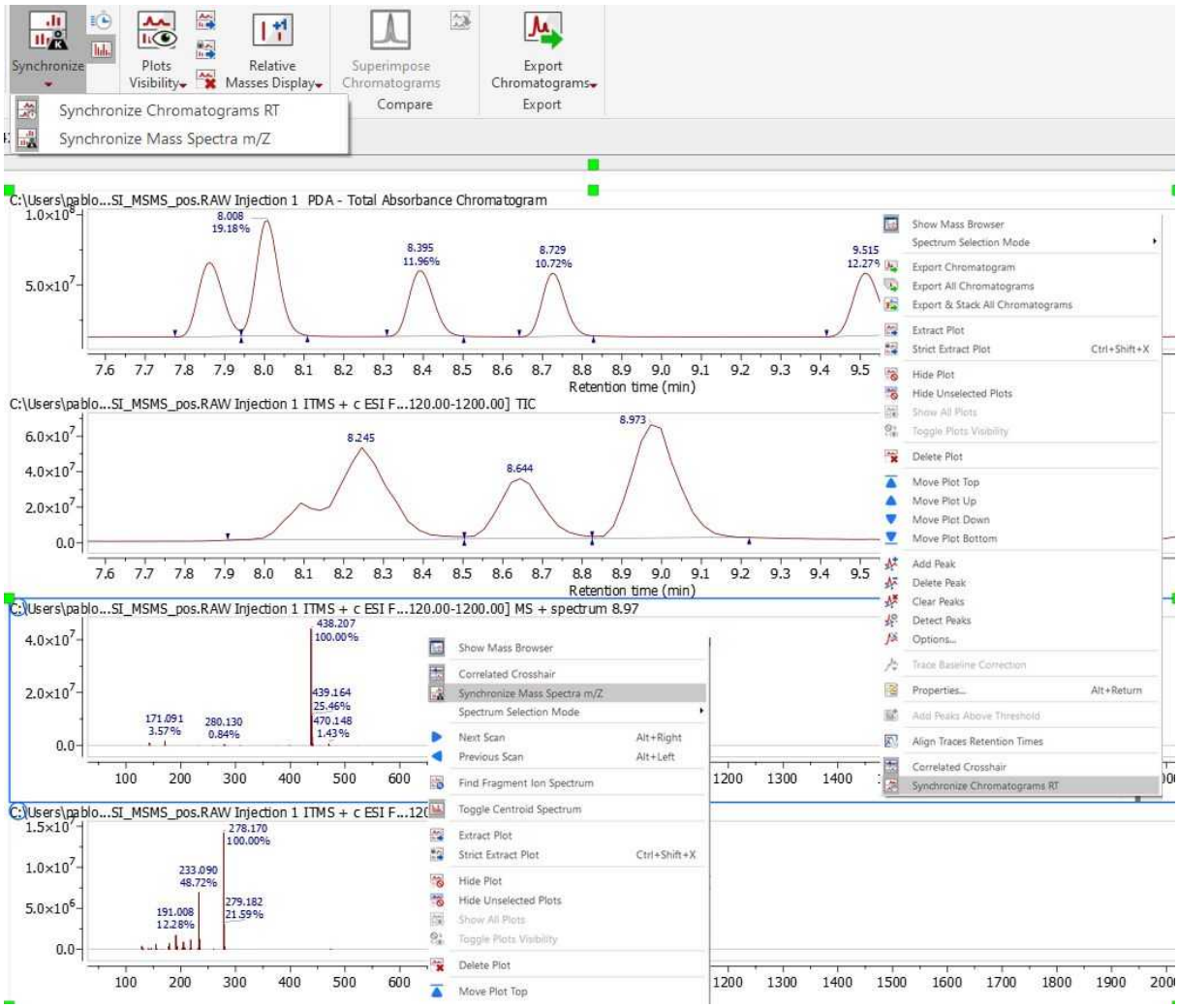
You can also subtract both TIC and DAD, just by selecting both subtractions:



14.2.2.4 Synchronize

The synchronization action can be applied/removed from the Syncronize button in Mass/Tools, or via context menu from chromatogram or mass spectrum plots.

© 2024 MESTRELAB RESEARCH

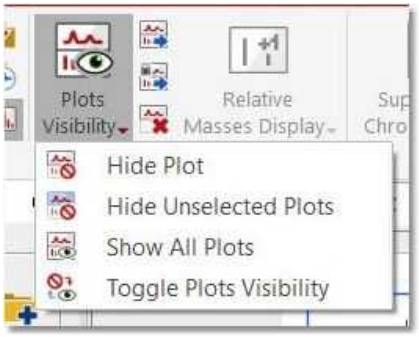


On enabling synchronization, all the chromatograms are synchronized to the X axis range of the current active chromatogram. Any modification in X axis of a chromatogram (zoom or panning) will be reproduced in the other chromatograms.

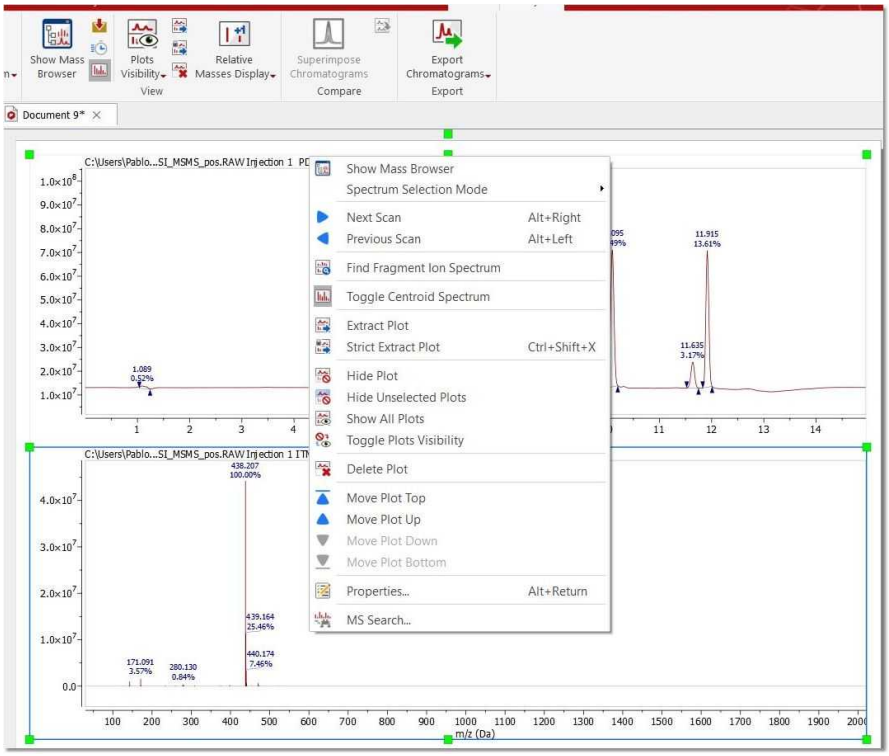
Appending new chromatograms or MS spectra to the spectral window also applies the current synchronization state.

14.2.2.5 Plots Visibility

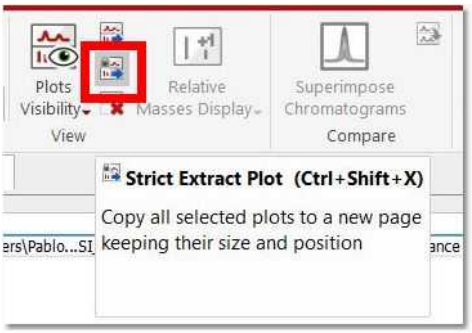
This part of the Mass Tools ribbon will allow you to customize the visibility of your chromatograms and spectra. From here you will also be able to extract plot as a new object or keeping the original size and position and to hide and delete plots.



These features will also appear in the context menu (displayed by right clicking on any spectrum or chromatogram):

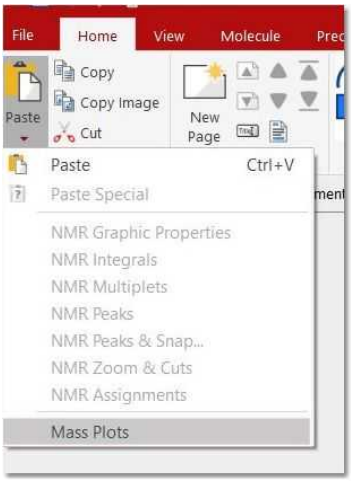


Strict Extract Plot: (shortcut Ctrl+Shift+X). It extracts the selected plots to a new page keeping their size and position.



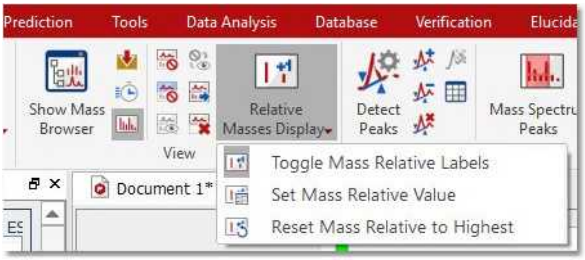
© 2024 MESTRELAB RESEARCH

The same result will be obtained by copying a mass item and then using the paste special action under the 'Home/Paste/Mass Plots' menu:



14.2.2.6 Relative Masses Display

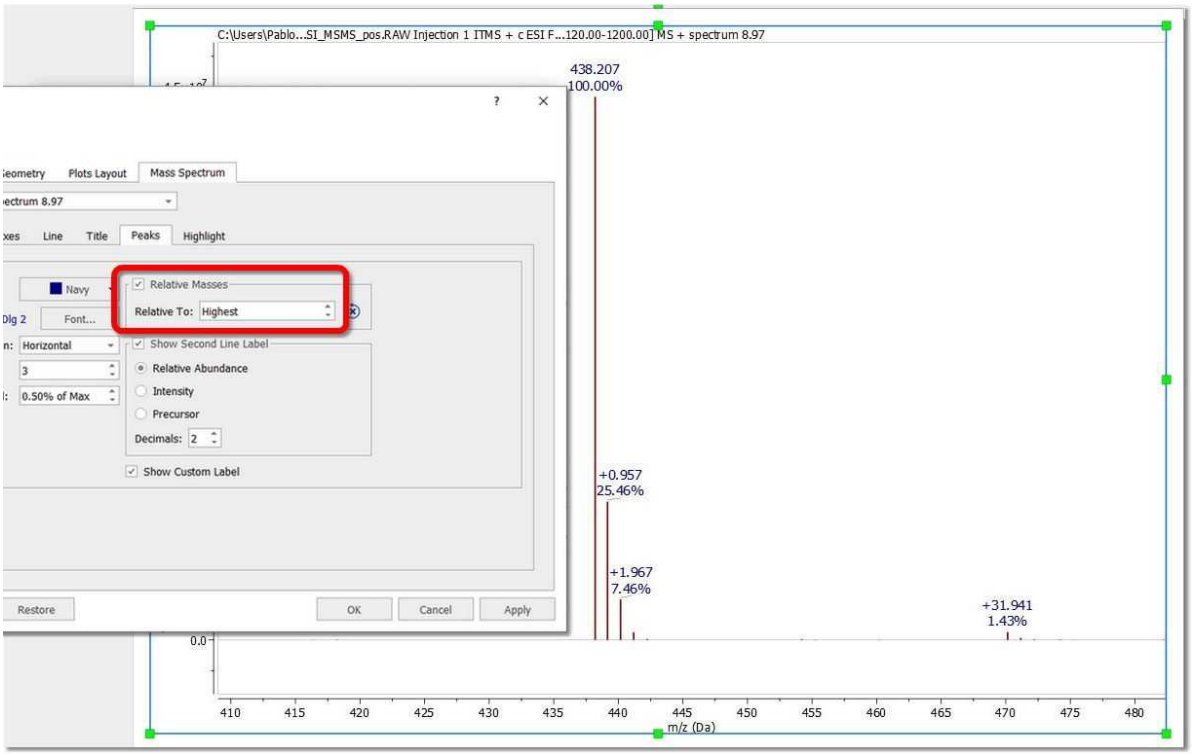
This feature will allow you to display the relative masses as peak labels instead of the absolute values.



The masses values will be relative to the position of the highest peak in the current view, but you can select any other peak by right clicking on the desired peak label and selecting "Set Mass Relative Pivot" from the context menu (or from the Mass Peaks Table).

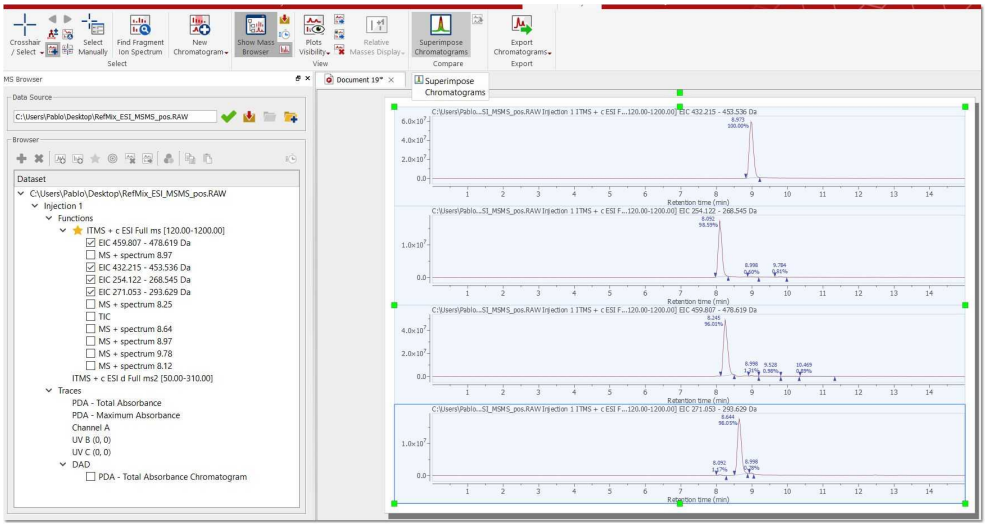
You could also manually enter a value by using the feature 'Set Mass Relative value' from the ribbon or from the Peaks Properties dialog box:

© 2024 MESTRELAB RESEARCH



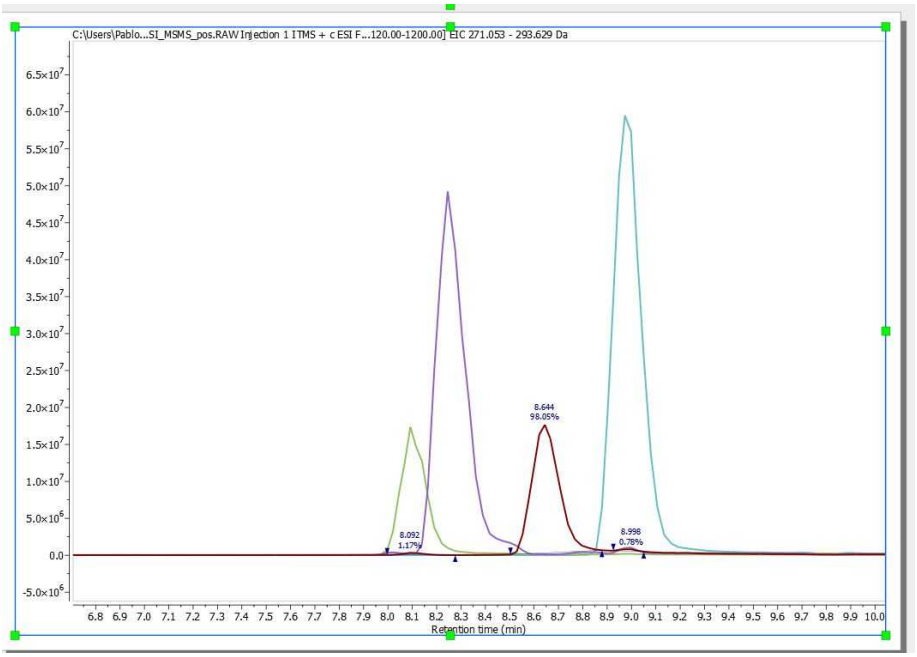
14.2.2.7 Superimpose Chromatograms

After having displayed several chormatograms on your page, (for example EIC from different m/z values), you can superimpose them by clicking on all the plots (while holding down 'Ctrl+Alt', or 'Shift+Alt' to select all) and pressing "Superimpose Chromatograms" button in Mass Tools menu.



You will get a superimposed plot like this (use Ctrl+Alt to deselect the plots):

© 2024 MESTRELAB RESEARCH



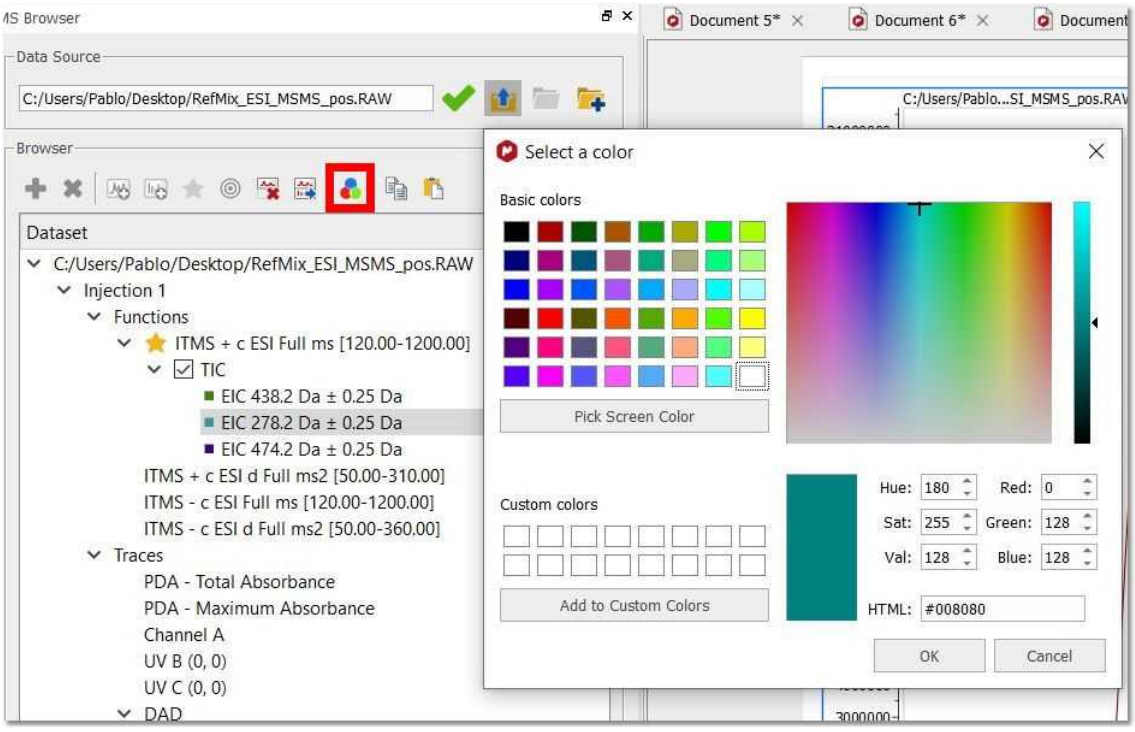
Shortcuts to select MS objects

- Alt+click: select/deselect a single object.
- Ctrl+Alt+click: add/remove object from/to selection.
- Ctrl+Alt+Shift+click: select multiple MS objects in between last selection.

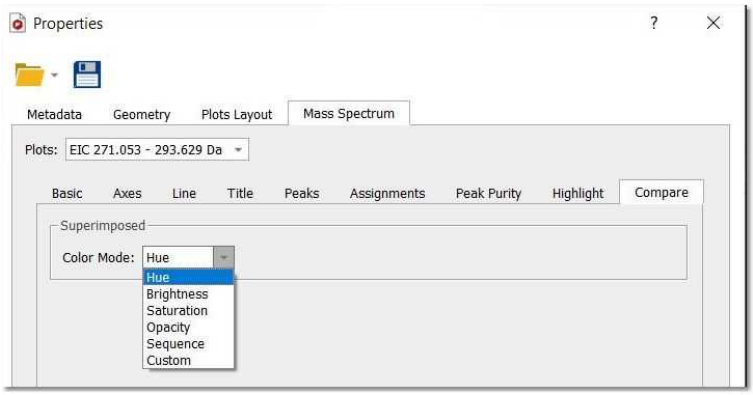
You get back to the separated spectra by making Undo (ctrl+Z) or by selecting the superimposed plot and clicking the "Separate Chromatograms" button.

You can change the colors from the MS Browser:

© 2024 MESTRELAB RESEARCH



The colors of the traces in the superimposed view can be customized via Mass Properties/Compare dialog:



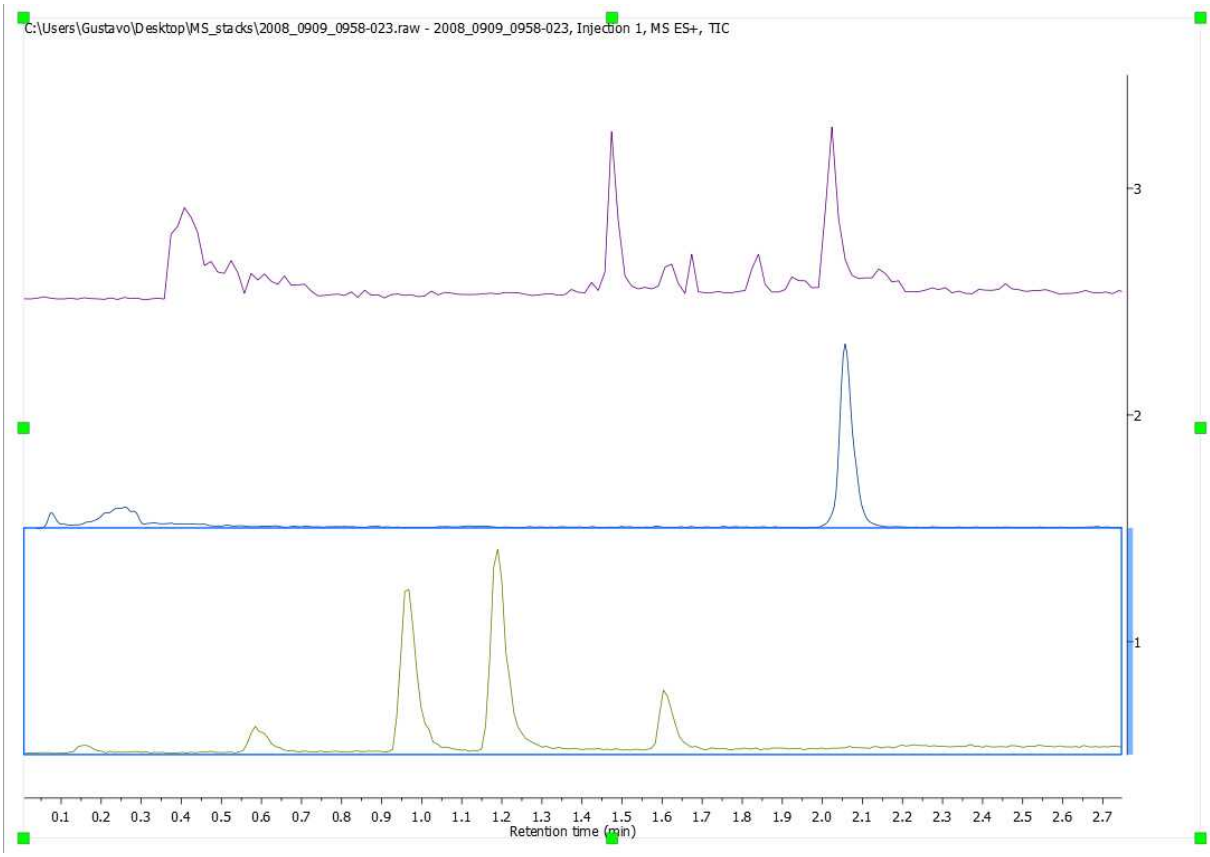
14.2.2.8 Chromatography

You can export TICs and chromatograms from the MS plugin with the Chromatography tool by selecting the MS spectra (in the 'Page Navigator') and following the menu 'Mass Analysis' to select the appropriate option.

© 2024 MESTRELAB RESEARCH



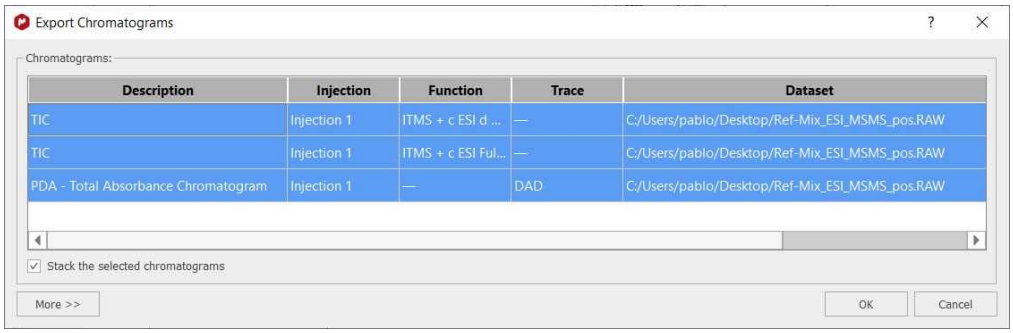
You can export only one chromatogram ('Export Chromatogram') or all the chromatograms in a MS spectrum ('export all Chromatograms'). You can also stack all the chromatograms by selecting 'Export&Stack All chromatograms' option:



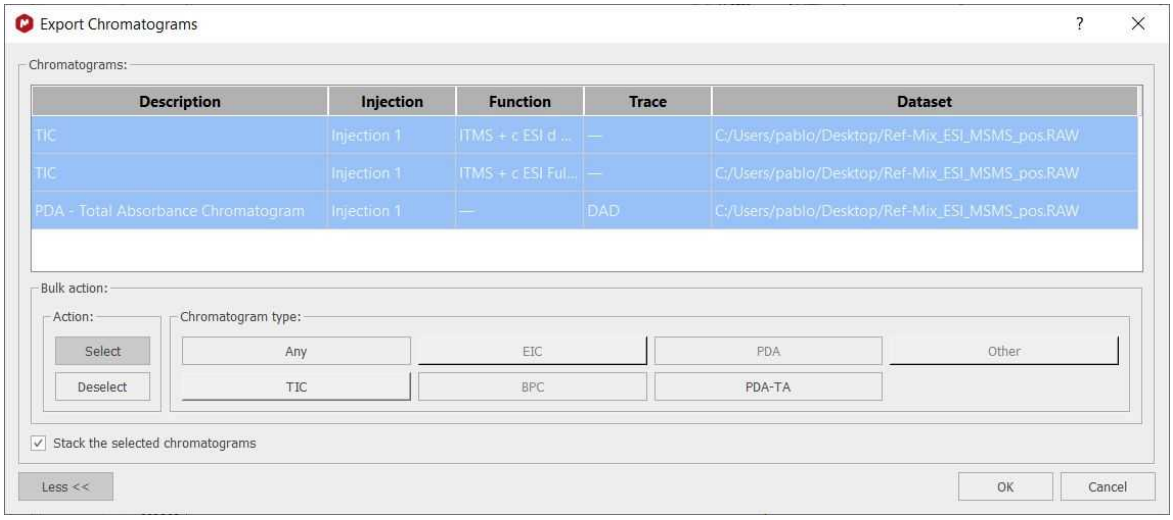
You can also stack chromatograms by selecting them in the 'Page Navigator' and following the menu 'Stack/Stack items'.

Selecting 'Export Chromatograms' will display a dialog to allow you to select the chromatograms to be exported.

© 2024 MESTRELAB RESEARCH



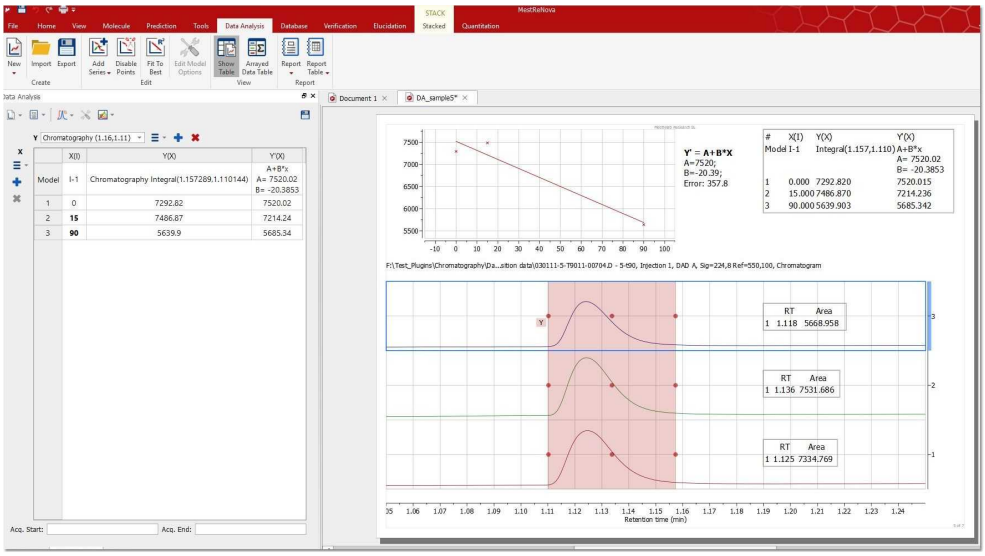
Alternatively, you can make a bulk selection/deselection by chromatogram type via clicking the "More" button. Only the buttons for the chromatogram types detected will be enabled. Any time "Select" or "Deselect" buttons are pressed, the Chromatogram Type selection is removed. The OK button is enabled only after at least one chromatogram is selected.



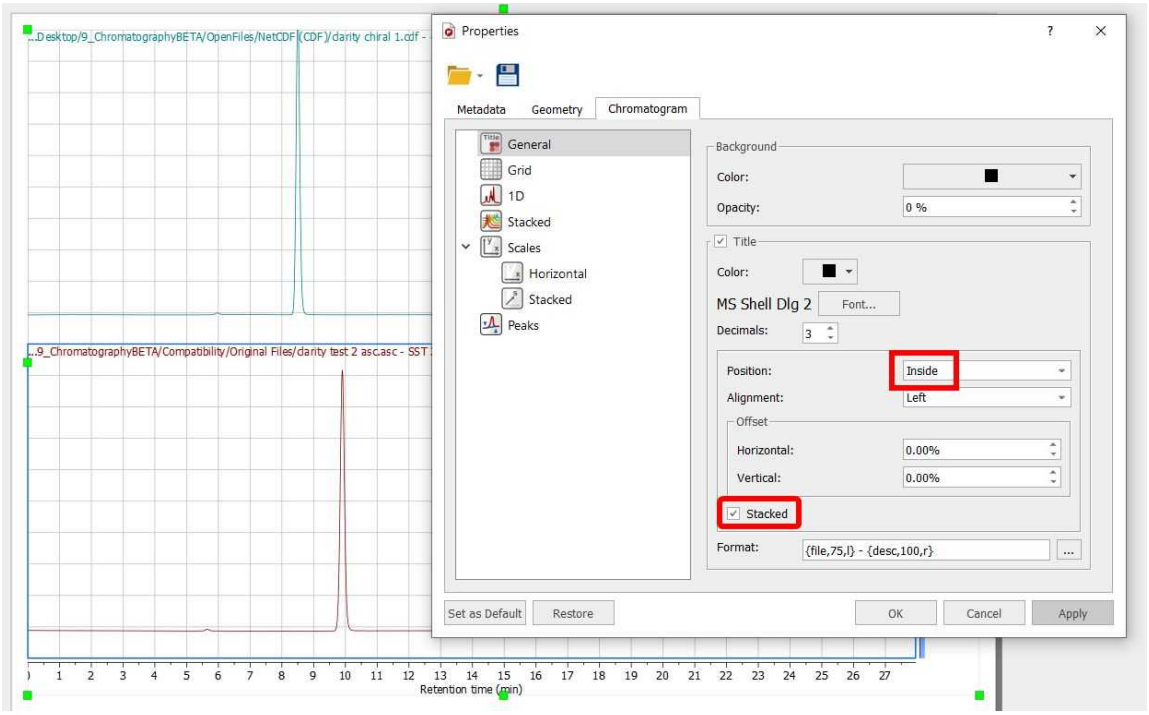
An additional option "Stack the selected chromatograms" allows to export and stack the selected chromatograms at once. If it's not checked, each chromatogram gets exported to a new page.

Once you have created the stacked plot, you could analyze how the integrals are changing by using the 'Data Analysis' feature. Here you can find an example:

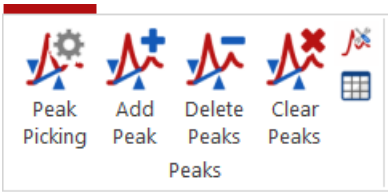
© 2024 MESTRELAB RESEARCH



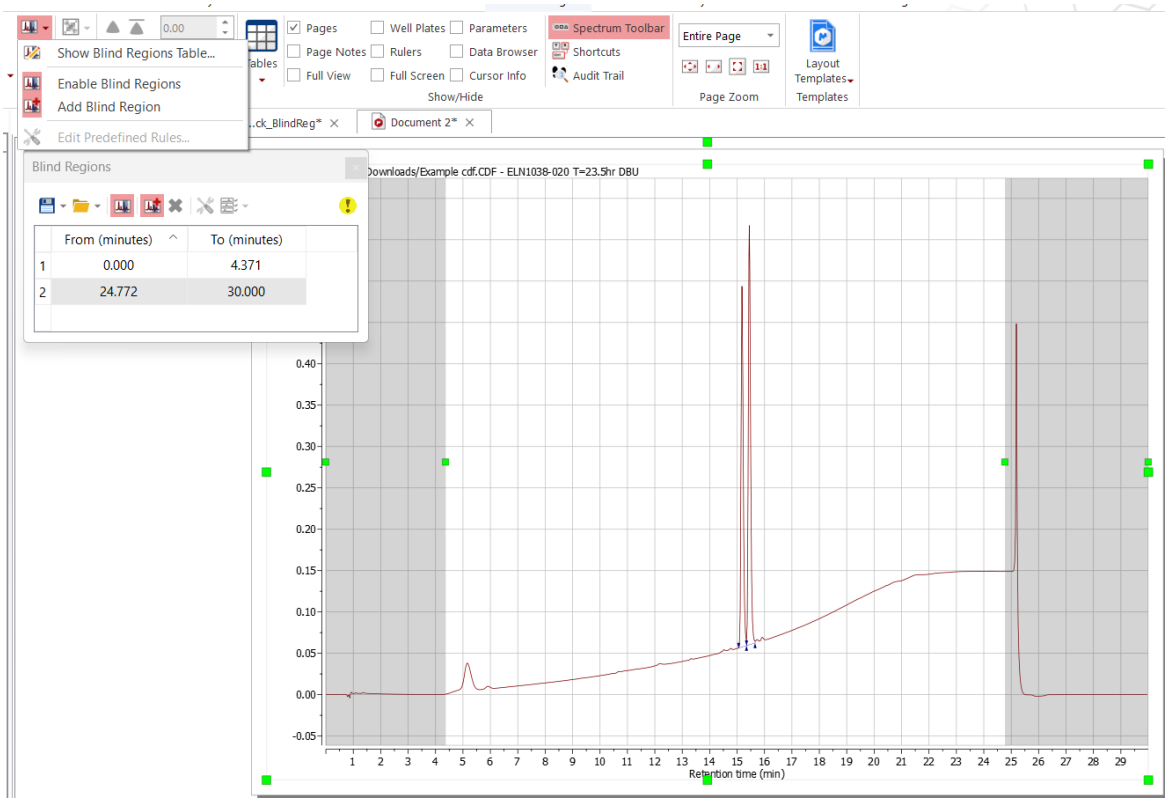
You can display the title of each trace from the Properties dialog box, by selecting "Position: Inside" and checking the 'Stacked' box:



You can use the Peak Detection algorithms included in the Chromatography ribbon. For further information about these features, please check this chapter:



Blind regions can be applied to chromatograms (from the View ribbon):



14.2.3 Mass Analysis

Mnova MSChrom allows the user to carry out fully automatic analysis of LC/GC/MS data. Easily obtain:

- Automatic TIC integration values and MS trace m/z values
- Automatically compute potential Elemental Compositions for a given mass ion peak
- Automatically match proposed molecular structures to a TIC spectrum

The Mass Analysis menu contains these useful features:



- **Peak Detection**
- **Mass Spectrum Peaks**
- **Charge State Deconvolution**
- **Calculate Peak Purity**
- **Elemental Composition**
- **Molecular Match**
- **Assignments**
- **Trace Baseline Correction**

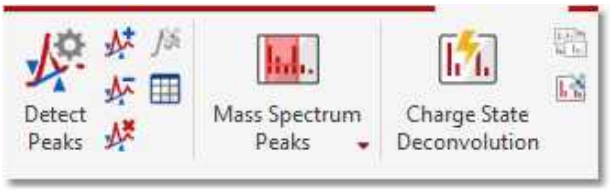
© 2024 MESTRELAB RESEARCH

- **Align Traces retention time**

Let´s take look at them one by one.

14.2.3.1 Peak Detection (Integration)

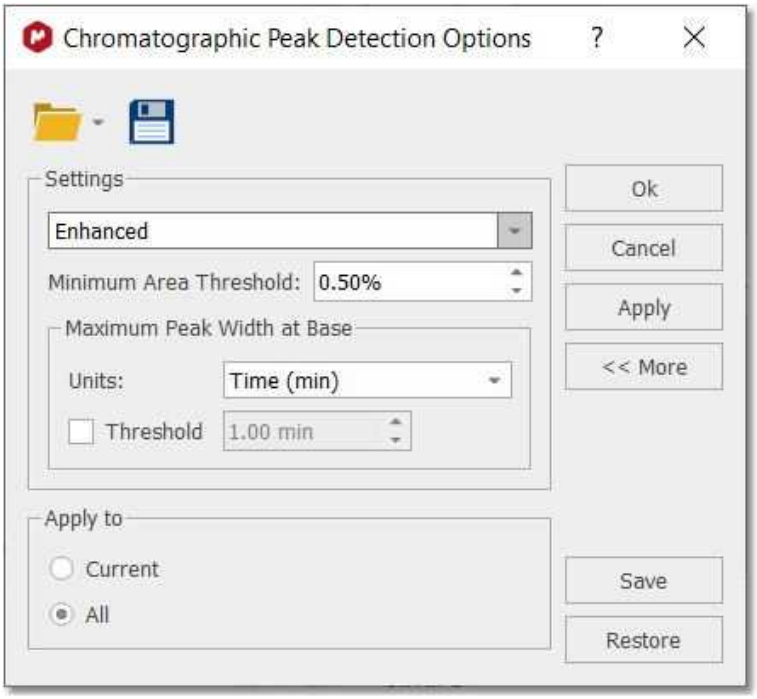
It is possible to integrate the desired peaks of your TIC chromatogram with Mnova MSChrom by following the menu 'Mass Analysis/Peak Detection' or by using the corresponding icon of the toolbar:



You will find several features under the 'Peaks Detection' menu:

Detect Peaks: applies an automatic peaks detection to your TIC.

Detect Peaks Options: from here you can select the Peak Picking algorithm (Enhanced will be the default one) and you can also customize the threshold of the minimum area of the peaks for the automatic detection.

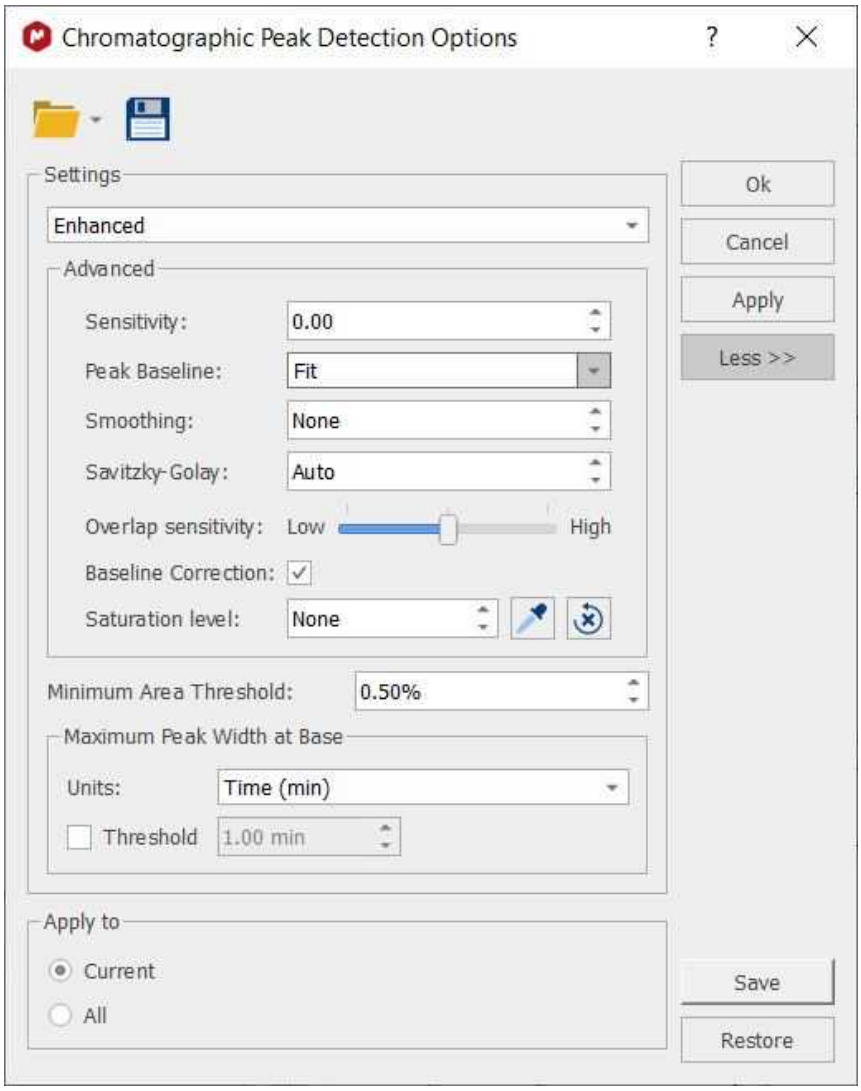


The maximum peak width at base is a filter which discards peaks with a width at base (x.end() - x.start()) greater than a threshold.

The threshold can be indicated as an absolute value (in minutes) or as a percentage of the total time of the chromatogram.

© 2024 MESTRELAB RESEARCH

Clicking on the 'More' button will allow to select other advanced options; such as Sensitivity, Peak Baseline, Smoothing, Savitzky-Golay, Overlap sensitivity, Saturation level and Baseline Correction.



Sensitivity can be -100 to 100. In general, it shouldn't go below 50 or above 80.

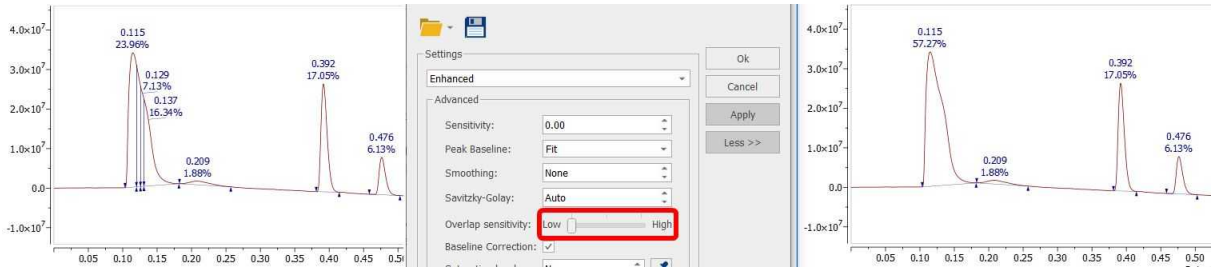
Peak Baseline: the scroll down menu will allow you to select between Fit or Absolute. Fit means that the baseline is built connecting the boundaries of the detected peaks. Absolute always uses zero as baseline (it is intended for baseline corrected signals). Anyway, the baseline of any peak is modified, if necessary, to ensure that the baseline will be always below the chromatogram signal. The default option is Fit.

The Savitzky-Golay option sets the window size (in points) for all the Savitzky-Golay (SG) filters (1st, 2nd and 3rd derivatives) that are internally used by the "Enhanced" peak detection process.

The smoothing option uses Moving Average filter to smooth the signal with the defined window size (in points). If you activate smoothing, the Savitzky-Golay option will be automatically set to "Auto".

© 2024 MESTRELAB RESEARCH

Overlap sensitivity: this option represents different levels of sensitivity for the search of overlapping peaks (by using the third derivative) of chromatograms with good definition. It may not work fine with noisy chromatograms.



High ("3rdDerivative"): equivalent to activating the deprecated "Sensitive overlap" option. This level uses 1st, 2nd and 3rd derivatives to search for overlapping peaks.
Medium ("2ndDerivative"): this is the default level. It uses the 1st and 2nd derivatives to search for overlapping peaks. Equivalent to disabling the deprecated "Sensitive overlap" option.
Low ("1stDerivative"): a new overlap sensitivity level that only uses 1st derivative to search for overlapping peaks. This option should fix the overdetection of overlapping peaks for those 'uggly peaks' as the one showed in the screenshot below:

Baseline Correction: This option performs a baseline correction of the signal just before the start of the peak detection process (but after the smoothing step, if present). This correction is for internal use of the peak detection process: it does not modify the signal intensity of the chromatogram.

Saturation Level: it allows you to set an abundance level (in height) to identify those peaks that might be saturated, to keep them out from results processes.
When using this option, all traces chromatogram points above the saturation level will be removed from the peak detection process. Therefore, these saturation ranges will always be integrated into the same peak: the imaginary peak constructed with the ramp and tail before and after the saturation range.

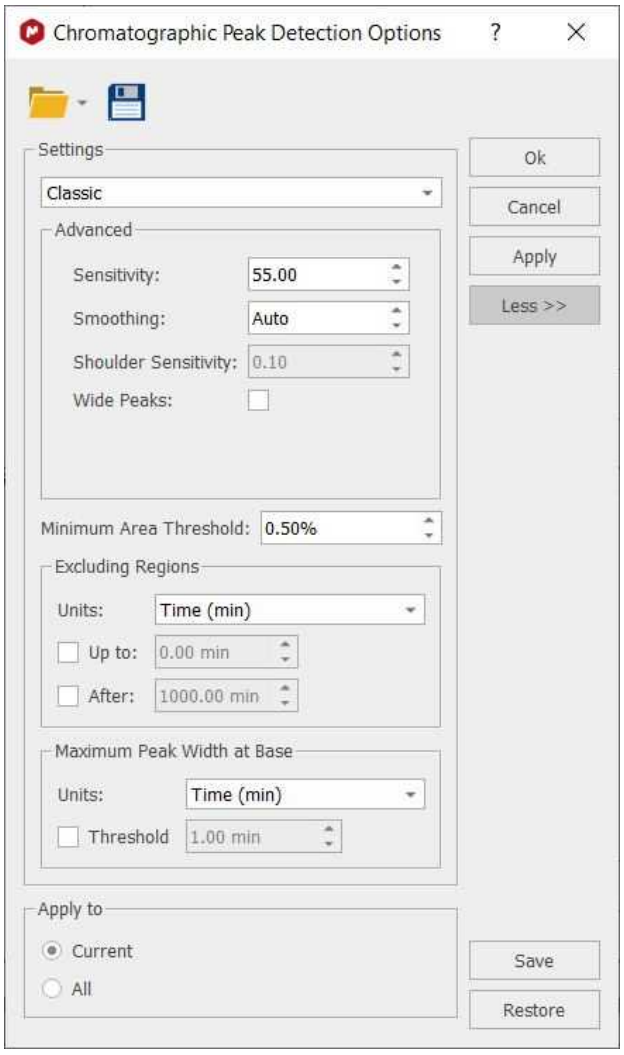
A value of 0 indicates that saturation detection is disable ("None"). You can type the value in the edit box or select it graphically in the chromatogram (by using this  button). To reset the saturation level, just click on the applicable button.

The Mass Peak table has a new column "Flags": the "Saturated" flag is set to those peaks that have been detected as saturated (the user can not modify this value). Also, the label "Saturated" is added to those peaks (this label is editable).

In general, the enhanced method shows better baseline for the peaks detected than the classic algorithm. To make sure that all the peaks and shoulders are automatically detected, you will need to play with the sensitivity or with the Savitzky-Golay smoothing parameters.

Selecting the classic method, will allow you to customize the threshold of the minimum area of the peaks for the automatic detection. You can also define the regions to be excluded and the Pick the maximum Peak Width at base.

© 2024 MESTRELAB RESEARCH



Sensitivity can be 0 to 200. In general, it shouldn't go below 50 or above 80.

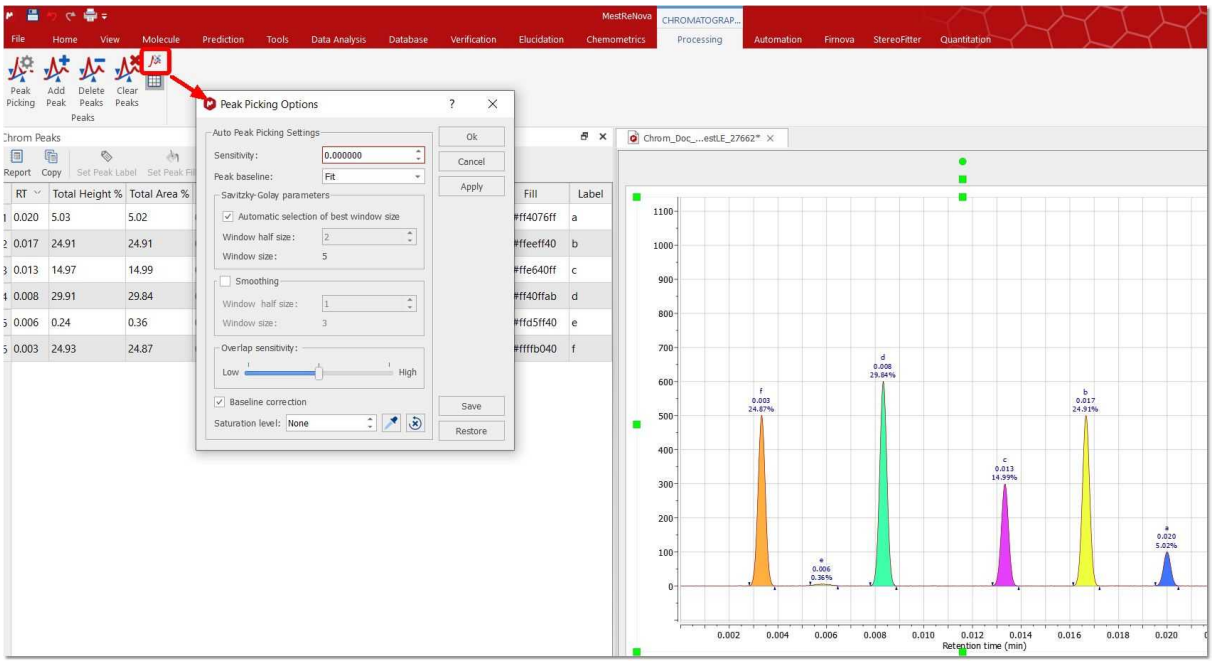
A smoothing Window size less than 5 means to automatically decide on a smoothing window based on the noise level of the chromatogram. If a nonzero window size is given, it must be an odd number between 5 and 151.

Shoulder sensitivity has an unknown maximum. It should not be changed from the default value of 0.1

When the wide peaks flag is set it indicates that adjacent chromatogram peaks should be combined into a single large peak.

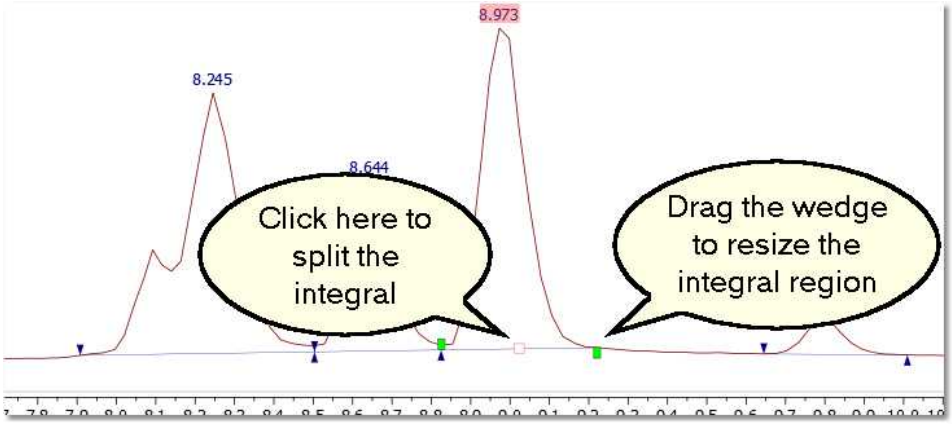
You can also select the Peak Detection options in the chromatographic plugin, by clicking on the highlighted button below:

© 2024 MESTRELAB RESEARCH



Add/Delete and Clear Peaks: these features are used to manually add or delete peaks to your TIC or to delete all the peaks.

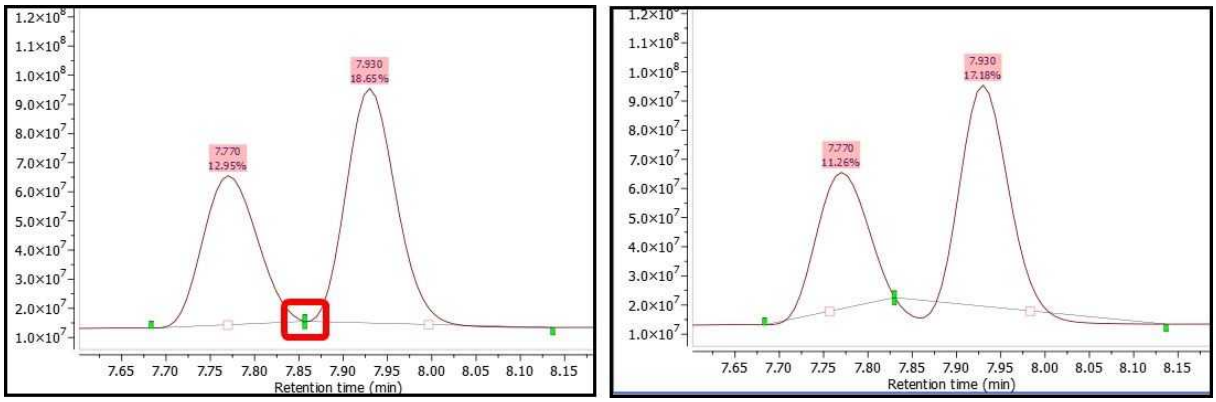
Please bear in mind that it is possible to change the integral region of any peak just by clicking and dragging on the wedges. You can also split the integral peak by clicking on the red square (and dragging).



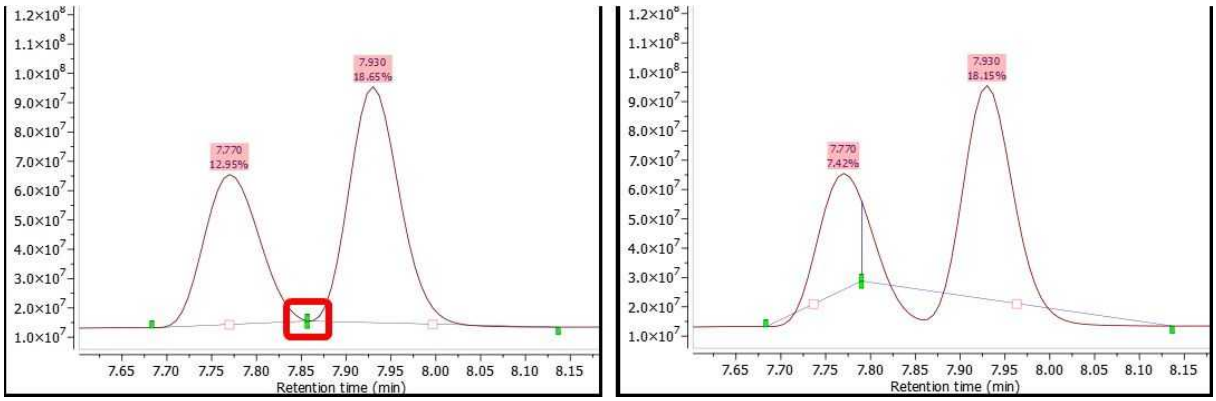
You can **freely** move the wedges by moving the integrals handler while shift is pressed. The same action can be used when splitting the peak to select the desired baseline.

If you have two adjacent peaks, you can hold down the Alt key and click&drag over the common point to modify the position of both wedges at the same time:

© 2024 MESTRELAB RESEARCH

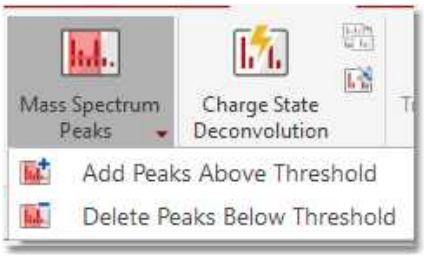


If you hold down Alt+Shift and click&drag, you will be able to freely move the common point (where the mouse is released):



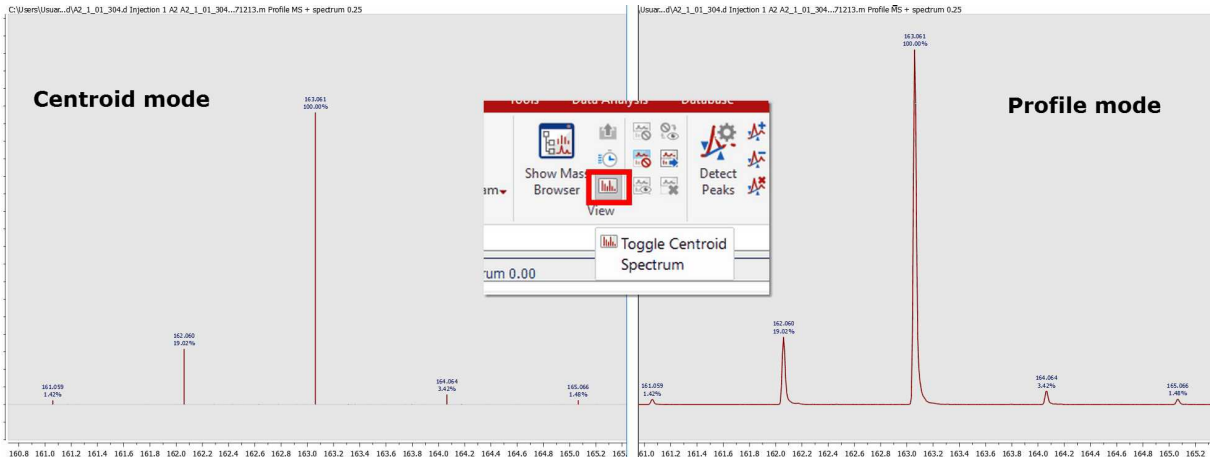
Add/Delete Peaks (Threshold)

You can graphically add peaks above a threshold and delete peaks below a threshold:

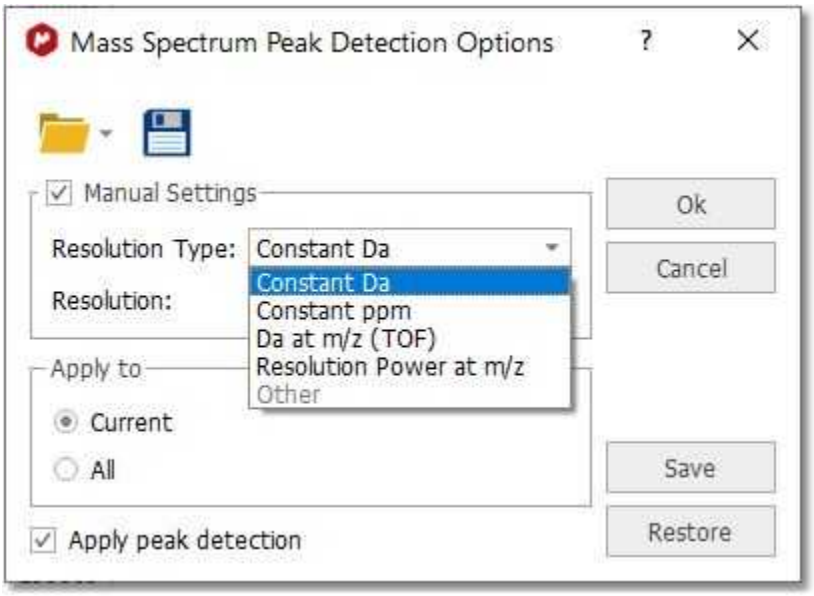


Toggle Centroid Spectrum: This is a toggle to switch between profile and centroid mode of the MS spectrum.

© 2024 MESTRELAB RESEARCH



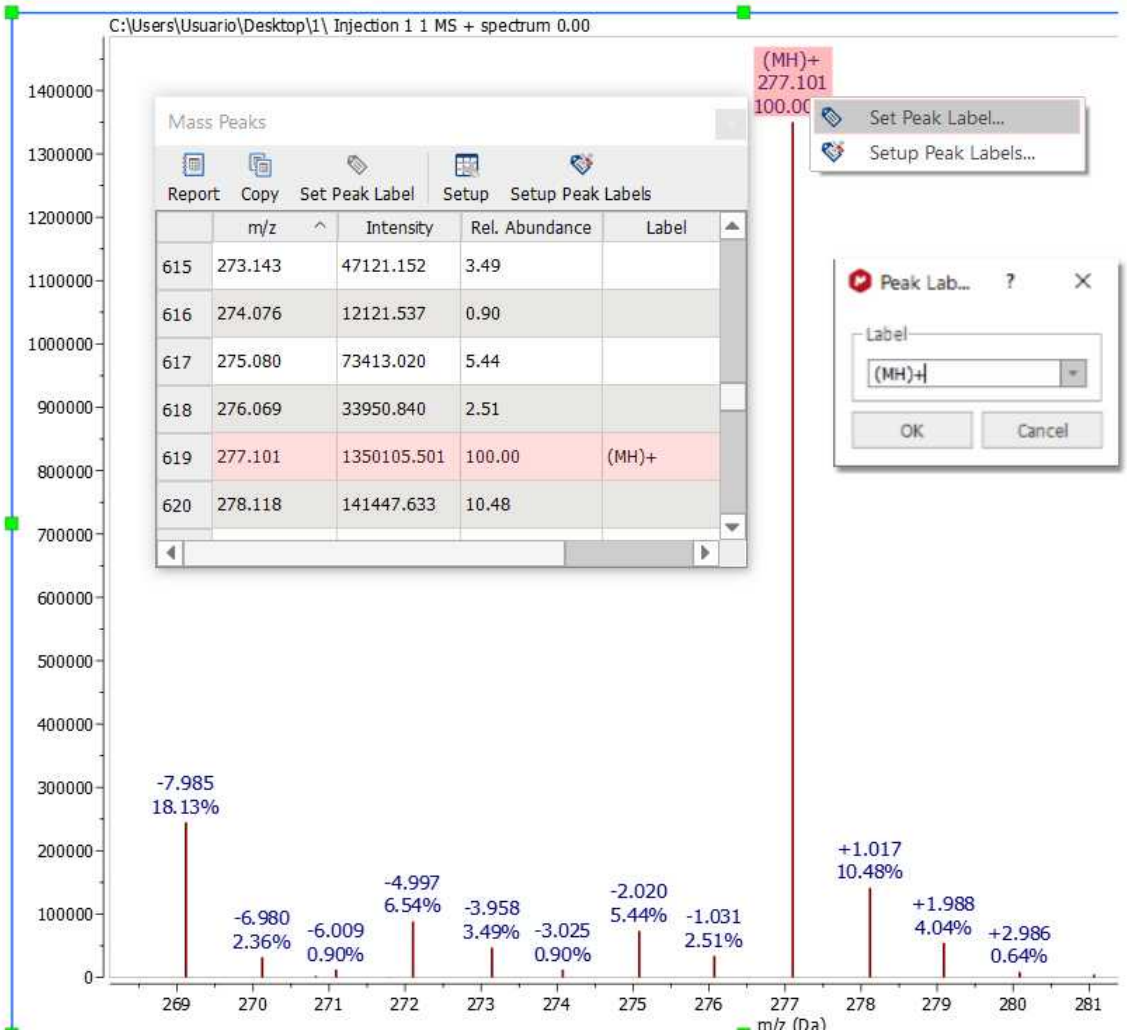
The user can manually adjust the peak detection settings, allowing to specify the desired resolution to build the centroid spectrum:



All peaks will be listed in the peaks table which will be displayed by following the menu 'View/Tables/Mass/Mass Peaks' (or by clicking on the 'Show Peaks Table' button of the ribbon).

You can label any peak by right clicking on the peak label (or from the Mass Peaks Table):

© 2024 MESTRELAB RESEARCH



Predefined labels for mass spectrum peaks can be configured by selecting "Setup Peak Labels" .



You can export mass peaks as ASCII by following the menu 'File/Save As' and selecting the option "Script: Mass Peak Table (*.csv *.txt)". You will get a list of m/z and Relative Abundance. You can follow the menu 'Tools/Export/MS Peaks (or MS Data for the chromatograms).

14.2.3.1.1 Blind regions

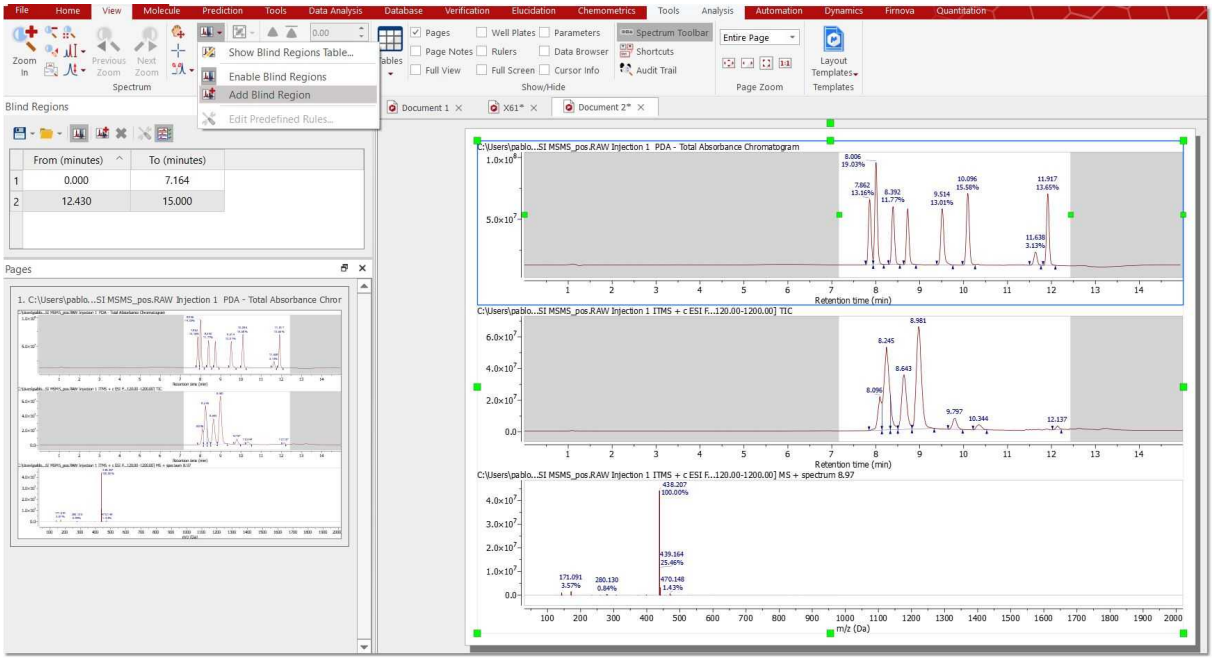
This feature will be used to select regions from your spectra which will be skipped by the calculation of the analysis algorithms (such as integration).

© 2024 MESTRELAB RESEARCH

Conversely, blind regions will not be saved or searched in the Database. This will allow users to ignore areas of the spectrum which present particular challenges, whilst still being able to use the rest of the information in the spectrum.

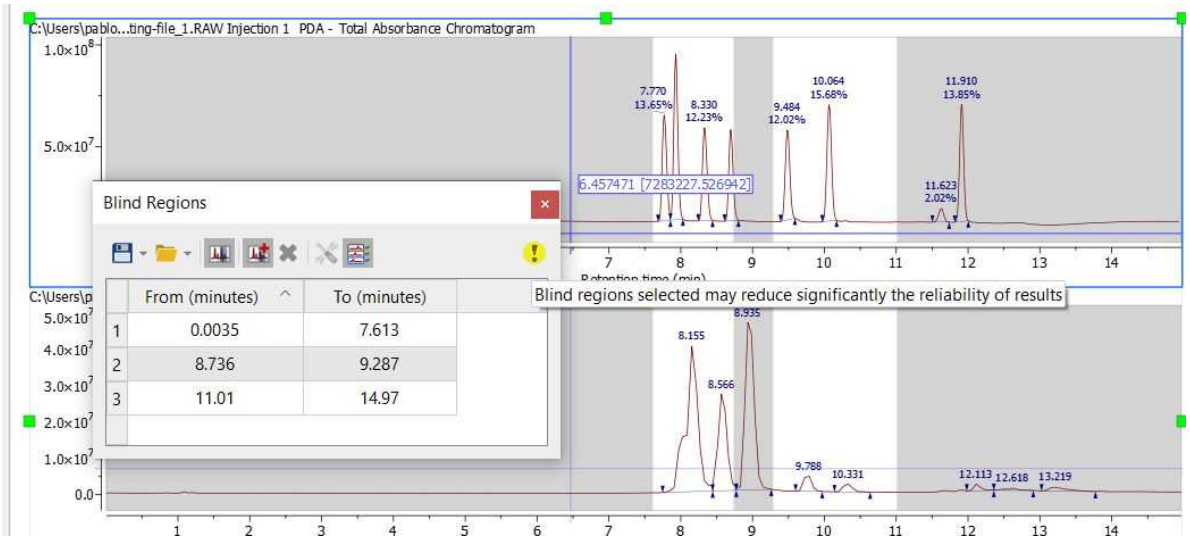
You will be able to select the Blind Regions by following the menu 'View/Blind Regions/Add Blind Region' and clicking&dragging on the regions of interest.

In the example below, you can see how we have added a couple of blind regions to discard the front and the end of the chromatograms:



Use the green handlers to graphically change the limits of the blind regions. Please bear in mind that overlapping two blind regions, will merge them.

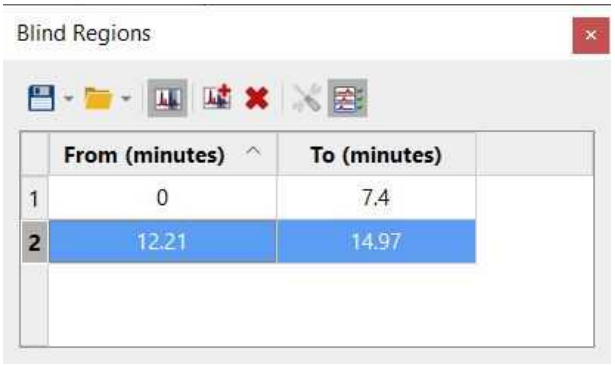
When setting up blind regions, a warning icon may appear in the upper right corner of the table, indicating that blind regions selected may reduce significantly the reliability of results.



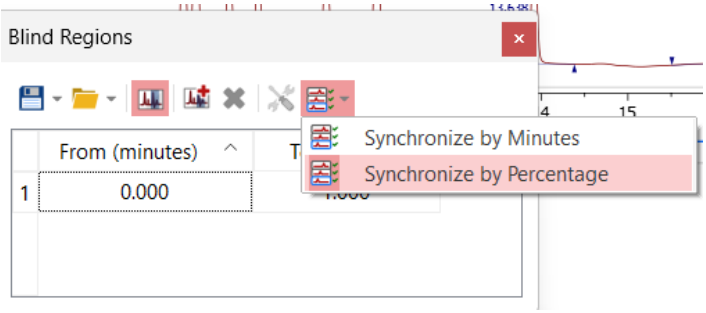
© 2024 MESTRELAB RESEARCH

This message could appear if:
The blind regions ranges extension is more than a 75% of the chromatogram range.
There is at least one not-blind region with a length less than a 25% of the chromatogram range.

Selecting the 'Show Blind Regions Table' will display a dialog box which will allow you to save the regions, to load another 'blind regions' file, to enable/disable the highlight of the blind regions, to add/remove blind regions and to synchronize chromatograms:

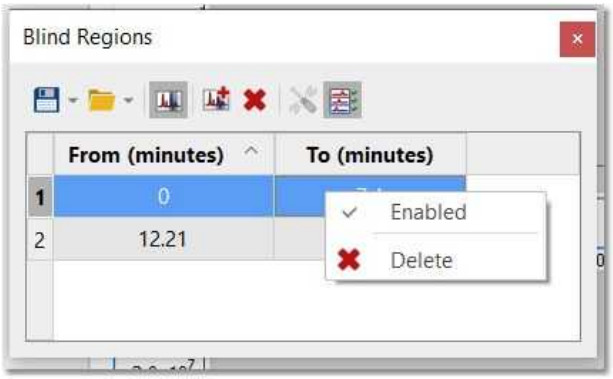


With the Synchronize Chomatograms feature enabled (which is the default mode), the user can select in only one chromatogram as many blind regions intervals as he wants and auto-set them for the rest chromatograms of the page. It will be possible to synchronize by minutes or by percentage:



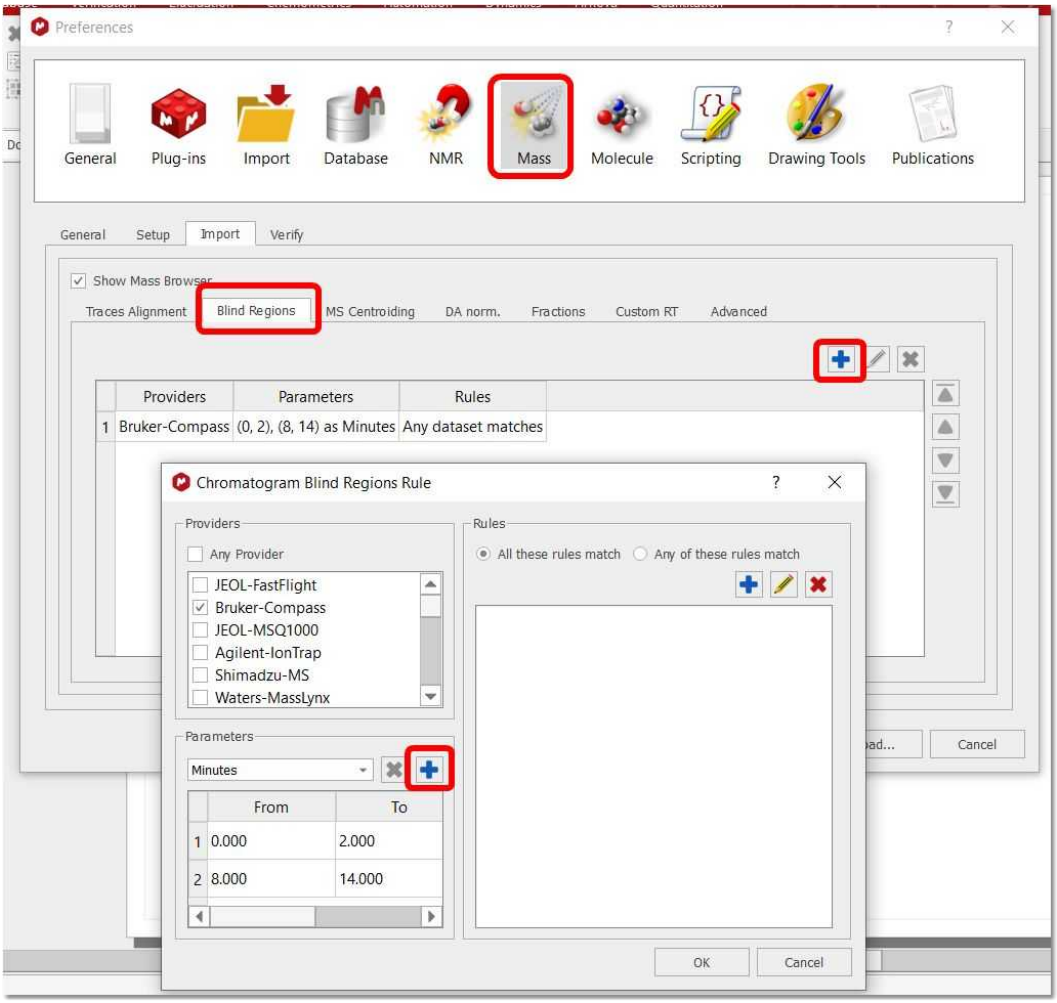
If you disable the Synchronize Chromatograms mode, you still can use it by holding down the Shift key while clicking&dragging the desired chromatogram time range.

Right clicking on any cell of the table will allow you to delete or disable the applicable blind region. You can also delete them by clicking on the red cross button 



© 2024 MESTRELAB RESEARCH

You can predefine blind regions rules from the 'Preference/Mass/Import/Blind Regions' dialog box:



14.2.3.2 Charge State Deconvolution

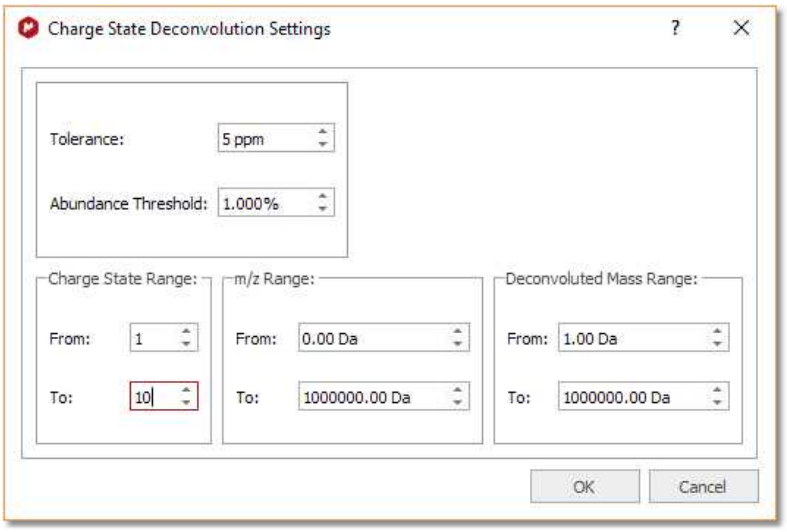


After extracting the MS spectra from each TIC peak, you can to obtain the deconvolved masses by pressing the button "Charge State Deconvolution". If the Append button is selected, the deconvolved MS spectra will be added to a new plot. Otherwise it will substitute the original MS spectrum.

There is an additional button to toggle between the original MS spectrum (showing one cluster for each charge state) or the deconvolved MS spectrum (showing only the zero charge state).

The settings for the deconvolution calculations can be modified via Charge State Deconvolution Settings button (see default settings below):

© 2024 MESTRELAB RESEARCH

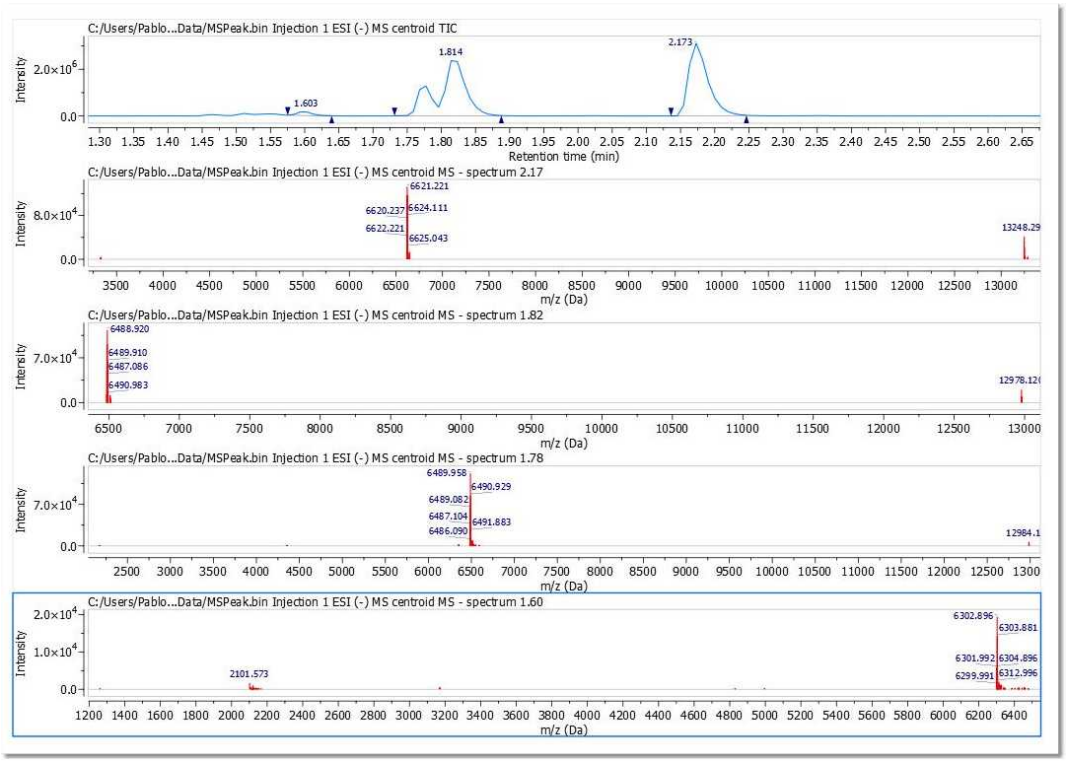


Basically, the main method takes a multiply-charged input spectrum and tries to convert each isotope cluster into a zero charge state cluster by determining the charge state (using isotope peak spacings) and assuming the adduct is H. It then multiplies each peak by the charge state and adds / subtracts the appropriate number of H+ / H-. Any peak for which the charge state cannot be determined will be left behind.

Here you can see an example; obtained after having applied the Charge State Deconvolution:

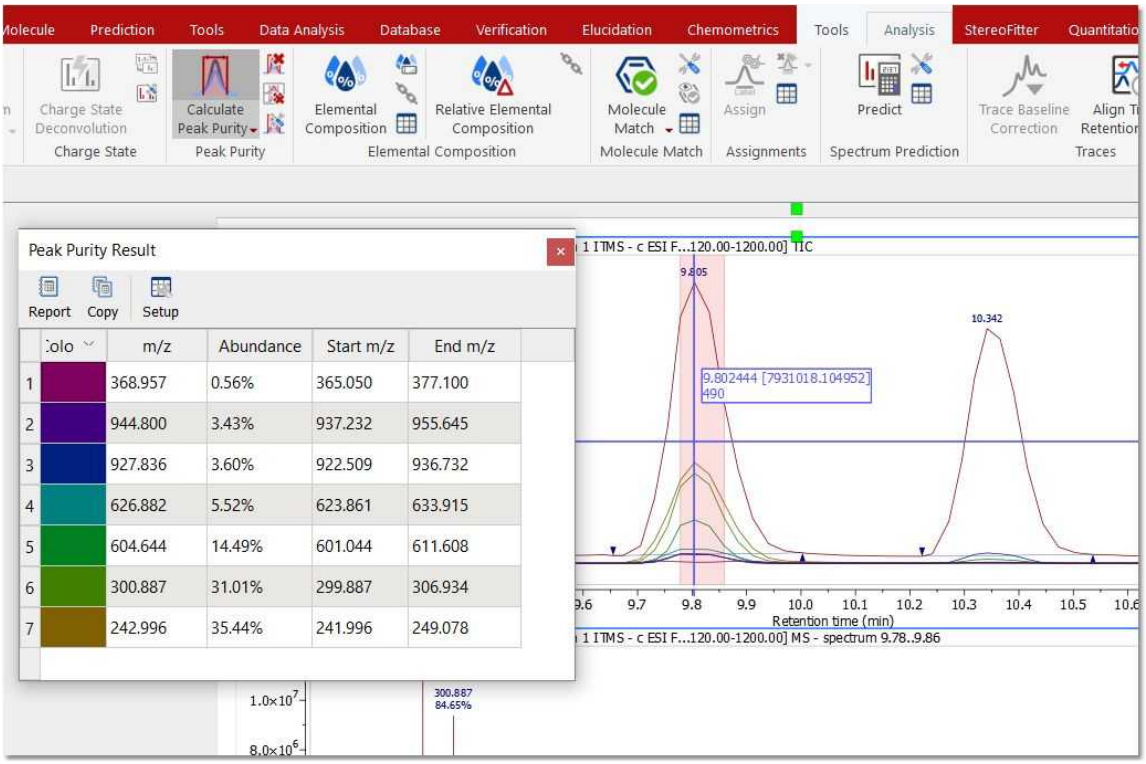
RT: 2.17 -> Combined mass around 6622.22
RT: 1.82 -> Combined mass around 6487.09
RT: 1.78 -> Combined mass around 6489.08
RT: 1.60 -> Combined mass around 6302.90

© 2024 MESTRELAB RESEARCH

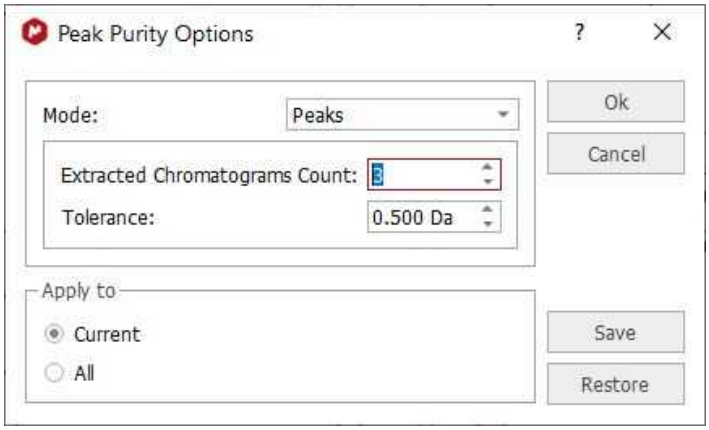


14.2.3.3 Peak Purity

This feature will calculate the chromatogram peak (or region) purity associated to the most abundant mass peaks which are displayed under the selected peak:



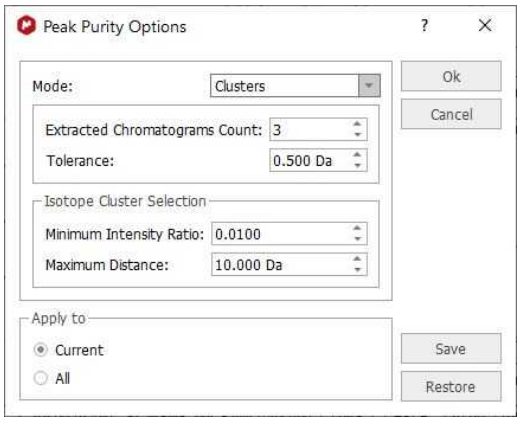
By default the number of curves are 3 and the tolerance is 0.5 Da, but it can be customized via 'Peak Purity Options'.



There are three different modes:

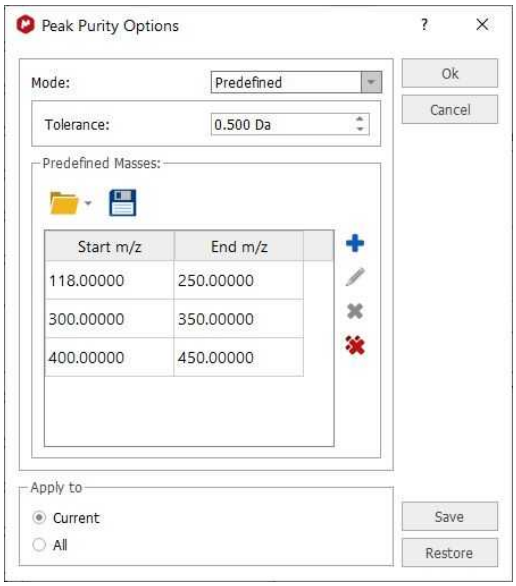
Peaks: will use the single chromatogram peak.

Cluster: will use isotope clusters instead of single peaks (with the option to select the minimum intensity ratio and the maximum distance):

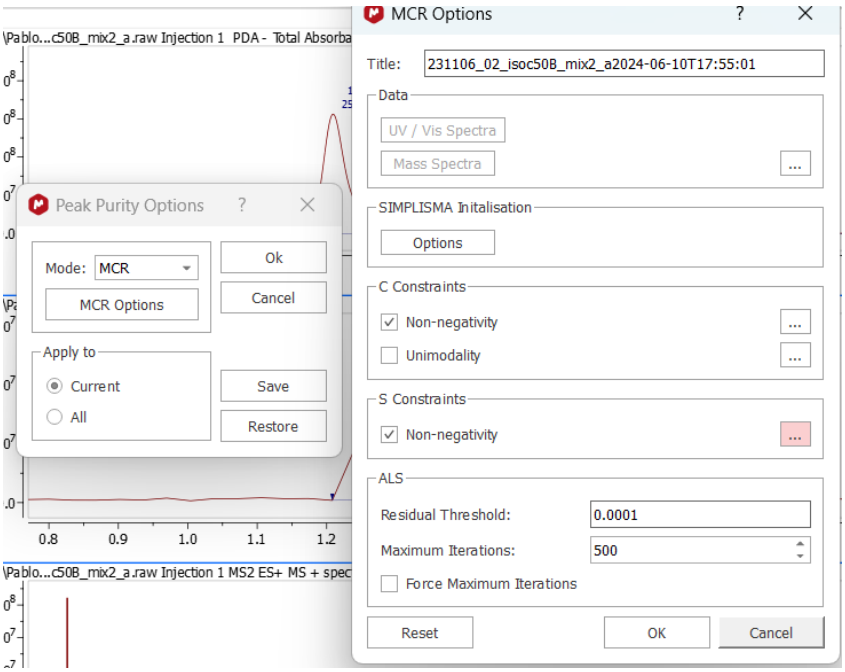


Predefined: will use the mass values or mass ranges provided by the user:

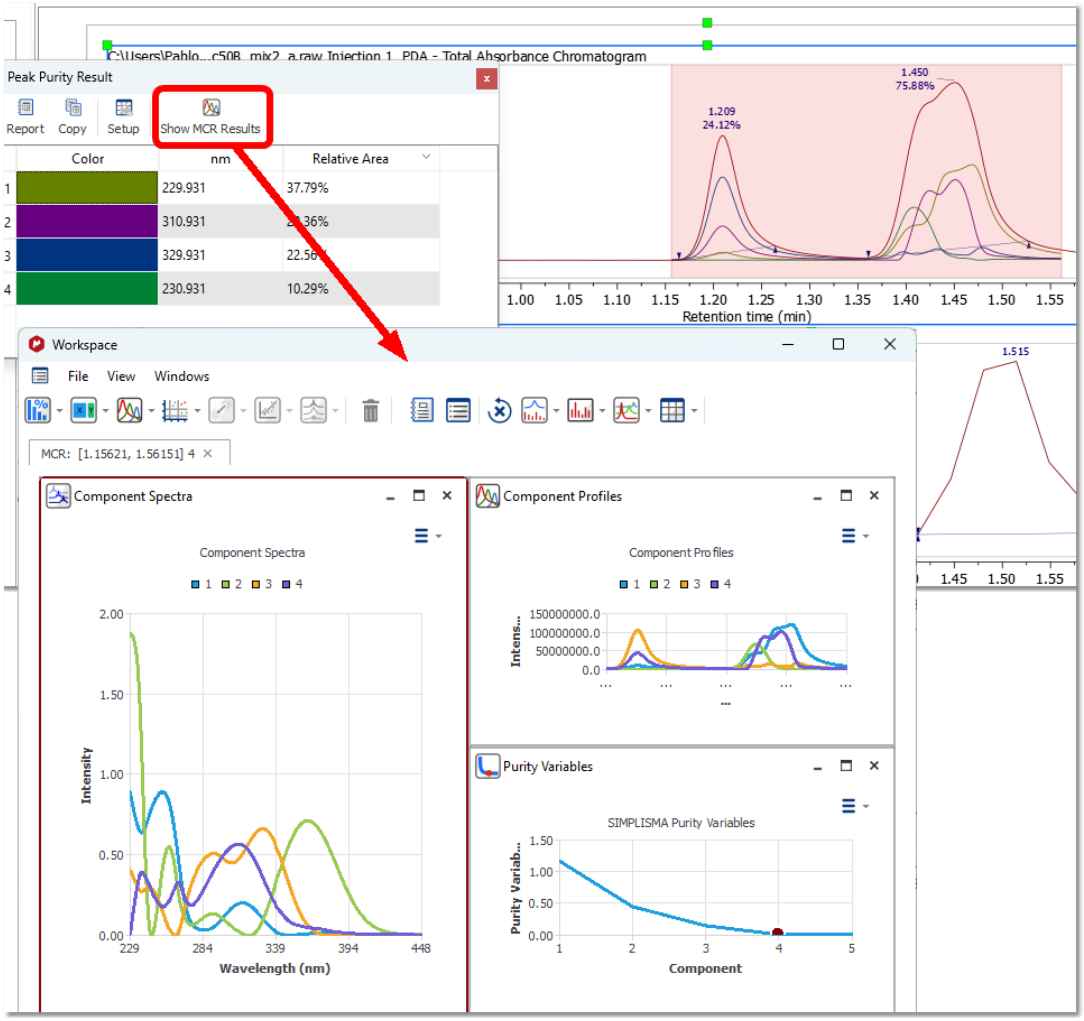
© 2024 MESTRELAB RESEARCH



MCR: it will allow you to apply the Multivariate Curve Resolution algorithm for the Peak Purity calculation in your chromatograms. For UV chromatograms, the MCR method will be the only available option:



If you have licenses for the 'Advanced Chemometrics' or 'MBioHOS' plugins, you will be able to display the results in the Workspace window, just by clicking on the 'Show MCR Results' button':



14.2.3.4 Elemental Composition

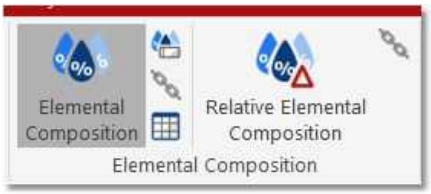
Elemental composition analysis is one of the most informative techniques in mass spectrometry. With appropriate mass spectral data, elemental composition can quite often determine the unambiguous elemental composition of a molecular ion.

This technique, however, only produces sensible results from high accuracy mass spectra. For ions less than or equal to m/z 200, a measured accurate mass within 5 ppm is often required to determine elemental composition. At higher m/z values, measured accurate mass values of significantly less than 5 ppm may be required.

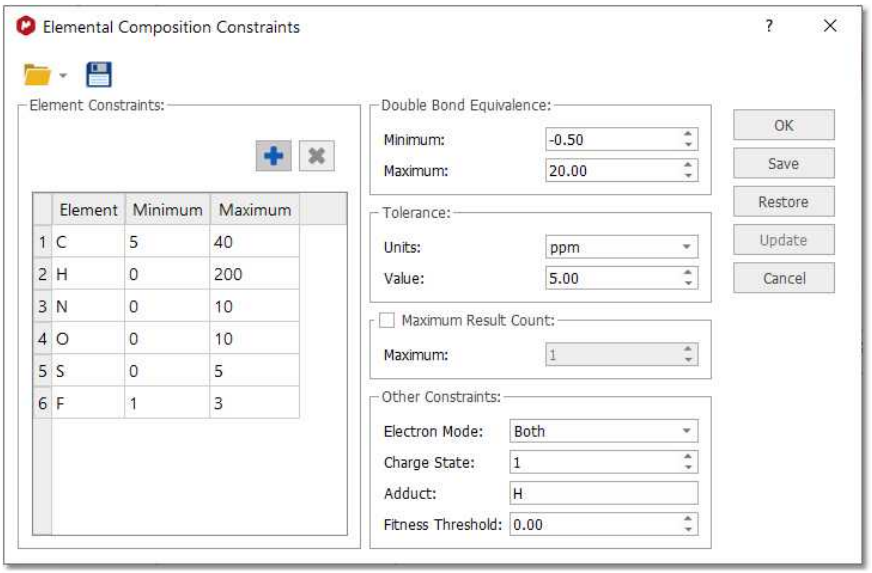
The algorithm that is used exhaustively computes all possible combinations of elemental formulae subject to the constraints provided by the user (see below). In some cases, millions of possible formulae are evaluated, but because of the speed of the algorithm, this happens very quickly.

This feature will be very useful to calculate the elemental composition of one or more mass peaks of a spectrum, if the m/z values are of high accuracy, typically 5 ppm mass accuracy or better.

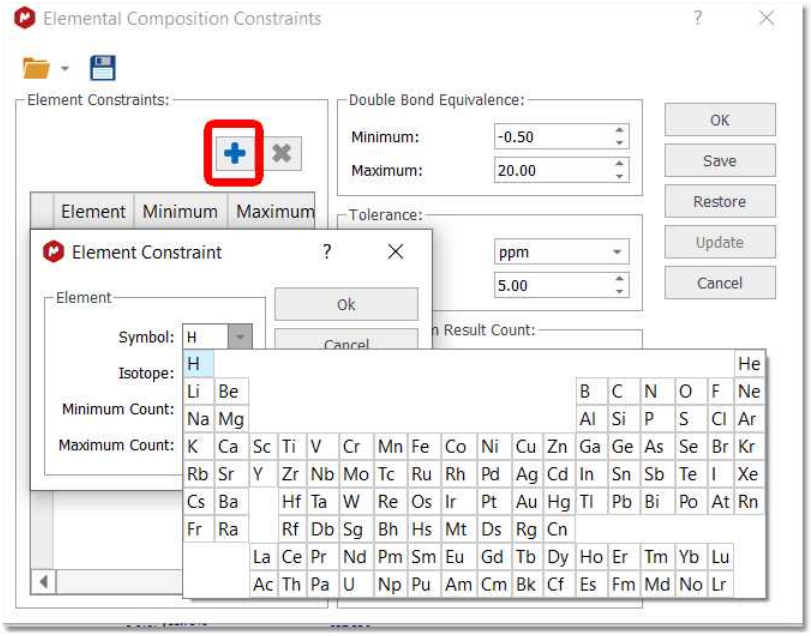
© 2024 MESTRELAB RESEARCH



First of all, you should establish the constraints of the calculation, just by clicking on the applicable option of the menu, which will display the dialog box below:



Click on this icon  to add more 'Element Constraints':



You can also select the 'Double Bond Equivalence', 'Tolerance', 'Maximum Result Count', 'Electron Mode', 'Charge State', 'Adduct' and Fitness Threshold'.

© 2024 MESTRELAB RESEARCH

Double Bond Equivalence (DBE): Rings + double bonds

Tolerance: Tolerance for matching computed composition to the target m/z. If spectral mass accuracy is expressed in ppm, then the tolerance for matching should also be in ppm. The typical values in Da should be 0.01 or less, or in ppm 5 or less. Tolerances much larger than this, generally give too many hits to be useful.

m/z: symbol used to denote the quantity, in units of Daltons (Da), formed by dividing the mass of an ion in unified atomic mass units by its charge number (regardless of sign).

Maximum Result Count: The maximum number of results to return.

Electron Mode: Electron mode can be even, odd, or both. Typically, EI (electron impact) produces positively charged, odd electron ions, whereas ESI (electrospray ionization) produces even-electron ions with either a positive or negative charge.

EI: Ionization of an atom or molecule by electrons that are typically accelerated to energies between 10 and 150 eV in order to remove one or more electrons from the molecule. The term electron impact is deprecated.

ESI: A process in which ionized species in the gas phase are produced from a solution via highly charged fine droplets, by means of spraying the solution from a narrow-bore needle tip at atmospheric pressure in the presence of a high electric field (1,000 to 10,000 V potential).

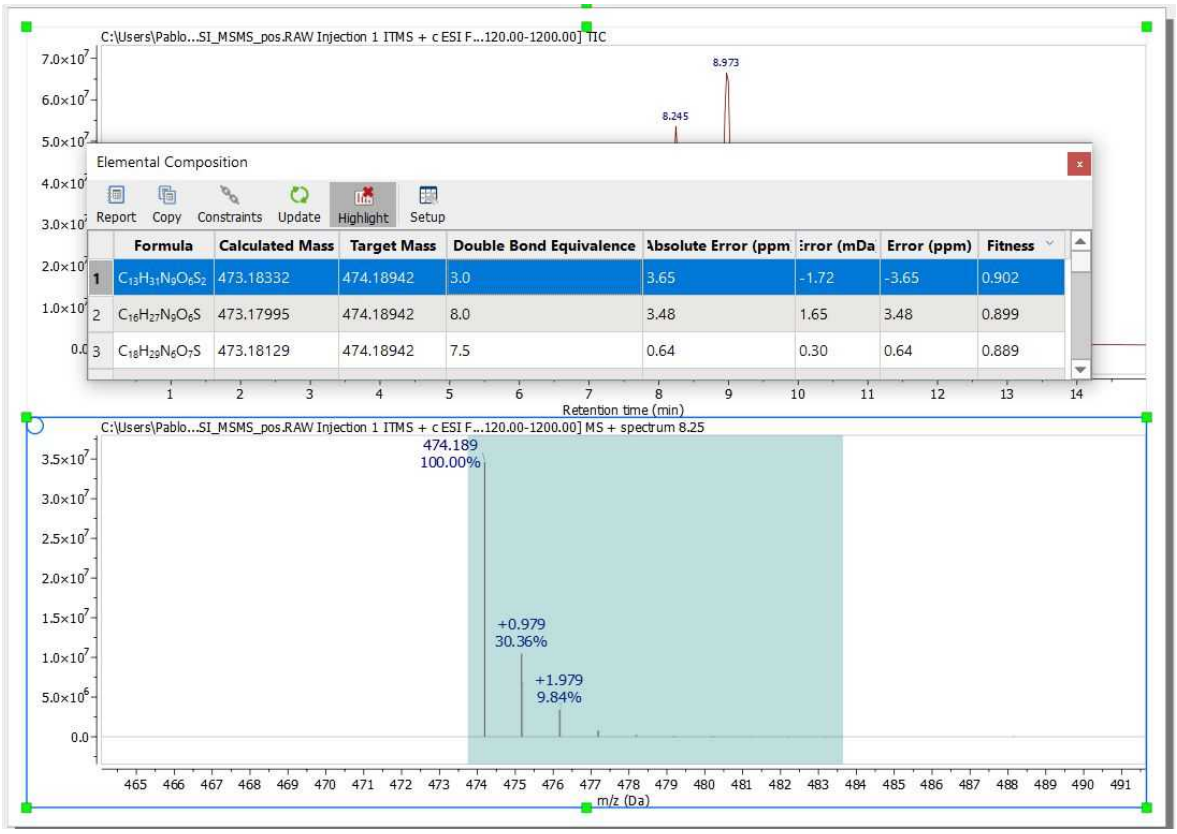
Charge State: is a signed value. The magnitude (1, 2, 3, ...) is a function of the experimental ionization technique, while the sign (+/-) is determined by the mode in which the experimental spectrum was produced and detected. For electron impact (EI) spectra, charge state is usually +1, while for ESI charge state is usually +1 or -1 for most organic compounds. Peptides may have higher charge states.

Adduct: Adducts are typically found in soft ionization techniques such as ESI. Proton (H) is most common for organic compounds, while H and/or Na (or other Group 1A alkali metal cation) may be found in protein, peptide or polymer spectra. NOTE: If an adduct is specified, the mass (* charge state) is subtracted from the target mass before compositions are computed. The composition calculation assumes a neutral (uncharged, non-adducted) mass.

Fitness Threshold: Results that match the other criteria but have fitness lower than this threshold will be discarded. Fitness ranges from 0.0 (no match to experimental spectrum) and 1.0 (perfect match). Fitness is computed by matching the experimental isotope cluster to the computed cluster for the formula, taking into account both / m/z and intensity.

Once you have finished with the constraints, click on the 'Elemental Composition' icon and select with the crosshair the desired peak of the Mass spectrum. A table with the results will be displayed (it is also possible to find this table under the 'View/Tables' menu). All the results obtained will satisfy the composition, DBE, and tolerance constraints, and will be ranked in decreasing order of fitness. Please bear in mind that a fitness value of 1 will be the perfect match.

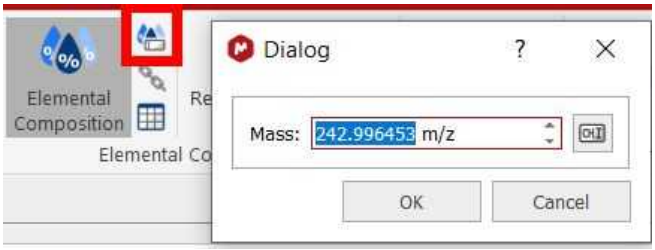
© 2024 MESTRELAB RESEARCH



The Update button will allow you to modify the constraints and apply these changes to the current Elemental Composition result.

Click on the applicable button of the Elemental Composition table to remove the highlighted area.

You can also calculate the elemental composition table from a given m/z value:



Please bear in mind that mass spectrometers measure monoisotopic m/z values which is different with the Molecular weight value (calculated using the average mass of each element weighted for its natural isotopic abundance). For example, for C20H23N, the chemical mw is 277.402, and monoisotopic mw is 277.182. Here you can see a list of the exact mass of some elements:

Element	Symbol	Exact Mass
Hydrogen	H	*1.00783*
	2H (D)	*2.0141*
Carbon	12C	*12*

© 2024 MESTRELAB RESEARCH

	13C	*13.0034*
Nitrogen	14N	*14.0031*
	15N	*15.0001*
Oxygen	16O	*15.9949*
	17O	*16.9991*
	18O	*17.9992*
Fluorine	F	*18.9984*
Silicon	28Si	*27.9769*
	29Si	*28.9765*
	30Si	*29.9738*
Phosphorus	P	*30.9738*
Sulphur	32S	*31.9721*
	33S	*32.9715*
	34S	*33.9679*
Chlorine	35Cl	*34.9689*
	37Cl	*36.9659*
Bromine	79Br	*78.9183*
	81Br	*80.9163*
Iodine	I	*126.9045*

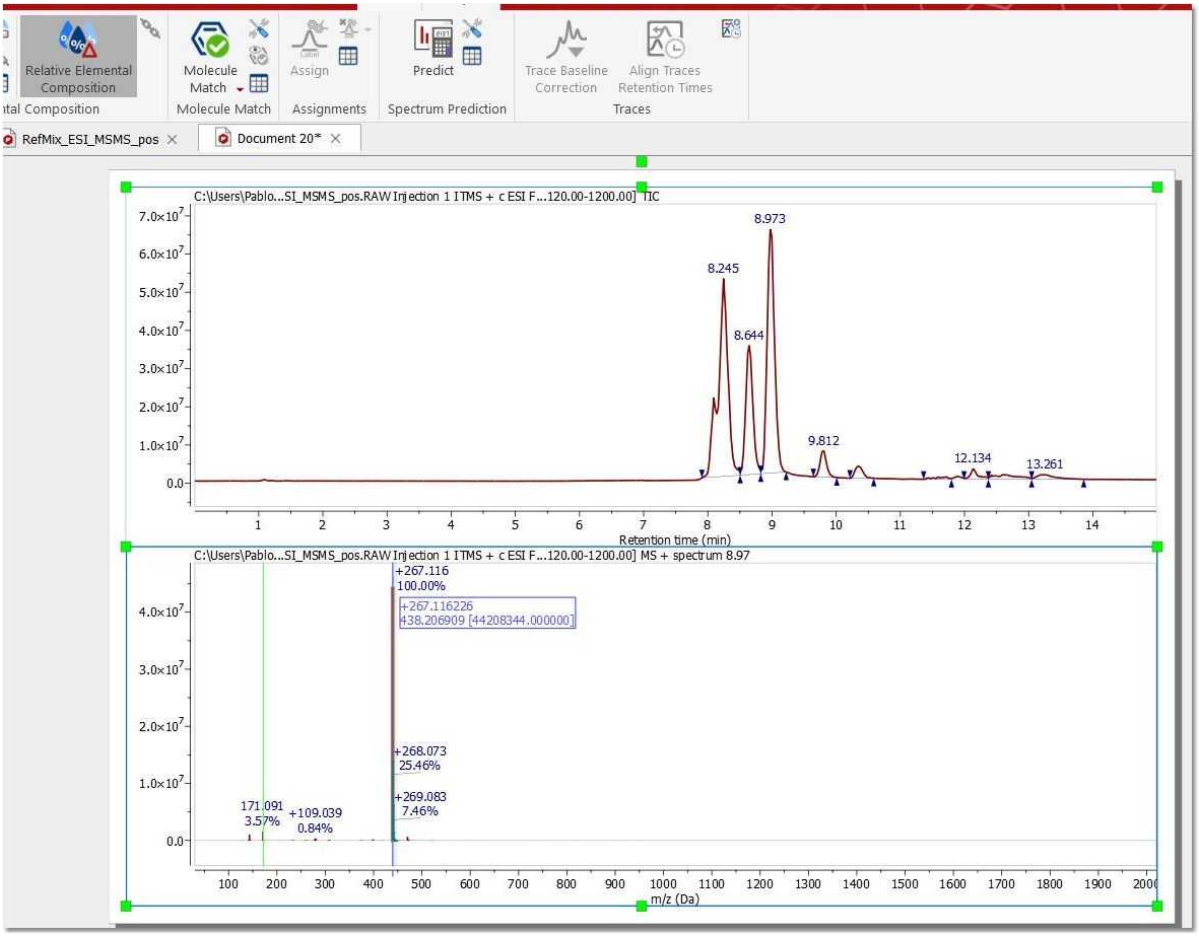
We use the table published by the US NIST: https://physics.nist.gov/cgi-bin/Compositions/stand_alone.pl

Their sources of data are described here: https://www.nist.gov/pml/atomic-weights-and-isotopic-compositions-relative-atomic-masses

14.2.3.4.1 Relative Elemental Composition

This is a new elemental composition mode which calculates the elemental composition for the relative mass value.

© 2024 MESTRELAB RESEARCH



In any of the elemental composition modes you can switch between them using the space key and in the relative mode you can set the pivot by pressing and holding down the CTRL key (it can be reset to the highest peak again by pressing the R key).

14.2.3.5 Molecular Match

Molecule match analysis determines the possible presence of a given structure or structures within a mass spectral data set. The molecular ion cluster of each structure is computed and compared to each spectrum in the data set. If one or more spectra contain the computed isotope cluster within reasonable matching constraints, the most intense spectrum within the most closely matched chromatographic peak is returned as a positive result. Structures not matched are assigned a “not found” result. The constraints dialog allows the user to select one or more adducts, specify ion polarity, and other parameters.

The algorithm makes use of fast isotope cluster calculations and a sophisticated spectral matching technique.

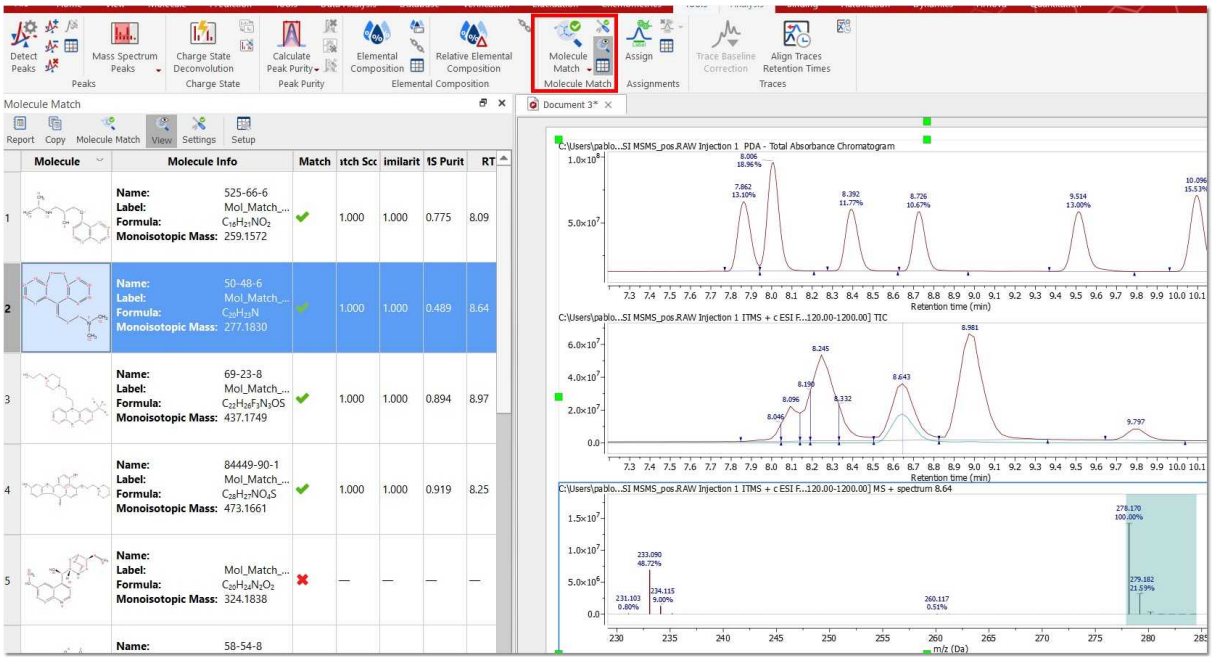
Molecule match analysis is very general purpose. Applications include small molecule confirmation and metabolite identification.

Limitations are few but include the requirement that the acquired spectra of the compounds being analyzed exhibit at least partial molecular ion clusters.

The 'Molecule Match' is very useful to see if a mass spectrum and a GC chromatogram match with one of the molecular structures of the document.

© 2024 MESTRELAB RESEARCH

Just load your spectrum (TIC and MS) and paste the molecular structures into any page of the same document (or load whole spectral libraries by opening an sdf file). Next click on the 'Molecule Match' button of the toolbar.



The 'Molecule Match' table with the results will be displayed (this table can also be found under the 'View/Tables' menu). This table will contain information about the 'Retention Time' (RT), Scan (number of spectrum), Match Score, Similarity, MS Purity, etc..

Similarity is the comparison of the computed isotope cluster spectrum of a given molecule's molecular formula, plus optional adduct - that is, the mass spectral peaks of the molecular ion - with the mass peaks of the acquired spectrum in that mass region. The similarity value can be from 0 to 1, and you will note that a threshold of 0.8 is used. For instance, a pesticide named Pydrin, with a molecular formula of C25H22NO3Cl, plus an adduct of H, has an isotope cluster of 420.137 - 100%, 421. 140 - 28%, 422.135 - 36%, 423.137 - 10%, 424.140 1.5%. This is compared with the mass peaks of the acquired spectrum in the mass range of about 420 to 425.

Match Score is the product of Similarity and MICC value (see below).

MICC is molecular isotope-cluster chromatogram. Which is the sum of mass chromatograms of all the significant peaks in the isotope cluster. Using the example above, m/z 420.137 + 421.140, and so on.

This is almost the same as the mass chromatogram, across several Daltons, in the case listed above, from m/z 420 425. The reason it is not the same is that for accurately measured mass spectral data, a small tolerance on the order of a few ppm might be used for matching.

For any given molecule, the MICC chromatogram is normalized to 1.0, and this value is what is used for Match score above.

So, basically, for each proposed structure's molecular formula, with the given ionization mode (such as protonation), a molecular ion cluster spectrum is computed. Each molecular ion cluster spectrum is then compared to every spectrum in the LC- or GC- MS run, and ranked according to spectral similarity, where 1.0 is identical within the m/z region of the molecular ion cluster. This ranking is further refined by taking into account the response of a molecular ion chromatogram (or chemical chromatogram) , which also has a maximum value of 1.0. The final match score is the product of the two metrics. The

© 2024 MESTRELAB RESEARCH

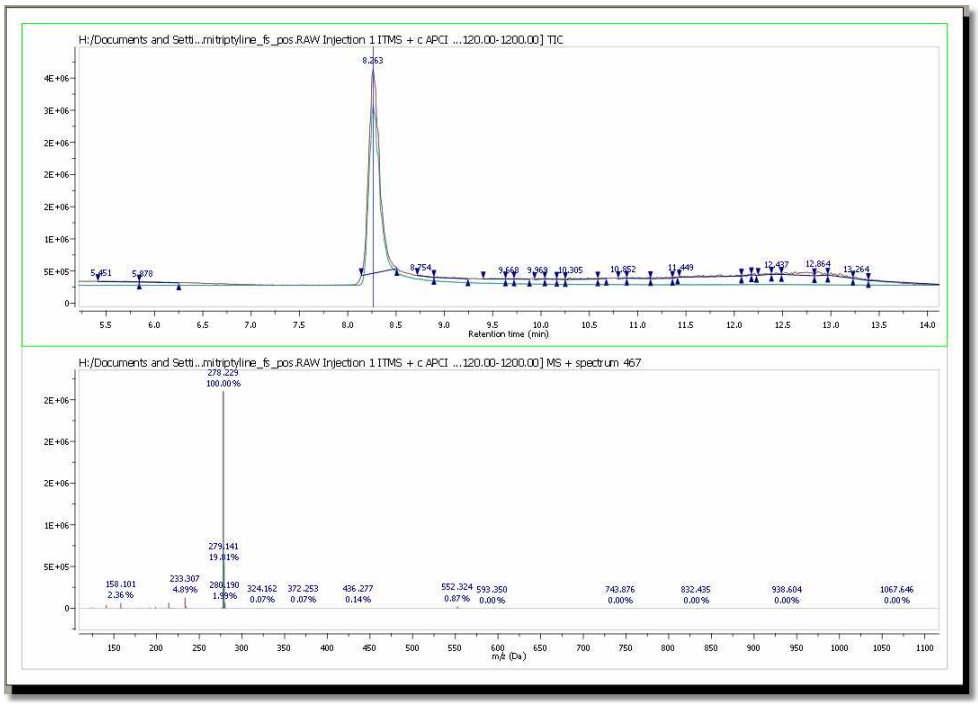
MS Molecule Match similarity measurements are derived from traditional dot product calculations and take into account a user specified m/z accuracy, to make full use of accurately mass measured mass spectral data. If MS/MS spectra are also present in the data set, the structures are used to predict fragment spectra, and these predicted fragment spectra are compared with the acquired MS/MS spectra.

MS Purity is the proportion of the total intensity of the mass peaks matched in the Similarity measurement relative to the total intensity of the whole spectrum. MS Purity is then, the fraction of the found molecular ion intensity versus the total intensity of the spectrum. This assumes that most of the intensity of the whole spectrum will be due to the molecular ion cluster, which is true for soft ionization mass spectra, which is true for most forms of ionization in use today. Which is not to be confused with MS/MS experiments, which have impart additional energy to produce fragmentation. So, using the MS purity as a metric generally provides a useful value that is a larger value with "pure" spectra, that is spectra without a lot of background (or overridden by the intensity of the molecular ion) or without a coelutant.

Purity is the ratio of the matched peak area (in the TIC) of the signals of matched compounds.

Clicking on any "matched molecule" will display the MICC (Molecular Isotope Cluster Chromatogram), overlaid with the TIC. The MICC shows the summed response of the isotope cluster for each MS spectrum over the entire data set. The MICC is the sum of the mass chromatograms of each m/z value of an isotope cluster.

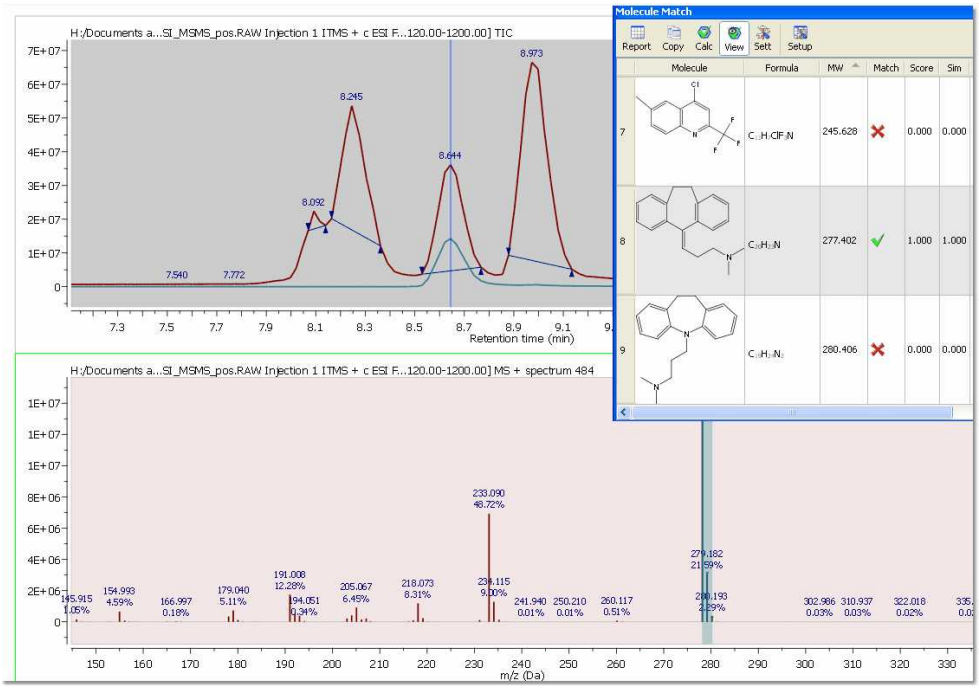
In this example, the best result was for the compound number 1 (C20H23N). You can see in the picture below the MICC (in green) over the TIC (in red):



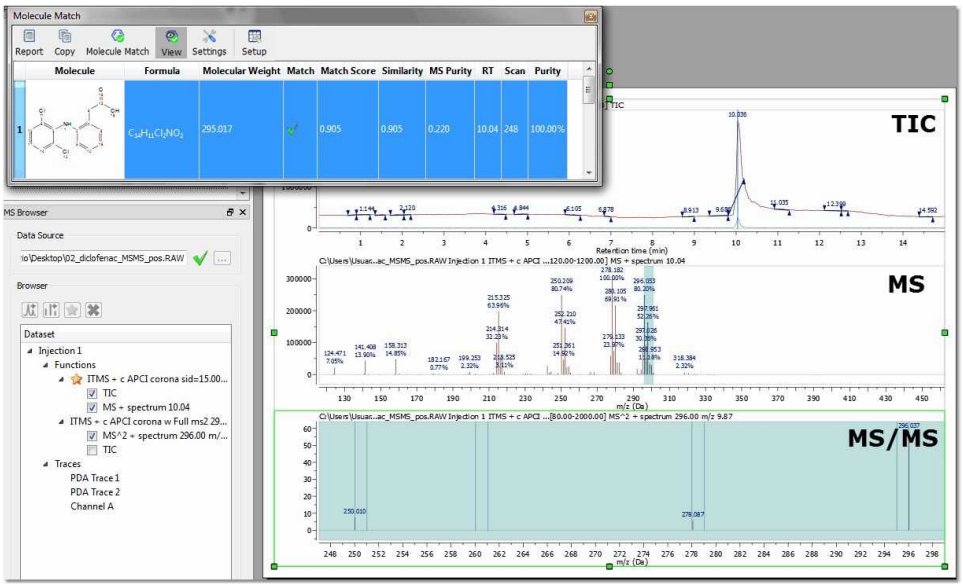
In addition, Mnova shows you in the chromatogram the retention time of the match (with a blue vertical line) and displays the corresponding MS spectrum (the number 467 in this case) overlaid with the theoretical one (in green at a M/Z around 278, in this case). It is possible to show/hide the MICC and the theoretic MS just by clicking on the 'View Molecular Match' icon .

© 2024 MESTRELAB RESEARCH

Here you can see another example, where the compound 8 (amitriptyline) is present in the mixture:



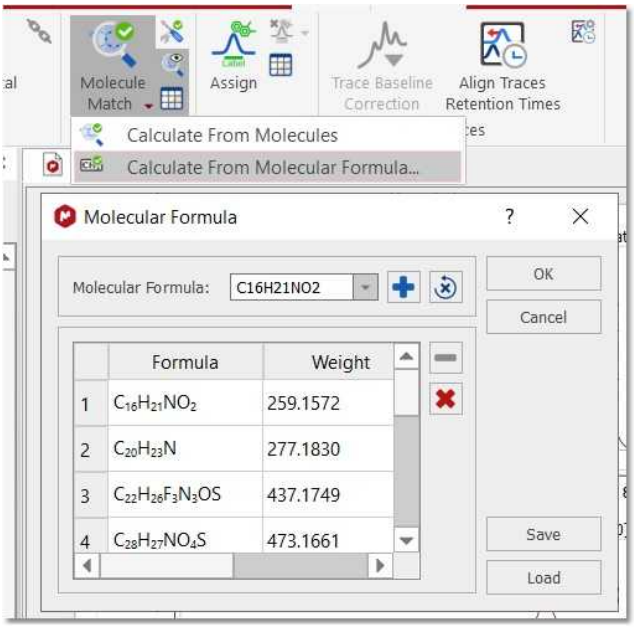
You can also run Molecular Matches with MS/MS spectra and see the result directly in the MS/MS plot. Here you can see an example:



Clicking on the 'Report' button will paste the content of the Molecular Match table into your Mnova document.

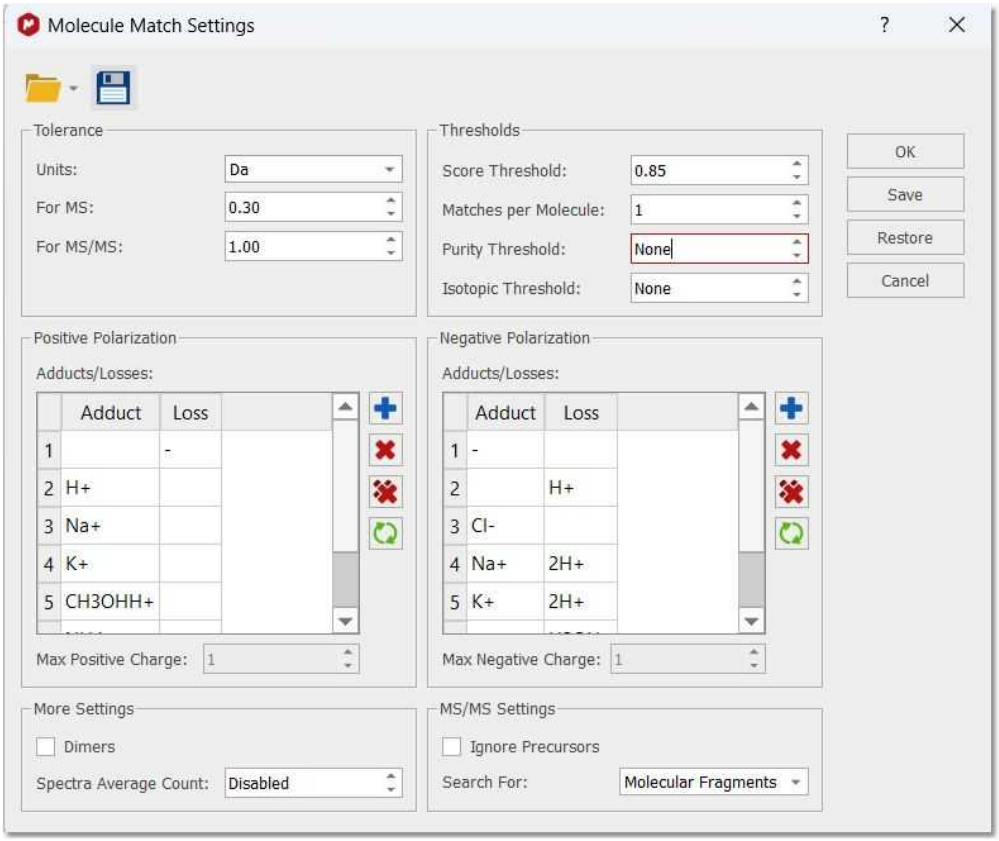
The user can also run the 'Molecular Match' feature just by entering a molecular formula after having clicked on the 'Calculate From Molecular Formula' in the applicable scroll down menu:

© 2024 MESTRELAB RESEARCH



You can save the list of molecular formulas by using the 'Save' button.

Select 'Settings' on the 'Mass Analysis/Molecule Match' to display the 'Molecule Match Settings' dialog box:



Tolerance: To set the m/z tolerance for matching predicted spectra to experimental; by default, MS spectra are matched to either 5 ppm or 0.5 Da, and MS/MS to 10 ppm or 1.0 Da, depending on whether the spectra are derived from high or low resolution instruments.

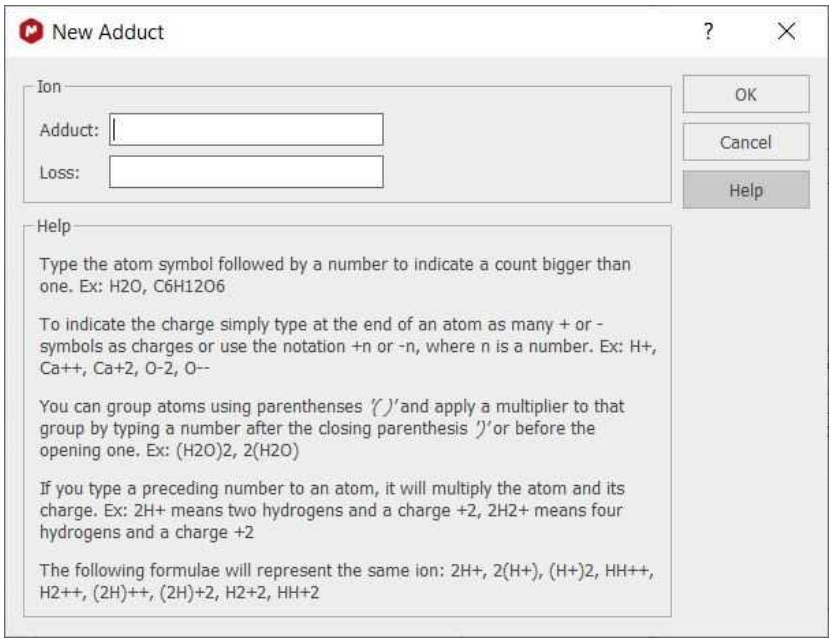
Thresholds: Set match score threshold for acceptable result; in most cases the default value of 0.5 is more than enough, however for weak / noisy spectra, the threshold may be lowered.

Matches per molecule: To set the maximum number of results per molecule; by default, a molecule is allowed to match only once, in which case the best result is returned. However, if no MS/MS spectra are available and the sample is known to contain isomers, then increasing this limit will allow the molecule to match every isomer peak.

Purity threshold: Molecule match algorithm will ignore any match with a mass purity less than that settings value.The default value is set to 30%.

Isotopic threshold: This parameter is in % units (by default, it is set to none). The usage of this parameter consists of ignoring/removing those peaks below the selected threshold value in the isotopic cluster.

Adducts: Set adduct/loss pairs using proper atomic or chemical formulas with charge notation. Clicking on the blue plus button will allow you add new adducts. Clicking on the Help button will display further information about the formula syntax:



Charges: Set maximum charge states to consider. By default, +/- 1 are the maxima. This option in Positive or Negative Polarization is enabled only when no adduct/loss is added in the list.

Note: If the molecule is charged with the same polarity as the MS data, then no ionization stage is done and the charged molecule is searched into the MS data: [M +n].
If the molecule is neutral or the charge is not the same as the polarity:

If there are adducts in the settings, those adducts will be used to search an ion composed by the M+A−L (M: molecule, A: adduct, L: loss)

If there are not adducts, then the max charge count setting is used and the ion is composed by simply charging the molecule from 1 to the number of maximum charges seleted: [M] + ... [M] +n

© 2024 MESTRELAB RESEARCH

Dimers: Set to enable consideration of dimers when matching; by default, dimer matching is disabled. A dimer (in this case) is a non-covalently bound molecule pair (e.g. bound by van der Waals, hydrogen bond, or electrostatic forces, not a covalent inter-atomic bond). If you have a molecule M, then in ESI+ it normally gives an [M+H]+ ion. If it forms a dimer in the ESI source, then the ion observed is [2M+H]+, and the m/z value we look for is twice the mass of M plus the mass of H.

If the Dimer setting is on, it will search in addition:
If the molecule is charged:

Twice the charged molecule: 2[M +n]

If neutral:

Twice the molecule plus the adduct minus the loss: 2M+A-L

Twice the molecule plus the charge from 1 to max charge state set in settings: [2M] + ... [2M] +n

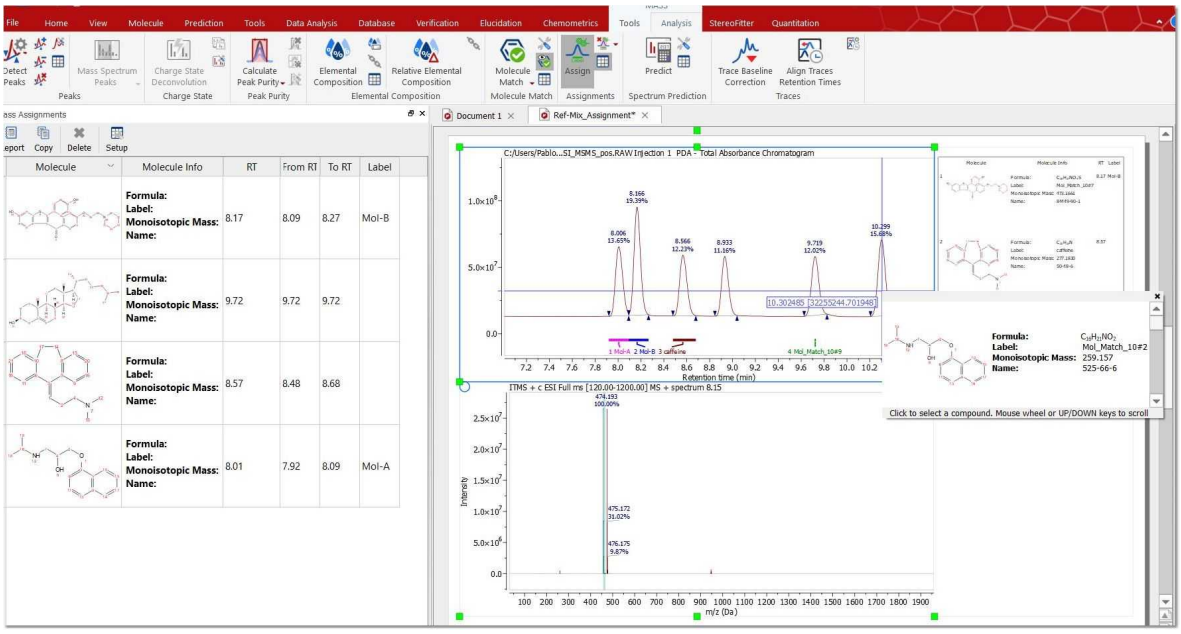
Spectra Average Count: It allows to co-add several scans when searching for a match instead of doing it with single scans. The number set corresponds to +/- n scans around a centered scan.

Ignore Precursors, when checked, it will ignore the precursor information when searching for fragments in MS/MS spectra. Until version 14, when matching the molecular fragments in MS/MS data for a given molecule, the precursor m/z for the MS/MS spectrum should match the molecular mass for the provide molecule. If this setting is set, then that check is not made, so the fragments will be searched in all the MS/MS spectra regardless its precursor. This setting must be used with care and only in very specific cases, because it could increase the false positives.

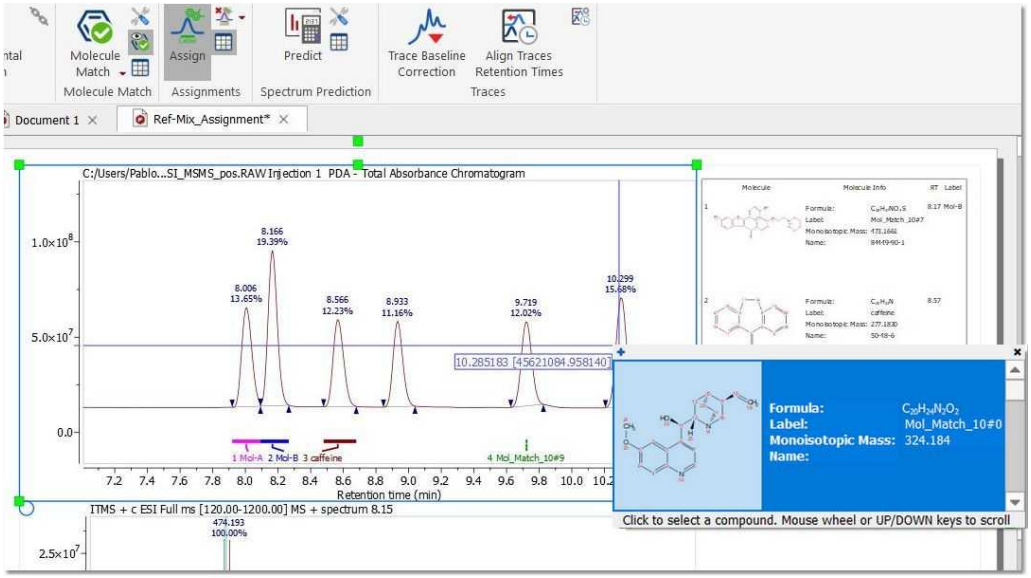
The *Search For* setting can be used when we have only MS/MS data. Until Mnova 14, we were searching always for fragments, but now we can instruct Mnova to search for the molecular ion in the MS/MS data (like we are doing for MS data).

14.2.3.6 Assignments

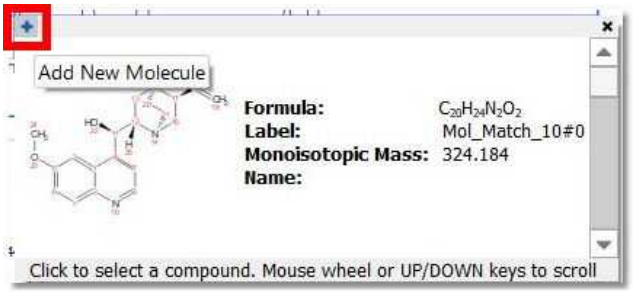
You can easily assign structures to chromatogram peaks by following the menu 'Mass Analysis/Assign' (or from the context menu of a mass assignment label).



Clicking on the 'Assign' button will allow you to load your molecular structures (if you do not have any). Next, click (or click and drag to select a range) on the chromatogram peak that you want to assign. If you have several molecules loaded, you will be allowed to select the desired structure by using your mouse wheel (or the up/down arrow keys).

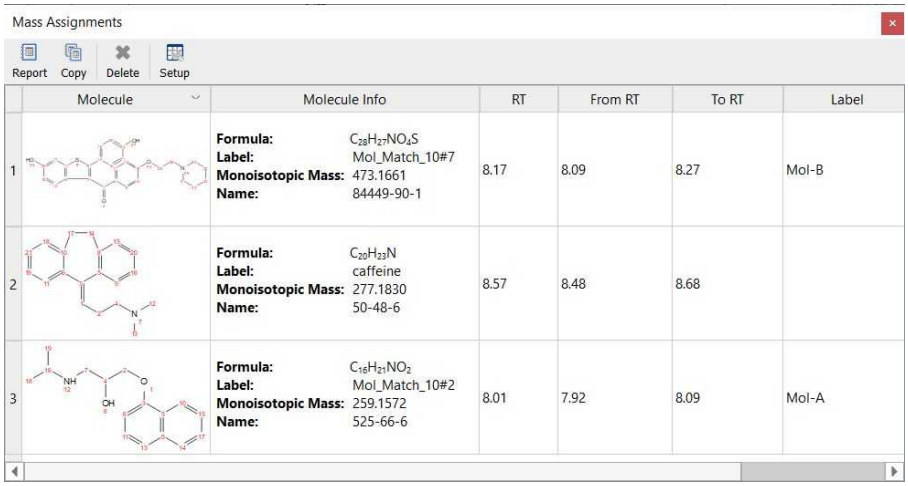


Clicking on the 'plus' button will allow you to load additional molecules:

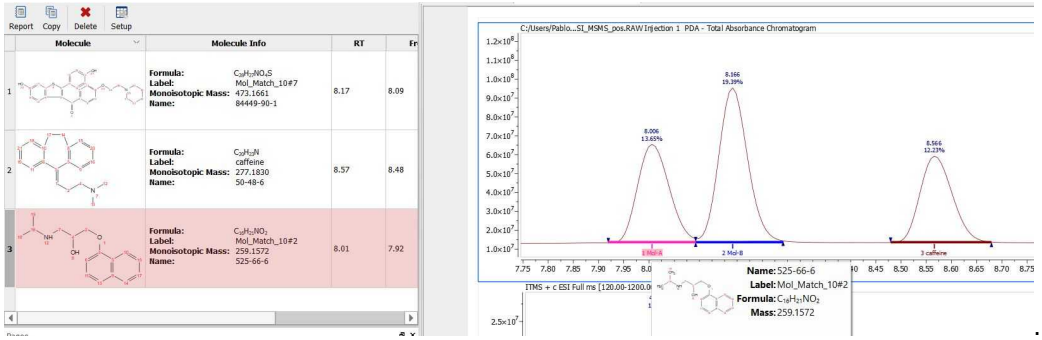


The assignments will be displayed in the MS Assignments table. By default, the assignment label in the chromatogram will show the Compound Label (which you could change from the Compounds Table), but the user could add a different label from the Mass Assignments table:

© 2024 MESTRELAB RESEARCH



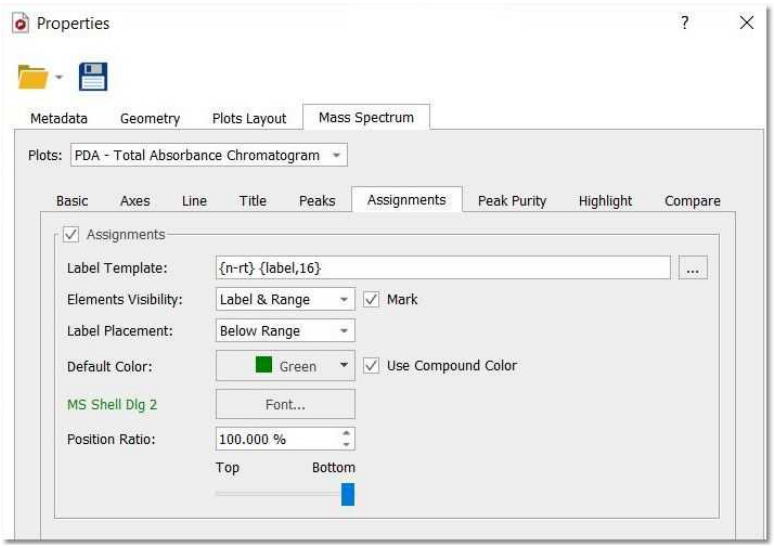
The table can be reported in the page or copied to the clipboard (to be pasted in a third party application). When hovering the mouse over a row in the table, the assignment label is highlighted on the chromatogram and viceversa (When hovering the mouse over the assignment label, the structure and molecule info shows in a pop up message):



Right clicking on the assignment label, will allow you to change the content of the label (which can also be edited from the MS Assignments table)

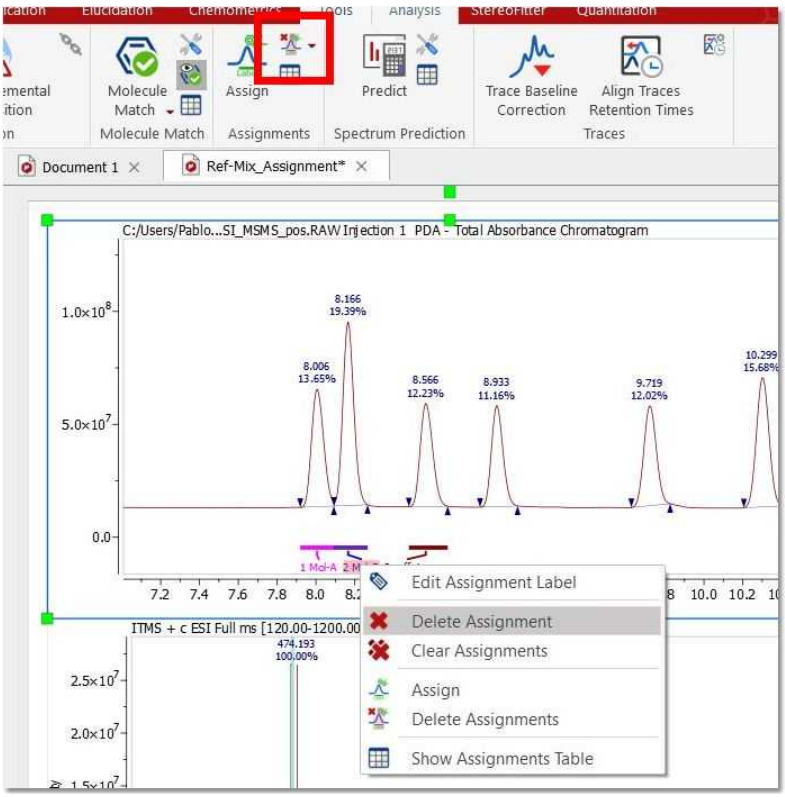
The label can be customized from the Assignments tab of Mass Spectrum Properties:

© 2024 MESTRELAB RESEARCH



When setting a color for each compound in the Compounds table, they could be used also for the assignment label.

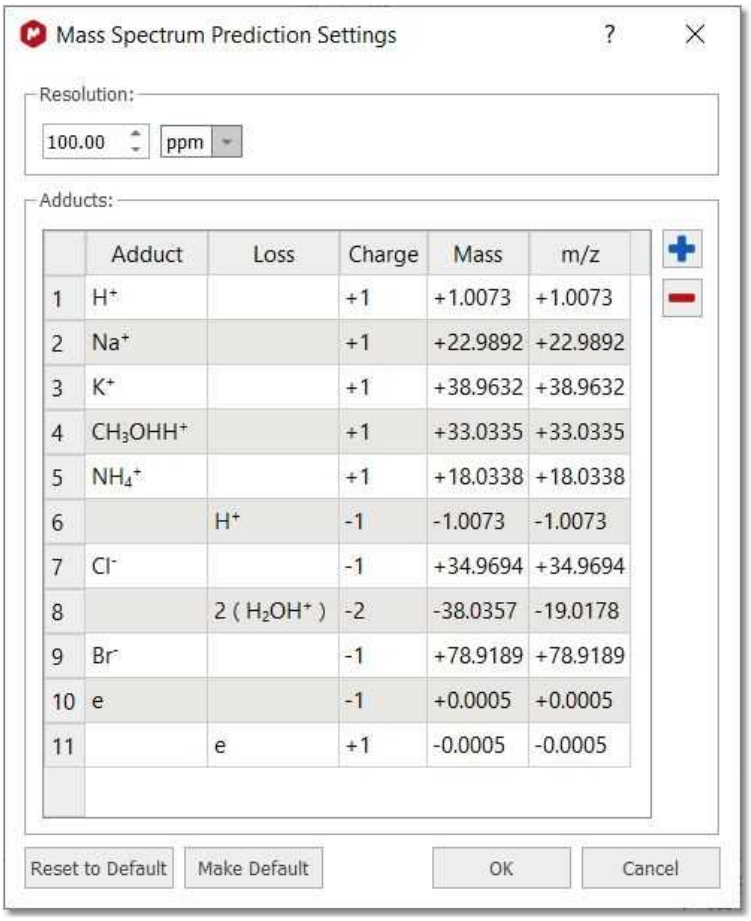
You can delete assignments by using the applicable button on the ribbon, from the context menu or from the Mass Assignments table:



© 2024 MESTRELAB RESEARCH

14.2.3.7 MS Spectrum Prediction

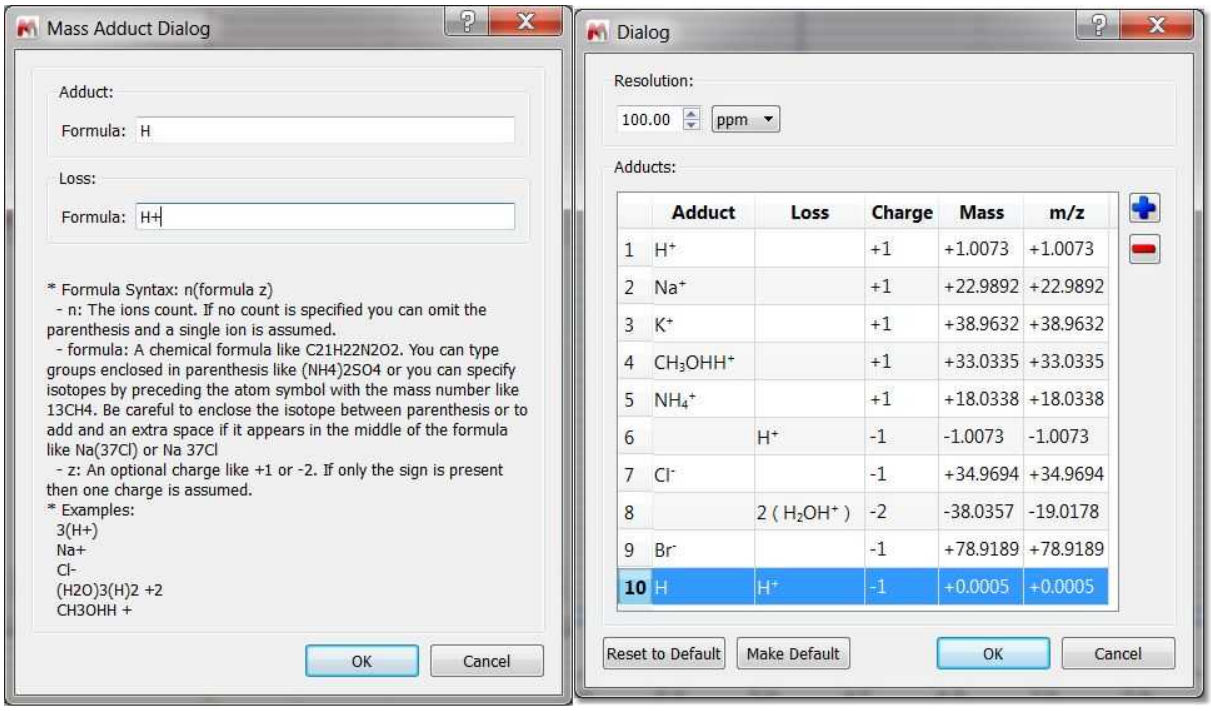
Follow the menu 'Prediction/MS Prediction/Settings' to choose the desired adducts and losses used for the predictions:



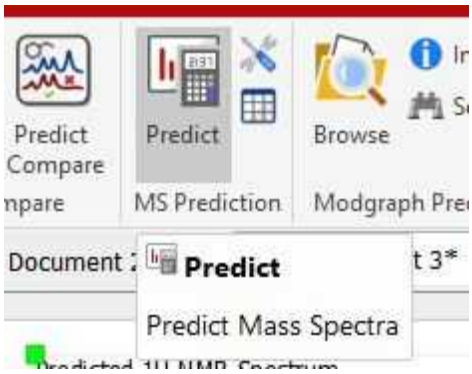
	Adduct	Loss	Charge	Mass	m/z
1	H^+		+1	+1.0073	+1.0073
2	Na^+		+1	+22.9892	+22.9892
3	K^+		+1	+38.9632	+38.9632
4	CH_3OHH^+		+1	+33.0335	+33.0335
5	NH_4^+		+1	+18.0338	+18.0338
6		H^+	-1	-1.0073	-1.0073
7	Cl^-		-1	+34.9694	+34.9694
8		2 (H_2OH^+)	-2	-38.0357	-19.0178
9	Br^-		-1	+78.9189	+78.9189
10	e		-1	+0.0005	+0.0005
11		e	+1	-0.0005	-0.0005

You can add new adducts by clicking on the 'blue plus' button. For example, to add an electron, just type 'H' for the adduct and 'H+' for the loss (the net result will be an electron):

© 2024 MESTRELAB RESEARCH

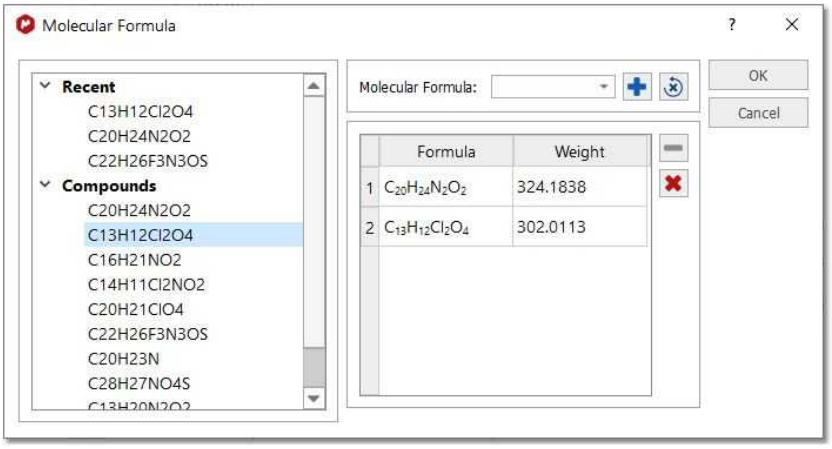


Select 'Predict' (under the prediction ribbon) to simulate the mass spectra with the desired losses and adducts:

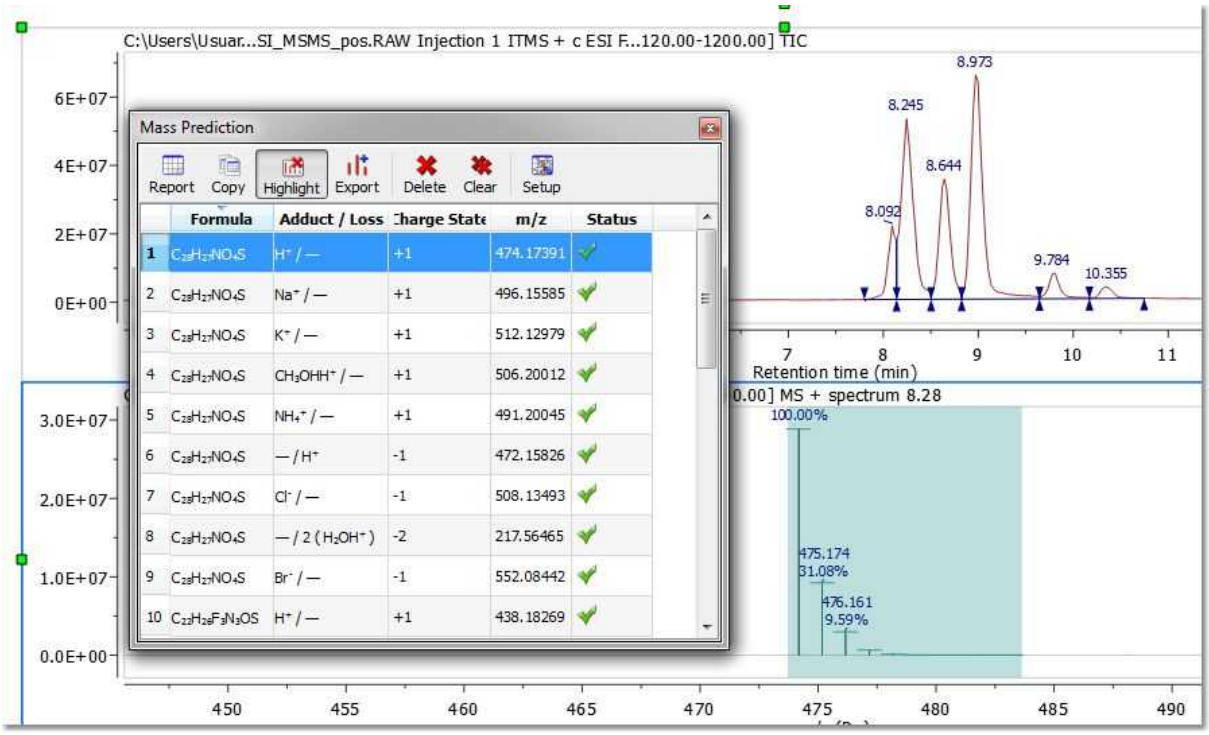


This will display a new window to enter the desired molecular formula or to select between the recent used or from the compounds available in the 'table of compounds':

© 2024 MESTRELAB RESEARCH



After having clicked on the blue plus button to add the desired formula and clicked OK, you will get the MS prediction table with all the applicable adducts:

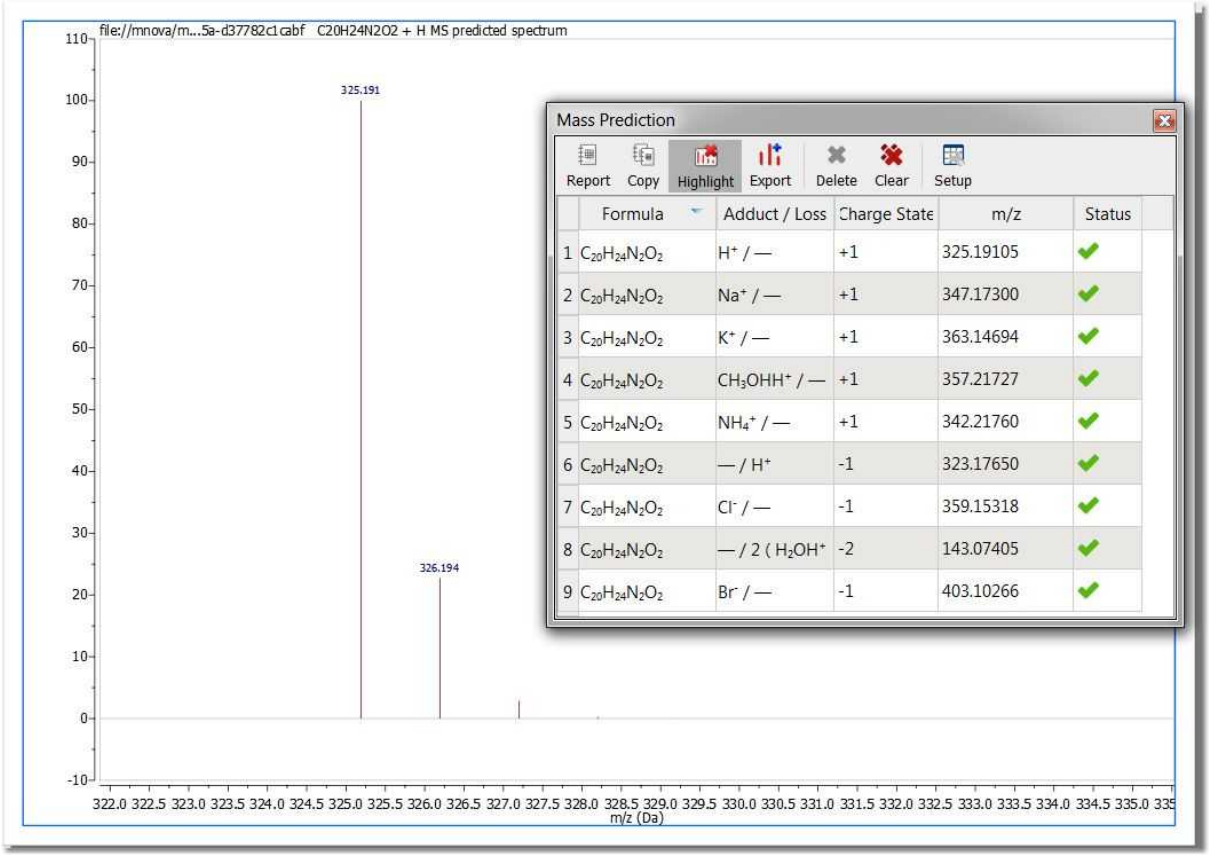


The simulated mass spectrum can be obtained even if there is no experimental spectrum present:

- if there is an experimental spectrum, the simulated spectra will be superimposed on the experimental spectrum by selecting each of them in the Mass Prediction table. Each of the predicted mass spectra can be exported to a new page by clicking on Export button from the table.

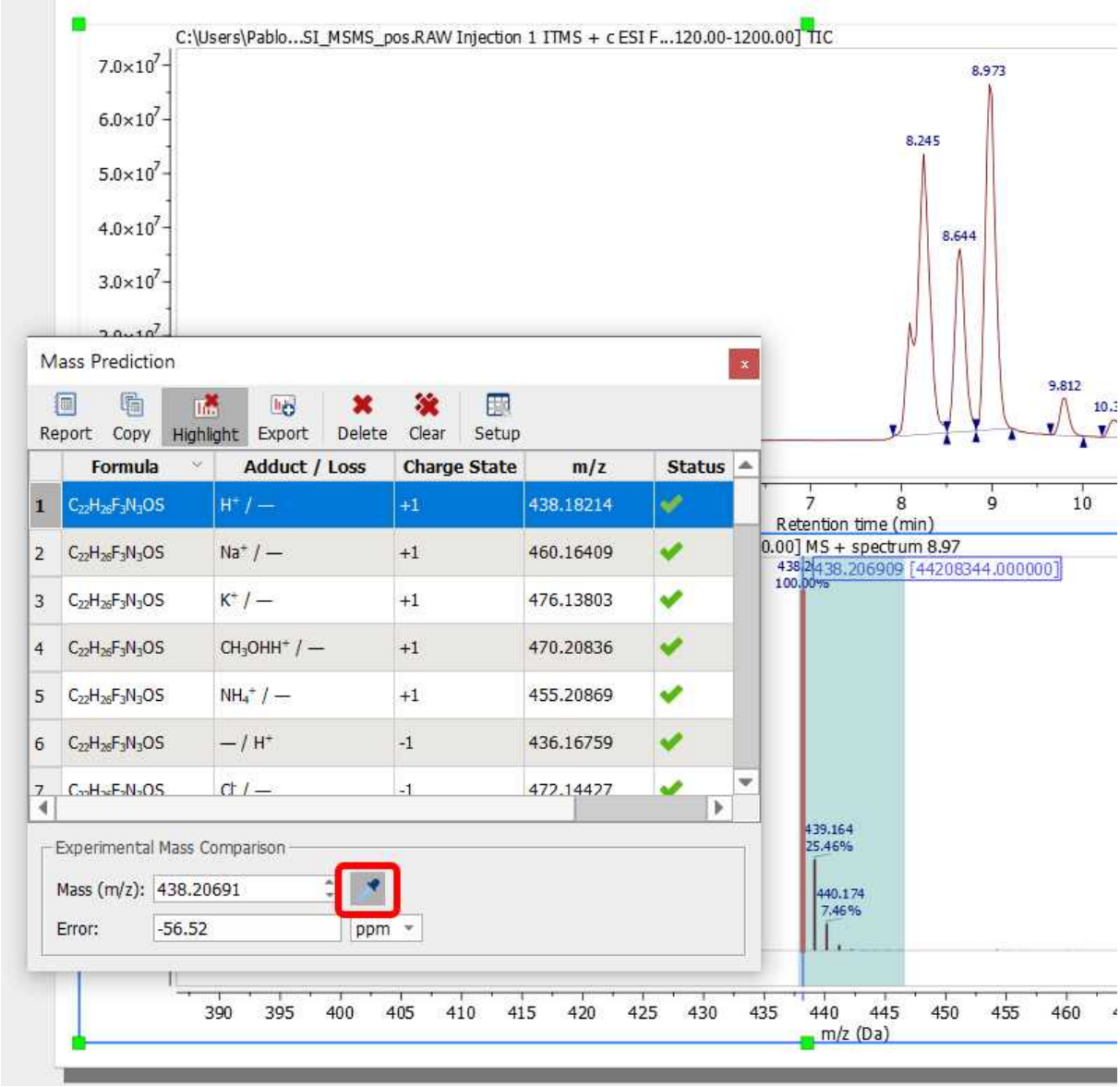
- if there is no experimental spectrum, the simulated spectra can be displayed just by exporting each of them using the Export button.

© 2024 MESTRELAB RESEARCH



You can obtain the error in ppm or in Da from a theoretical m/z value and the experimental one. The experimental value can be introduced graphically by selecting the peak in the experimental mass spectrum or by entering the mass value.

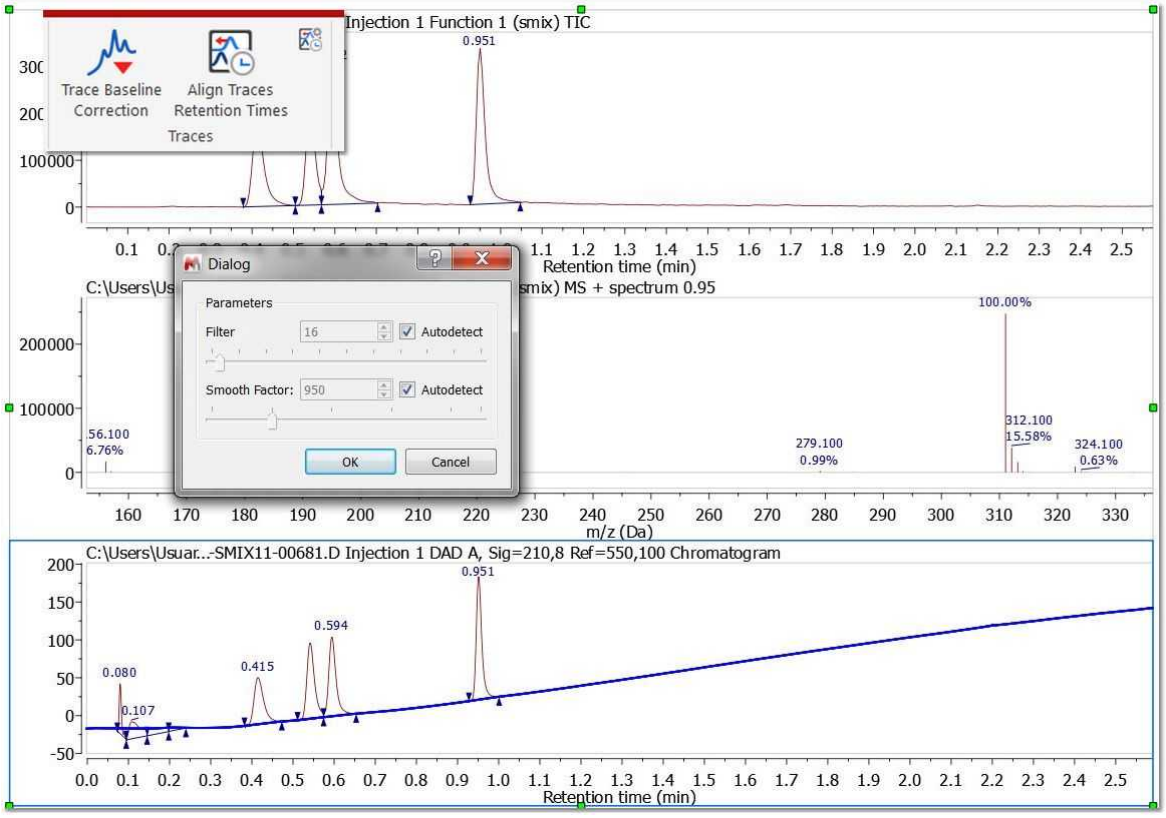
© 2024 MESTRELAB RESEARCH



See also this video

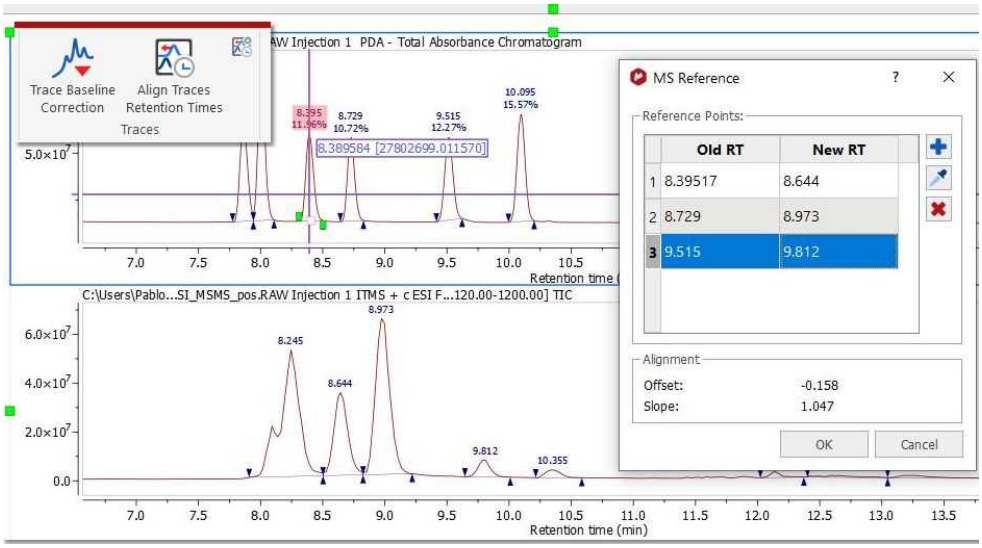
14.2.3.8 Trace Baseline Correction

It is possible to correct the baseline of DAD traces just by following the menu ‘Mass Analysis/Trace Baseline Correction:



14.2.3.9 Align traces retention times

For some technical reasons, the TIC peaks can be delayed compared to the UV peaks. This feature will re-calibrate the chromatograms so that the peaks match across them. You will only need to click on a peak in the selected chromatogram and enter the new value(s) to change the X scale (time).

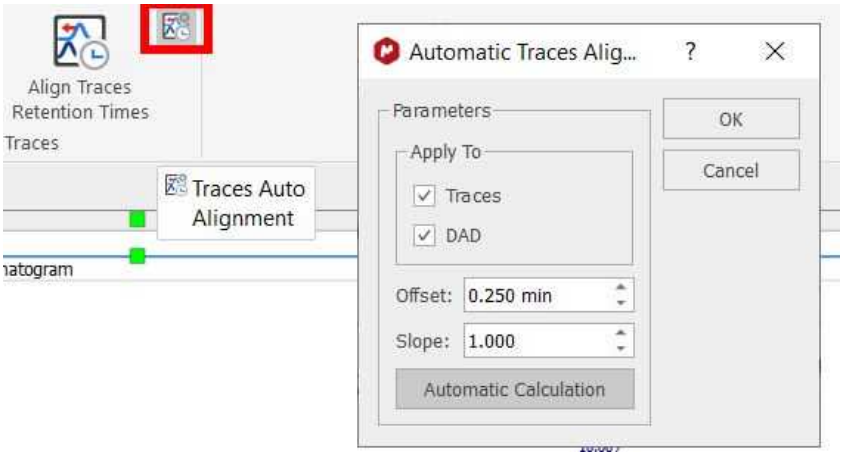


You can introduce more than one value and the alignment will do it calculating a linear regression to obtain the slope and an offset. These values are recalculated every time a new value is added or removed from the list.

© 2024 MESTRELAB RESEARCH

Additional points can be introduced manually (by using the blue plus button) or selecting graphically the “Old RT” (pick button). The more points are added, the better result is obtained in the alignment.

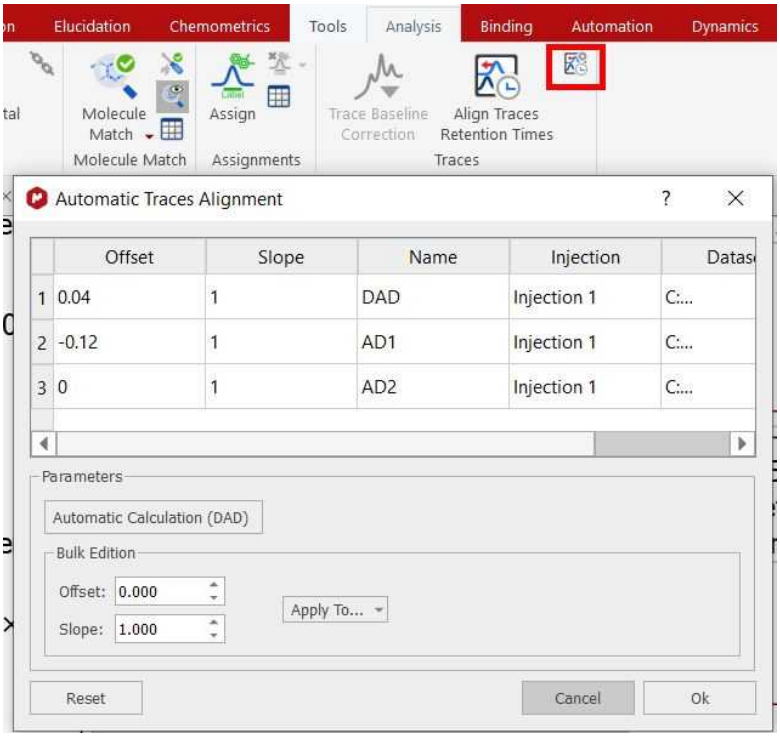
You can also apply an automatic alignment by clicking on the 'Traces Auto Alignment' button and manually typing the offset and the slope (or by clicking on the Automatic calculation button to get the correct values):



14.2.3.10 Traces autoalignment

This feature will be useful to align UV/DAD and MS traces by using a correction value.

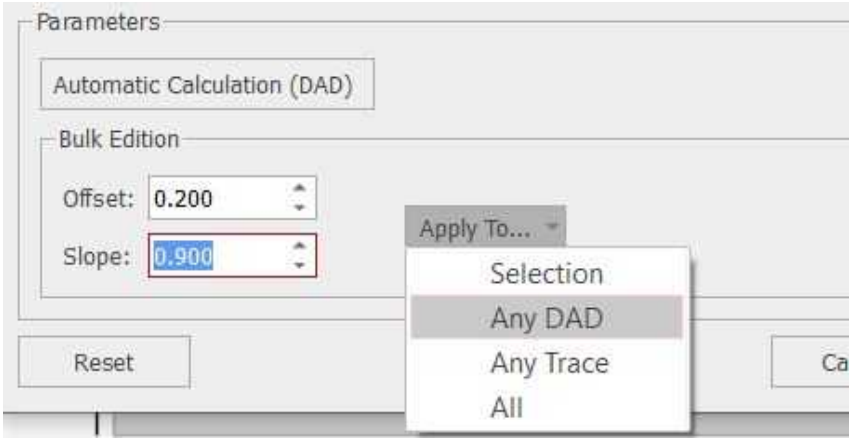
All the mass items have automatic trace alignment parameters that can be set independently by following the menu 'Mass Analysis/Traces Auto Alignment':



The dialog will display a table with all the traces present for each dataset. Clicking on the offset or slope cells will allow you edit the applicable values:

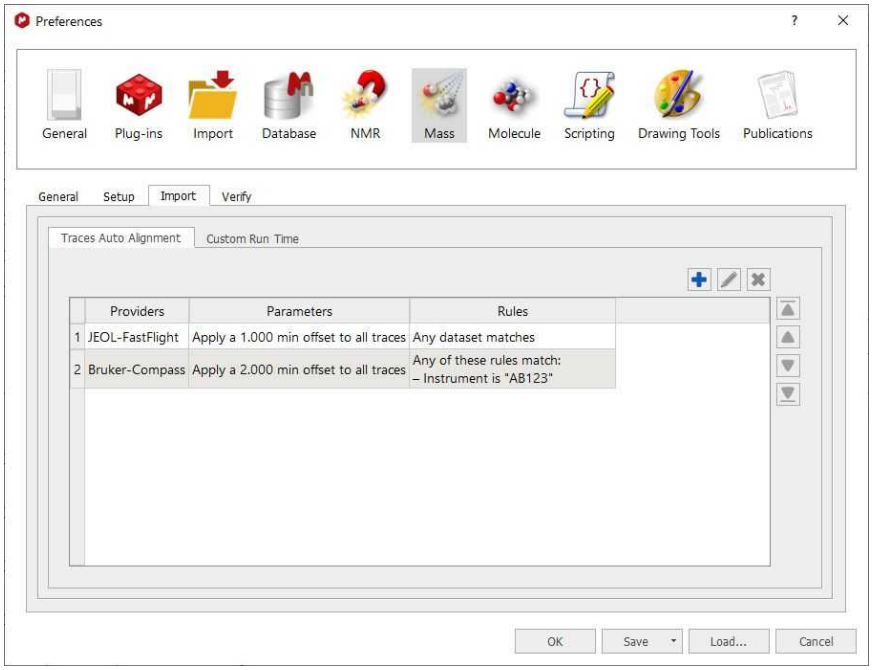
Clicking on the 'Automatic Calculation (DAD)' button will allow you to compute the time delay offset for the UV trace.

In addition, you can edit the offset and slope in batch mode by selecting the applicable values and the scroll down menu to apply the changes to the selected traces in the table, to any DAD, to any Trace, or to All:



The 'Reset' button will restore the Offset and Slope parameters to their default values.

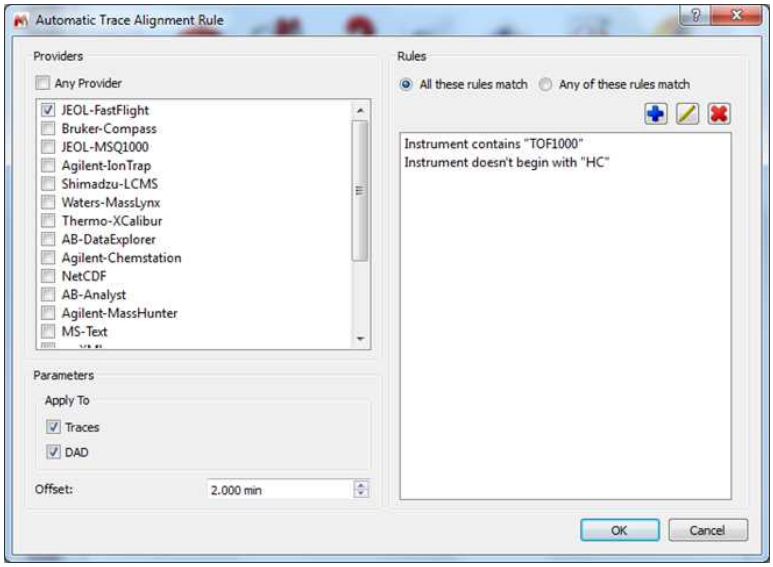
Those parameters can be predefined from the 'File/Preferences/Mass/Import' menu:



The trace alignment parameters are automatically applied to any new trace or DAD chromatogram and the default value for those parameters is determined by the selected preferences. When importing a dataset, if any of the rules match, then the parameters present in the matched rule are copied to the new item.

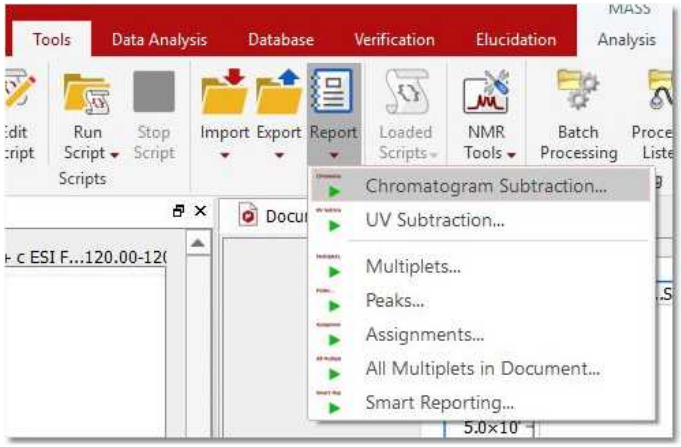
© 2024 MESTRELAB RESEARCH

For the rules, you can specify to which provider (or any provider) the rule applies, and a set of parameter rules based on pattern matching:

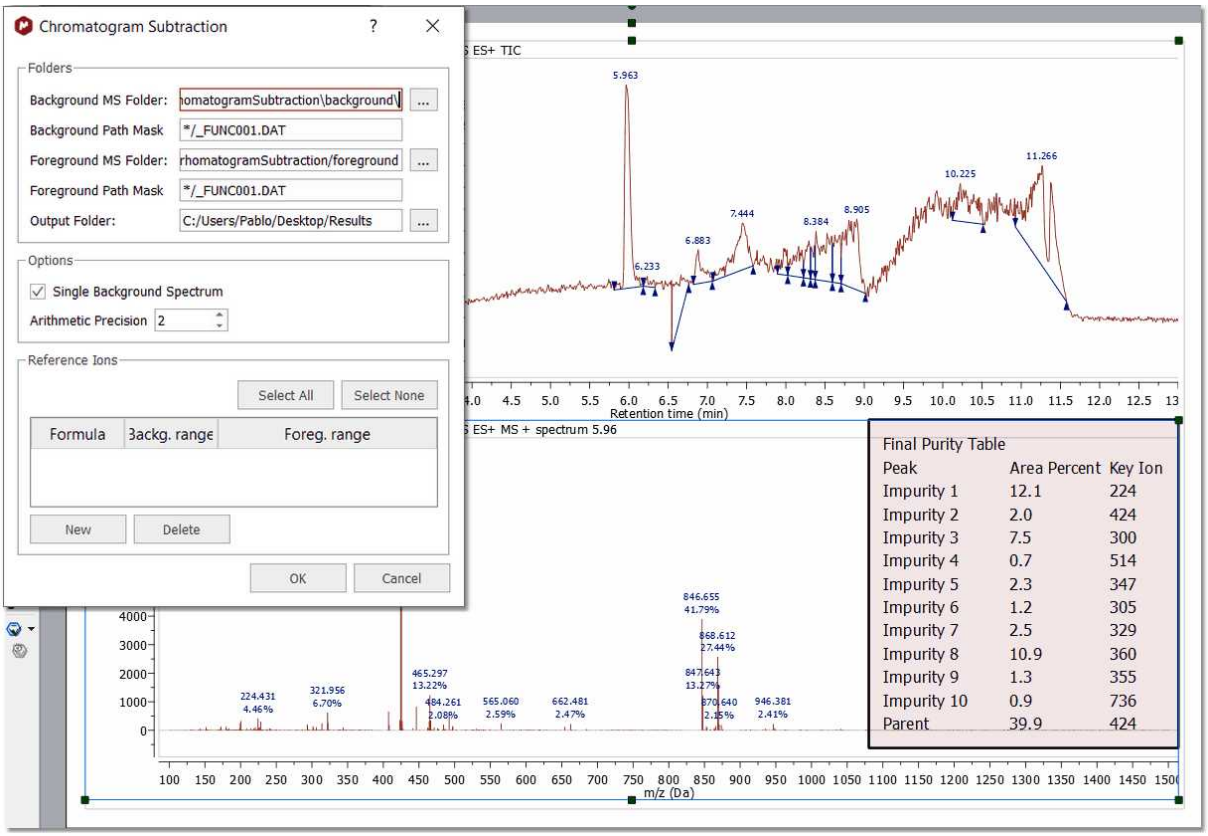


14.2.3.11 MS Scripts

This script will subtract a blank background run from the run of interest, for example to ensure a good baseline for quantitation purposes, or to accurately measure differences between two or more runs. See also this chapter in the case that you have acquired the foreground and background in the same run.



Just follow the menu 'Tools/Report/Chromatogram Subtraction' and select the folders containing the datasets (foreground and background) and the applicable path masks. Once the script has run, it will generate a report with information about the impurities found:



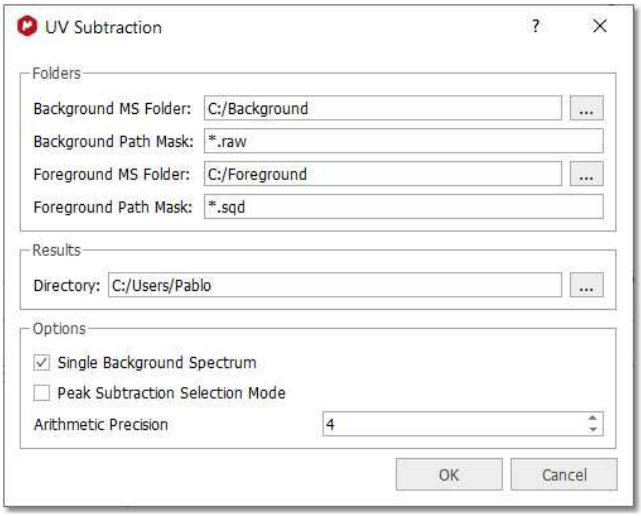
First thing the scripts does is subtraction of the Analysis chromatogram (foreground) minus the blank (background), this will give us the real chromatogram we are interested in, which will only show peaks which are not in the background chromatogram.

To do this subtraction, it is necessary to align the spectra, and this will be done by introducing known peaks at the beginning and end of the run (they will use compounds with certain known very short and very long retention times, which they will introduce in the sample). Finally in the resulted chromatogram, the scripts does a peak picking and integration, which will allow to identify impurity peaks (assumption is that this should be a pure compound, therefore the big peak is the compound, the others are impurities). The script continues until all impurities are check, and repeat process for every analysis chromatogram and its respective blanks. The script generates a Mnova document with the result in the Users folder and an output which is a set of tables as follows:

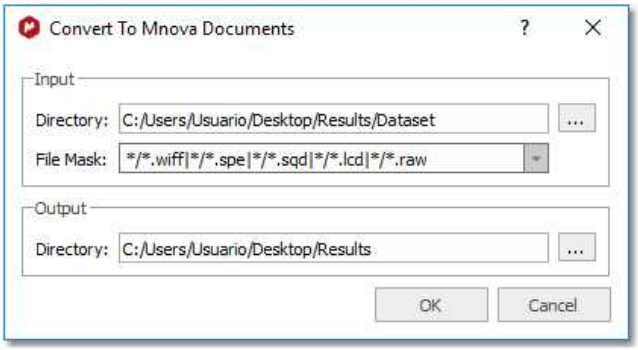
- Table with parent compound and impurities for analysis 1, with retention times and relative areas, as well as with the key ions which identify the impurity or parent compound
- Same table for subsequent analysis (2, 3, 4, etc.)
- Summary purity table, with averages from all analysis for each one of the peaks

Similarly to chromatogram subtraction, there is another script for UV subtraction (under the 'Tools/Report/UV Subtraction' menu). So you will subtract a background UV chromatogram from a foreground one:

© 2024 MESTRELAB RESEARCH

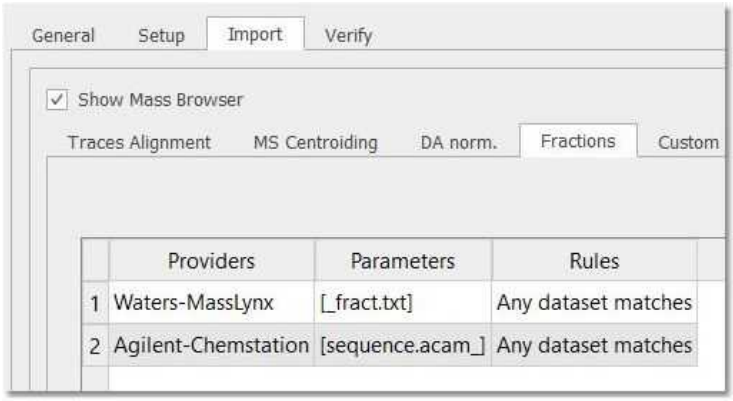


The script located under the menu 'Tools/Export/Convert to Mnova Document" will allow you select the folder containing your datasets spectra, define a mask to set the name and extension of the target files and the folder where the Mnova documents will be saved. This script will be useful to convert MS formats (not compatible in Mac or Linux) from a Windows computer

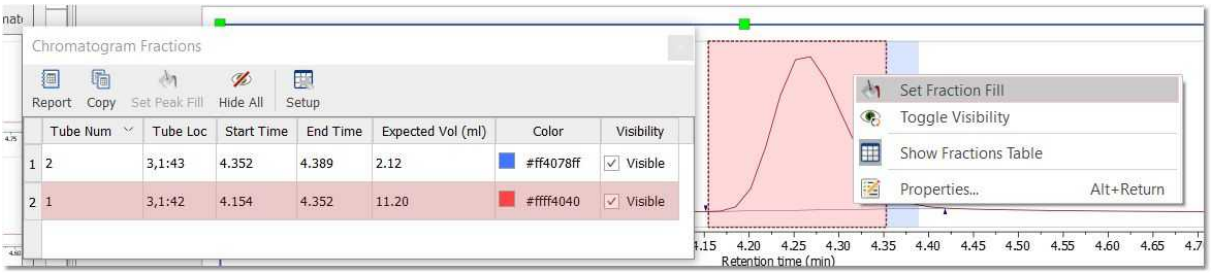


14.2.3.12 Fractions

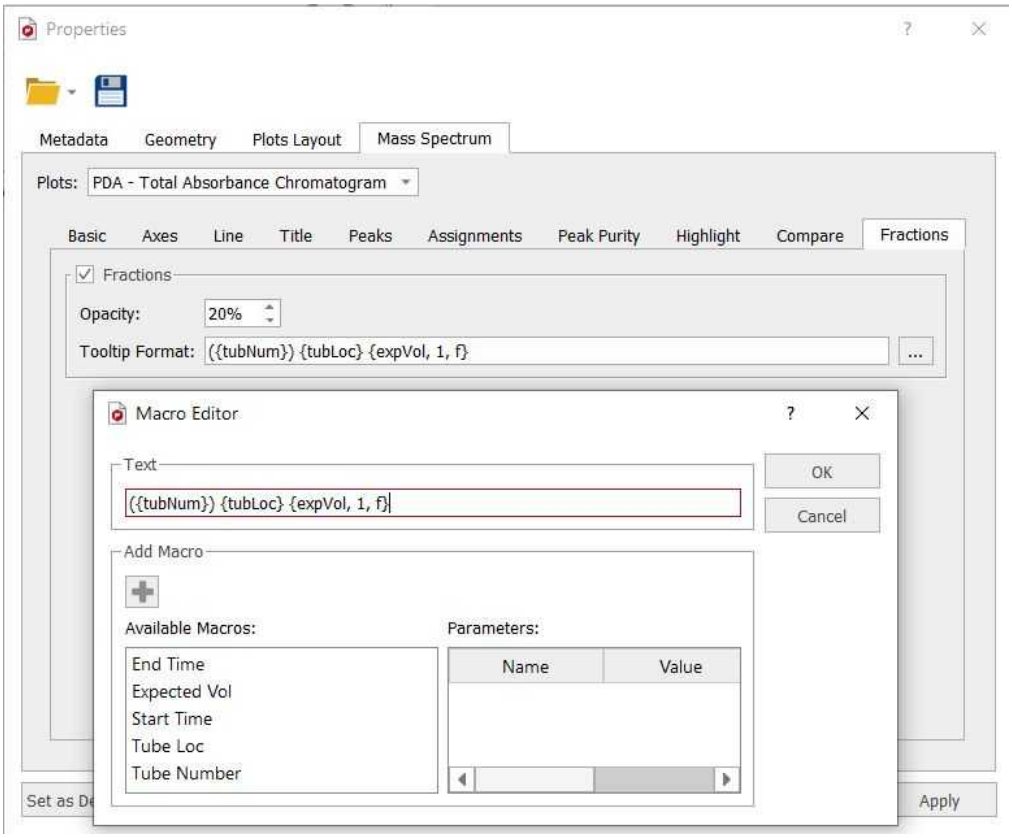
For Waters Masslynx datasets, you can highlight the fractions imported from the _fract.txt file:



Once you have loaded the Masslynx dataset, the imported fractions will be highlighted. Follow the menu 'View/Tables/Mass/Chromatogram Fractions' to change the color or toggle the visbility. The same can be also done from the fraction context menu:

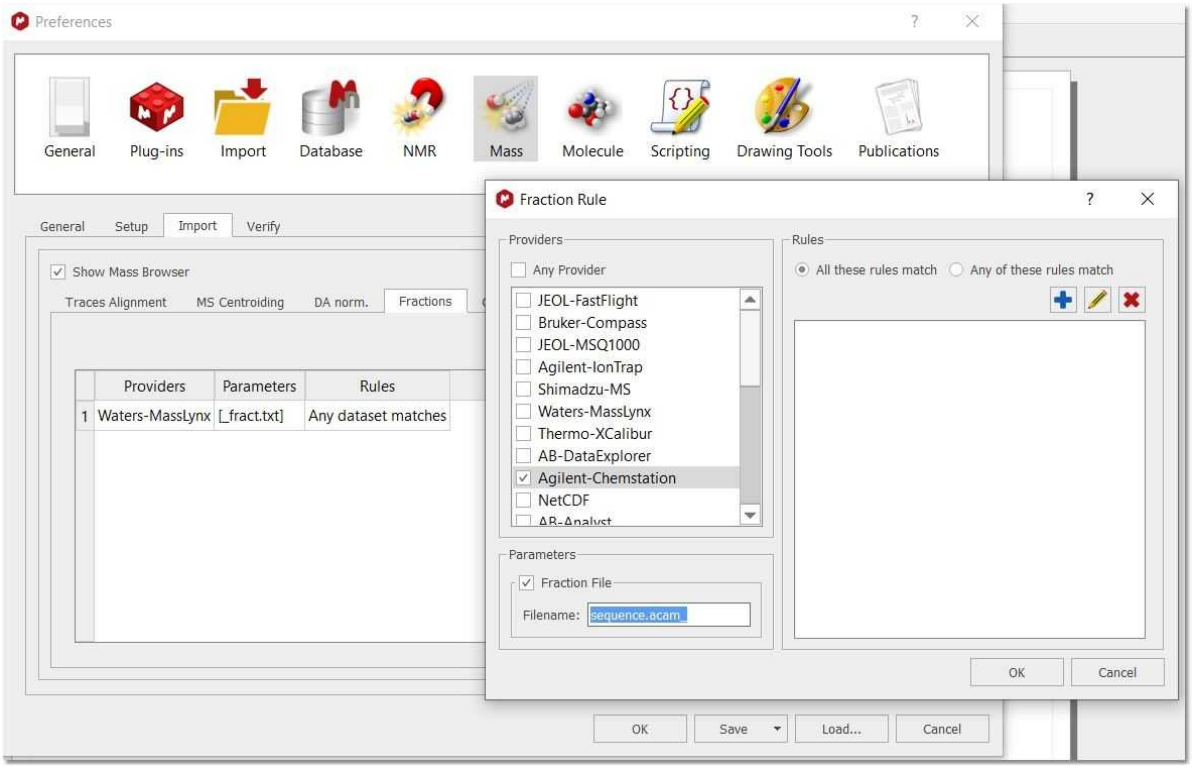


The highlighted regions have a tooltip with some relevant information (such as tube number, tube location, start and end time, expected volume, etc). From the properties dialog box, you can disable the fractions, edit the tooltip format and modify their opacity:



You can also manually include Agilent-Chemstation as fractions provider by following the menu 'Preferences/Mass/Import/Fraction' and clicking on the blue plus button:

© 2024 MESTRELAB RESEARCH

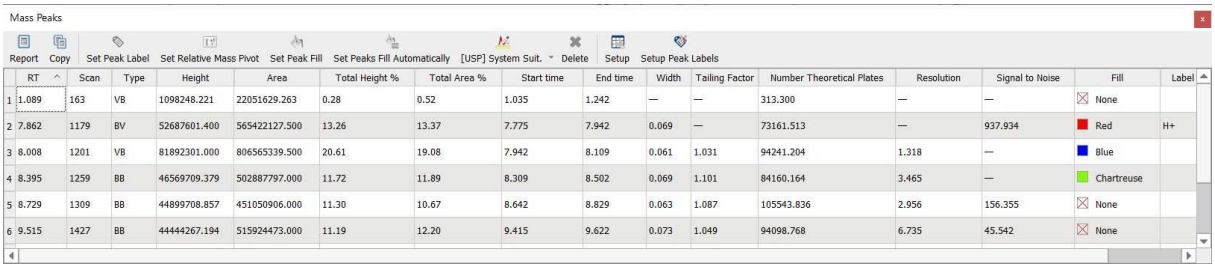


A providers list will be displayed where you can manually select the desired one (for the same filename, you can include more than one provider). In this case, Agilent-Chemstation will be added. You should enable “Fraction file” and manually type the filename that contains the fraction information (sequence.acam_ for this particular provider).

14.2.4 MS Tables

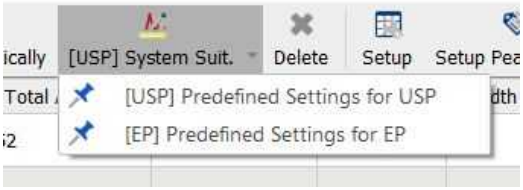
You will find all the information about the peaks of your chromatograms and mass spectra by following the menu 'View/Tables/Mass Peaks' (or by right clicking on any toolbar and selecting 'Mass Peaks'):

You can see below an example of a 'Peaks Table' of a chromatogram; which includes information about the *'Retention Time'*, the number of the *'Scan'*, *'Type'* (to describe peak ends. Possible values: 'Unknown', BB (Baseline to baseline), 'BV' (Baseline to valley); 'VB' (Valley to baseline); 'VV' (Valley to valley) and 'Shoulder'), 'Height', 'Area' 'Start', 'End Time', 'Width', 'Tailing Factor', 'Number of Theoretical plates', 'Resolution', 'Signal to noise ratio', etc:

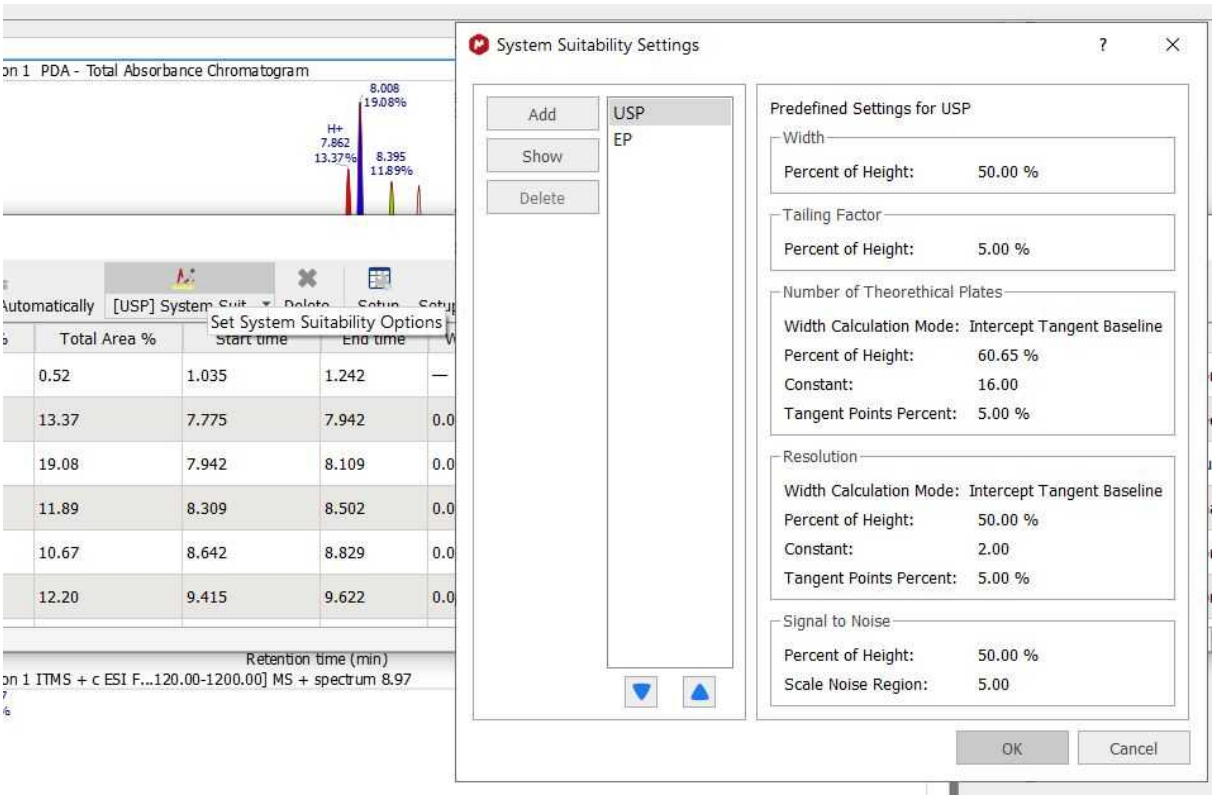


	RT	Scan	Type	Height	Area	Total Height %	Total Area %	Start time	End time	Width	Tailing Factor	Number Theoretical Plates	Resolution	Signal to Noise	Fill	Label
1	1.089	163	VB	1098248.221	22051629.263	0.28	0.52	1.035	1.242	—	—	313.300	—	—	None	
2	7.862	1179	BV	52687601.400	565422127.500	13.26	13.37	7.775	7.942	0.069	—	73161.513	—	937.934	Red	H+
3	8.008	1201	VB	81892301.000	806565339.500	20.61	19.08	7.942	8.109	0.061	1.031	94241.204	1.318	—	Blue	
4	8.395	1259	BB	46569709.379	502887797.000	11.72	11.89	8.309	8.502	0.069	1.101	84160.164	3.465	—	Chartreuse	
5	8.729	1309	BB	44899708.857	451050906.000	11.30	10.67	8.642	8.829	0.063	1.087	105543.836	2.956	156.355	None	
6	9.515	1427	BB	44444267.194	515924473.000	11.19	12.20	9.415	9.622	0.073	1.049	94098.768	6.735	45.542	None	

Clicking on the scroll down menu of “System Suit” button will allow you to choose betewen: US or European Pharmacopoeia.



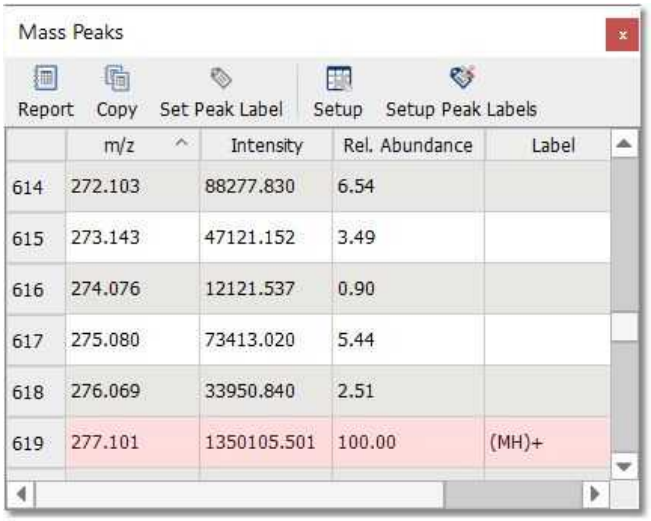
Clicking on the USP button itself will display the predefined settings for USP and EP. This predefined sets cannot be modified, however you can create a custom method by clicking on the“Add” button.



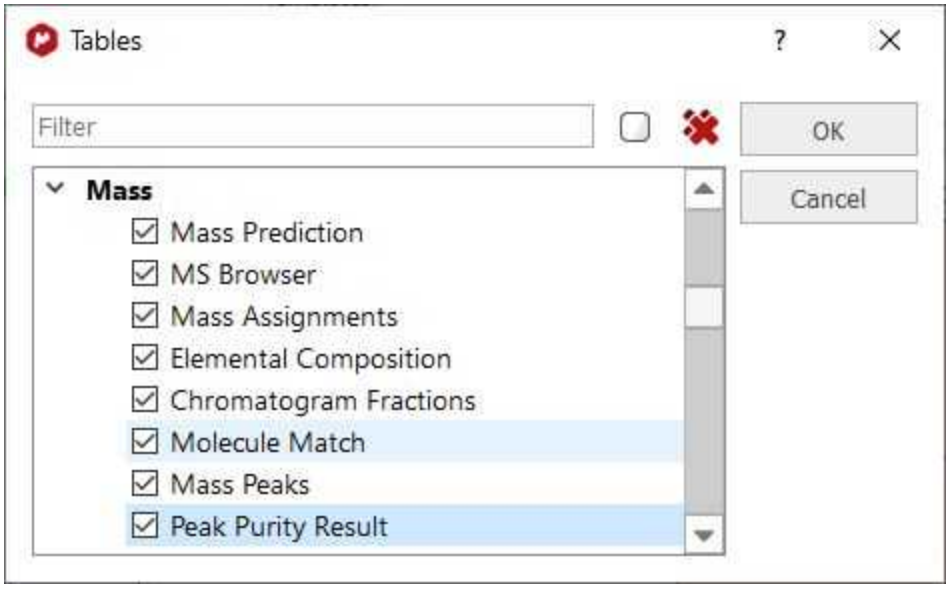
Chromatogram peaks can be filled with a color by manually selecting a color for a chromatogram peak with the "Set Peak Fill" button or by double clicking the "Fill" cell. The "Set Peaks Fill Automatically" button will automatically fill all the peaks. Color and opacity can be modified via "Set Peak Fill" button.

You can also add peak lables from the table. A table like the below one will be obtained in the case of a 'Mass' spectrum:

© 2024 MESTRELAB RESEARCH

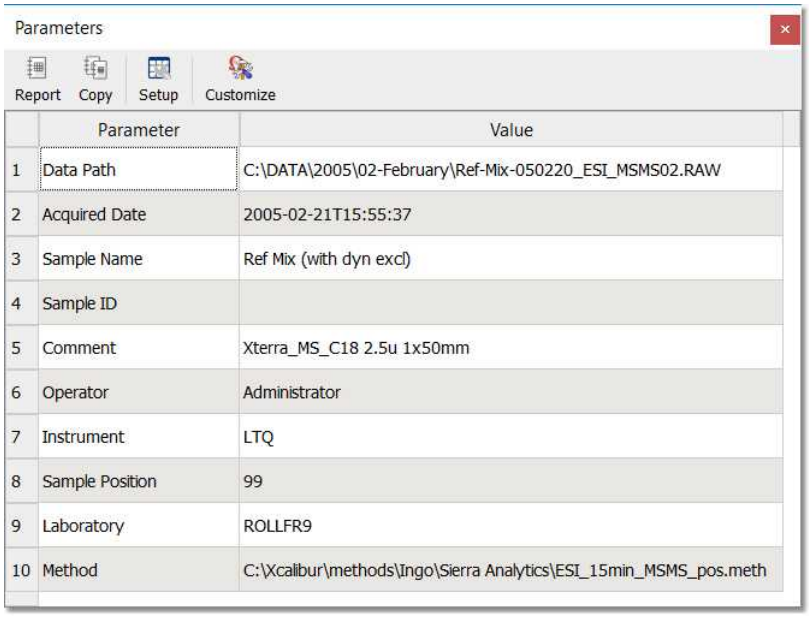


From the View/Tables/Mass', you can also open the other Mass tables:



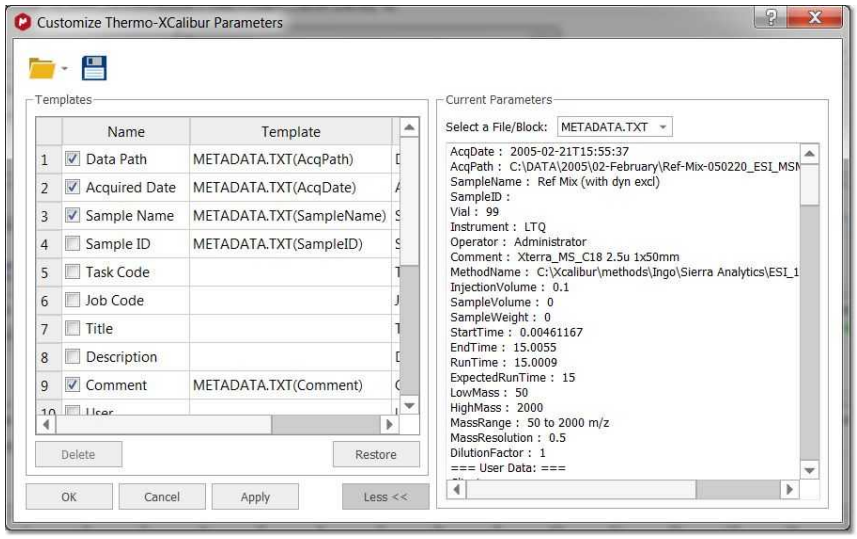
Following the menu 'View/Tables/Parameters' will display the Parameters table:

© 2024 MESTRELAB RESEARCH



	Parameter	Value
1	Data Path	C:\DATA\2005\02-February\Ref-Mix-050220_ESI_MSMS02.RAW
2	Acquired Date	2005-02-21T15:55:37
3	Sample Name	Ref Mix (with dyn excl)
4	Sample ID	
5	Comment	Xterra_MS_C18 2.5u 1x50mm
6	Operator	Administrator
7	Instrument	LTQ
8	Sample Position	99
9	Laboratory	ROLLFR9
10	Method	C:\Xcalibur\methods\Ingo\Sierra Analytics\ESI_15min_MSMS_pos.meth

You can customize the content of the 'Parameters' table by clicking on the applicable button (as you can do with the NMR parameters)



If you need to concatenate several values from parameter files (e.g. a _HEADER.TXT file from Waters) into a single parameter, you can do it via scripting by following the example below:

Set the parameter as =someScriptFunctionName(_HEADER.TXT(Conditions)). You can rename as you wish.

Create a script file with the function scriptFunctionName, for example, to get the first word:

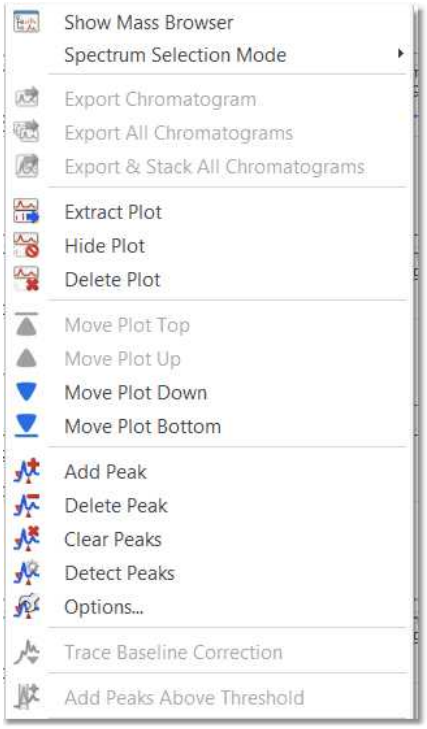
```
function someScriptFunctionName(aConditionsValue) {
    // function to get first word
      var firstWord = aConditionsValue.trim().match(/^\S+/);
      return firstWord ? firstWord[0] : "";
}
```


© 2024 MESTRELAB RESEARCH

Now setup Mnova to load that script file on load by following the menu 'File/Preferences/Scripting' and adding it either to a known scripts folder or to a new folder.

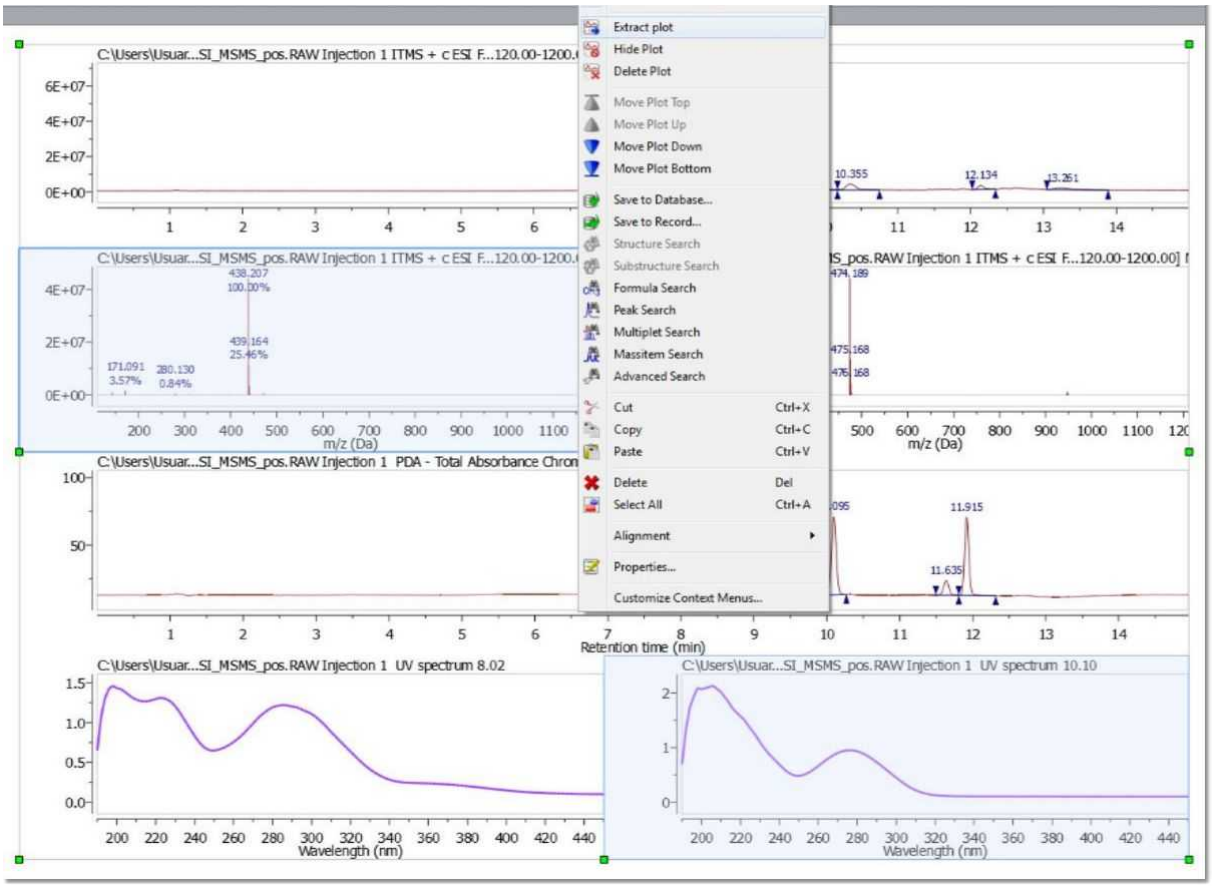
14.2.5 Context menu

You can access to the context menu just by right clicking on the spectral window:



From here you can show the Mass Browser, select the spectrum selection mode, extract the active(s) plot to a new page and hide or delete the plots. You can select several chromatograms or spectra in the same page, for example to extract all of them to a new page. To do it, just hold down the 'Ctrl+Alt' ('Cmd+Alt' in Mac) key and click on the desired plots, next right click and select 'Extract plot'.

© 2024 MESTRELAB RESEARCH

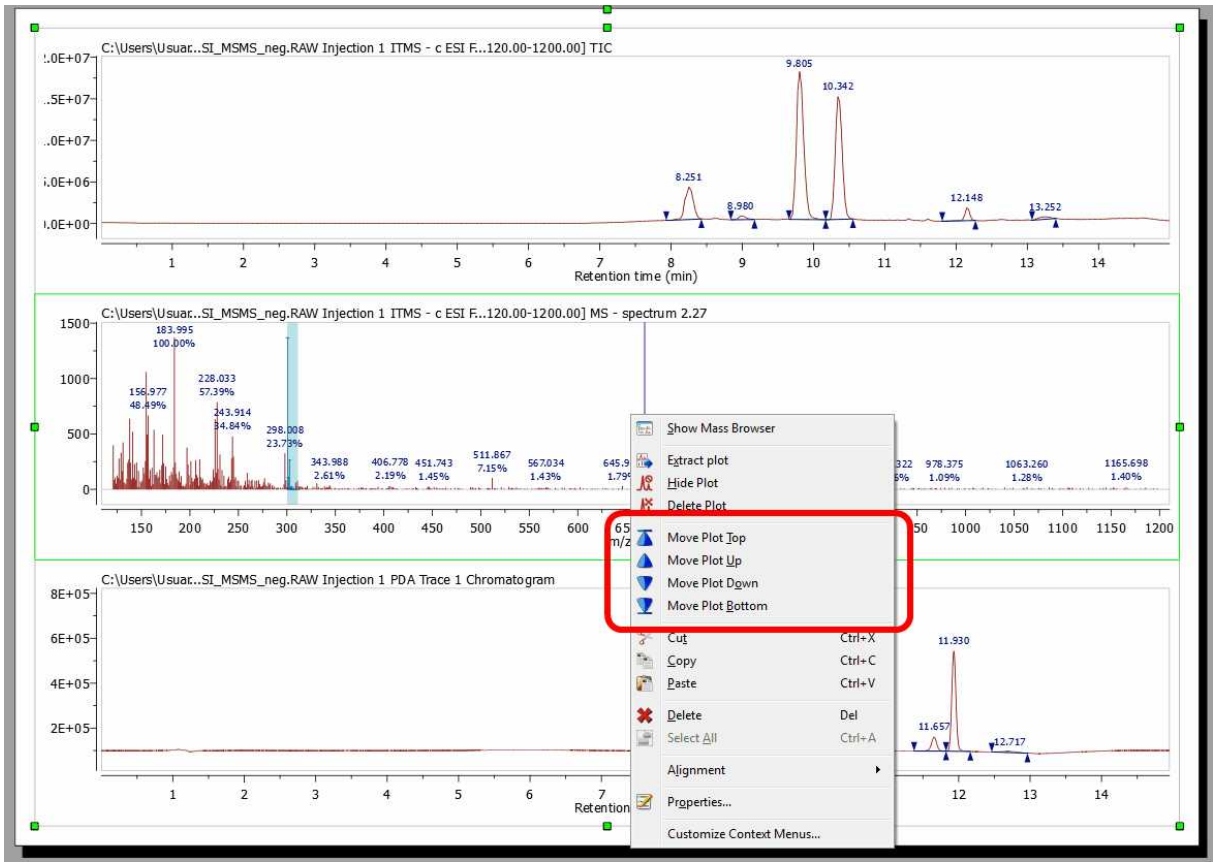


Shortcuts to select MS objects

- Alt+click: select/deselect a single object.
- Ctrl+Alt+click: add/remove object from/to selection.
- Ctrl+Alt+Shift+click: select multiple MS objects in between last selection.

You can also freely move up and down the plots just by right clicking on them and selecting the desired option (Move Plot Top, Up, Down or Bottom):

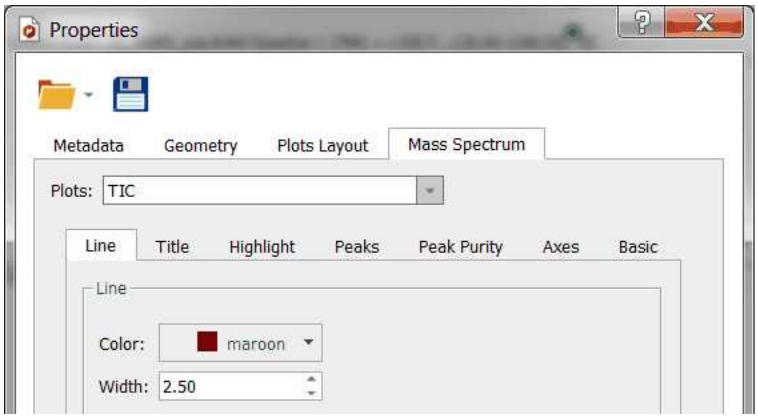
© 2024 MESTRELAB RESEARCH



14.2.6 Properties and layouts

Mnova allows you to customize nearly all the attributes of the spectrum. You can select the properties of the spectrum by following the menu 'Home/Properties' or by double clicking the left mouse button or pressing the right mouse button on the spectrum display and selecting '*Properties'* from the *pop-up* menu.

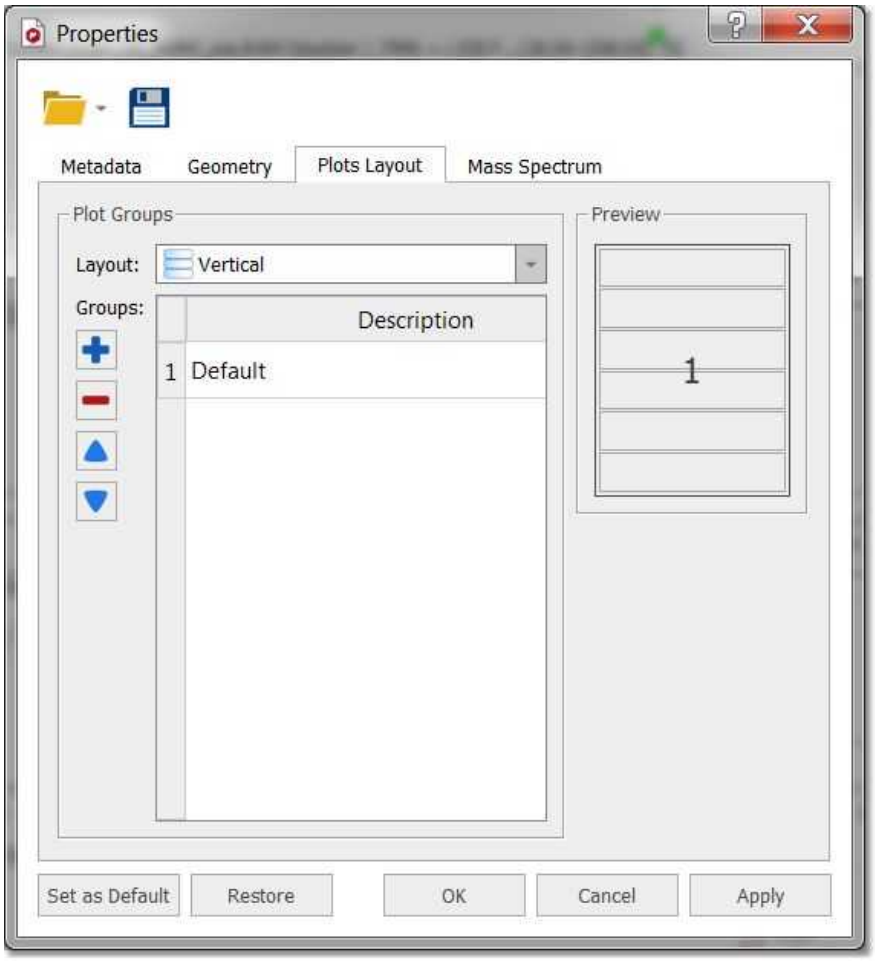
Double clicking on the spectral window will display the properties dialog box. From here you can modify the Line, Title, Peaks, Highlight, Axes and Basic properties of the TIC and MS spectrum by selecting the corresponding tab in the dialog box.



The majority of these properties are similar to NMR spectral properties. The most important difference will be in the 'Plots Layout' and 'Peaks' tab.

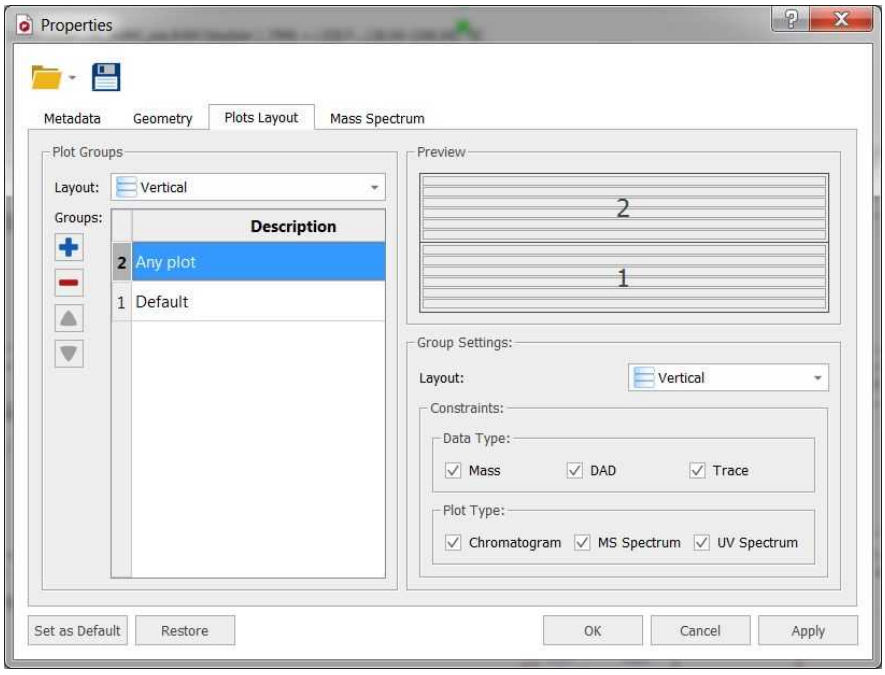
Plot Layouts

You can see below the dialog box for the 'plots layout' properties:



Clicking on the 'Add new group' button , will allow you to add another plot group. If you click on the new created group (called 'Any plot'), you will be able to select some settings (highlighted in red in the picture below):

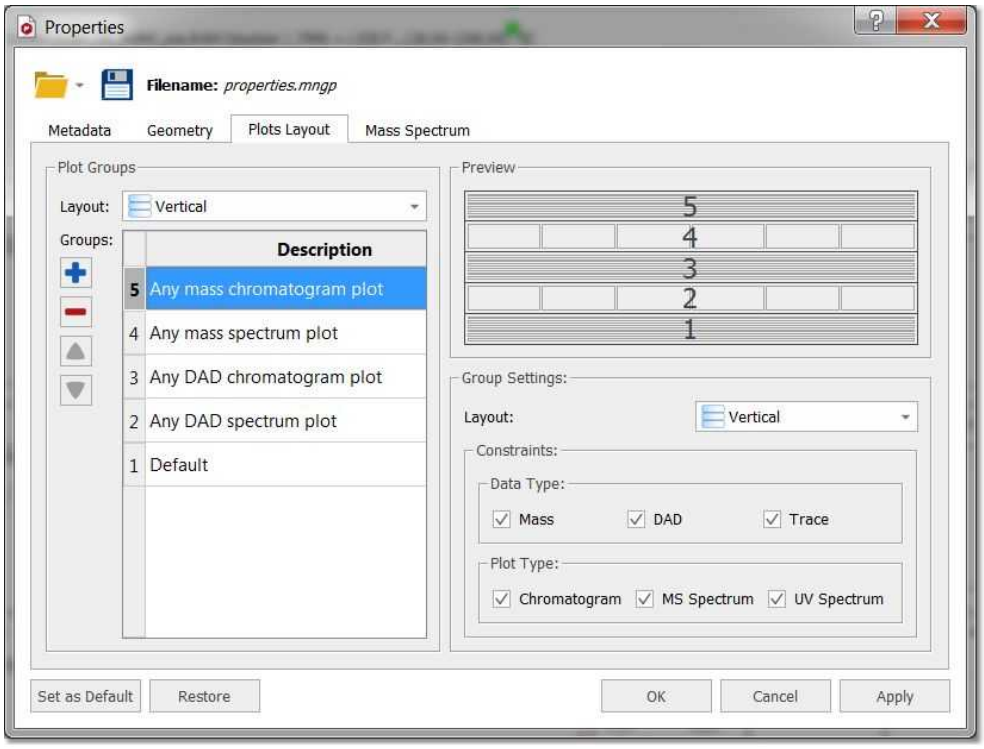
© 2024 MESTRELAB RESEARCH



You can select the layout of the group (vertical, horizontal or grids) and several constraints about the Data Type (Mass, DAD or Traces) and Plot Type (Chromatogram, MS or UV spectrum). You can check the boxes for the applicable constraints or combine several options.

In the example below, it is shown how to create plot with 4 new groups. One group (number 5) for any Mass chromatogram plot (with any polarity or order), other group for any Mass spectrum plot with a horizontal layout (number 4), other group for any DAD chromatogram plot (number 3 with vertical layout) and another for any DAD spectrum plot (number 2; UV spectra with horizontal layout). The default group will be used in the case of any other object not present in the other groups.

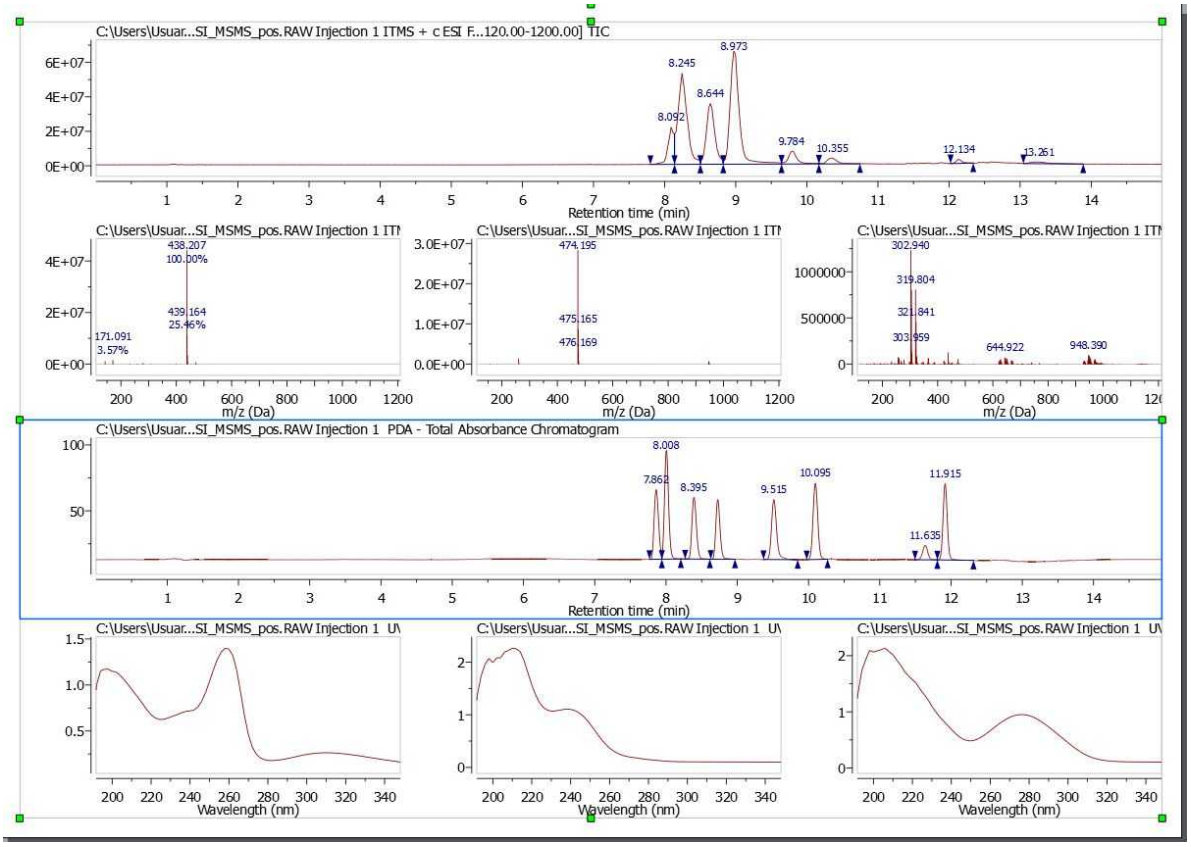
© 2024 MESTRELAB RESEARCH



You can download the 'Property' file used above from here. Next load it, by clicking on the 'Load' button of the 'Properties' dialog box and open a MS dataset. Use the MS Browser to display the DAD chromatogram and the crosshair to select the applicable MS or UV at a given retention time in the chromatogram. To get the additional spectra, you will need to click on the 'Append' button  of the toolbar.

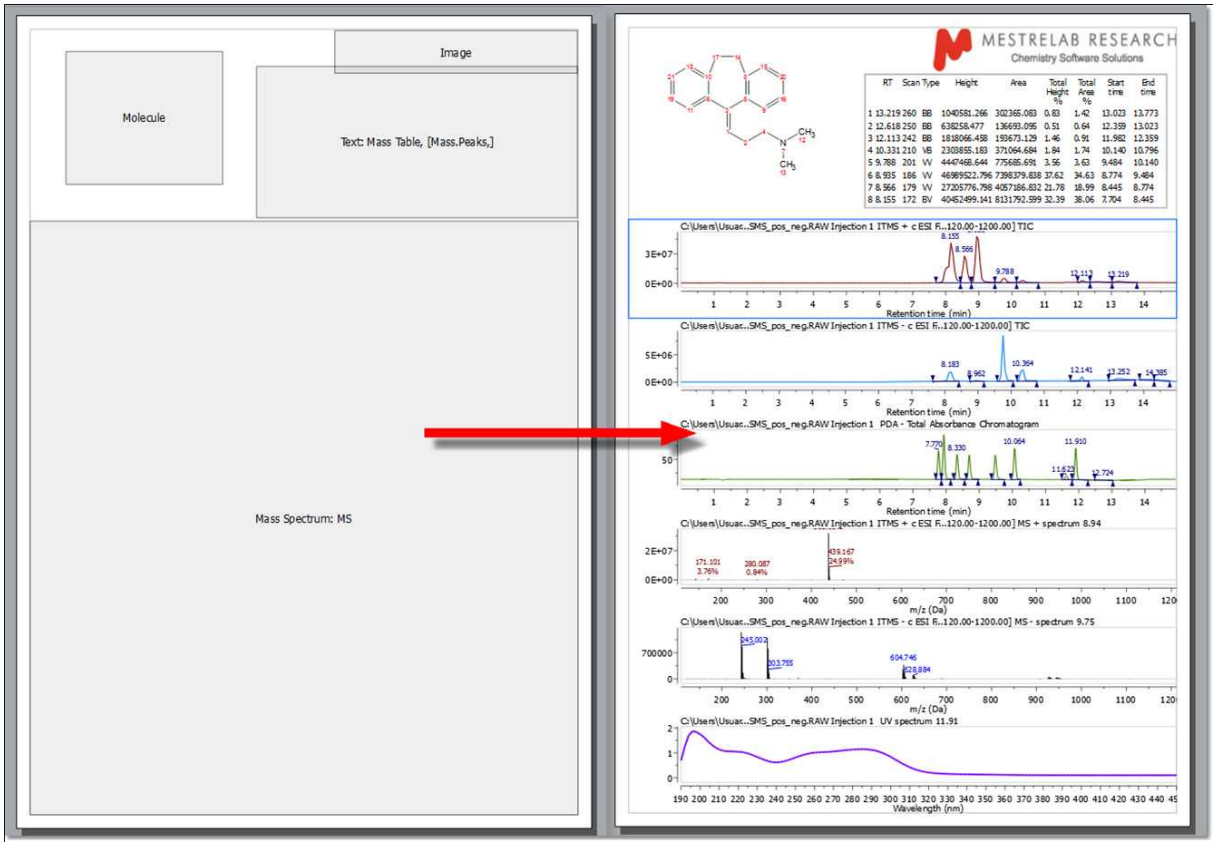
Here you can see the result with the TIC on top followed by several MS spectra, the Total Absorbance Chromatogram (as a DAD) and several UV spectra:

© 2024 MESTRELAB RESEARCH

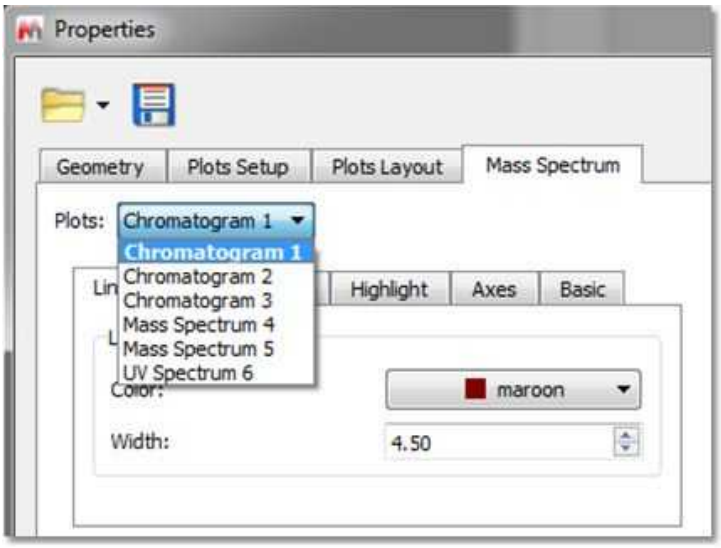


You can load/save and select the plot layout as default by using the applicable options in the 'Properties' dialog box.

You can generate layout templates from your MS documents. Here you can see an example:

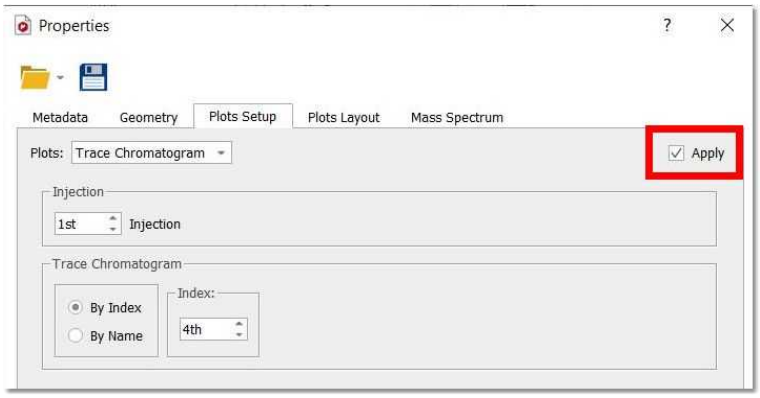


Of course, the layout templates will include the desired 'Properties' embedded, including the selected 'plots layout'. You can see the Properties embedded in a layout template, just by double clicking on each item. Double clicking on the MS box of the layout template will show you the properties of the layout for each plot (spectra and chromatograms):

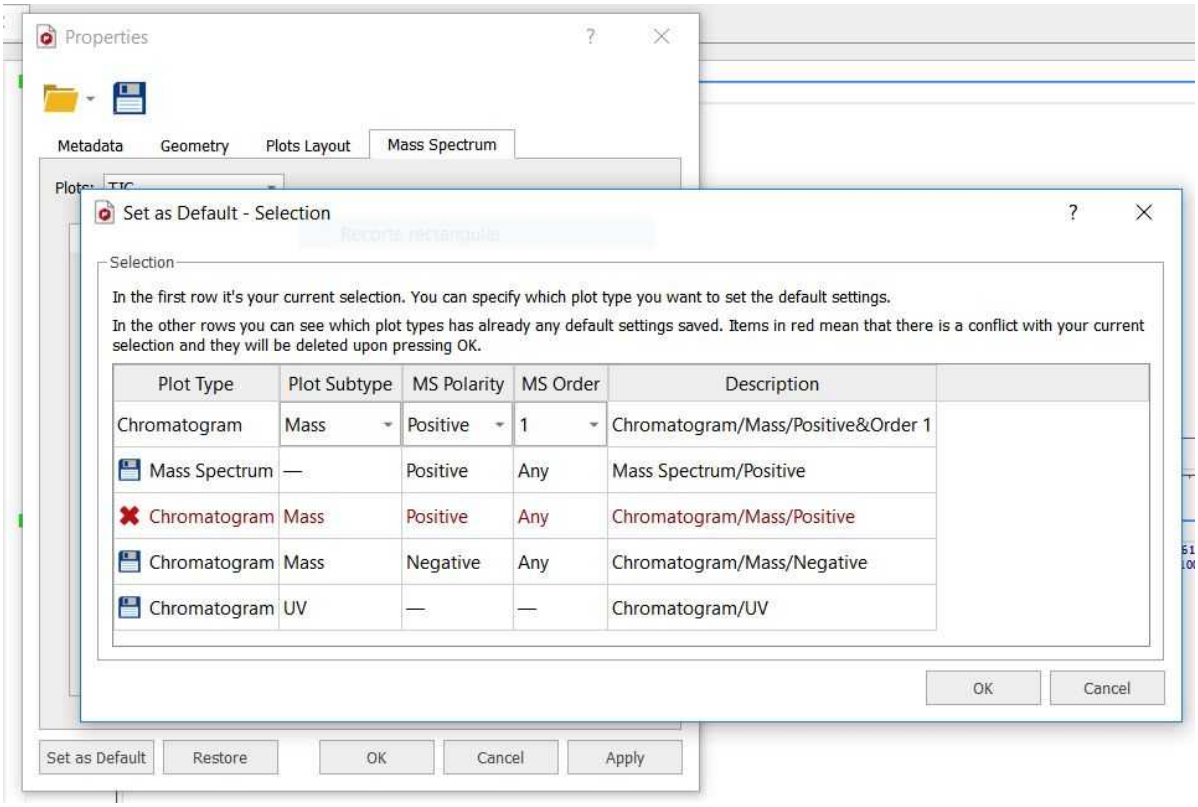


© 2024 MESTRELAB RESEARCH

In the MS layout template documents, you can switch ON/OFF the application of the processing specified in Plots Setup tab (very useful for Mgears bricks to avoid to reprocess the chromatograms):



When setting as default the properties of any chromatogram/spectrum, you can choose the type of plot affected (Plot subtype, MS polarity, MS order).



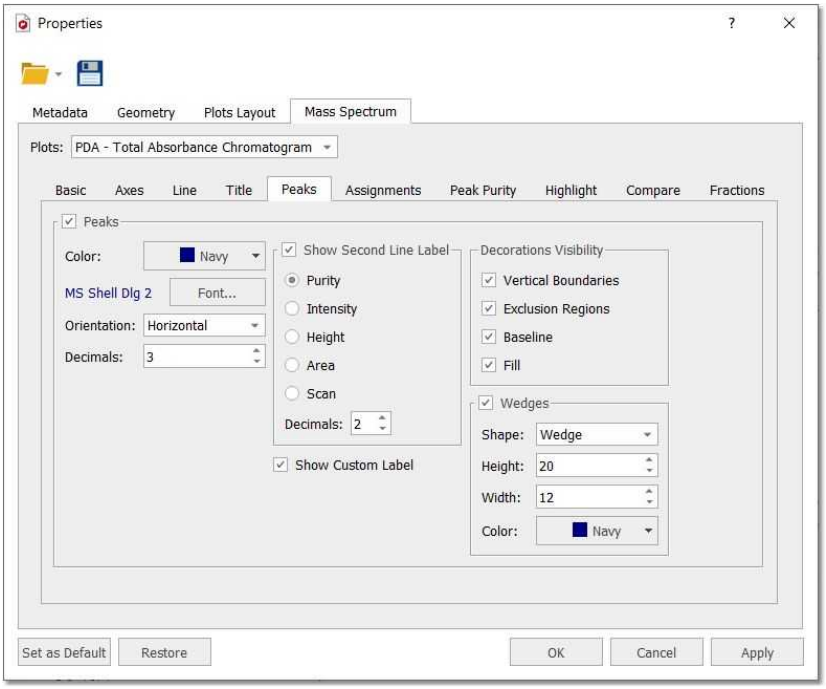
When there is a conflict:

- a more general setting overwrites an existing more specific one.
- a more specific setting overwrites the general settings in the specific plot. The properties of the others will be restored.

Peaks Properties

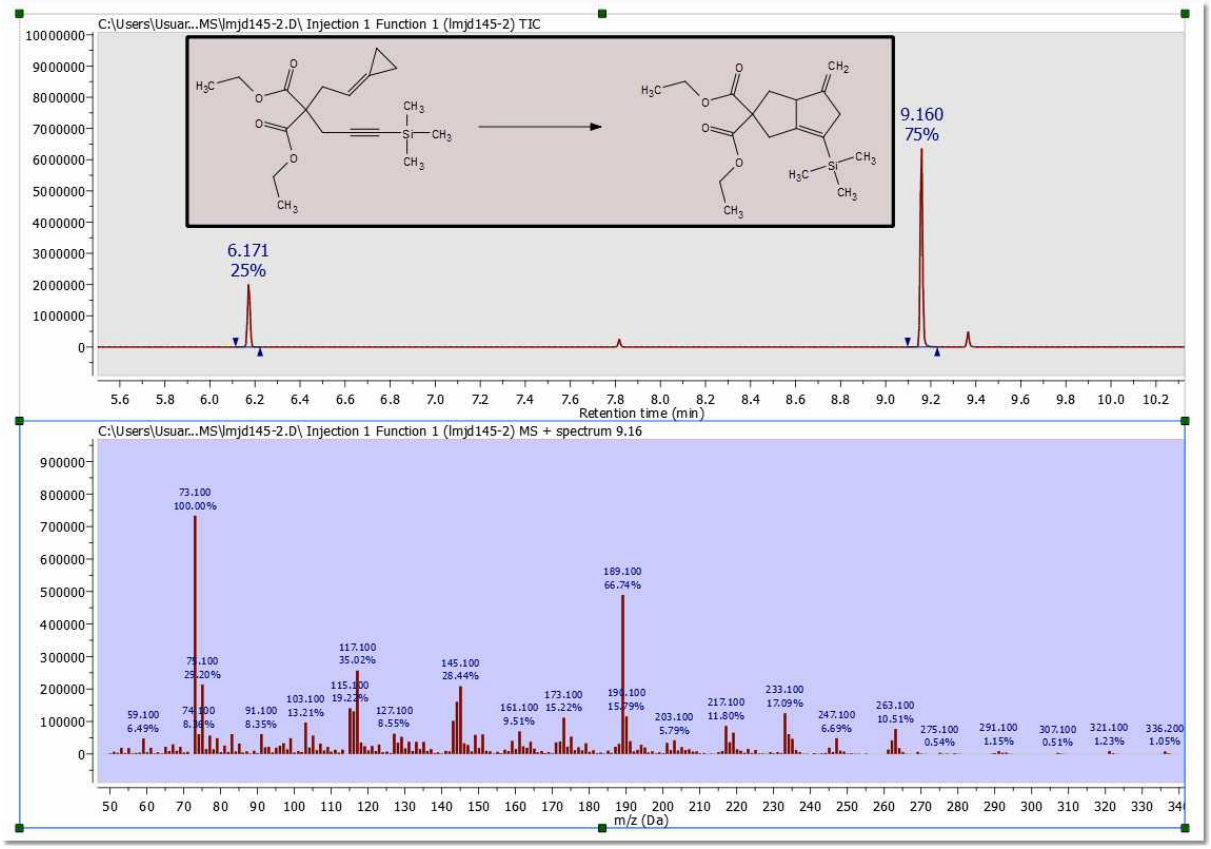
You can see in the picture below the 'Peaks properties' of the TIC. From here, you can 'show the wedges' and the 'baseline' (and select the threshold) or add a second line in the peak label, with information about the Intensity, the Height from the baseline, the Area, or the Purity (which will show the

integral of each peak as the percentage of all integrated peaks). Please bear in mind that the Area can not be used as a quantitative value.



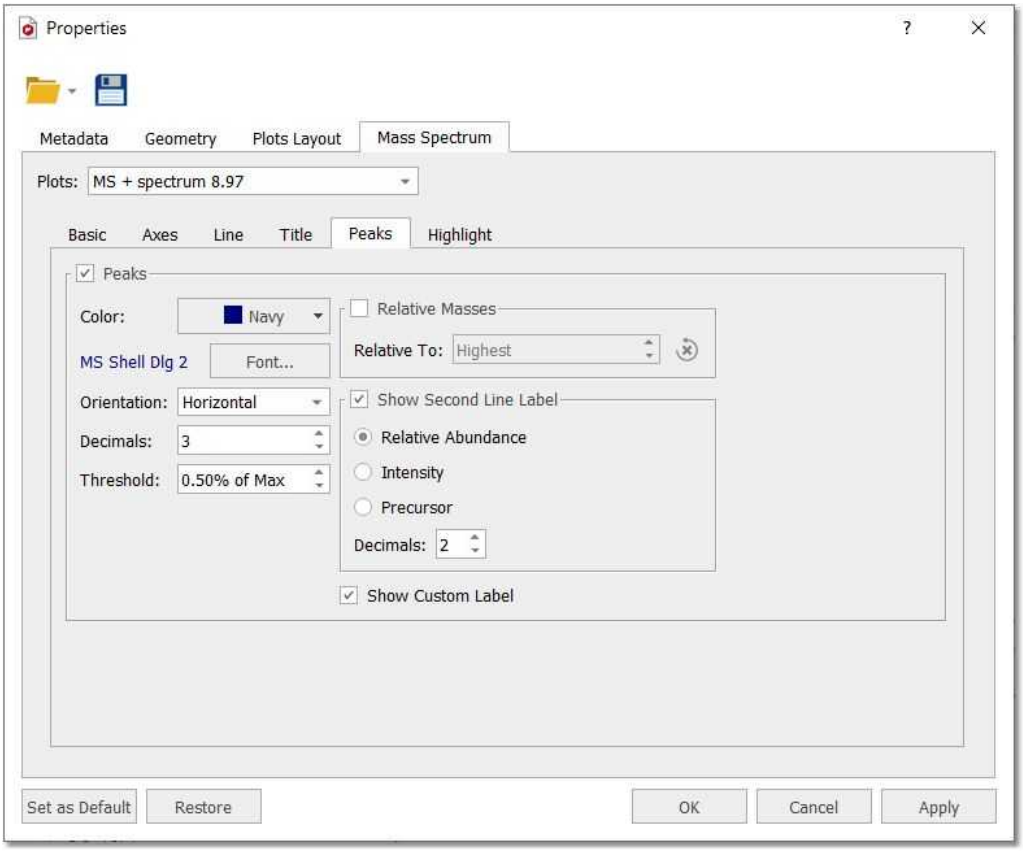
Here you can see an example of a TIC with the Purity values in the second line:

© 2024 MESTRELAB RESEARCH



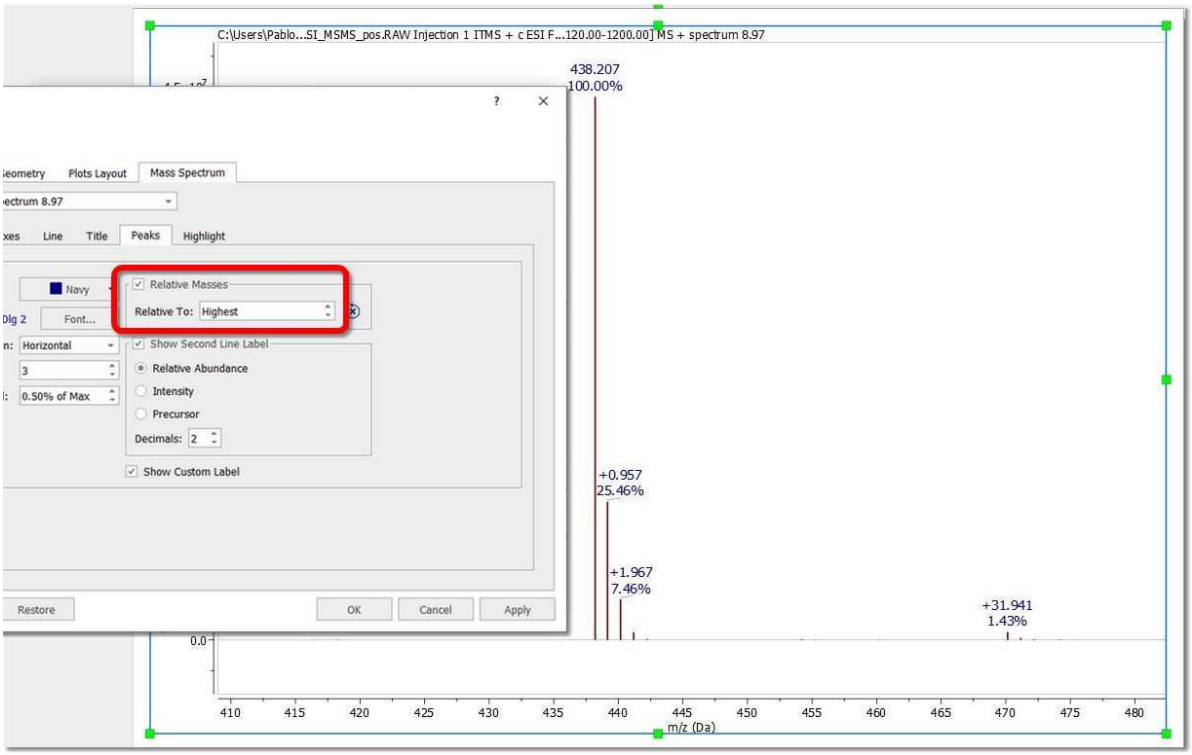
You can see below the 'Peaks Properties' dialog box of the MS spectrum. In this window, you can change the Color, Font and Number of Decimals of the Peak label and you can also show/hide the 'Second Line Label (which can contain information about the 'Intensity' or the 'Relative Abundance').

© 2024 MESTRELAB RESEARCH



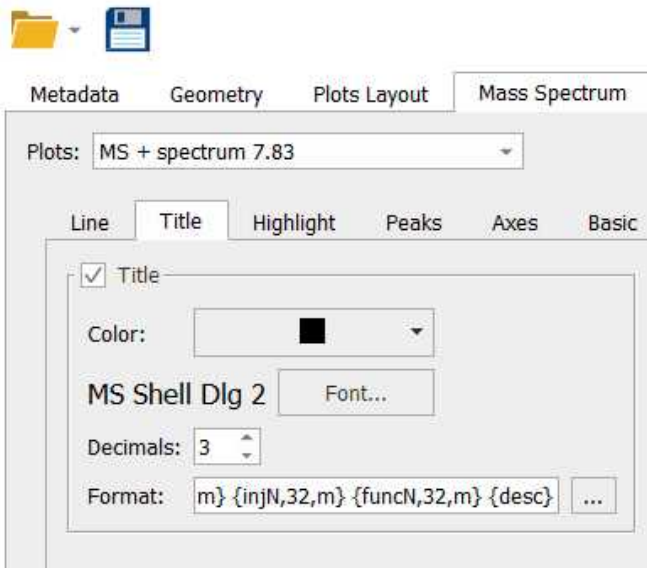
You can enable the display of relative masses instead of absolute ones by checking the applicable box. The masses will be relative to the position of the highest peak in the current view. So if the user wants to see the relative masses for one specific peak he must ensure that that peak is the highest one in the current view. Such property will be disabled by default.

© 2024 MESTRELAB RESEARCH

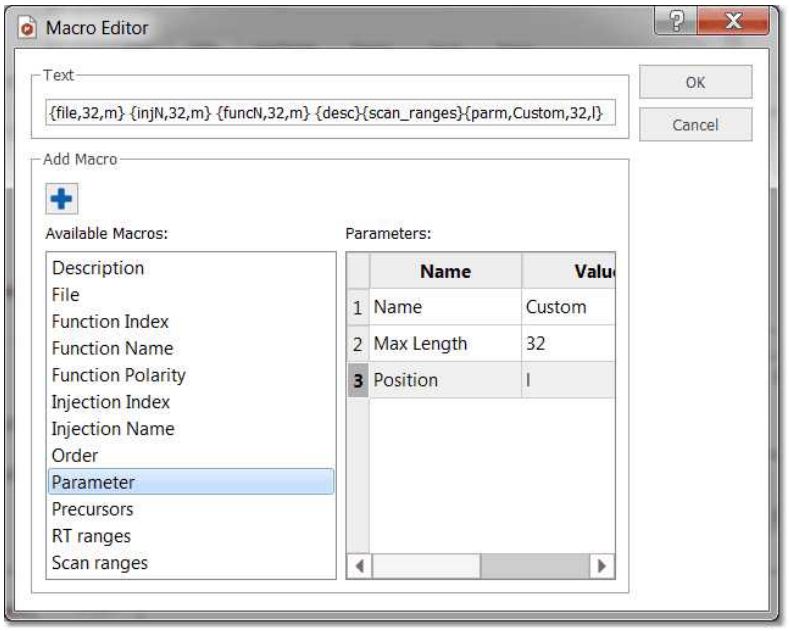


From 'Properties/Peaks' dialog, it will also be possible to change the orientation of the peak labels (horizontal/vertical).

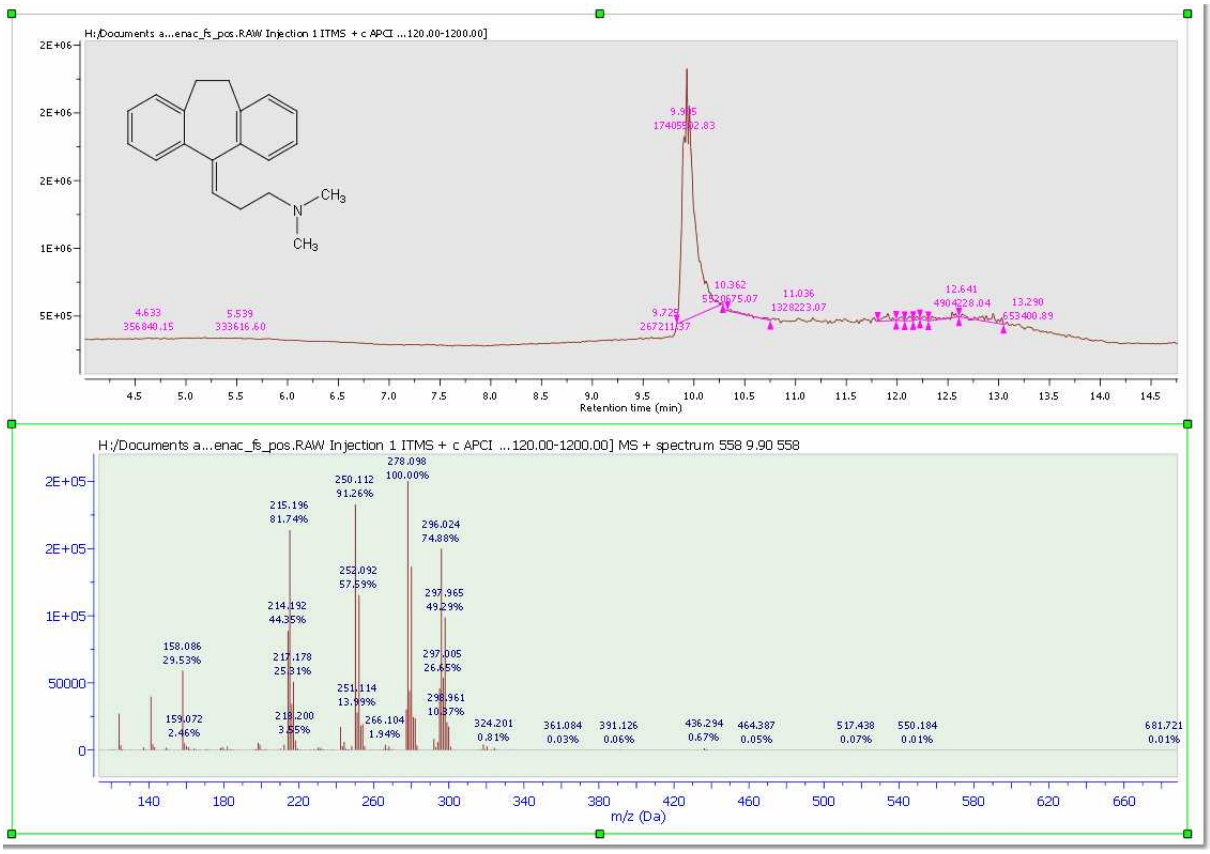
Selecting Title in the tabbed menu, will display the below window:



Clicking on this icon  will allow you to customize the format of the title; for example to display the 'RT' or 'Scan ranges' or to select the number of characters (Max Length; 32 by default) or the position (n: none, l: left, r: right, m: middle):

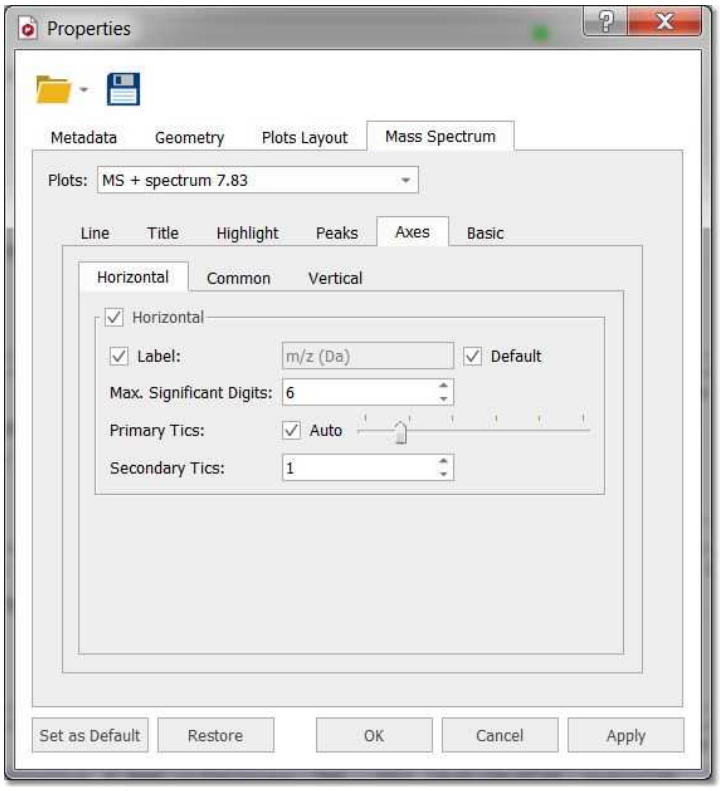


Here you can see an example of a TIC and MS after having changed some properties:

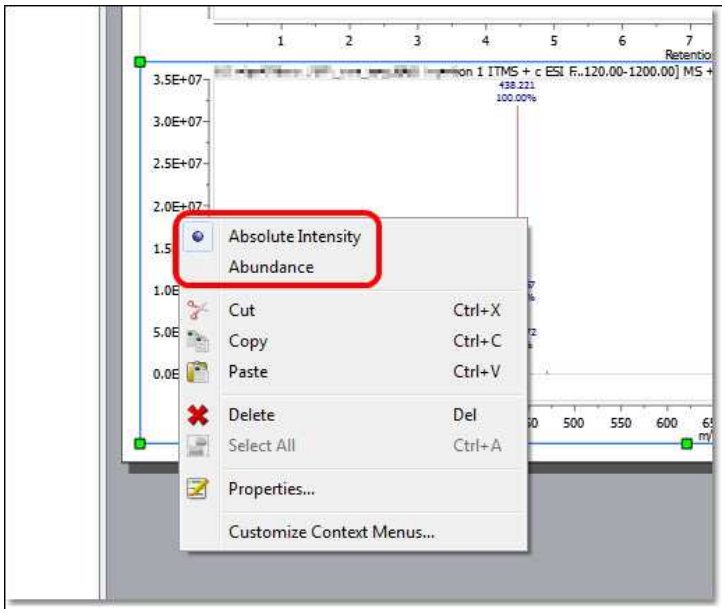


Selecting the Axes tab will allow you to change the Units individually for each plot:

© 2024 MESTRELAB RESEARCH



If you want to change the units for all the Mass spectra, youy would need to use the context menu (by right clicking on the vertical axis):



Label: When the default box is checked, it means that the label will be set by Mnova to the default value for that kind of plot (so the label line editor remains disabled). If it's unchecked, then the label will be set to whatever the user sets in the line editor.

The default values are:
Horizontal axis:

© 2024 MESTRELAB RESEARCH

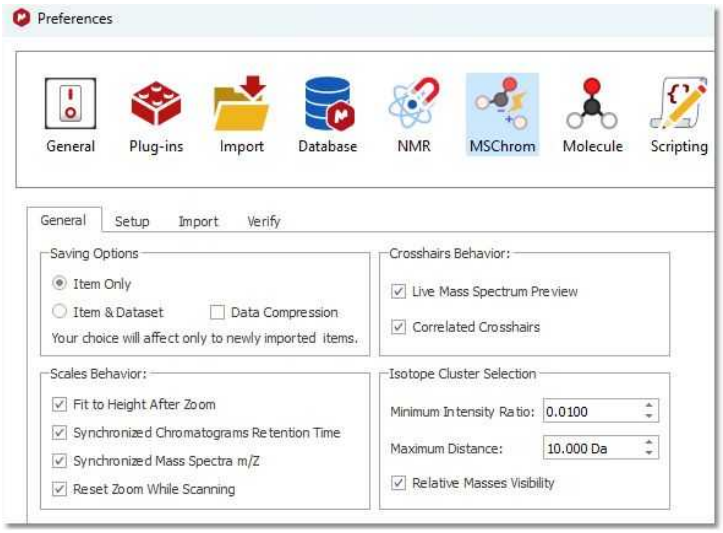
Mass spectrum: m/z (Da)
UV spectrum: Wavelength (nm)
Chromatogram: Retention time (min)

Vertical axis:
Mass spectrum: Intensity
UV spectrum: Absorbance
Chromatogram: If the current data provider, provides an axis label, that will be used. If not, either Absorbance or Intensity regarding if it is a UV chromatogram or any other kind of chromatogram respectively.

You can read more about general Mnova properties here.

14.2.7 Preferences

Follow the menu File/Preferences/MSChrom to change your MS preferences:



The user will be allowed to select the saving options (item only or item+dataset).

You will find also an option to fit to height after zoom (selected by default). When this option is checked, a fit to height is applied after making a horizontal zoom in a mass spectrum or a chromatogram. In case of the other zoom modes (vertical or in both dimensions), a fit to height is never applied.

When the option 'Synchronized chromatogram's Retention time' is selected, you can zoom, pan and cut to all showing chromatograms. An analogous action for synchronization of the m/Z scale of mass spectra is selected by default. When checked, the "Synchronize Cromatograms RT" and "Synchronize Mass Spectra m/Z" buttons in Mass/Tools/Syncronize show enabled by default, for example when loading a raw Mass file. When unchecked, the "Synchronize" buttons in Mass/Tools/Syncronize show disabled, for example when loading a raw Mass file.

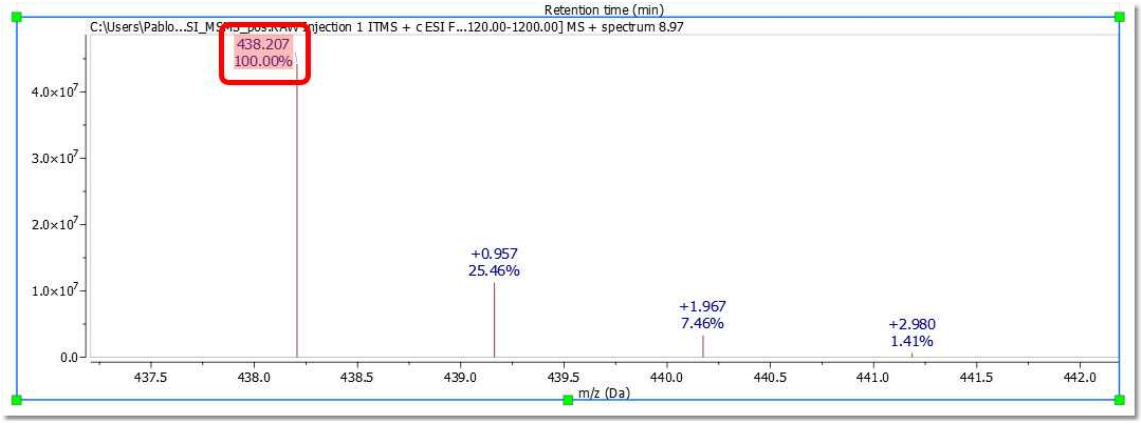
Check the 'Reset Zoom with scanning' box, to enable or disable resetting the zoom in a MS plot while scanning (or when using the 'live crosshair') through the spectrum. When the option is disabled, the zoom in the mass range is preserved when switching to a different scan in the MS plot or scanning through the chromatogram.

The "Live Mass Spectrum Preview" will allow you to show the extracted mass spectrum *on the fly* when you hover the crosshair over the chromatogram (or trace). After having clicked at the desired retention time, you will get the expected MS spectrum displayed.

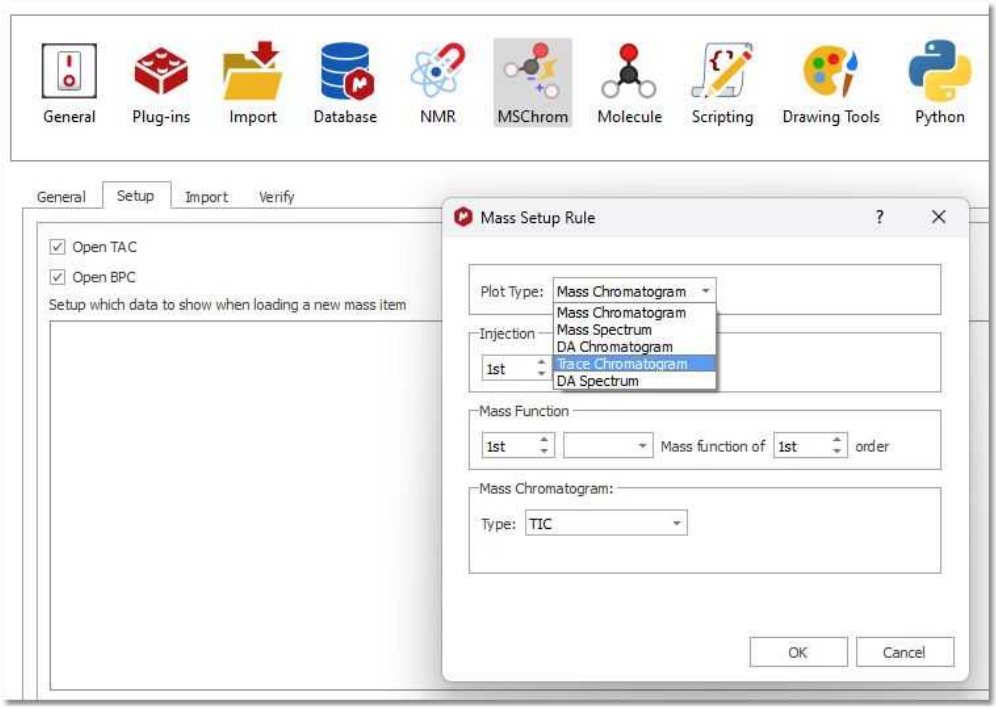
© 2024 MESTRELAB RESEARCH

The 'Correlated Crosshair' mode will display the crosshair as usual in the active plot and also as a semi-transparent crosshair in the none-active plots of the same type (chromatograms, mass spectra or UV spectra).

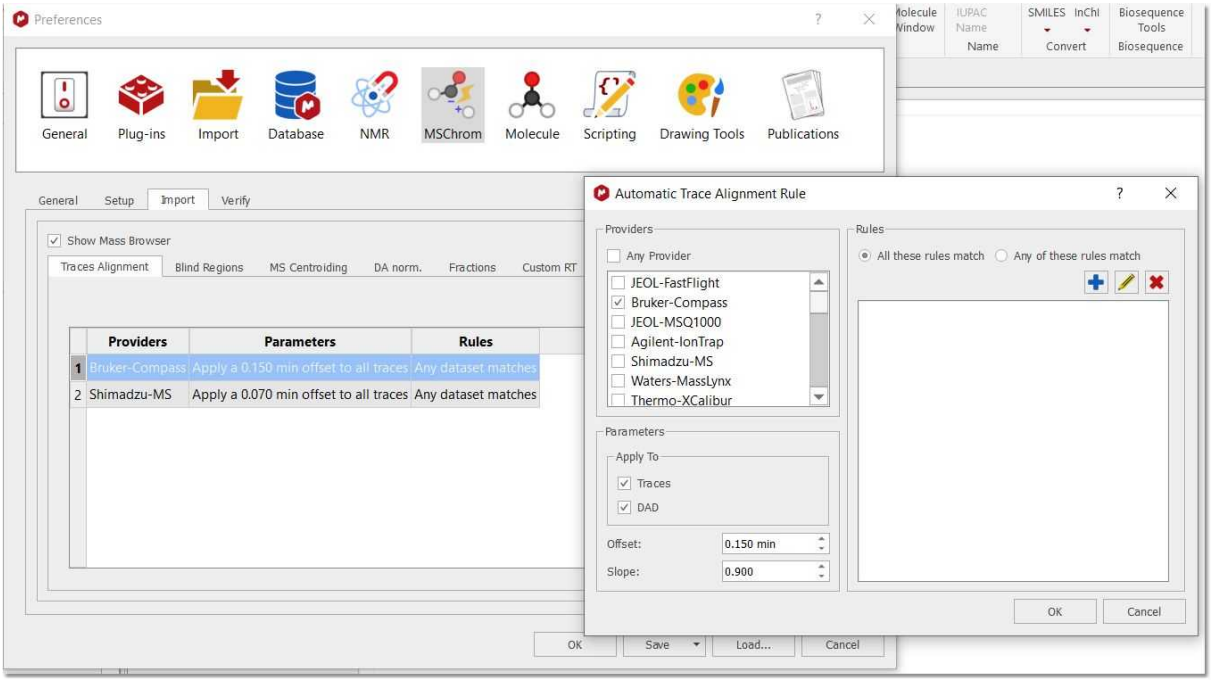
"Isotope Cluster Selection": After having extracted a MS spectrum if you click on a mass peak label, a zoom is automatically applied trying to show the isotope cluster. This zoom can be tuned by changing the values of the *maximum distance* (between the peaks forward and backward) and the *intensity bigger ratio.*



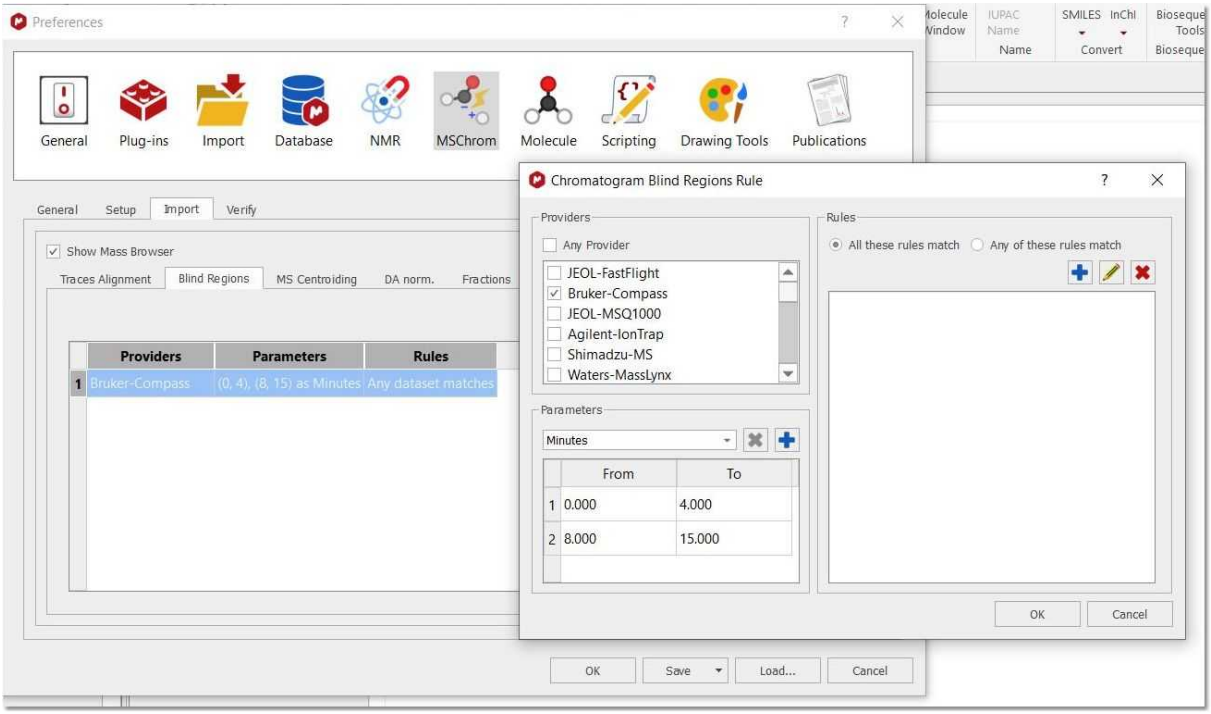
It is possible to choose which chromatogram and spectra are displayed when loading an MS dataset via File/Preferences/Mass/Setup. This mass setup will be stored when saving the preferences. You can also check the 'Open TAC' or 'Open BPC' if you are always interested in loading any of those chromatograms:



You can define rules to apply 'Traces Auto Alignment' or 'Custom Run Time ranges' automatically when importing a mass dataset:

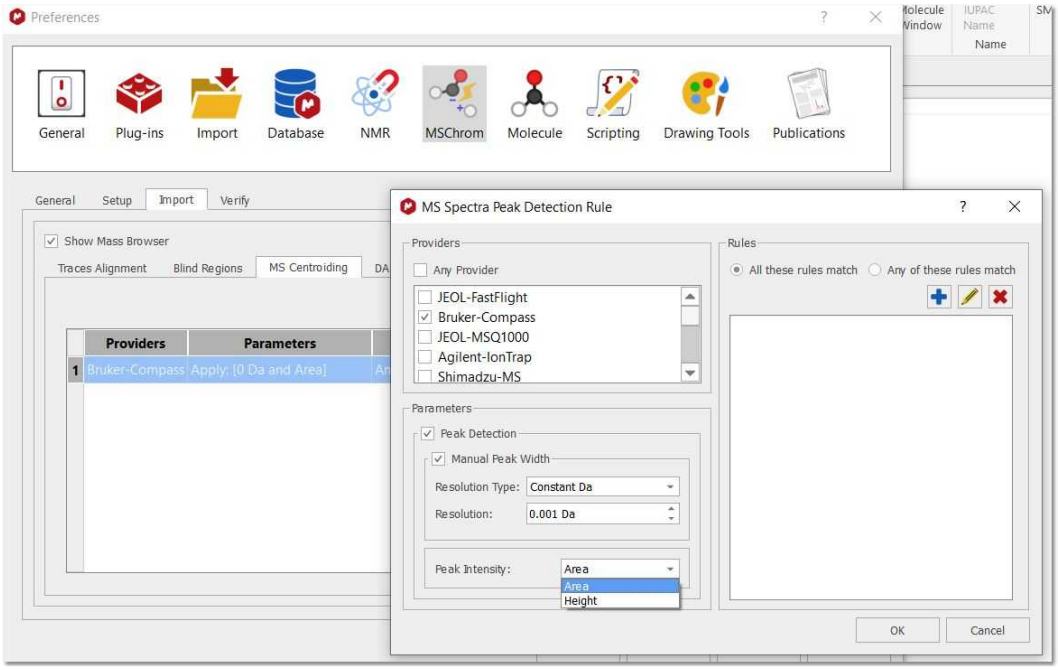


You can define default blind regions from the applicable tab:

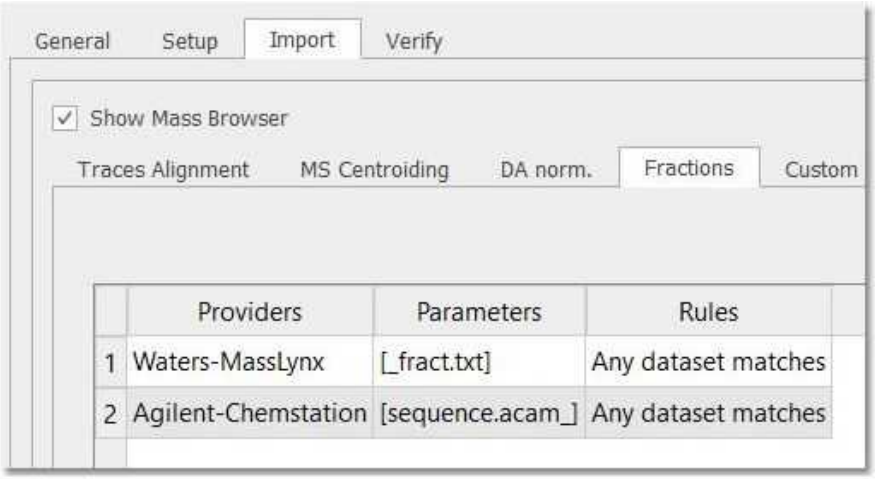


© 2024 MESTRELAB RESEARCH

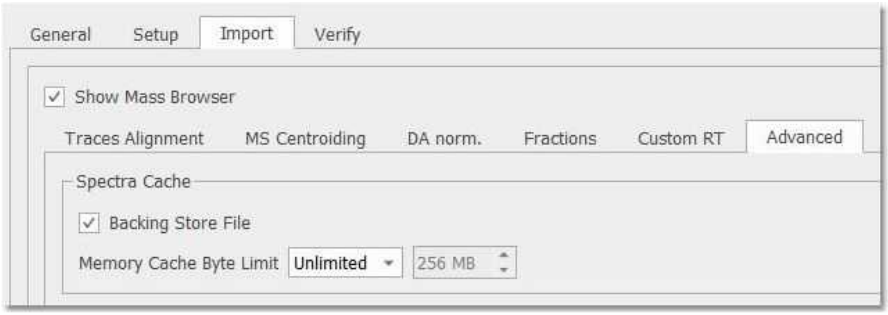
From the MS centroiding tab, you can add rules for peak detection, to be height or area based according to the selected provider (make sure that you restart Mnova after having applied the change):



For Waters Masslynx datasets, you can highlight the fractions imported from the _fract.txt file. The same can be done for Agilent-ChemStation datasets, by importing the fractions from the sequence.acam_ file.



From the advanced tab, you will be able to customize the cache settings for your spectra collections:



Backing Store File: When enabled, all the spectra read from a dataset file, will be stored in a temporal file, so the next time we want to get the same spectrum, Mnova will get it from that file instead of reading it from the dataset by using the vendor libraries. This would benefit those providers using 32-bit libraries, like Advion and Varian.

Memory cache: In addition to the backing store, you can keep the spectra in memory (which would have an impact in memory usage):

- Disabled: No memory caching is done.
- Unlimited: The spectra will be cached in memory without limitation, until you run out of memory.
- Limited: The spectra will be cached in memory, but only the last spectra that do not exceed the set memory amount.

© 2024 MESTRELAB RESEARCH

Part



15 ElViS

Mnova 14 comes with a new ElViS plugin designed to aid in analysis of various optical spectroscopy data including ultraviolet and visible (UV/Vis), near and mid infrared (NIR/MIR), Raman, fluorescence, and other spectroscopic methods operating in the whole region of wavelength from 100 nm to 100 µm (100 000 – 100 cm^{-1}). Covered by the practical optical spectroscopy and actually beyond.

The name of ElViS (Electronic Vibrational Spectroscopic) abbreviates the main optical techniques and spectral ranges covered by the software. The product concept takes into account the main modern trends in optical spectroscopy, in particular, growing data volumes, multivariate quantitative analysis of spectra, distinct shift from laboratory to industrial analysis, development of multi-spectral and hyphenated techniques, and recent advances in optical sensing.

Practical application areas of Mnova/ElViS include:

- Spectroscopic analysis of various samples from pure substances to complex mixtures;
- Analysis of spectral peaks and features, their chemical interpretation;
- Data preparation and preprocessing for advanced multivariate analysis (chemometrics);
- Investigation and monitoring of chemical reactions and industrial process;
- Analysis of hyphenated data, such as HPLC-DAD and TGA-IR;
- Building spectral data banks; and
- Professional reporting of experimental data and results.

15.1 Formats supported

Here you can see a list of some of the main formats supported by Mnova ElViS:

ASCII (.txt, .csv). This format must be loaded by following the menu 'File/Open' and selecting the ElViS CSV (*.csv; *.txt) converter from the scroll down menu. The CSV files could contain two XY columns (being the first column: wavenumber/wavelength/arbitrary) or three or more XYY columns in case of stacked plots.
JCAMP-DX (.jcamp, .dx, .jdx, .jcm)
OPUS (.0, .1,...)
Thermo Nicolet Omnic (.spa)
Thermo Galactic GRAMS (.spc)

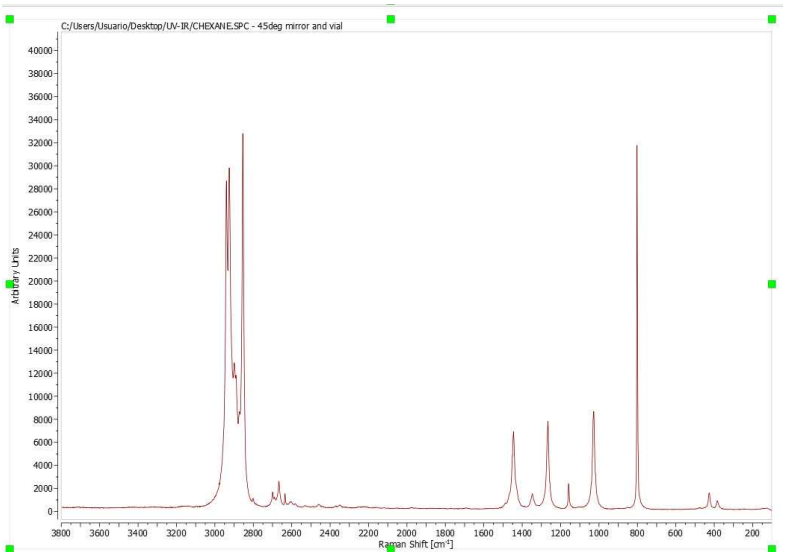
Mnova will allow you to export your ElViS datasets as .csv, JCAMP-DX, .txt and .mnova and different image formats; by following the menu 'File/Save As'.

15.2 Fast Visual Guide to process routine datasets

Go through the following procedure to process a routine ElViS spectrum (you can download the examples from here):

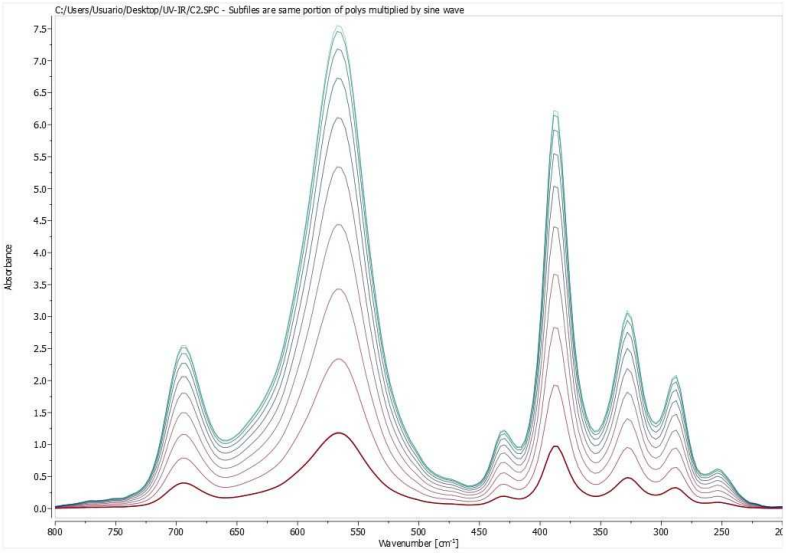
1. Drag and drop the file into Mnova to get your spectrum fully processed. (You can also follow the menu 'File/Open')

© 2024 MESTRELAB RESEARCH



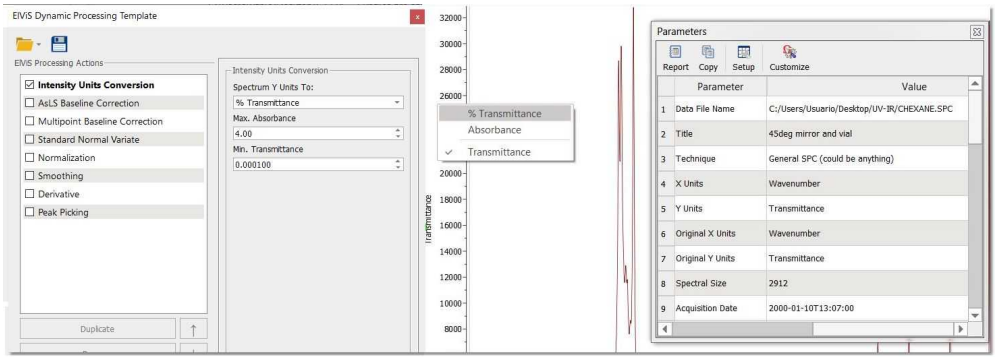
If you have several datasets, you can load of all them in your Mnova document, to generate a stacked plot (after having highlighted them on your Page Navigator and followed the menu 'Stack/Stack Items')

You can also load spectra acquired in arrayed mode:

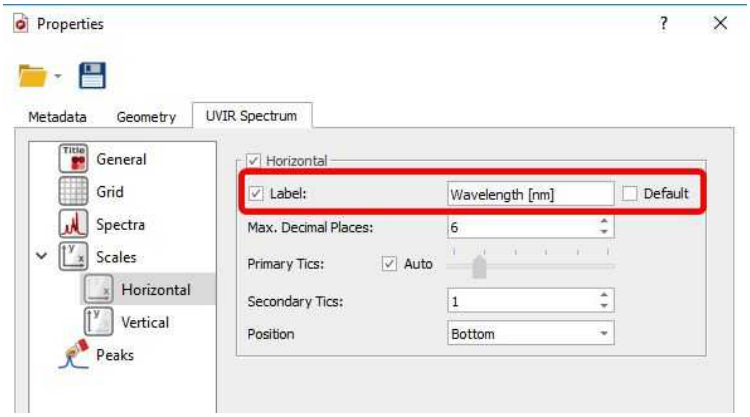


2. You can convert the spectral X-scale into another units by right clicking on the scale and choosing another compatible unit type from the drop-down list. Similar operation is available for the Y-units (intensity). The intensity units can also be converted from the Processing Template. If the units were not recognized by the import function or were absent in the source data format, they appear as ‘Arbitrary units’ in spectra. You can reset ‘Arbitrary units’ (as well as any other wrong original units) from the 'Parameters Table' (which can be displayed by following the menu 'View/Parameters') Double click to ‘Original X units’ or ‘Original Y units’ field values and choose an appropriate unit type from the drop-down list of available unit types. (Note, this operation is generally discouraged, because it can lead to data visualization and analysis mistakes! Avoid its application unless you are confident).

© 2024 MESTRELAB RESEARCH



Besides, independently on the current and original X- and Y-units, you can define any custom labels on the respective axis using the Properties dialogue:

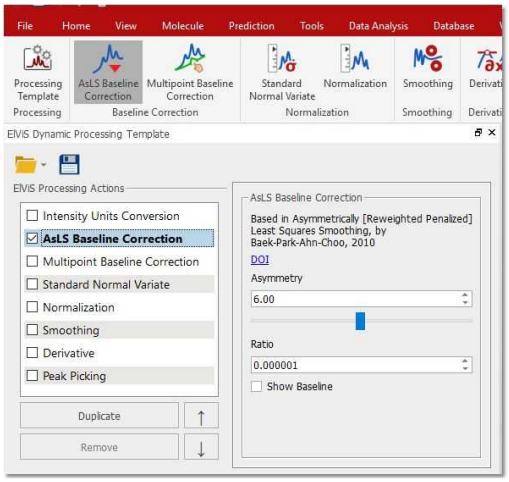


3. You can zoom in by using the applicable button from the View ribbon (or the shortcut: Z). With active 'Zoom In' mode press Z to switch between horizontal, vertical and rectangular zoom. From that ribbon, you will find different zoom modes and a tool to create expansions (shortcut: E):



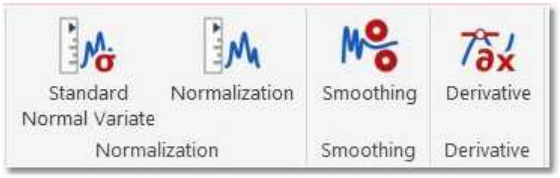
4. Click on the 'AsLS Baseline Correction' icon to correct the baseline of your spectrum automatically. The Dynamic Processing Template panel will appear to allow you to change the ALS baseline correction parameters:

© 2024 MESTRELAB RESEARCH



A manual multipoint baseline correction mode can also be selected, when fine tuning of the corrected baseline is necessary.

5. If spectrum processing is needed, to improve the data to subsequent analysis,you can apply a normalization, smoothing or derivative from the main toolbar (or from the 'Dynamic Processing Template'):

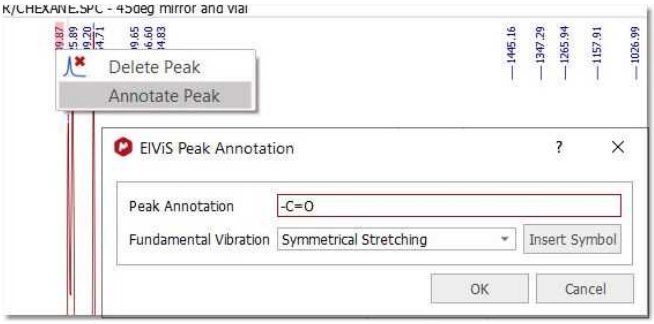


6. Clicking on the Automatic Peak Picking button will label the peak in a spectrum on screen in accordance with the options.

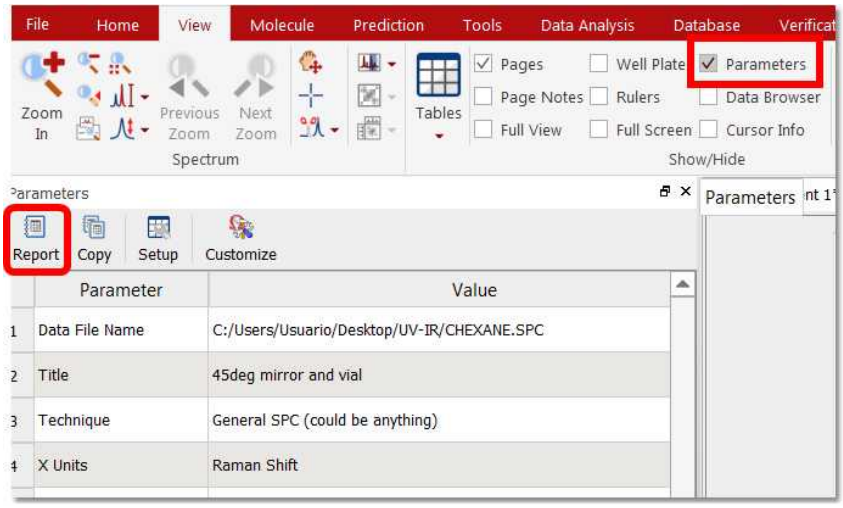


Manual Peak Picking can also be applied by selecting the 'Manual Threshold mode' and clicking and dragging over the desired region of interest to label the peaks within it. The 'Peak by peak' mode also allows setting or removing a label in an arbitrary position one by one to select unresolved peaks.

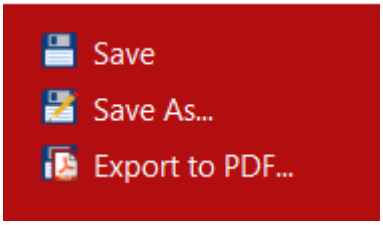
Right clicking on any peak label will allow you to annotate it:



7. To paste the Parameters Table on the spectral window, just follow the menu: 'View/Parameters' and click on the **'Report'** icon.



8. Finally, you will be able to save, print or export the document to PDF by clicking on a respective icon under the 'file ribbon' (or on the toolbar):



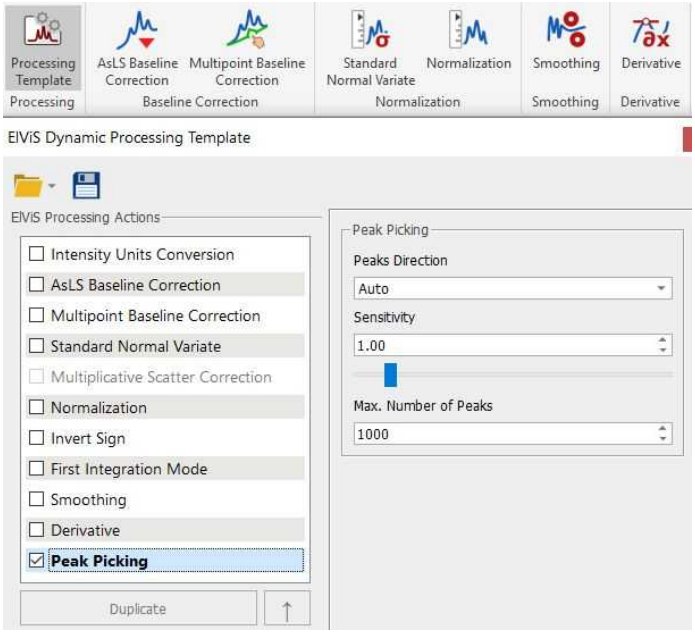
You can also export your datasets as ASCII (*.txt or *.csv), JCAMP-DX or any image format by selecting 'Save As'.

15.3 Basics Processing

Our aim in developing Mnova has been to make the opening, processing, handling, analysis, saving and printing of data simpler than it has ever been before, so that even the novice can enjoy the software and obtain excellent results from the very start.

All the processing features can be applied directly from the ribbon or from the Dynamic Processing Template:

© 2024 MESTRELAB RESEARCH



The main idea behind the template is to apply the tools and their combinations “on the fly”, to remember favorite processing patterns and quickly apply them, also automatically to a new opened data.

15.3.1 Baseline Correction

Spectra suffering from high background intensity may need baseline correction to improve their quality prior to analysis.

The UVIR plugin of Mnova offers two algorithms that can handle even very complex baselines:

- Asymmetric Least Squares (AsLS) Baseline Correction
- Multipoint Baseline Correction



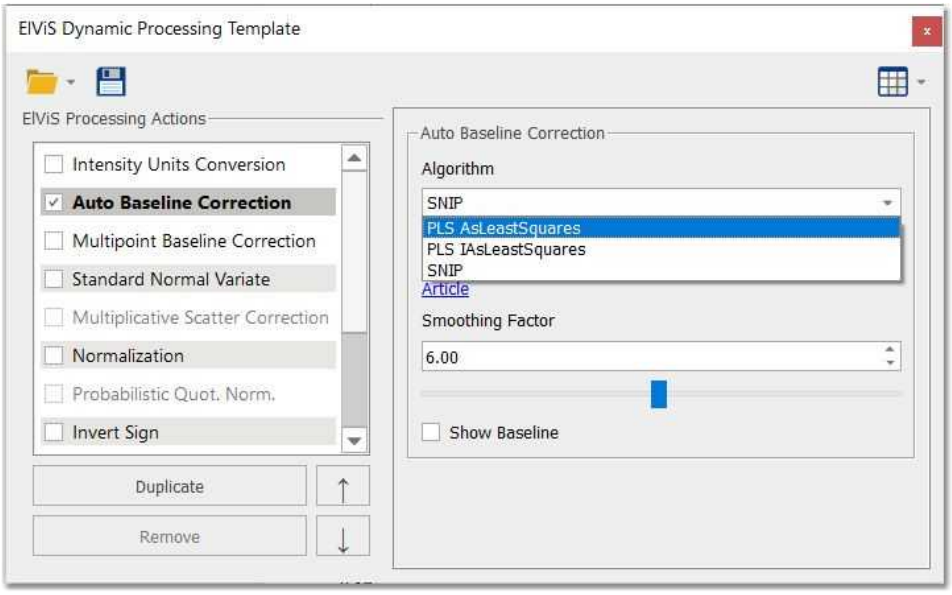
Asymmetric Least Squares Baseline Correction:[1,2] Combines a smoother with asymmetric weighting of deviations from the smooth trend to get an effective baseline estimator.

There are two algorithm parameters helping to optimize the results; “AsLS Logλ” (from 0.1 to 12; being 6 the default value) and “AsLS asymmetry” (0.000001 - 0.999999; being 0.000001 the default value).

[1] *P.H. Eilers, H.F. Boelens, Leiden University Medical Centre Report 1, 5,* ***2005.***

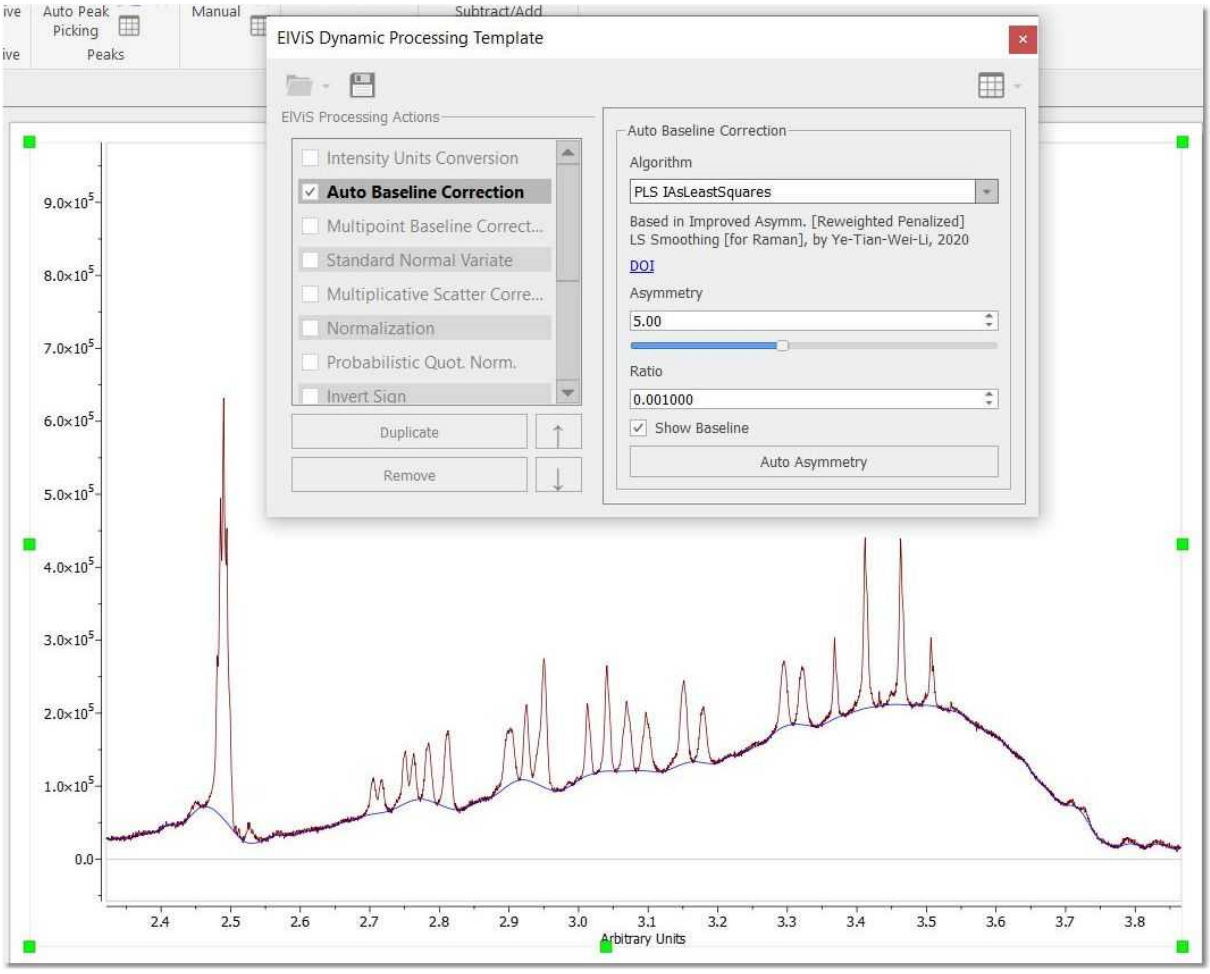
[2] *Journal of The Institute of Electronics and Information Engineers Vol.53, NO.3,* ***2016.***

© 2024 MESTRELAB RESEARCH



Checking the 'show Baseline' box will display the baseline in your spectral window with the option to compute the initial values for assymmetry (for PLS algorithms) or smoothing factor (for SNIP algorithm) and ratio parameters. Unchecking the 'Show Baseline' box will apply the baseline correction with the selected parameters.

© 2024 MESTRELAB RESEARCH



In the IAsLeast Squares algorithm, the parameters Asymmetry and Ratio have similar influence in the construction of the baseline, though the Ratio tends to have a less critical influence (i.e. in the number of needed internal iterations for convergence). For further information about his algorithm, please check the reference below:

https://doi.org/10.1364/AO.404863

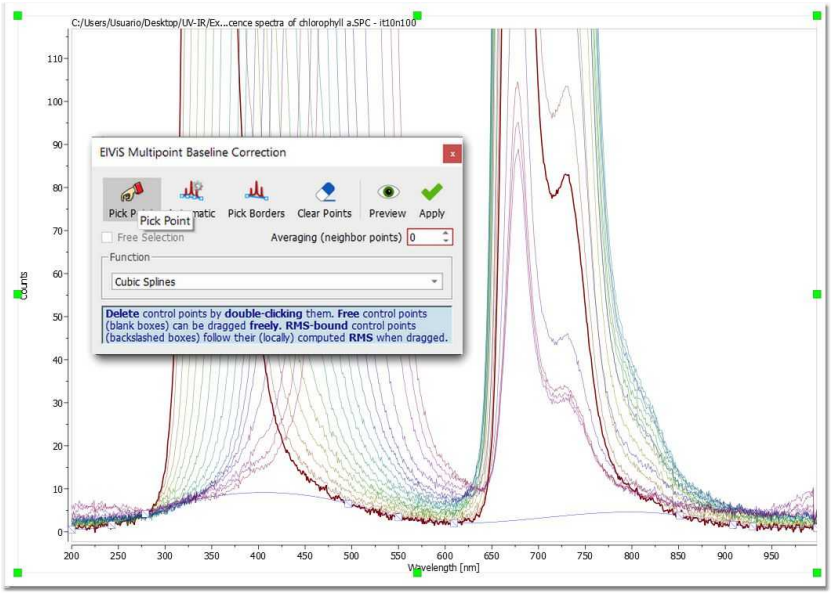
For further information about SNIP algorithm, please check this article:

https://doi.org/10.1016/S0168-9002(97)01023-1

Multipoint Baseline Correction: This method provides a way of modeling the baseline by selecting a well-distributed set of points that fall on the baseline and then interpolating between those points to complete the model.

Multipoint series baseline correction is a very useful algorithm of spectral (pre)processing that eliminates intensive spectral backgrounds, such as a fluorescence signal in Raman spectra, in a simple and efficient way. Although the algorithm can be applied to single spectra, its main value is the possibility of processing spectral series with essentially different individual baselines using a set common baseline points.

© 2024 MESTRELAB RESEARCH



After having clicked on the 'Multipoint Baseline Correction button'; you will get a dialog box which will allow you to put down points along the baseline (by clicking on the 'Pick Point' button) to help the program to find the correct coefficients for the baseline correction equation that it will subtract from the spectrum. If you want to remove any undesired point, just double click on it (click and drag if you want to change the location of the point).

Let´s see the functionality of each button of the "Multipoint Baseline Correction' dialog box:

'Pick Baseline points': Allows the user to pick the points. To remove any selected point, just double click on it.

'Automatic': Automatically add points for the baseline correction.

'Pick Borders': Allows the user to easily pick both the first and the latest points, if they need a correction.

'Clear Points': Click on this button to delete all the selected points (restart your work).

'Preview': to see a preview of the baseline correction prior to apply the changes.

'Apply': to apply the correction.

'Free Selection': Check this box to be able to pick points anywhere (otherwise their vertical positions are strictly defined by the baseline curve).

'RMS Calculation span (points): This option takes spectrum noise into account at the baseline construction. The value of 1 means that any picked point is picked exactly on the spectral curve. Otherwise, the point will be set into a vertical position corresponding to a virtually smoothed spectrum. The virtual smoothing is performed by simple averaging of points within a window where the selected point stays in the centrum and the present option indicates the number of neighbors from each side to be included into the averaging window. Therefore, the full window width is 2**n*+1 (*n* is the option value). For example, if you set this option to 3, the ordinates of each selected baseline point will be calculated as an average of ordinates of the spectral points within a window of 7 points with the selected point in the middle.

© 2024 MESTRELAB RESEARCH

'Function': from this drop-down menu, we can select the baseline construction function on the basis of selected points:

Linear Segments: Simply connects the points with lines.

Cubic Splines: Uses the method of splines to connect the points.

Witthaker: Fits the baseline points using the Witthaker Smoother (the points do not generally belong to the resulting baseline!).

15.3.2 Normalization

Normalization is a re-scaling of the spectral intensity that may be necessary to compensate for various experimental effects for better presentation, comparison, and quantitative or qualitative analysis of the data.

Mnova ElViS offers two different algorithms of spectrum re-scaling:



- Standard Normal Variate
- Normalization

Standard Normal Variate

Standard normal variate (SNV) is a popular transformation that treats each spectrum as a vector (*S*) in accordance with the following equation:

$$\tilde{S}_i(t) := (S_i(t) - \bar{S}_i)/\sqrt{\mathrm{var}(S_i)}$$

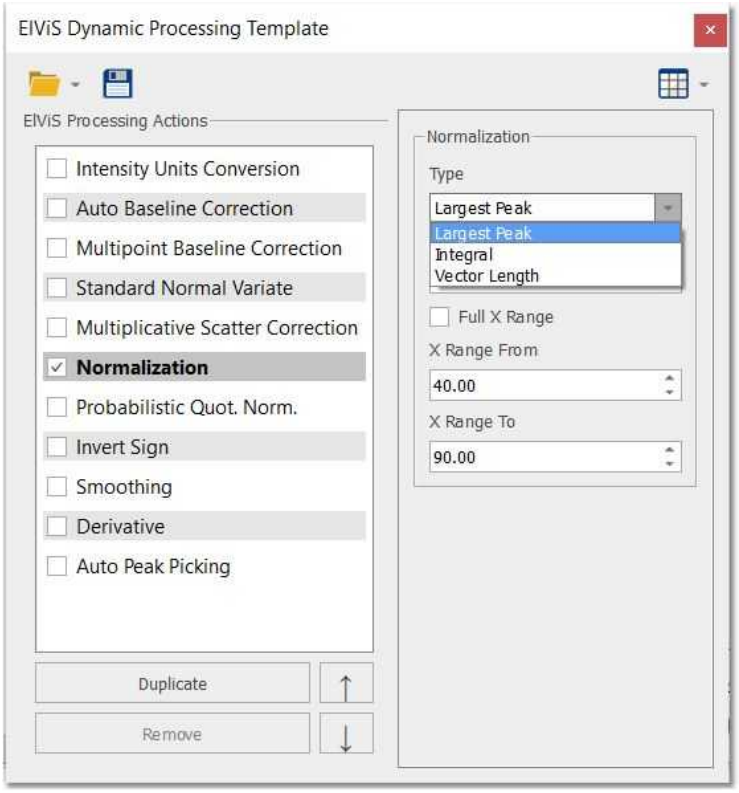
SNV is particularly useful when the sample volume (physical or virtual, as for on-line analysis) is not stable and may change from measurement to measurement. It is also applied to eliminate the so-called „scatter effect“ often observed in Vis/NIR spectra of solid and powder materials obtained in diffuse reflectance mode. SNV-correction is typically applied to spectral series prior to quantitative analysis.

Normalization

Simple Largest Peak normalization to 1 is a standard (pre)processing tool for UVIR spectral type. It is typically applied in small series to make spectra acquired at different conditions better suitable for comparison, peak interpretation and qualitative analysis. In databases all spectra are typically normalized to the unit intensity.

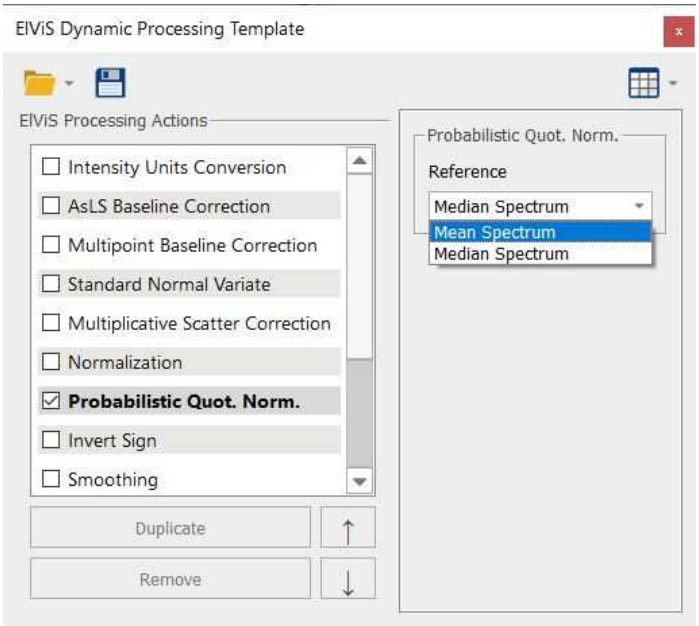
You can also apply spectrum wise integral and vector length normalizations by selecting the appropriate option from the Processing template scroll down menu:

© 2024 MESTRELAB RESEARCH



Check the Full X Range box to apply the normalization to the entire dataset. If you need to apply the normalization to a selected region of interest, just select the range values from the edit boxes.

For Raman datasets, you can also apply a spectrum set dependent probabilistic quotient normalizaton (PQN). The reference spectrum computed internally can be chosen to be the mean or median spectrum of those selected (from the stacked items table). For further information about it, check this paper: Anal. Chem. 2006, 78, 4281-4290.



© 2024 MESTRELAB RESEARCH

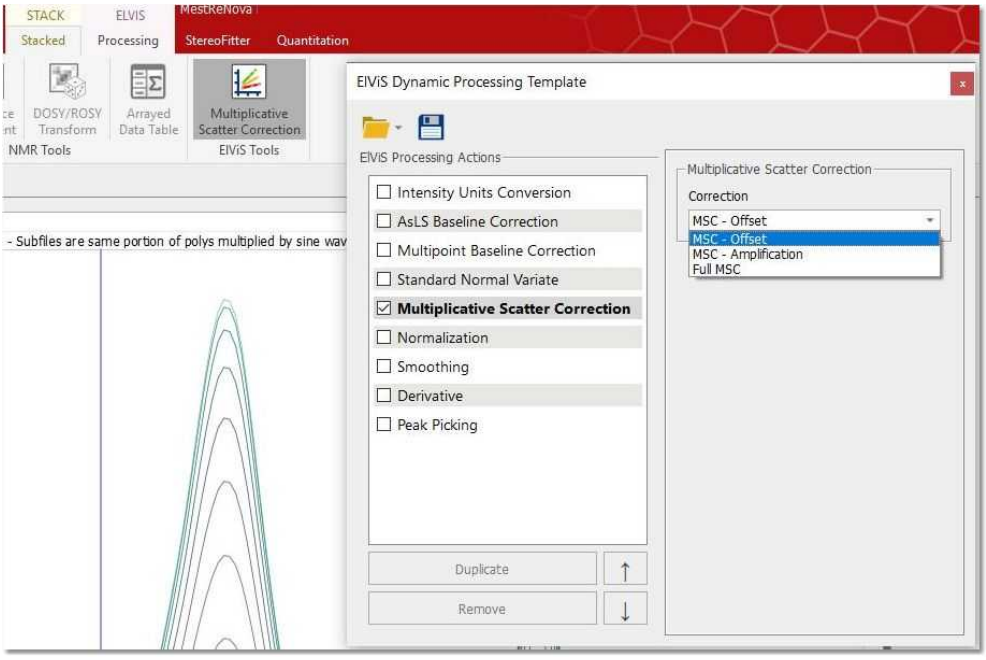
15.3.3 Multiplicative Scatter Correction

The multiplicative scatter correction is a good method in NIR spectroscopy to reduce the noise and background effects which cause baseline shifting and tilting in stacked plots.

The MSC function replaces every element in the original X-matrix according to one of the equations below:

Common offset	$M_{new}(i,k) = M(i,k) - A(i)$
Common amplification	$M_{new}(i,k) = \frac{M(i,k)}{B(i)}$
Full MSC	$M_{new}(i,k) = \frac{M(i,k) - A(i)}{B(i)}$

You can select any of the methods above from the Dynamic Processing template:

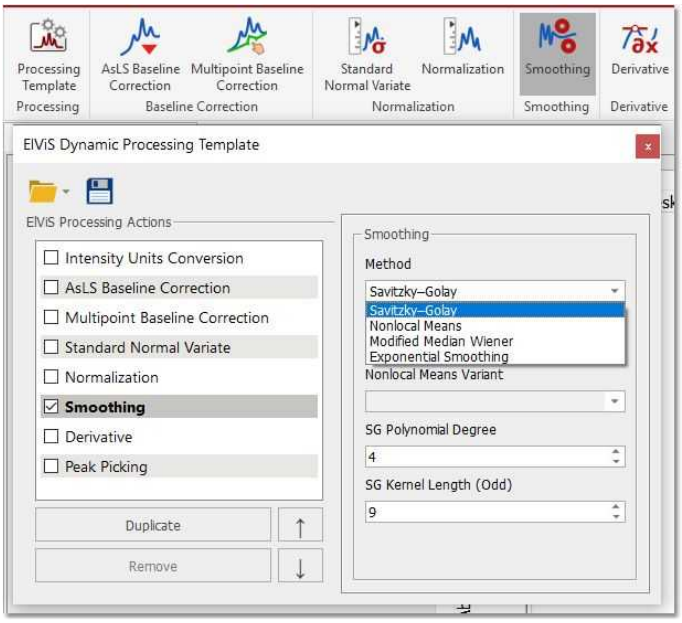


15.3.4 Smoothing

The signal to noise ratio (S/N) of a spectrum can be enhanced by smoothing (or filtering) technique. The noise contains high-frequency (compared to the spectral features) fluctuations of the signal. Smoothing applies a low-pass filter to the spectral data to remove the noise while having the informative spectral signals preserved.

Mnova ElViS incorporates some of the most used signal smoothing algorithms.

© 2024 MESTRELAB RESEARCH



You can select a desired smoothing algorithm from the Method drop-down list: Nonlocal Means, Modified Median Wiener, Exponential Smoothing and Savitzky-Golay.

'Savitzky-Golay algorithm': This approach performs a least squares fit of a set of consecutive data points to a polynomial and takes the calculated central point of the fitted polynomial curve as the new smoothed data point.

Savitzky and Golay has shown that a set of integers (A_{-n}, $A_{-(n-1)}$ …, A_{n-1}, A_n) could be derived and used as weighting coefficients to carry out the smoothing operation. The use of these weighting coefficients, known as convolution integers, turns out to be exactly equivalent to fitting the data to a polynomial, as just described, and it is computationally more effective and much faster. Therefore, the smoothed data point $(y_k)_S$ by the Savitzky-Golay algorithm is given by the following equation:

$$(y_k)_S = \frac{\sum_{i=-n}^{n} A_i y_{k+i}}{\sum_{i=-n}^{n} A_i}$$

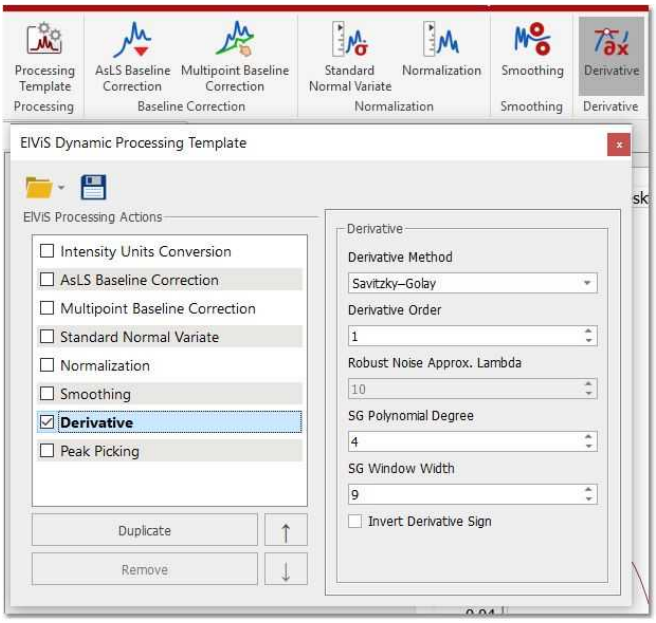
Sets of convolution integers, instead of the smoothed signal, can be used to obtain directly, instead, its 1st, 2nd, …, mth order derivative, therefore the Savitzky-Golay algorithm is very useful for calculation of the derivatives of noisy signals consisting of discrete and equidistant points.

15.3.5 Derivative

Differentiation of spectral data is a useful tool to enhanse the data information content. First or second derivatives are typically used to improve the interpretation by revealing unresolved peaks or to correct spectra for a baseline (offset or slope, respectively) prior to quantitative or qualitative analysis.

The Derivative tool is available under the ElViS processing ribbon and in the dynamic processing template:

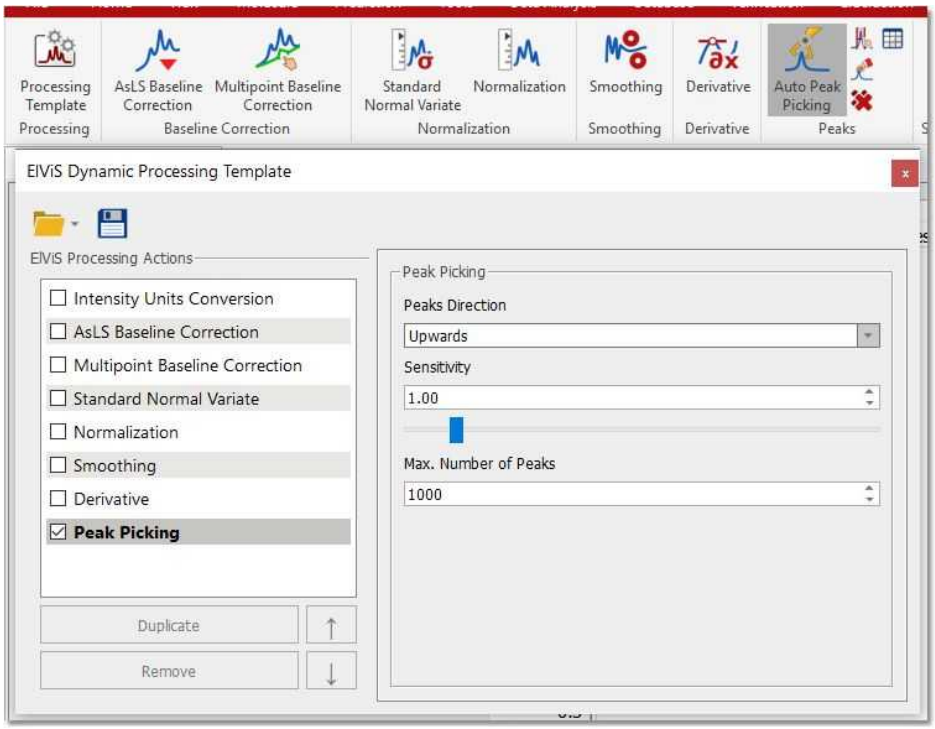
© 2024 MESTRELAB RESEARCH



Mnova ElViS offers three algorithms for the calculation of spectral derivatives: Savitzky-Golay, Robust Noise and Centered differences.

15.3.6 Peak Picking

Mnova ElViS implements a series of peak picking algorithms which can be accessed from the Processing Ribbon and from the Dynamic processing template:



From the ribbon, you will be allowed you to use **Automatic**, **Manual** (or Manual Threshold), **Peak by Peak** methods of Peak Picking, to **Delete all peaks in visible range** and to display the peaks table.

© 2024 MESTRELAB RESEARCH



NOTE

You can annotate and delete peaks by right clicking on the peak label.

Automatic Peak Picking

The Automatic Peak Picking option sets labels all spectral peaks in accordance with the selected options. It can be launched by simply clicking on the **'Auto Peak Picking'** icon on the toolbar.

Manual Threshold

The Manual Peak Picking allows the user to set an area of the spectrum where the software will carry out the Peak Picking, by simply left clicking and dragging the mouse over the area.

Peak by Peak

The Peak by Peak option will allow you to select peaks to be labeled (unlabeled) one by one by hovering the mouse over a peak of interest and clicking on it. The procedure is very simple: just point to the peak you want to pick so that it is highlighted and then left click on the mouse.

To pick a peak at an arbitrary position (e.g. for a non-resolved peak), press <Shift> and click.

From the Dynamic Processing Template, you can select the 'peaks direction' (upwards, downwards or both), the algorithm sensitivity (to avoid picking the noise) and the maximum number of peaks.

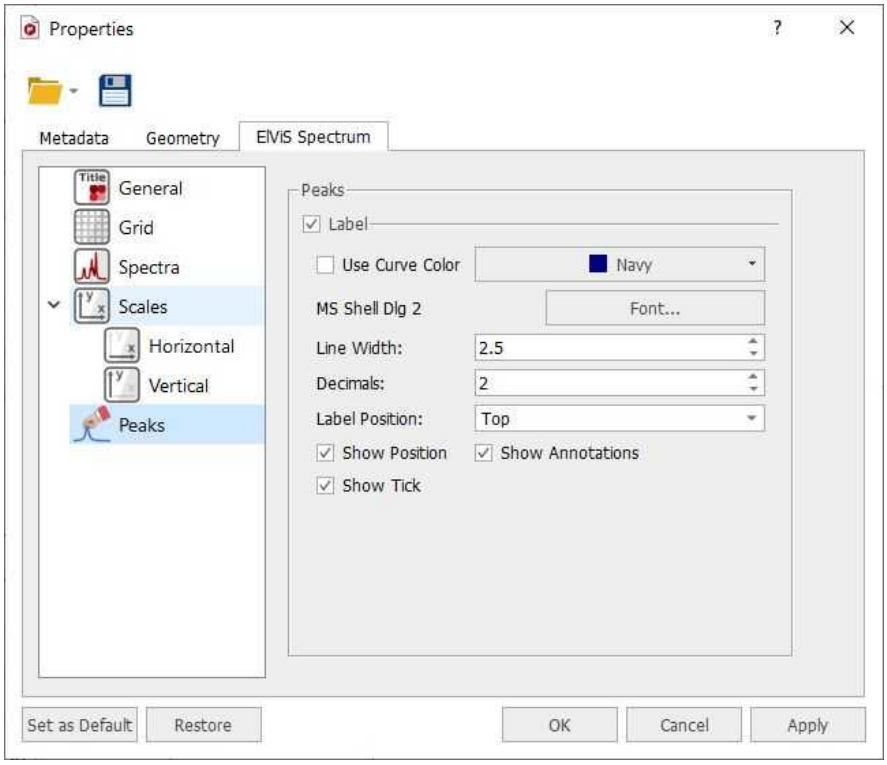
Sensitivity: it is basically a kind of smoothing parameter. For example, in noisy spectra, large values of this parameter will smooth the spectrum in the background so that less peaks will be detected.

The ElViS Peaks table will be displayed just by clicking on this button of the ribbon 

15.3.6.1 Peak Picking Properties

You can modify the properties of the **Peak Picking** by following the menu 'Home/Properties/Peak Picking' or by double clicking the left mouse (or pressing the right mouse) button on the spectrum display and selecting *Properties/Peak Picking* from the *pop-up* menu.

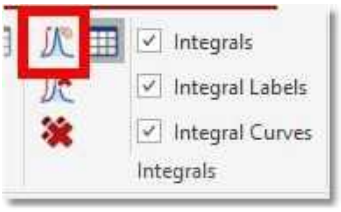
© 2024 MESTRELAB RESEARCH



From here, the user can display or hide the peaks labels and the Position, Annotations and Ticks by checking (or unchecking) the applicable check box. You can select the color, the font, the number of decimal figures to be shown on screen and the position of the 'peak labels'.

15.3.7 Integration

Mnova ElViS implements a manual integration feature which can be applied over one single spectrum or to a stacked plot. You can use the 'Manual Integration' button from the ribbon (or the shortcut: I) and next click and drag over the region of interest:



You can use the check boxes of the ribbon to hide/display the integrals, labels and curves.

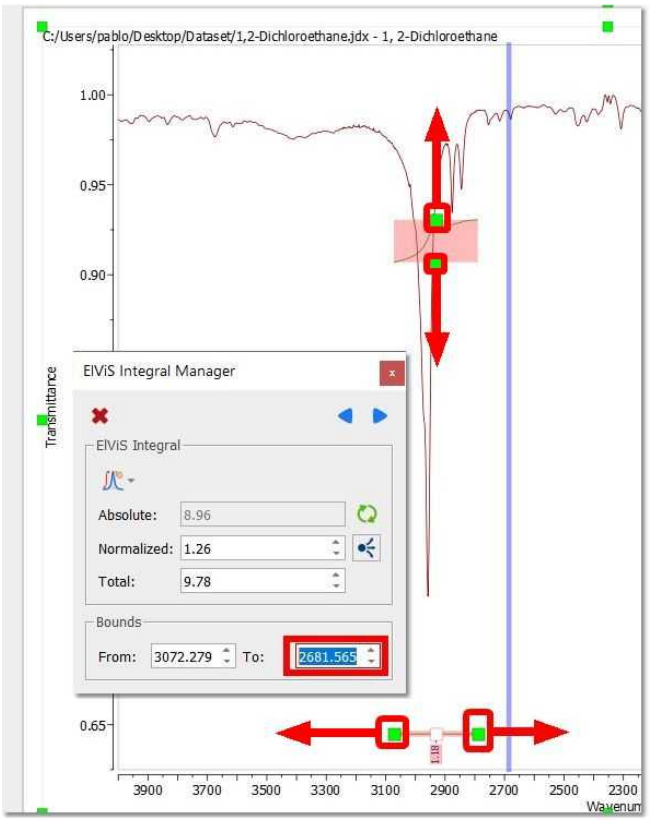
The first integral will be normalized to 1.0. All following integrals will be referenced relative to the first integral.

All the integral curves are mouse-sensitive and they respond to usual mouse operations. If you want to move up or down all the integrals, just click and drag (with the left mouse button) over anyone of the integrals (notice that hovering the mouse over the integral will highlight it in red). If you keep pressed the SHIFT key at the same time, the height of the integral curves will be changed.

The same effect will be obtained if you hover the mouse over integral curve, click on one of the green squares and drag the mouse up or down.

© 2024 MESTRELAB RESEARCH

If you want to change the range of the integral, do the same with the integral label (notice that once you hover the mouse over the green squares of the integral curve or label, the mouse pointer turns to vertical or horizontal arrows, respectively). The integral regions can also be resized the 'Integral manager' which can be accessed by double clicking on the integral curve:

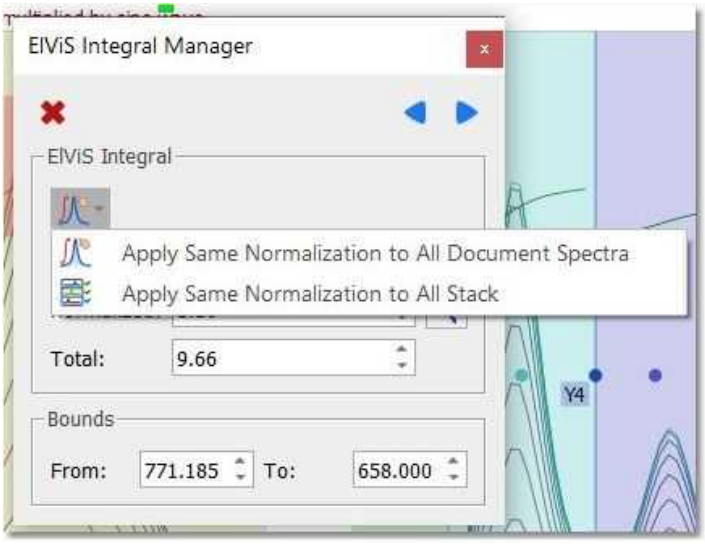


Bear in mind that you are able to navigate over the integrals by using the **'Previous or Next'** icons. You can delete an integral one by one by clicking on the 'Delete' button.

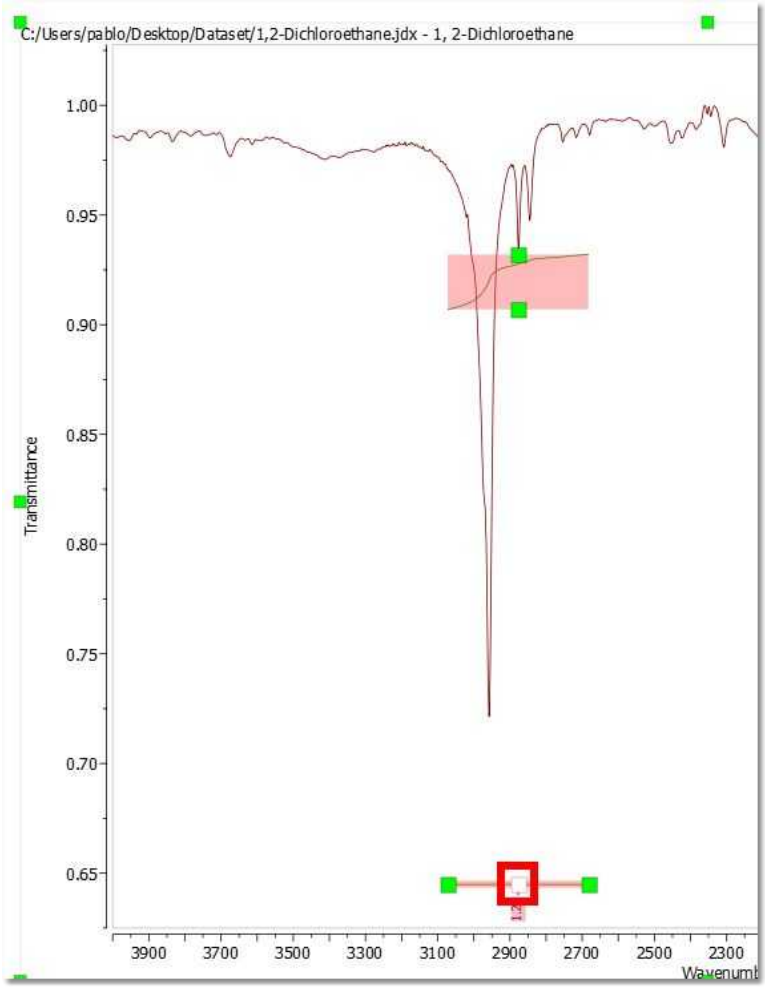
If you want to normalize the integrals, just overwrite the desired value on the 'Normalized box' and press OK, all integrals will update with reference to the chosen one.

You can apply the same integral normalization to all the spectra (in the current document) or to all the traces of a stack plot by selecting the applicable option, as you can see below.

© 2024 MESTRELAB RESEARCH

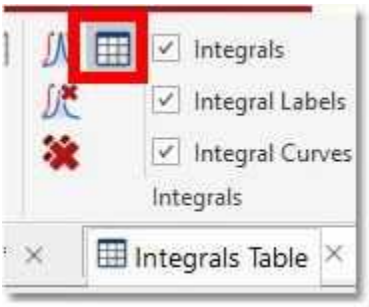


Integral regions can be split by clicking (or clicking and dragging) on the 'red square' of the integral segment:

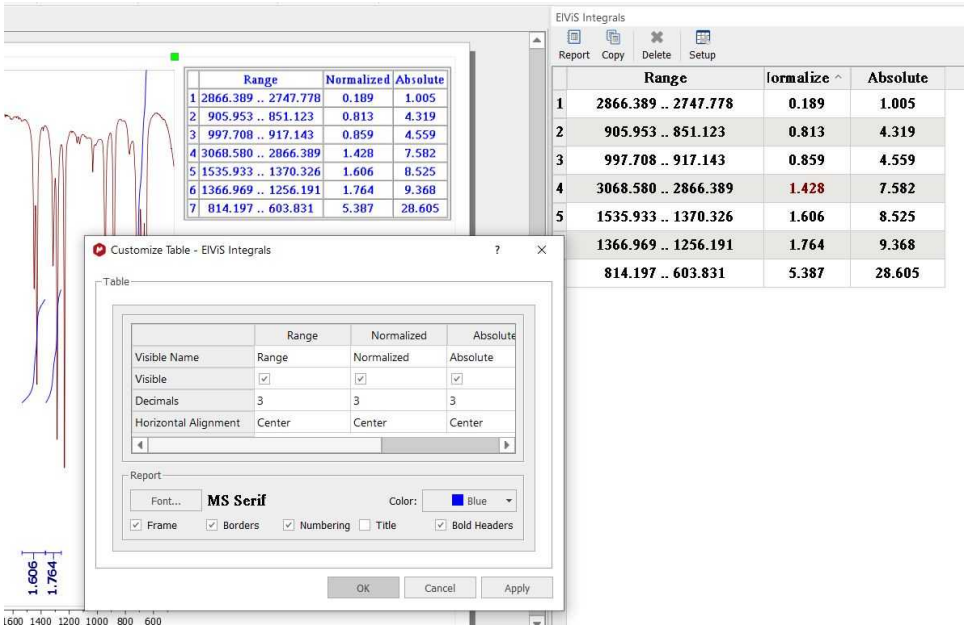


All the integrals will be stored in the integrals table which can be displayed by clicking on the applicable button of the ribbon (or by following the menu 'View/Tables/ElViS/ElViS Integrals'):

© 2024 MESTRELAB RESEARCH



The information displayed in the table can be customized by clicking on the 'Setup' button. As usual, you can report and copy to clipboard the table:



15.3.8 Processing Templates

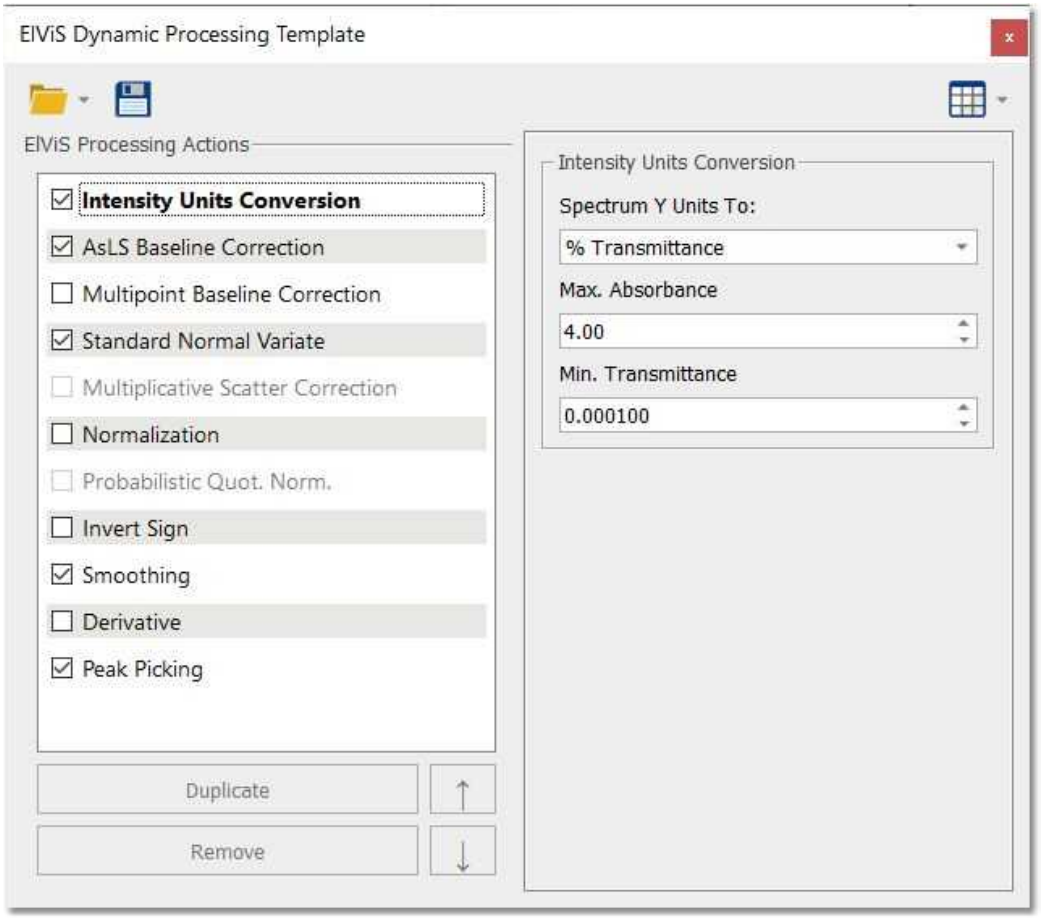
Mnova includes a powerful and flexible feature which allows the user to easily automate the full processing of ElViS data sets. This is ideal for the batch processing of spectra of the same type, and can also incorporate analysis operations.

For example, let's say you have acquired 20 datasets which you want to process using the same processing operations. The procedure is:

1. Load one spectrum and process it as desired.

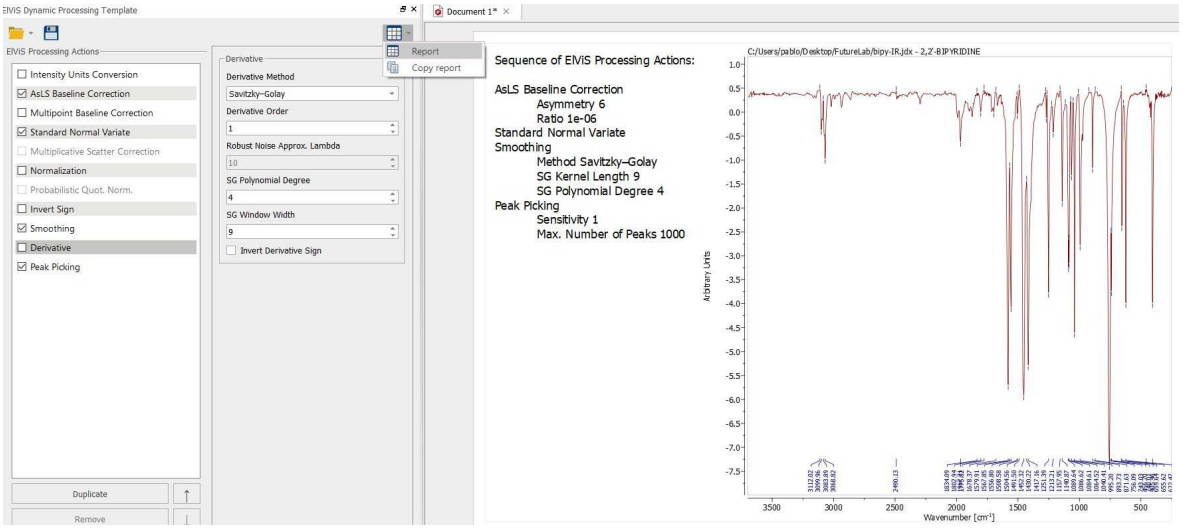
2. Go to ***Processing/Processing Template***. The following dialog appears:

© 2024 MESTRELAB RESEARCH



This dialog includes all the main processing features implemented in Mnova ElViS. From here, you can also change the units of your spectrum (Transmittance, Absorbance). You can choose the required options by selecting the applicable check boxes.

Clicking on the Report button will paste in your spectrum, the sequence of the processing features that you have applied:



3. Once you are happy with the result, save the script file to a Mnova Processing file (*.mnp) by clicking on the save button in the dialog box.

4. You can now apply this processing script file to any other spectrum/spectra by selecting the spectra you wish to process following this procedure (one or several spectra), and then opening the previously saved file by using the open command in the Processing Template dialog box.

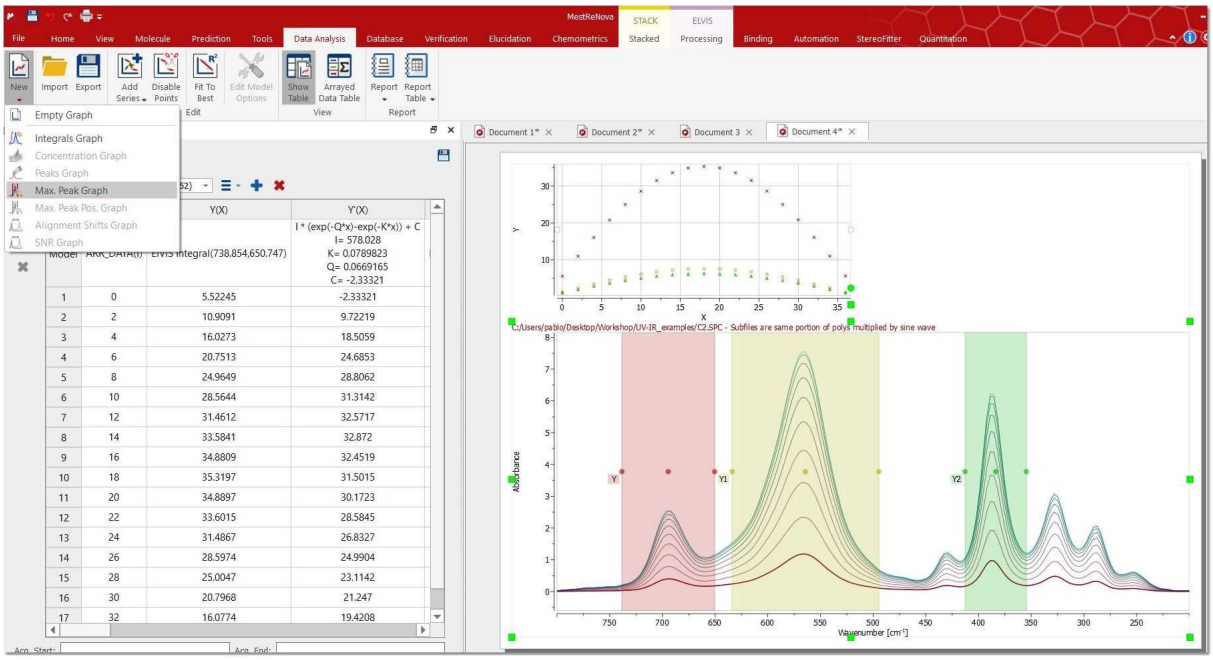


A few helpful tips ...

1. If you want to apply a processing script to all the spectra opened in one document, first select all the spectra in all the pages.
2. Next you can simply go to ***Processing/Recent Processing Templates*** and select the appropriate processing file script.
3. Remember that only spectra acquired using the same conditions should be processed using a processing script file.

15.3.9 Data Analysis

You can use the Data Analysis feature with the ElViS module, just by loading your stack plot and following the menu 'Data analysis' to select the desired action from the 'New Graph' scroll down menu:



1	0	5.52245	-2.33321
2	2	10.9091	9.72219
3	4	16.0273	18.5059
4	6	20.7513	24.6853
5	8	24.9649	28.8062
6	10	28.5644	31.3142
7	12	31.4612	32.5717
8	14	33.5841	32.872
9	16	34.8809	32.4519
10	18	35.3197	31.5015
11	20	34.8897	30.1723
12	22	33.6015	28.5845
13	24	31.4867	26.8327
14	26	28.5974	24.9904
15	28	25.0047	23.1142
16	30	20.7968	21.247
17	32	16.0774	19.4208

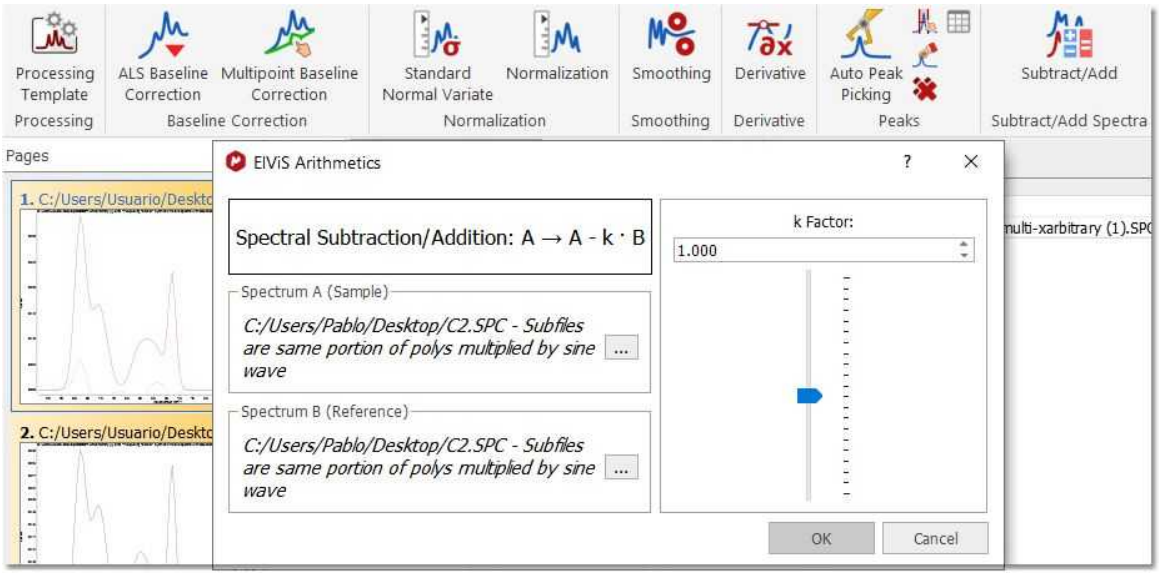
For further information about this feature, check this chapter.

15.3.10 Arithmetics

The ElViS arithmetics feature is available from the ElViS Processing tab ribbon (Subtract/Add button) or from the context menu (by right clicking on your ElViS spectrum).

When selected, a dialog is displayed with the ability to select the Sample Spectrum A (which can be a single spectrum or a stacked plot) and the reference B (which must be a single spectrum) and to adjust the factor *k* (coefficient). You can also select both pages from the page navigator before having clicked on the 'Subtract/Add' button and the Sample and Reference datasets will appear in the dialog:

© 2024 MESTRELAB RESEARCH



Please bear in mind that both spectra must have the same number of points and units.

When working with a stack for Spectrum A (Sample), the subtraction will be applied to all the traces unless you have selected some spectra from the 'Stacked Items' Table.

NOTE

Use negative k to add a spectrum to the Sample one.

Part XVI

16 Mnova Database

This plugin is not included in Mnova Suite. If you want to try it, please email us to support@mestrelab.com

This plugin of Mnova was born as a new concept to the shared storage for molecules, Mnova objects (texts, drawings, images, etc...), NMR, MS and Optics analysis data.

Please bear in mind that you will also need a server to store your databases. You will find here the instructions to set up that server. See also this video for further information.

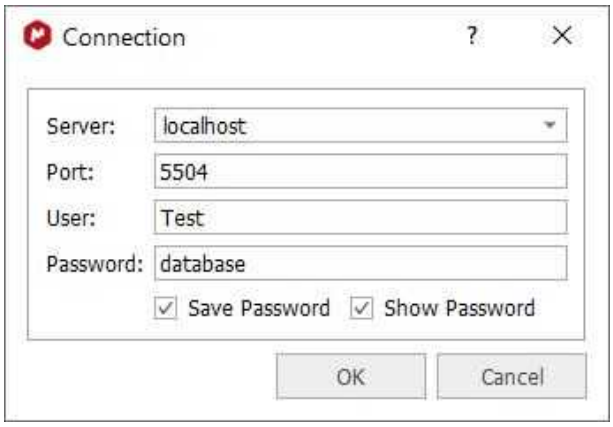
Check also this *application note* about Mnova DB MyData.

16.1 Connecting to the Server

Firstly, you will need to connect with the database server by following the menu 'Database/Connect' and entering the corresponding password:

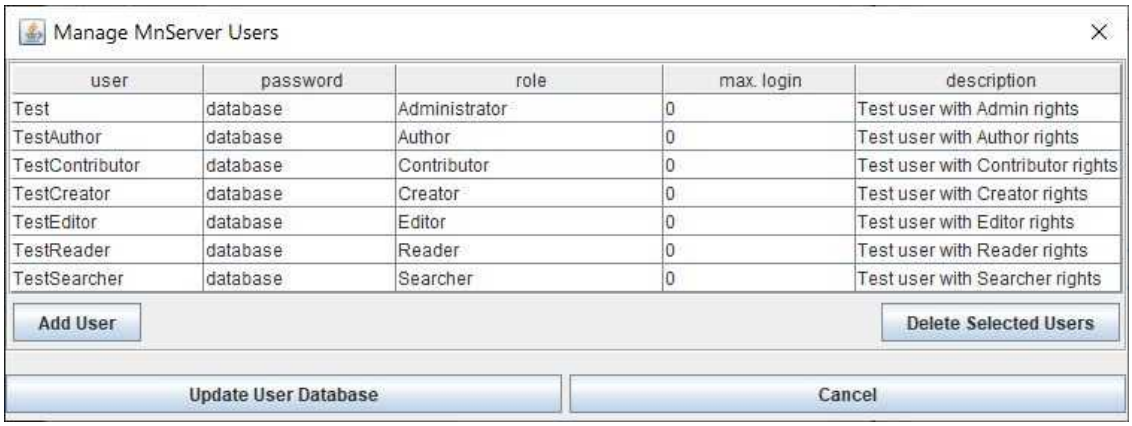


If you have not created any user, you could use the default credentials below for an administradot user:



You can create more users by double clicking on the MnServerAdmin.exe file (from your MnServer installation folder).

Here you can find the list of the default users and passwords for each role:



Further information about MnServer can be found here.

If you do not have any Database created, you will need to create one. In that case, please go to the applicable chapter.

16.2 Create a Database

If you do not have any previous database, you will need to create one. To do it, just follow the menu 'Database/Manage/Create Database':



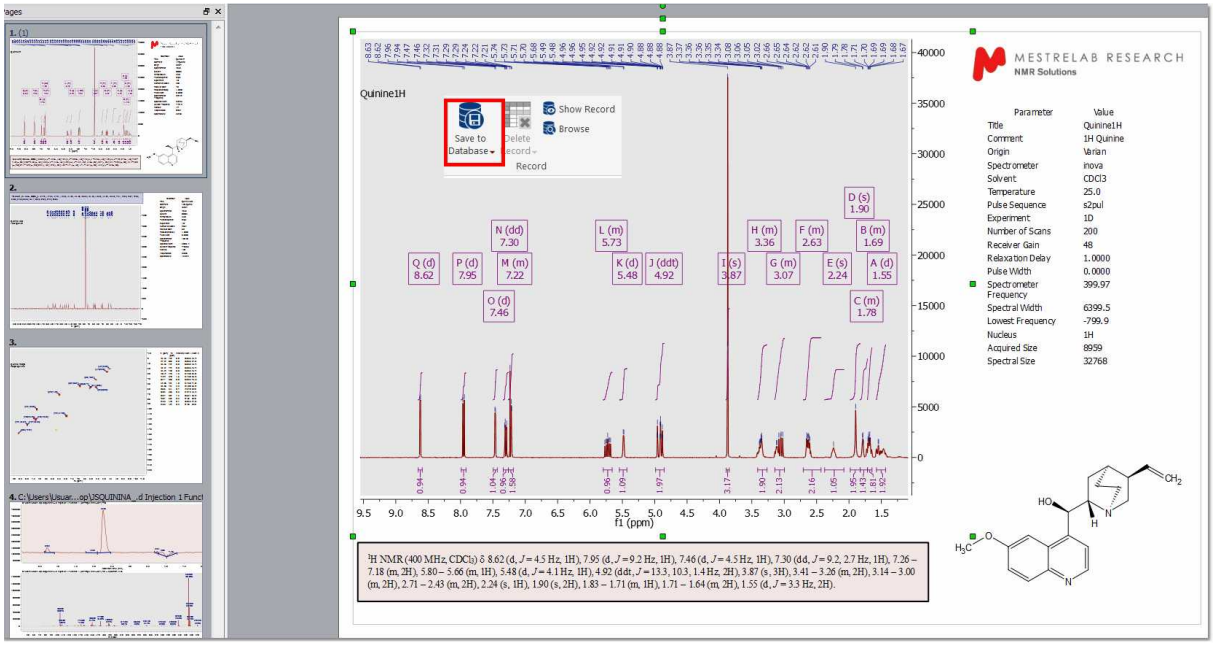
For further information about it, please go to this chapter.

16.3 Saving items to Database

You will find the quinine datasets under the 'File/Help/Download Example datasets' menu)

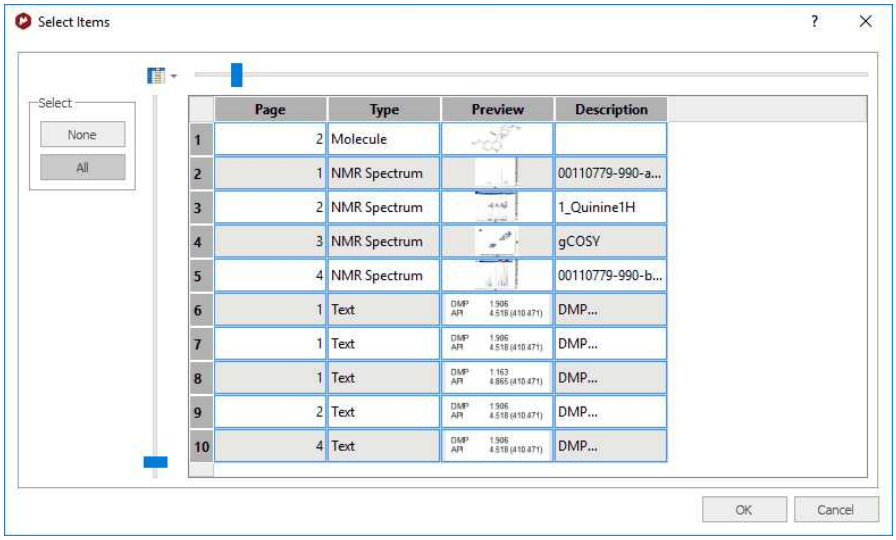
It will be really easy to add objects (spectra, molecules, etc...) to a database; just open your Mnova document (containing for example a 1H-NMR spectrum, a molecule and some objects) and follow the menu 'Database/Save to Database'

© 2024 MESTRELAB RESEARCH



You can also 'Save to Database' by right clicking on the spectrum to display the spectrum context menu.

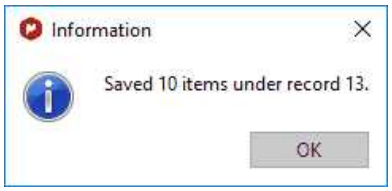
After having selected 'Save to Database', you will get a new dialog box where you will see how the spectrum and the molecule(s) are selected by default to be added to the database. If you want to add also the other objects, you will need to check the corresponding boxes or click on the 'All' button.



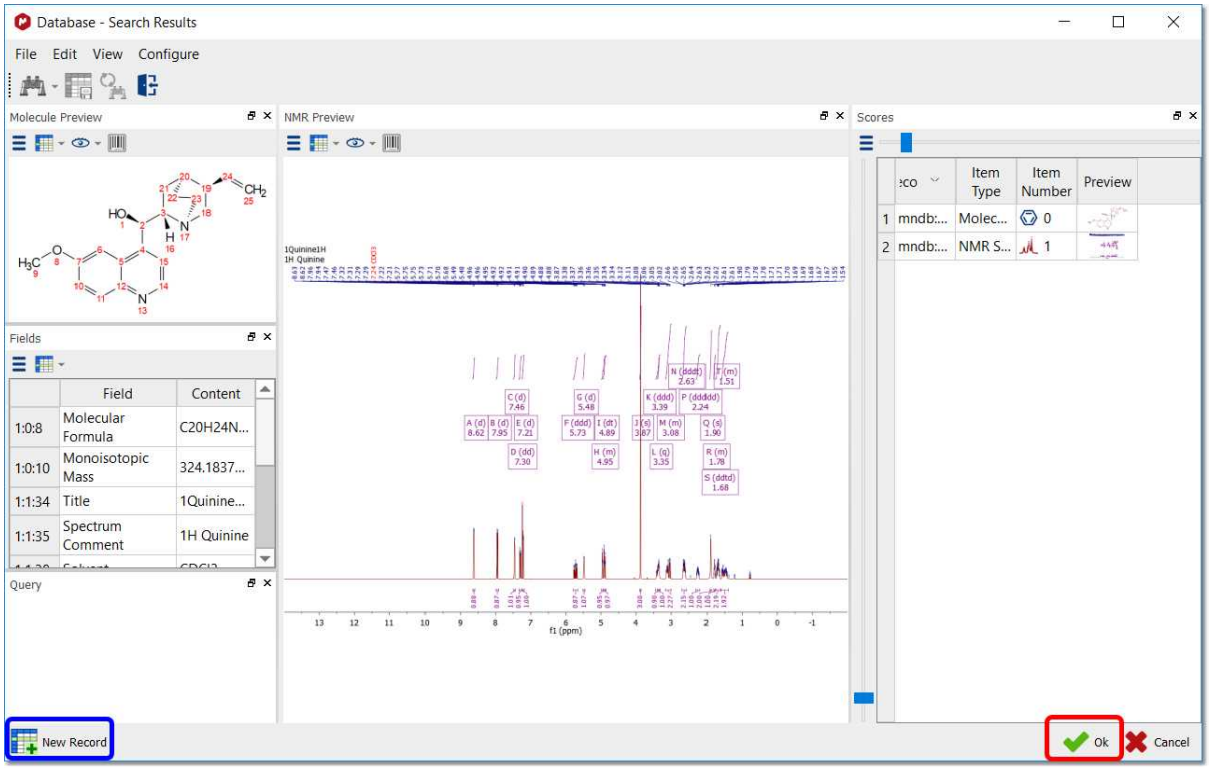
Once you have done the selection just click on OK to save to the database. During this procedure, Mnova will check the database to find similar spectra or molecules.

If your dataset is new in the database, you will only get a 'Save Complete' message and your dataset will be added to the database:

© 2024 MESTRELAB RESEARCH



If it finds a match during the saving procedure, it will ask you to add the new items to the compound (to avoid duplications). If you want to add a new item to an existing record, you will only need to check the OK button (highlighted in red in the picture below).



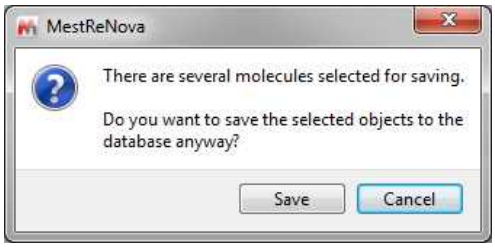
After having clicked on the OK button, a new dialog box will be displayed to ask you whether to add or replace (when existing items have been found matching those being saved).

If for some reasons, you do not want to add the 13C spectrum to the compound 1, just click on the 'New Record' button (highlighted in blue in the picture above).

You can add 2D NMR or MS datasets to the database. In the case of 2D datasets, if the file size is too big, we recommend to use the 'Compression' feature (under the Processing menu) before uploading the experiments to the database.

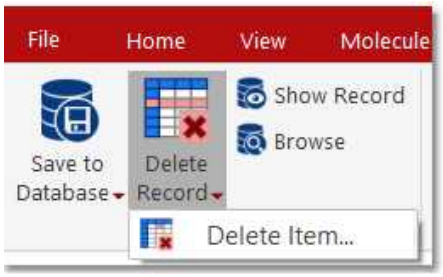
If your document contains more than one compound and several molecules are selected for saving, a warning message will be displayed:

© 2024 MESTRELAB RESEARCH



When the user chooses to continue, several records (one for each selected molecule) are created in db and each of the records will contain all of the selected spectra.

You can delete any existing record by clicking on the 'Delete Record' button:



Selecting 'Delete Item' from the scroll down menu above, will allow you to delete the undesired item from that particular record.

16.4 Displaying database contents

In this chapter you will find information about how to display the content of your Database.

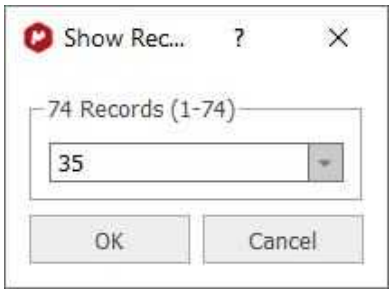
16.4.1 Operations

Once you have created the database, you will be able to search for any record just by using the 'Show Record' or the 'Browser'.



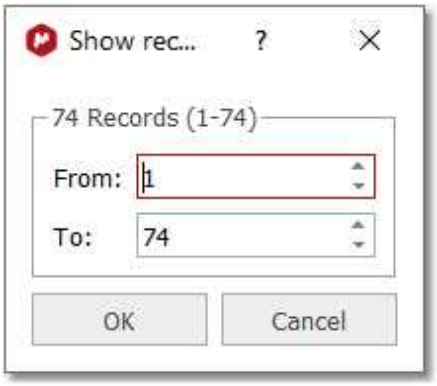
Show Record

This feature will allow you to find compounds by using their ID. The first record added to the database will have the ID number 1; the second record will have the ID number 2 and so on:



Browser

The Browser will allow you to show a range of records:



16.4.2 Searching in the Database

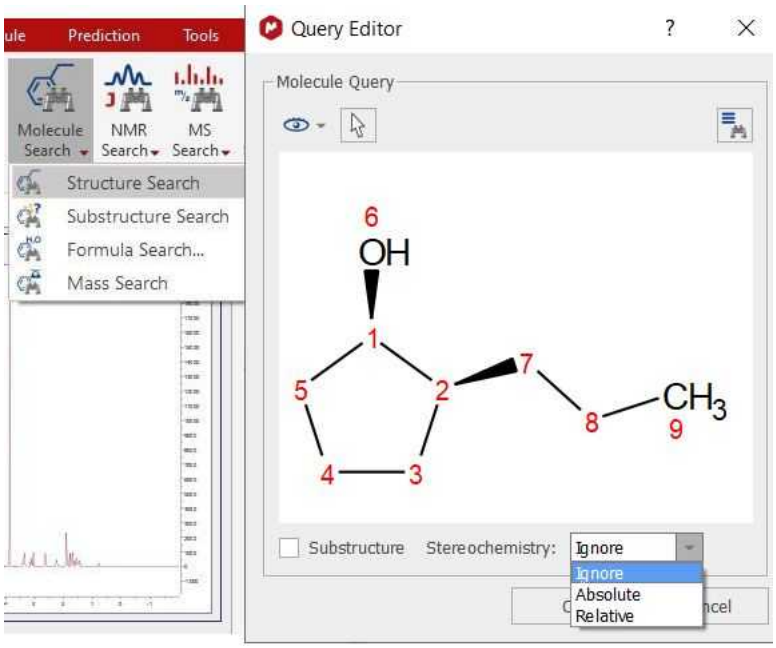
You can use the applicable commands to search 'structures', 'substructures', 'formulas', 'peaks', 'multiplets', 'Massitems', 'numbers', 'texts', etc':



Structure Search

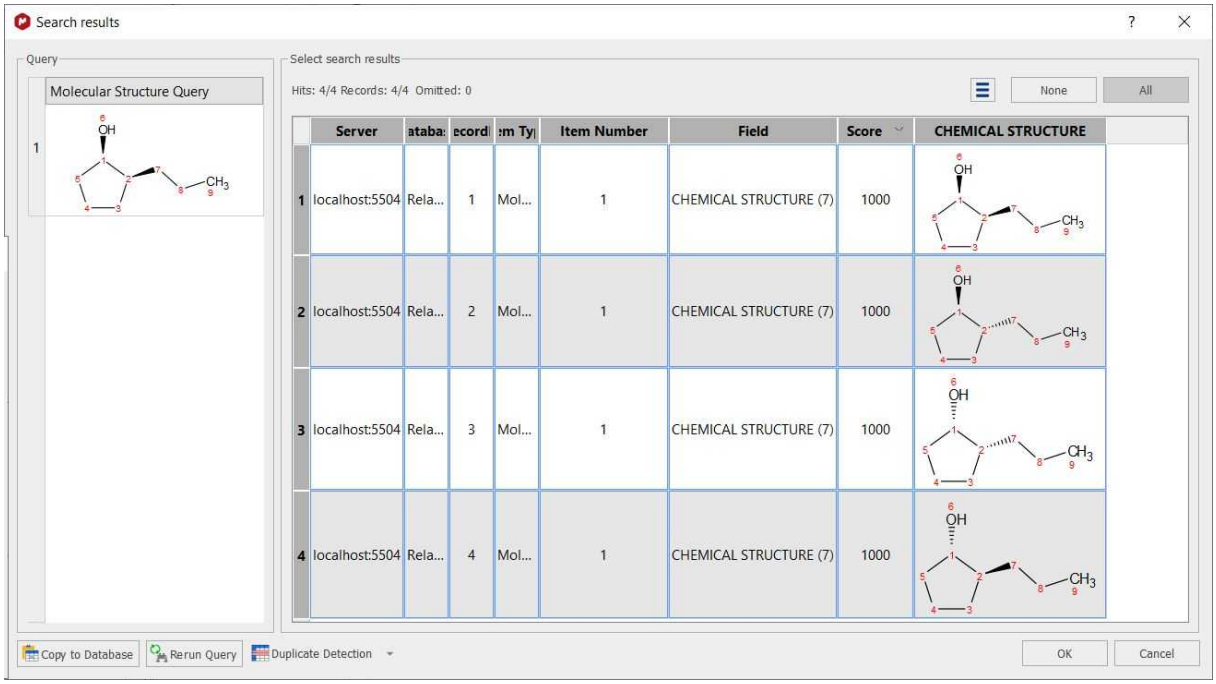
You can search for any molecular structure in the database by selecting 'Structure Search' (which will display the Query Editor). Click on OK to run the search.

© 2024 MESTRELAB RESEARCH



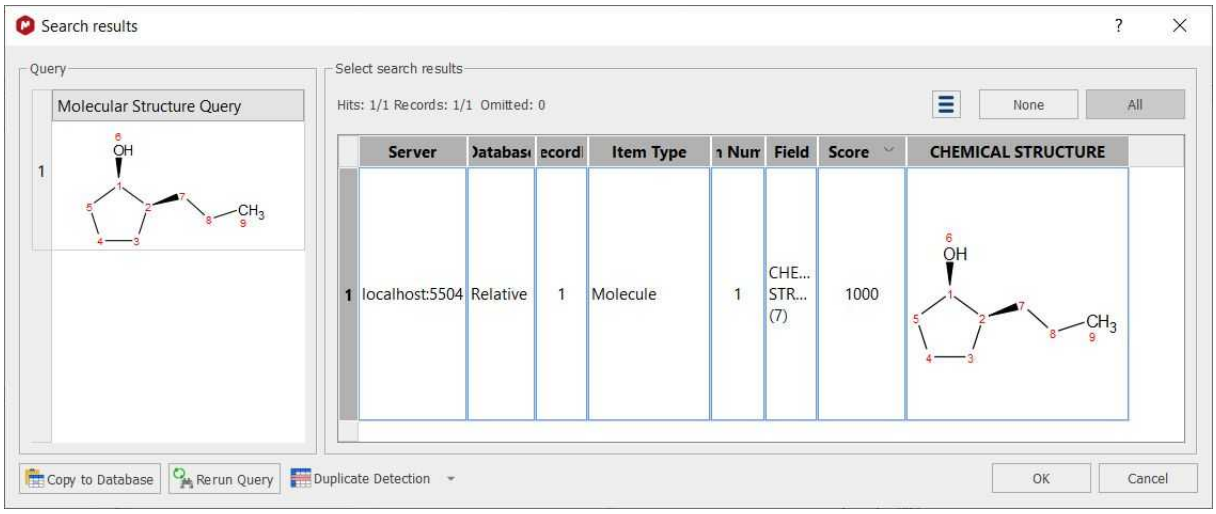
From here, you will be able to run also Substructure searches just by checking the applicable box.

Selecting ignore from the StereoChemistry scoll down menu will not take into account the stereobonds for the search. Imagine a database with 4 compounds RR, RS, SR and SS, if you search for one of the 4 compounds (i.e. RS), you will get 4 hits:

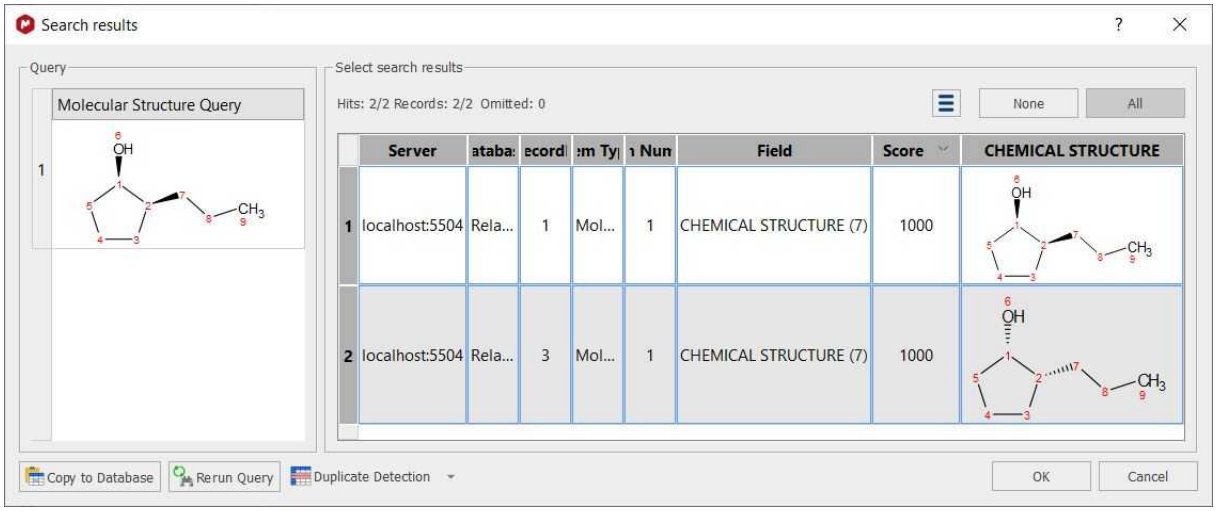


Selecting 'Absolute' will take into account all the stereocenters present in the structure (so if you search for a molecule with a RS configuration, the hit will be only the molecule RS).

© 2024 MESTRELAB RESEARCH



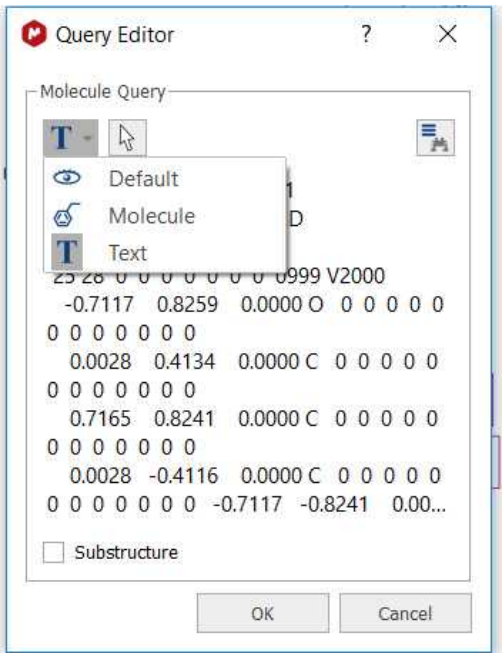
Whilst selecting 'Relative' will only take into account the relative stereochemistry, that's it; if you search for a molecule with a RS configuration, the molecules RS and SR will be hits:



Clicking on the 'Convert to advance query' button will allow you to convert it to an advanced search so that you can add new queries and combine them.

You can also see the mol file as plain text, by selecting the applicable option in the 'Select Display Mode' scroll down menu:

© 2024 MESTRELAB RESEARCH



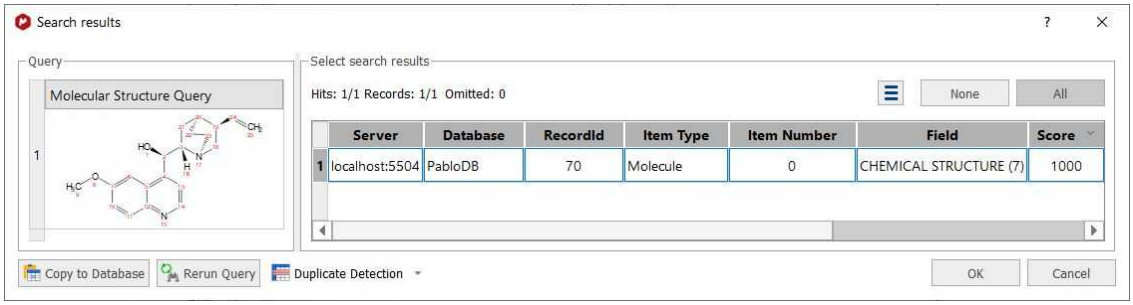
This button  will allow you to select which molecule you want to search in the case that you have several molecules in the same document.

After having clicked on OK, Mnova will search for the molecule in the database.

- If the molecule is not in the database you will obtain this message:



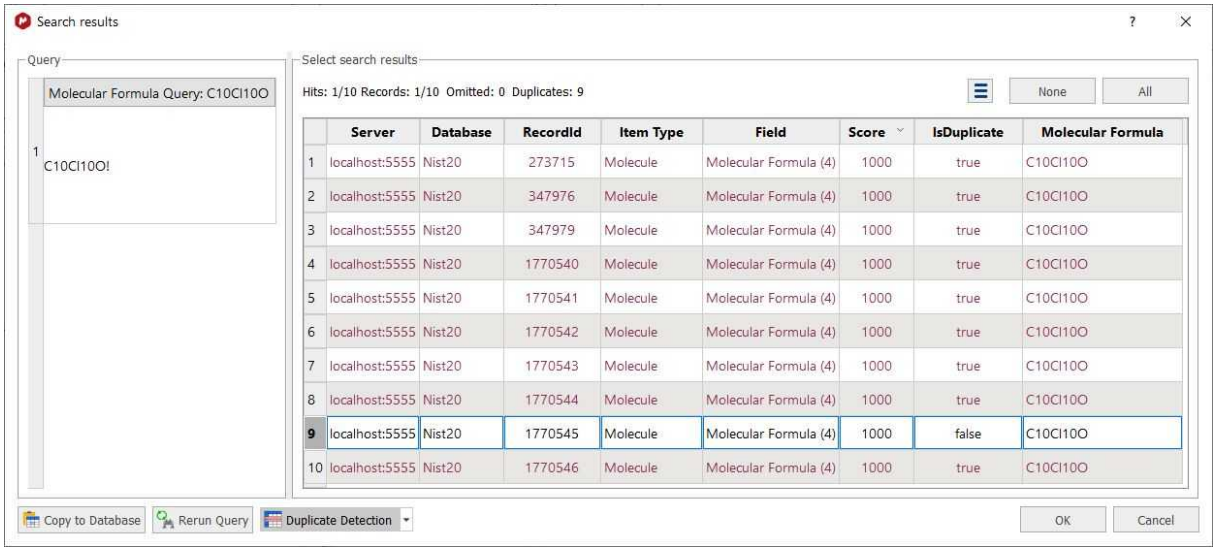
- If the molecule structure is stored in your database, Mnova will show you the information which appears in the database about this compound. For example, imagine that you load the molecule structure of the quinine and you select 'Structure Search'. In that case, you will obtain a window like this one (with a Match Score of 1000 which is the maximum value):



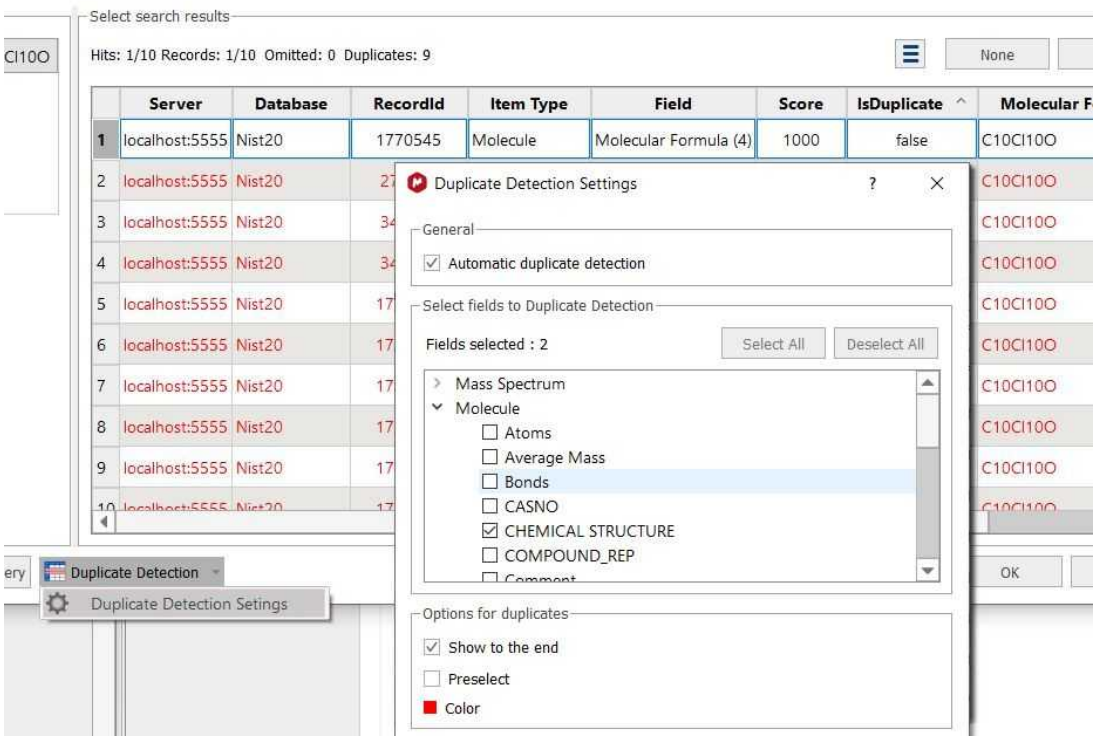
Note: Clicking on the Copy to database button, will allow you to copy the result to another database. Clicking on the 'Rerun Query' button will allow you to refine your search.

© 2024 MESTRELAB RESEARCH

Clicking on the Duplicate selection button, will highlight the duplicated results (in the case that you have them).

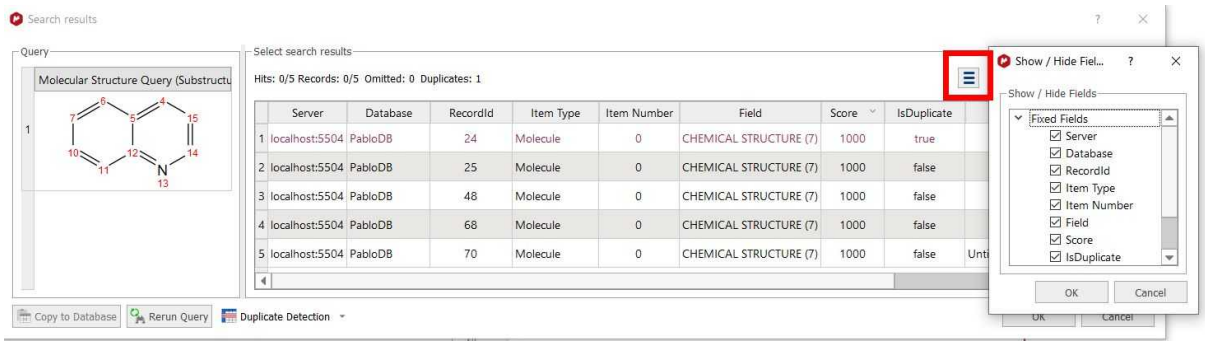


You can customize the settings for the duplicate detection feature by selecting the appropriate option from the scroll down menu. By default Mnova detects duplicates using the "Molecular Formula" field, but you could use any other combination of fields:

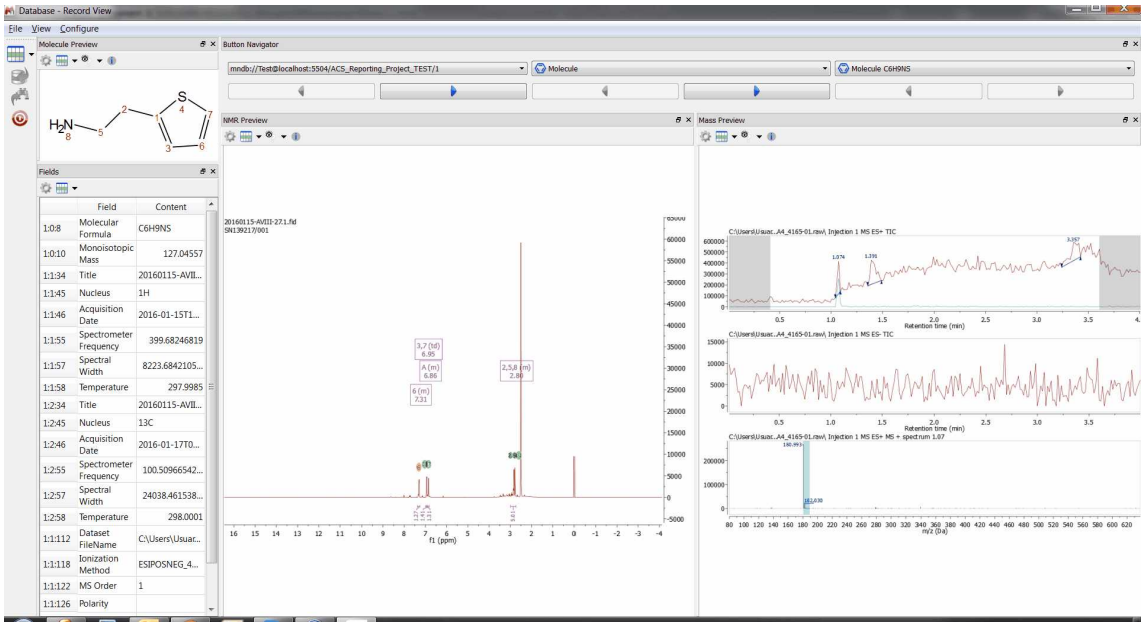


Clicking on this button  will allow you to 'show/hide fields' in the search results panel:

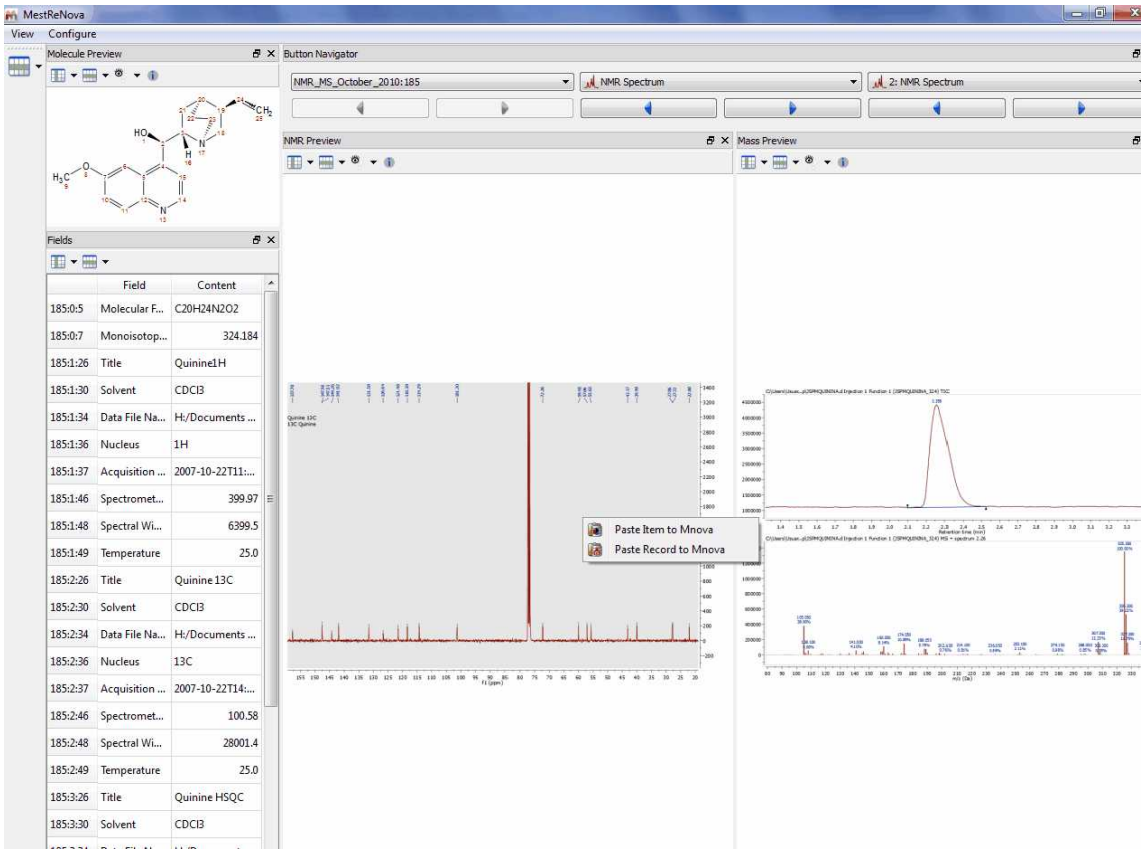
© 2024 MESTRELAB RESEARCH



Click on OK to get a window like this one:



Please bear in mind that you can also check any other spectrum (such as a 13C NMR) if we have previously added to the database, just by clicking on the 'blue arrow' (highlighted in red in the picture above):

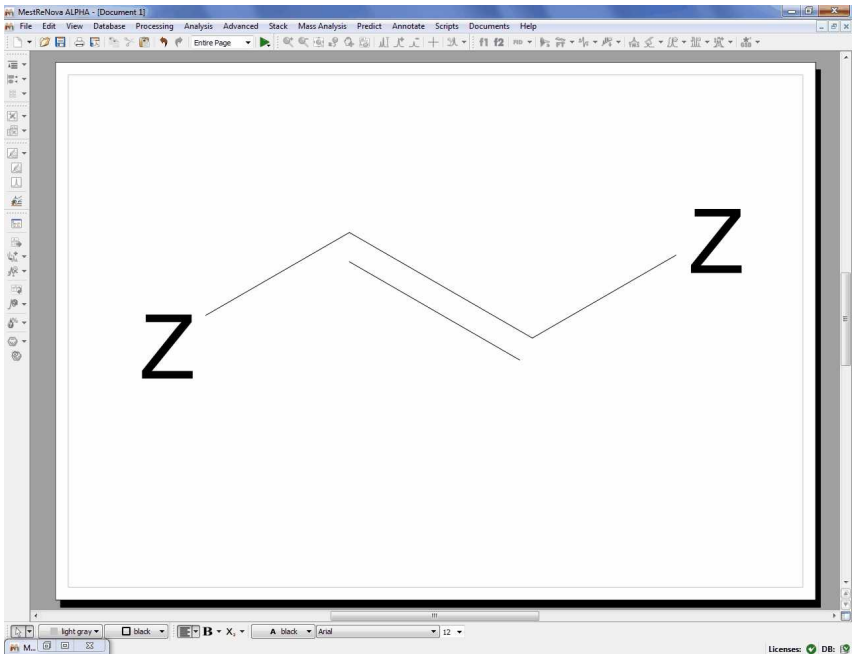


You can also copy the spectra or the molecules to a Mnova document just by double clicking on it (or by right clicking and selecting 'Paste Record or Item to Mnova').

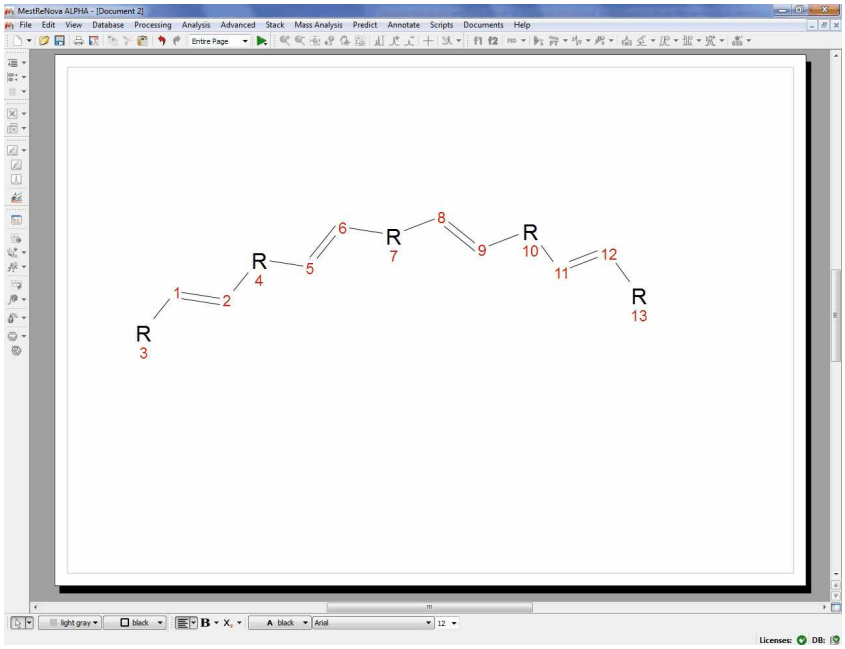
You can also use generic atoms like R (any chain, except H, D and T), Z (any chain; including H, D and T), A (any atom, except H, D, T), X (any halogen), Ht (any heteroatom) in the molecular structures. For example, to search for alcohols or amines, draw the corresponding group (and anything else you want the results to contain), add Zs (Z-NH_2, Z-OH), and do a structure search.

Here you can see an example to find compounds containing C=C double bonds:

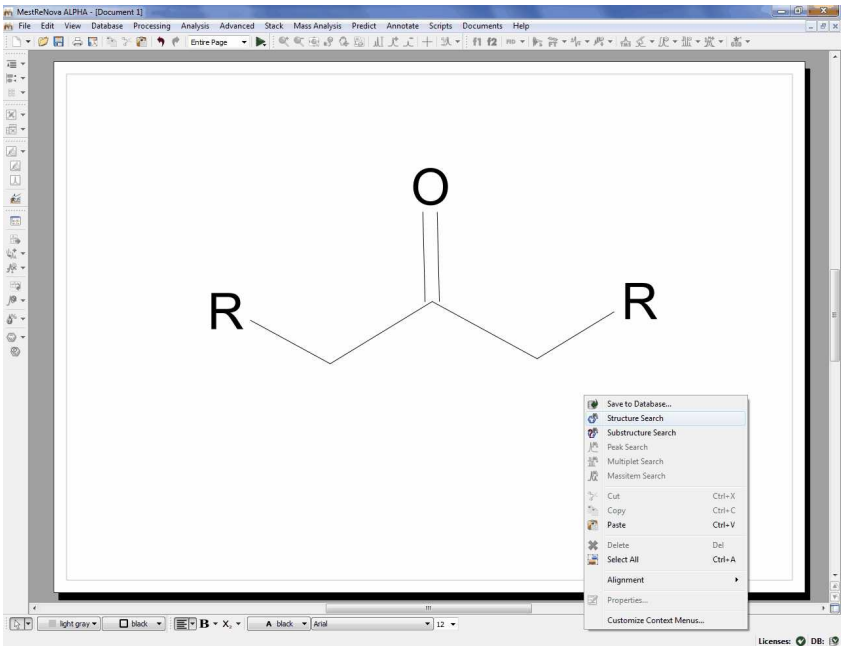
© 2024 MESTRELAB RESEARCH



If you want for example to find compounds with at least four C=C double bonds, you can draw this:

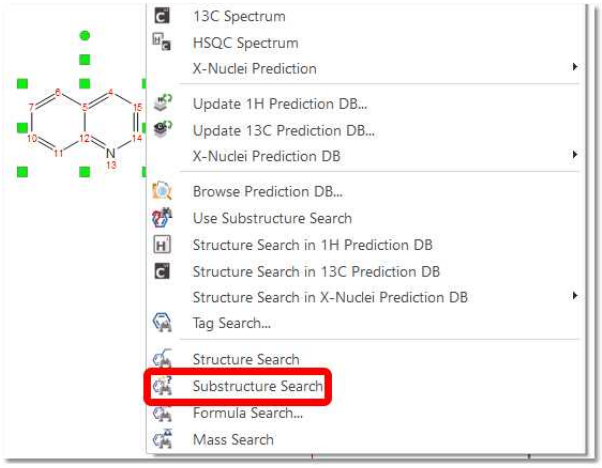


If you wan to search for ketones, you can draw this molecular structure:



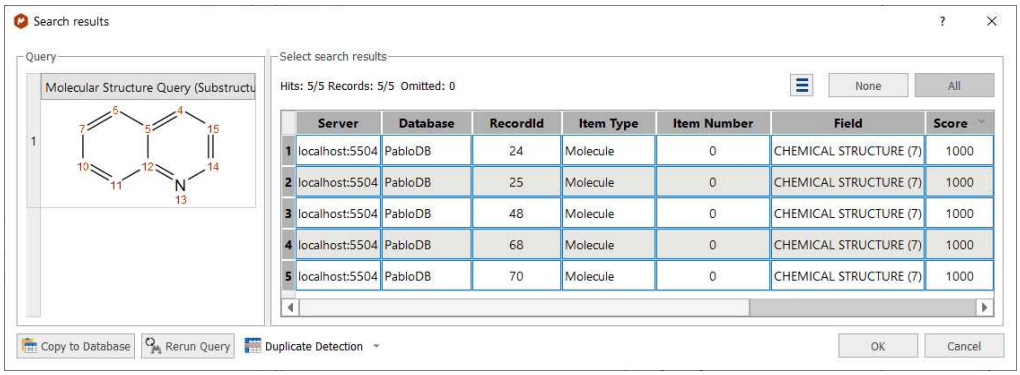
Substructure Search

The SubStructure Search feature is used to locate products by chemical structure. Simply copy a structure fragment, right click on it and select 'Substructure Search':



In that case we obtained 5 hits (for the records 24, 25, 48, 68 and 70):

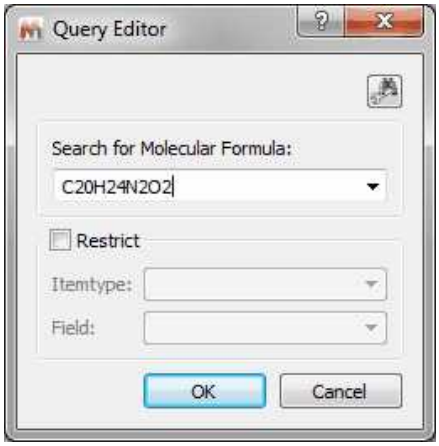
© 2024 MESTRELAB RESEARCH



You can also use generic atoms like R (any chain, except H, D and T) ,Z (any chain; including H, D and T) ,A (any atom, except H, D, T) ,X (any halogen) ,Ht (any heteroatom) in the molecular structures. For example, to search for alcohols or amines, draw the corresponding group (and anything else you want the results to contain), add Zs (Z-NH_2, Z-OH), and do a substructure search.

Formula Search

This feature will allow you to search for any specific molecular formula of your database. To do that, just type the molecular formula in the 'edit box' and click on OK. You can restrict (it is not mandatory) the search by using the 'ItemType' and 'Field' options:



The format for a **molecular formula search** is quite flexible. It can be explained with some examples:

- If you type: C1,4 H3,8 N1,1; the program will search for molecular structures with 1 to 4 carbons, 3 to 8 H and exactly one N; other elements are allowed but not necessary. Note that spaces can be omitted.

- Elements can be in lower case, as long as there are not 2 without space, so “c1,4h3,8n1,1” would be OK, whereas “c4,7no” is not, since “no” will be interpreted as “No”.

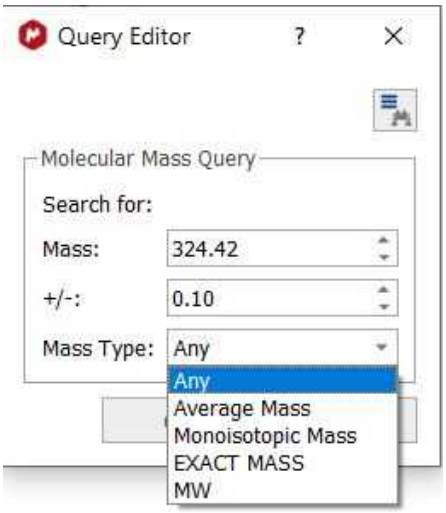
- Ranges can be omitted, so “C” is the same as “C1,1”, “C3” is the same as “C3,3”, “C,4” is the same as “C1,4” and “C2,” is the same as “C2,∞”

- The meta symbol “HT” can be used for hetero atoms, “X” for halogens, and “!” (exclamation mark) at the end of the formula to only allow specified elements. Thus, “C1,4 Ht3” searches for 1 to 4 carbons and exactly 3 hetero atoms, whereas “C3,5 H6,10!” would search for hydrocarbons with 3 to 5 C and 6 to 10 H.

© 2024 MESTRELAB RESEARCH

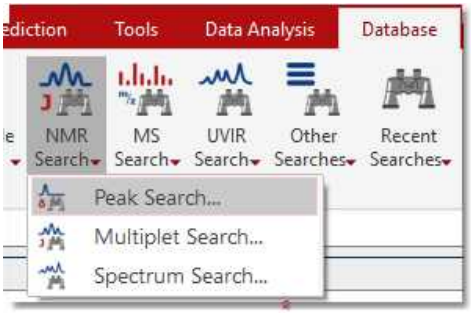
Mass Search

This feature will allow you to search in the Database (by using the Cosine method) for the Mass value of your molecular formula, with the capability to select a range and also the Mass Type (Average, monoisotopic, exact mass or Molecular weight)



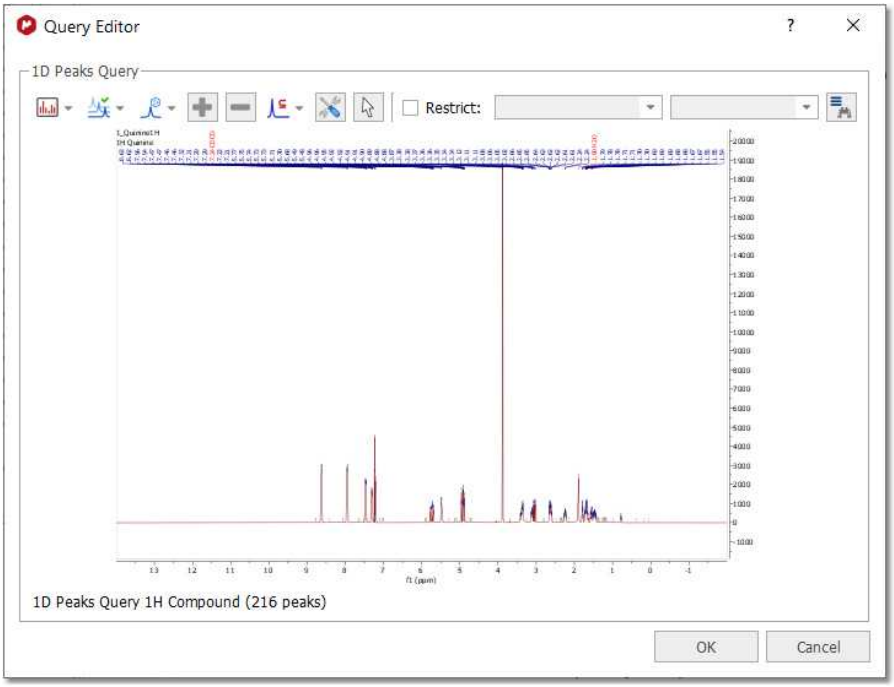
Peak Search

If you have a spectrum loaded in Mnova (this is not mandatory) and you select 'Database/NMR Search/Peak Search' (or right click/Peak Search'), the application will look for these peaks in the database. If you have expanded any area of the spectrum, it will only look for the peaks of the expanded area (and not for the whole spectrum). Please bear in mind that if you have forgotten to apply the Peak Picking to the expanded area, Mnova will do it (by using the automatic Peak Picking).



After having selected the Peak Search feature, the Query Editor dialog box will be displayed:

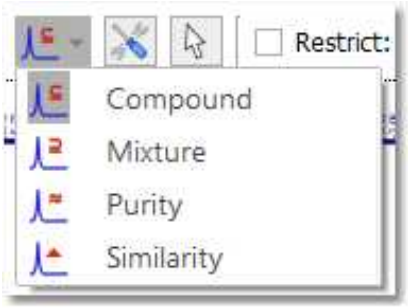
© 2024 MESTRELAB RESEARCH



Clicking on the 'Convert to advance query' button will allow you to convert it to an advanced search so that you can add new queries and combine them.

In the dialog box above, you will find a toolbar with different buttons:

- Select Display Mode: it will allow you to show the peaks table or the spectrum preview
- Peaks: to show 'Full Range' or only 'Visible Range' of the expanded region
- Select Peaks by Compound: to search only for some specific compound peaks
- Add/Remove: To add or remove peaks from the table
- Compound/Mixture/Purity/Similarity:



The peak and multiplet search dialogs have a tool button for changing the search mode. The default mode is "compound", which penalize peaks which are in the query but not in the db spectrum. The default mode can be set in the user preferences.

The scoring by using 'compound search' assumes that the sample is one compound and is based on how well a stored spectrum fits the total query.
Score (compound) = 1000*[number of peaks matching in query/total peaks in query spectrum]
Peaks or multiplets that are in the query, but not in the stored spectrum, count against the score. This works well for spectra composed primarily of a single compound, but can lead to problems and excess “bad” hits when searching against a mixed sample. Another way that would be useful for binary, tertiary, etc. type mixtures would be a “content” score in which any potential hit is scored based on how well it’s peaks compare back to the queried spectrum. To get a “1000” for a

© 2024 MESTRELAB RESEARCH

"content" score all peaks in the stored spectrum must be present in the queried spectrum, but there is no penalty for extra peaks in the query spectrum. It is almost a reverse grading of the spectra

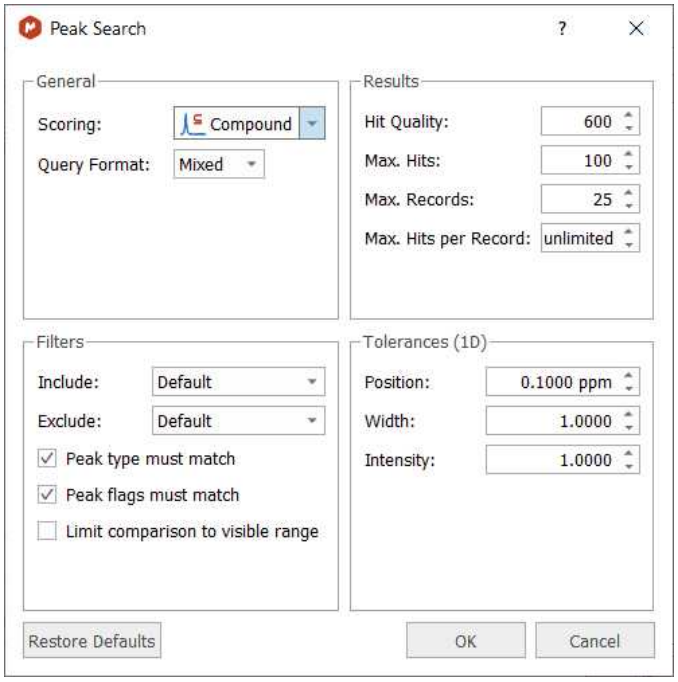
For example: If you have a ~50:50 mixture of compound A and compound B and run a query, you get what would be approximately a "500" scores for both compounds using the traditional scoring method. If you were to additionally display the scoring method proposed above, each stored spectrum would get "1000" scores for a "content" grade. This way of scoring would better indicate the presence of particular compounds in the analyzed sample. This would also additionally help to completely rule out compounds that get low grades in the traditional scoring method, but show up in the scoring due to coincidental overlap of multiplets/peaks.

Mixture means that peaks in the query spectrum but not the library spectrum will be ignored.
Score (mixture) = 1000*[number of peaks matching in DB spectrum/total peaks in DB spectrum]
This is for cases where the query spectrum might be a mixture containing additional compounds and the database has built with pure compounds. It will penalize peaks that are in the database spectrum but not in the query.

Purity Search: unclean spectra in the db (with many spurious peaks) will get low search scores.It will search directly for the best match of the query spectrum.
Score (purity) = 1000*[number of peaks matching in query / [(total peaks in query spectrum + total peaks in DB spectrum)/2]

Similarity: intelligent algorithm to search 1D spectra by comparing the area behind the curves.

- Parameters: to set the Peak Search method (Compound, Mixture, Purity or Similarity), some settings for the Results, Filters and Tolerances:



The 'Query Format' could be Legacy, JSON or Mixed:
Legacy: Used for simple peak search requests in a simple line-oriented format. Number of peaks to search for, followed by one peak per line
JSON: Used for simple peak search requests in JSON format. MnServer <r420 only understands legacy format. MnServer >r420 understands both legacy and JSON format.

Hit Quality: Minimum score for search hits to be included in the results.
Max. Hits: Maximum number of hits returned by a search.
Max. Records: Maximum number of records returned by a search
Max. Hits per Record: Maximum number of hits returned per the same record.

© 2024 MESTRELAB RESEARCH

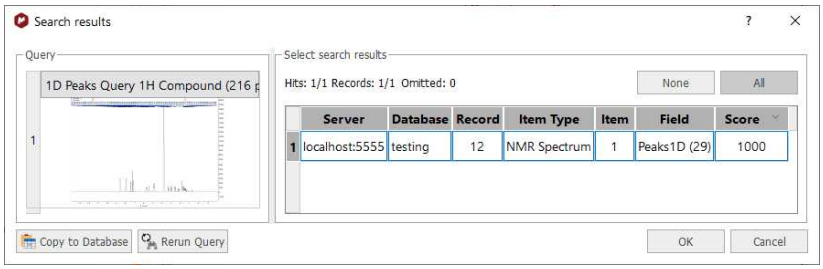
Use the filters scroll down menus to select what kind of peaks type you want to include (Compound, artifacts, impurity, solvent, etc) and what type of peaks flags you want to exclude (hidden, weak, rotational, labile, etc). Check the applicable boxes to take into account the 'peak type' and/or the 'peak flag' for the match. You can limit the filters to only the visible range, just by checking the applicable box.

From the Tolerances section, you will be able to select the search tolerance for the peak position (as an absolute value).

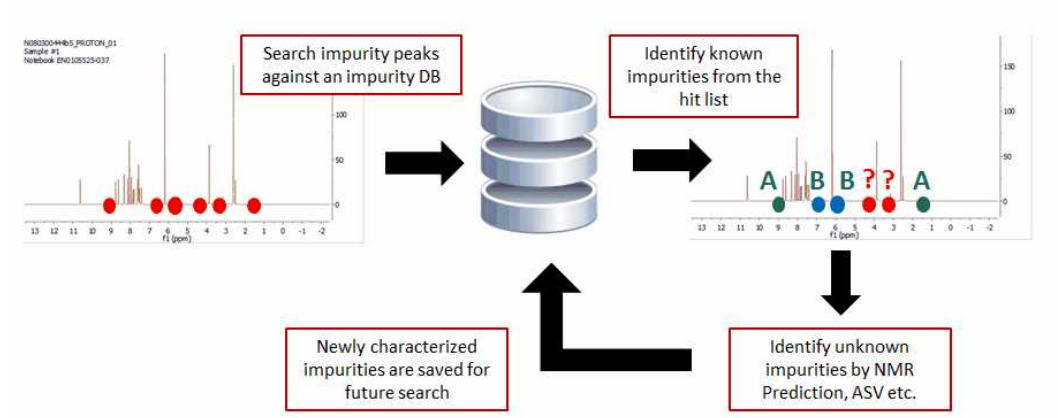
The intensity and width are multiplicative tolerances, so for example if you include a tolerance width value of 0.8 and the peak query has a width value of 5; you will get a hit if in the database you have a peak with a width value between 5-0.8*5 and 5+0.8*5 (that is, between 1 and 9). A tolerance value of 1.0 would be a 'special case', where the intensity and width will be ignored to get a match.

- Select from document: to select the spectrum if you are in a document with several datasets.

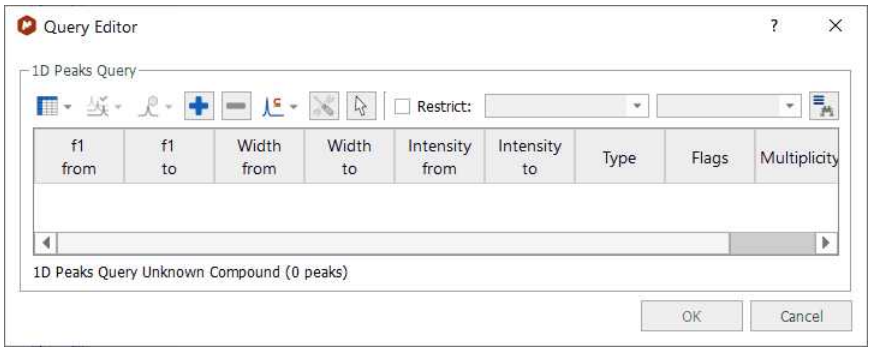
After having clicked on the OK button, the peak search will be run. In the example below, we have obtained a match for the compound number 25, with a score of 1000 (maximum value):



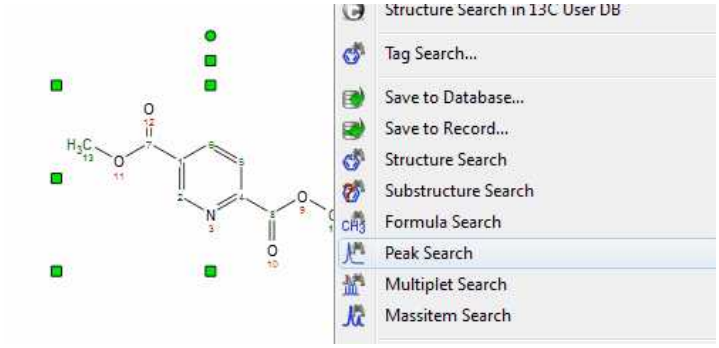
The Peak Search feature (combined or not with the Verification plugin) can be very useful to identify impurities in the datasets.



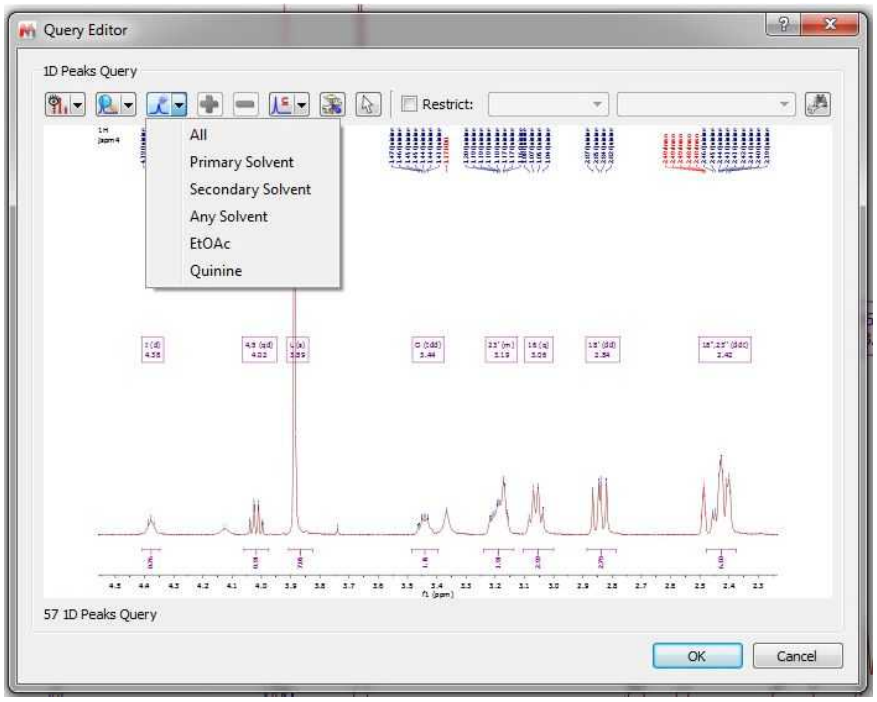
Please note, that you can also use the 'Peak Search' feature without any spectrum loaded. In that case, you will need to type in the table, the information of the peaks that you want to find in your database:



Alternatively, you can also use the 'Peak Search' feature from a molecular structure with assignments:



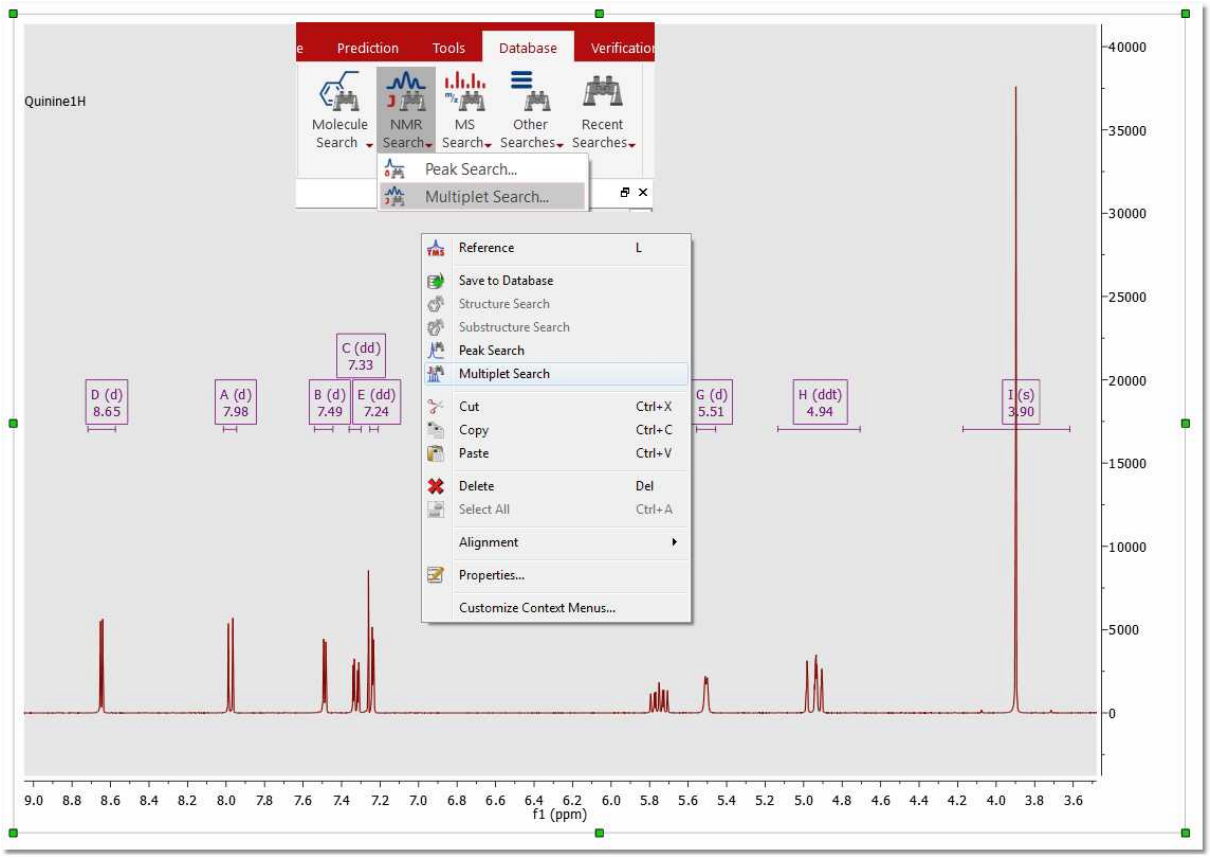
If your spectrum has more than one compound, you can run peak searches of only one of the compounds:



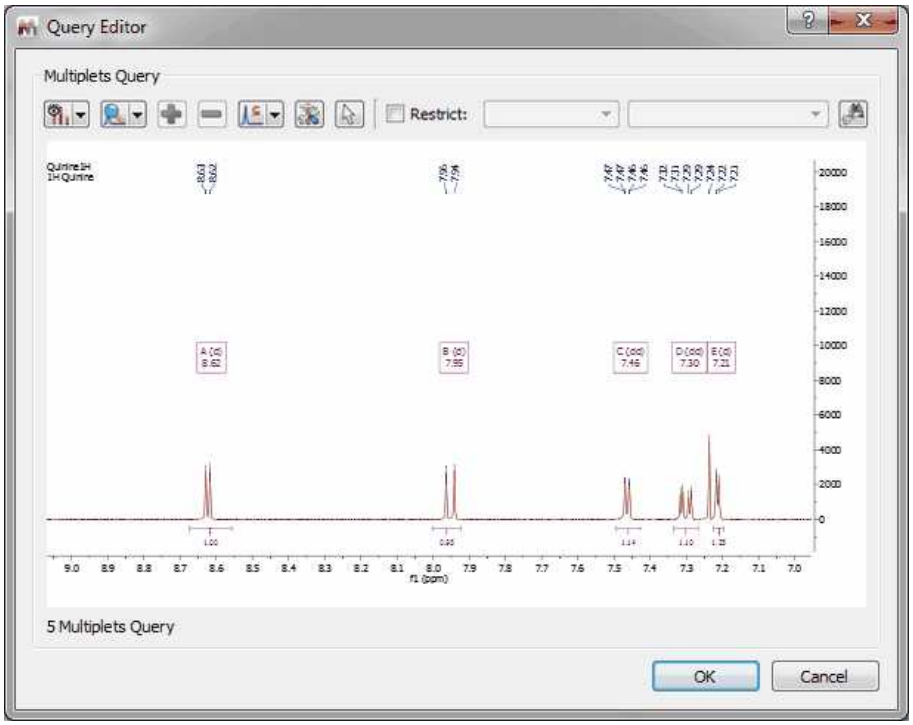
Multiplet Search

© 2024 MESTRELAB RESEARCH

To search for multiplets, you will only need to open a spectrum with the desired multiplet analysis and finally select 'Multiplet Search' (after having right clicked on the spectral window):

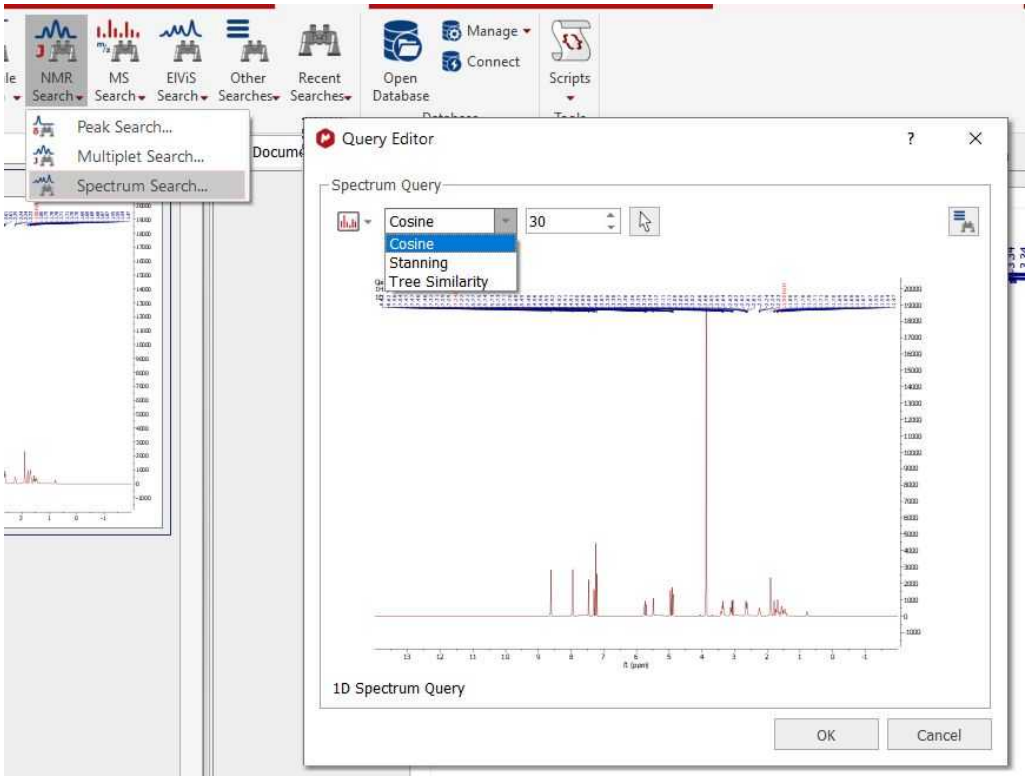


After that, the query editor will be displayed, to how you which multiplets will be searched. This dialog box is similar with the 'Peaks Query Editor' (explained above)



Spectrum Search

Spectrum search allows to find spectra in the database which are "similar" to the query spectrum, based purely on shape of the spectrum curve.



© 2024 MESTRELAB RESEARCH

Note that this is not the optimal or recommended method to find spectra in the database. We recommend to do a rigorous peak analysis on any spectra saved to database as well as the query spectrum, and then use the Database/Peak search to identify similar spectra.

The methods under spectrum search are experimental and only for unusual situations where a full peak analysis may not be feasible.

The following similarity measures are available:

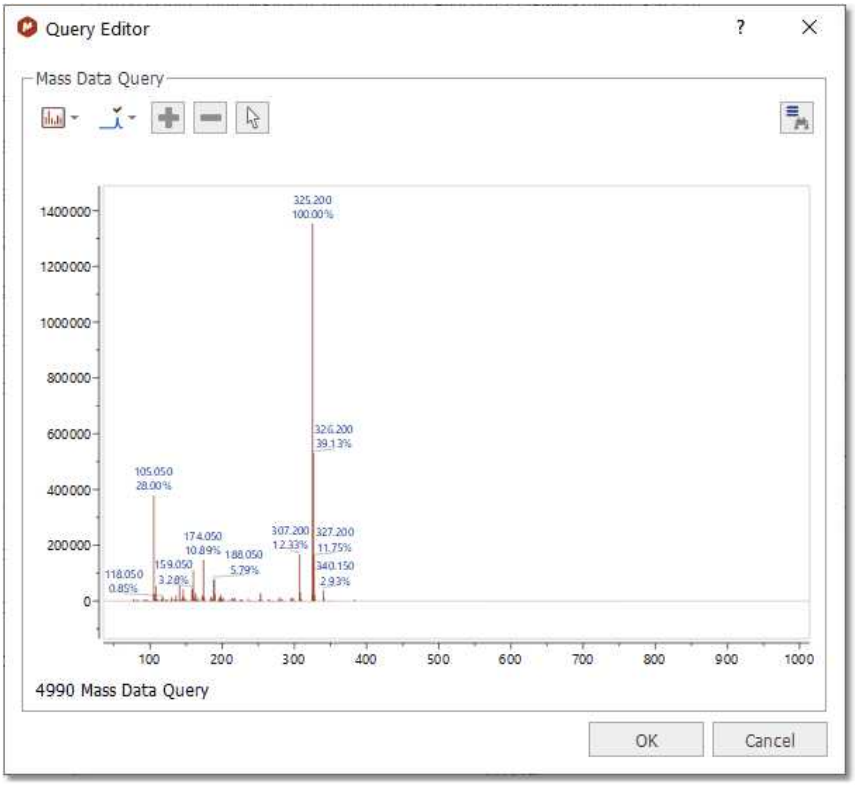
- Cosine similarity: The classic cosine of the angle between two vectors. The default parameter 30 refers to the number of bins to be used for the query. Higher values mean better discrimination at the cost of lower speed
- Stanning: A mathematical sound method to calculate the distance / similarity between two 1D spectra comparing the areas under their curves to each other.
- Tree similarity: A tree-based method for measuring similarity between NMR spectra.

MS Search

If you have a MS dataset loaded in Mnova and you select 'Database/MS Search' (or right click/MS Search'), the application will look for these MS peaks in the database.



After having selected the 'MS Search' feature; the query editor will be displayed:



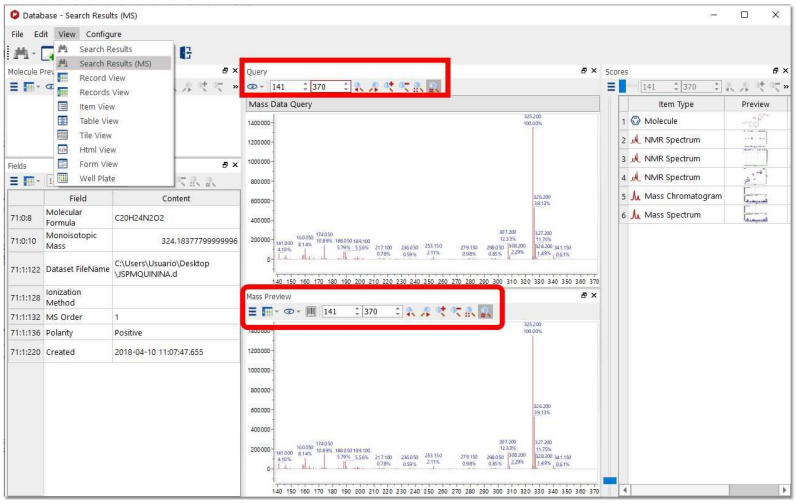
From here, you will be able to change the view (preview or plain text), or to select the tolerance of the Mass Item Searches.

Finally, click on the OK button and wait for the result:

© 2024 MESTRELAB RESEARCH



For MS searches, we recommend to use the Search Result (MS) view, which will allow you to compare the Query and the Match and easily apply zoom in, zoom out or to synchronize the retention time ranges by using the zoom toolbars:

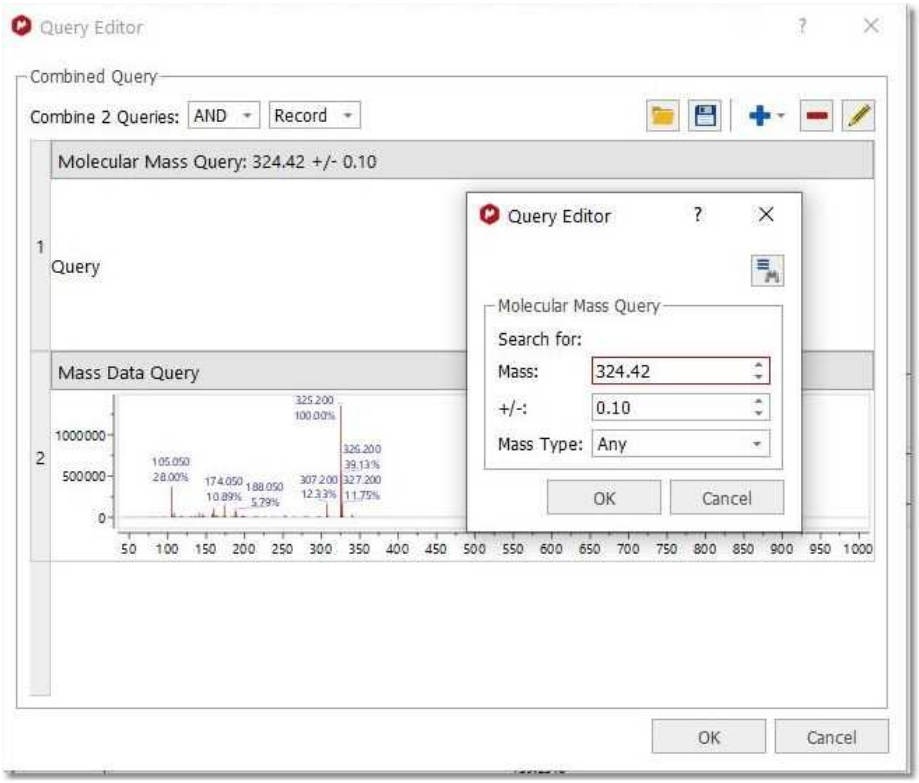


NOTE: The Database definition within the MassItem, will include information about: RT, Scan, Type, Height, Area, Total Height %, Total Area %, Start Time, End Time, for each chromatographic peak corresponding to that particular MassItem in each Chromatographic run. The database definition accommodates up to 8 traces (TIC, Total UV (or DAD or PDA), ELSD, CNLD + 4 UV traces as specific wavelengths).

© 2024 MESTRELAB RESEARCH

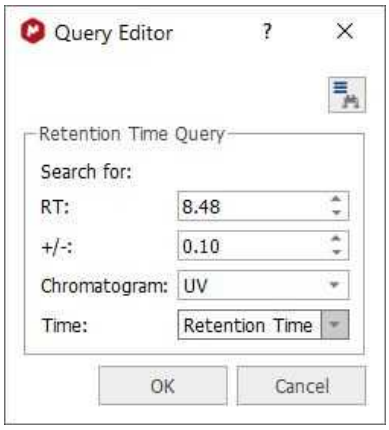
MS + Molecular Mass Search

The MS+ Molecular Mass Search will allow you run a combined mass spectrum and molecular mass value query.



Retention Time Search

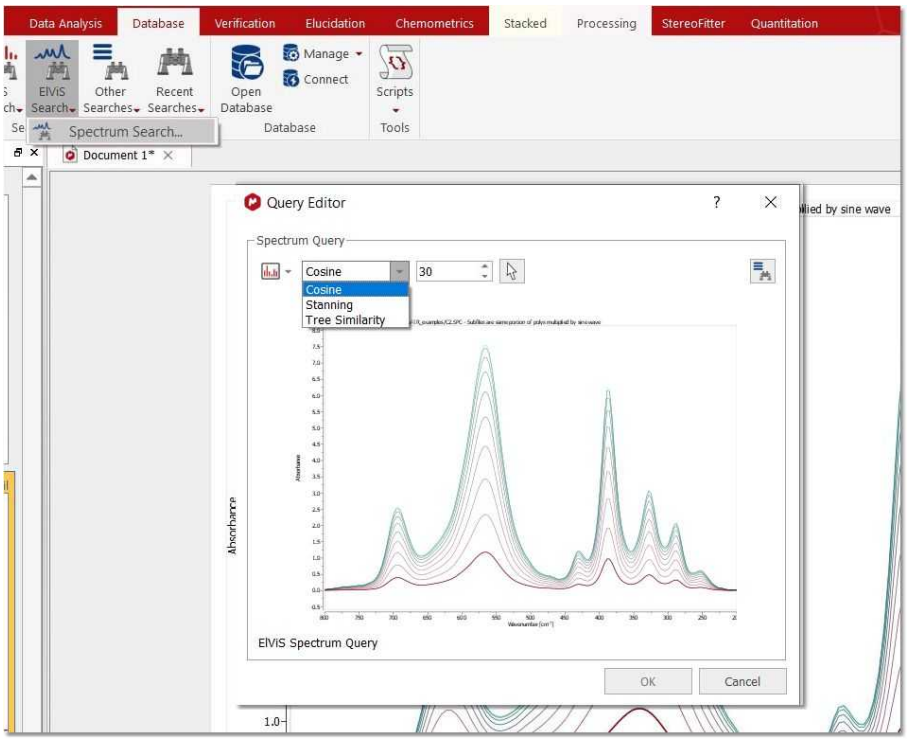
The´Retention Time Search´ will allow the user to enter a range of retention times and choose to work within the ´RT´, ´Start time´ or ´End time´ fields of the DB, within the Itemtype MassItem.



Elvis Search

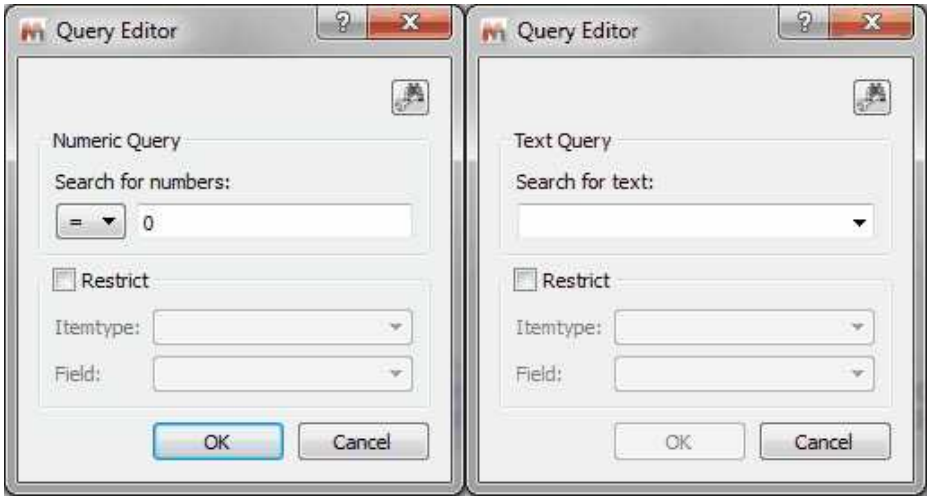
Use this option to search for your UV, IR or Raman datasets by using 'Cosine', 'Stanning' or 'Tree similarity' methods:

© 2024 MESTRELAB RESEARCH



Numeric and Text Search

You can search for numbers or for any text in the database just by selecting one of these options:

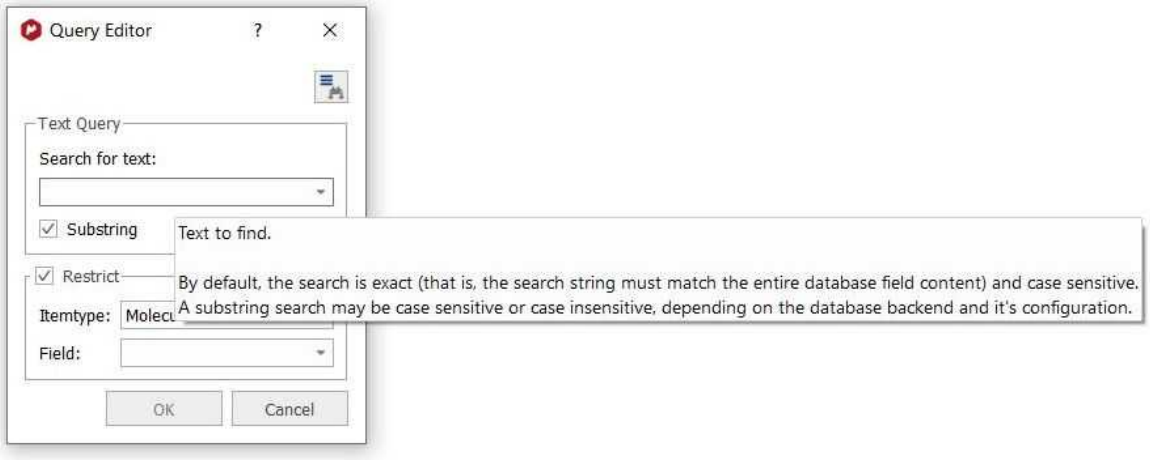


Please bear in mind that the numeric search can contain ranges like “10 < 20” or “10.2 <= 20.5”.

To search for all the items of your database, type "-" (without the quotation marks) in the search dialog.

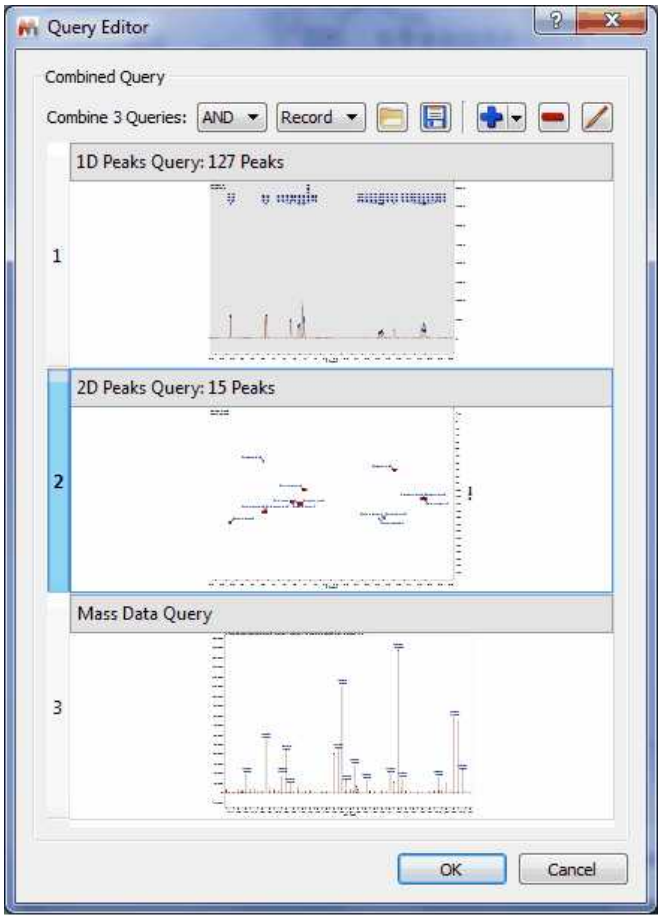
Please bear in mind that by default the text search will be exact and case sensitive.Checking the substring box could allow you to do a case sensitive or insensitive search depending on your DB backend.

© 2024 MESTRELAB RESEARCH



Advanced Searches

This option will allow you to combine several queries (in the same document) for the same search. In the example below, you can see how we are combining the peaks of 1D and 2D NMR datasets with the Mass spectrum.



You can look for any of the 3 queries (by selecting *'OR'* in the applicable scroll down menu) or for all the queries (by selecting *'AND'*). You can also load/save combined queries in XML format, or to add/remove/edit the queries by using the applicable buttons of the dialog box.

© 2024 MESTRELAB RESEARCH

The settings specifies how the search results from the subqueries are combined into the single result of the advanced query.

Let A be the results returned by subquery a, and B the results returned by subquery b. Let R be the final search scores of the advanced query. Then, With Combinator = "AND", score(R) = min(score(A), score(B)) With Combinator = "OR", score(R) = max(score(A), score(B))

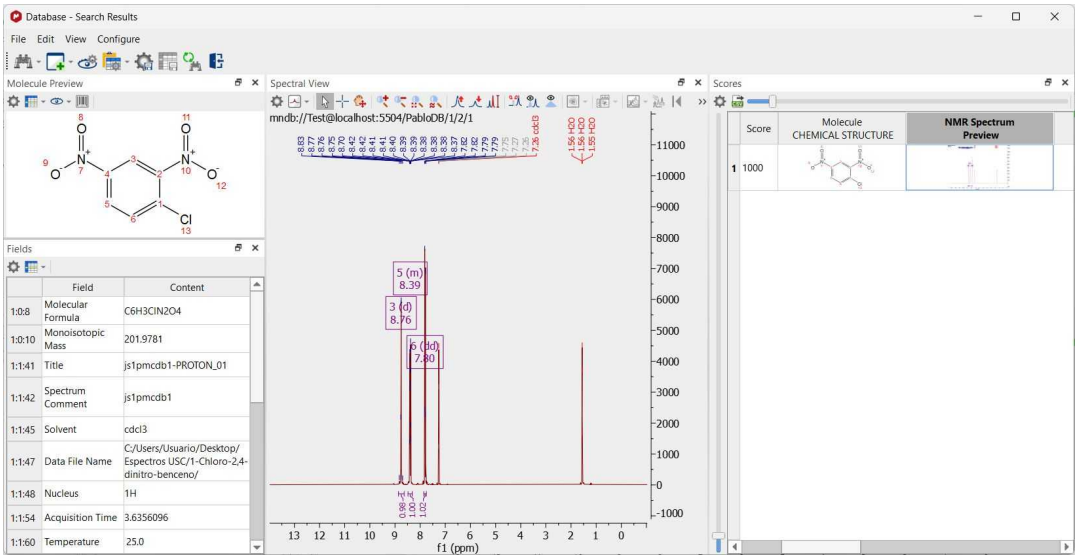
With combination = records, A and B must be matches within the same record for the subquery results to be combined.
With combination = items, A and B must be matches resulting from the same item (in the same record) for A and B to appear in the final result.

So, with your advanced query where for example subquery a="1D Peaks>2" and subquery b="1D Peaks<3", when you select combinator "Records", you will get matches for records which may contain one msitem with 1D Peaks 5 (5>2, match for a) and another msitem with 1D Peaks 1. (match for b). When you select combinator "Items", these matches are excluded as A and B need to occur in the same item for the match to appear in the final result, so you will only get items where both conditions hold true for the same item, which is in effect msitems with both 1D Peaks>2 and 1D Peaks<3.

Combinator "Records" is useful if you want to combine conditions from different items, for example: search for records where "Methyl" appears in the molecule name and which have an NMR spectrum with 1D Peaks between 1 and 3.

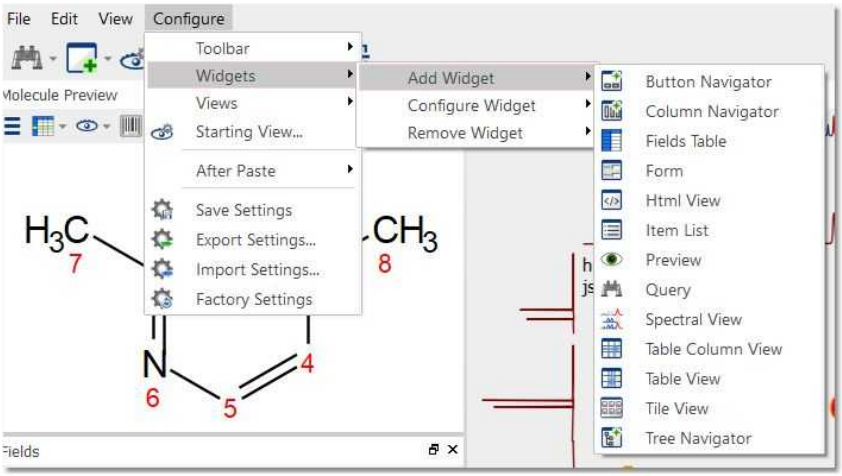
16.5 View Results

Once you searched your items in the database, you will get this view by default with the results:

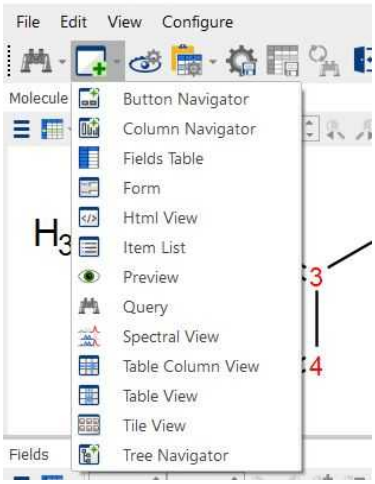


The default view contains a preview of the molecular structure, a table with several fields, a spectral view and the Scores list, which will allow you to change the compound or the spectrum of the preview (just by clicking on the applicable item of the list).

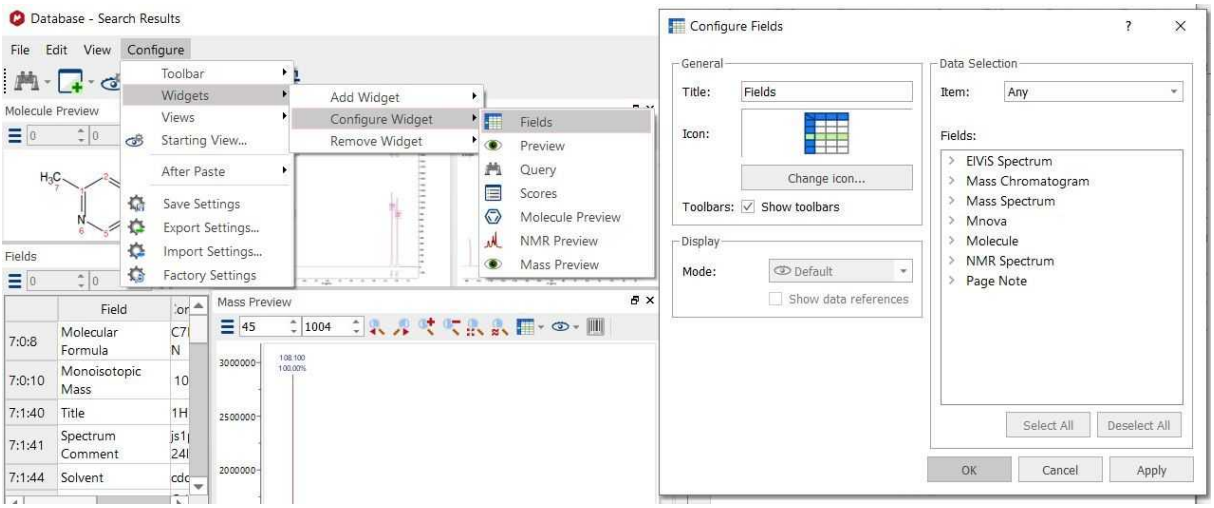
You can add new widgets to the 'Search Results View' by following the menu 'Configure/Widgets/Add Widget'.



You can also add widgets from the toolbar:

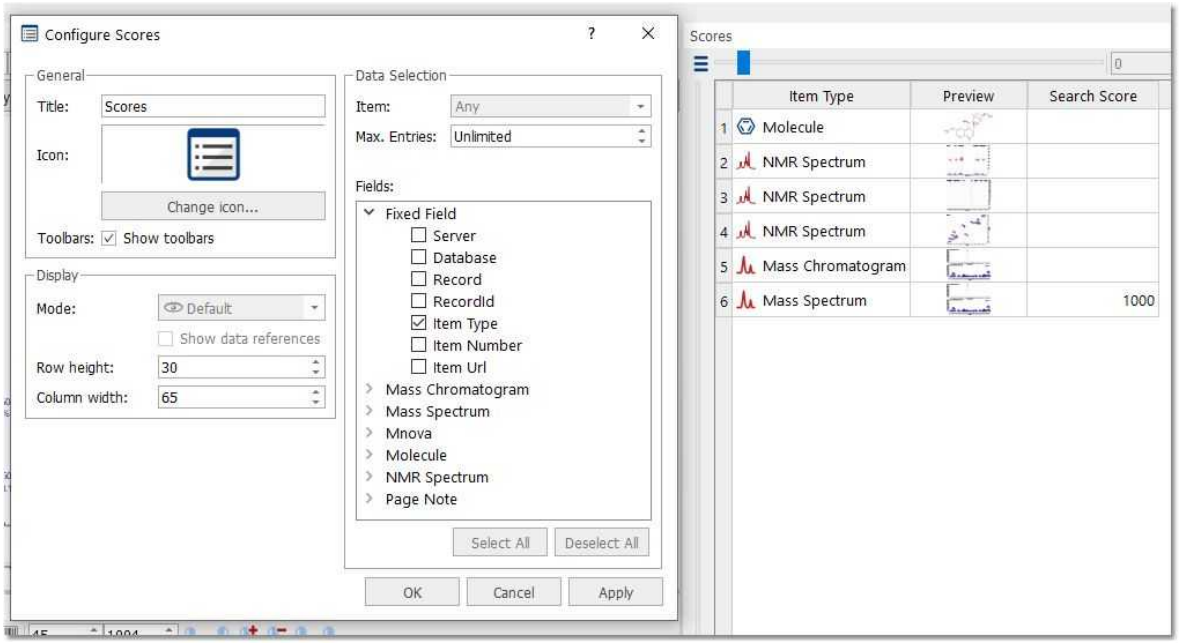


You can select the desired fields to be displayed by clicking on the 'configure button' or by following the menu 'Configure/Configure Widget/Fields' and checking the desired fields from the list:

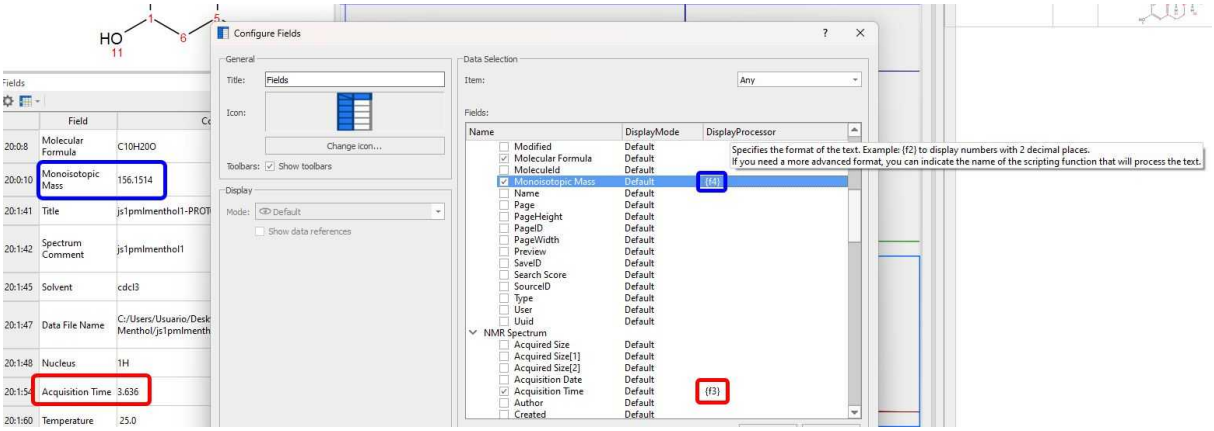


© 2024 MESTRELAB RESEARCH

You customize the 'Scores list' to select the maximum number of items that are displayed by record. By default is set "Unlimited" (show all the existing items per record), but you can change it from "Configure Widget\Scores". From here you could also select the desired fields to be displayed on the Score list:



The number of significant digits displayed in the DB Browser can be customized from the 'Configure Fields' dialog, under the 'DisplayProcessor' column by typing {f#} (i.e. {f2} for 2 decimal places, {f3}, for 3 decimals, etc) :

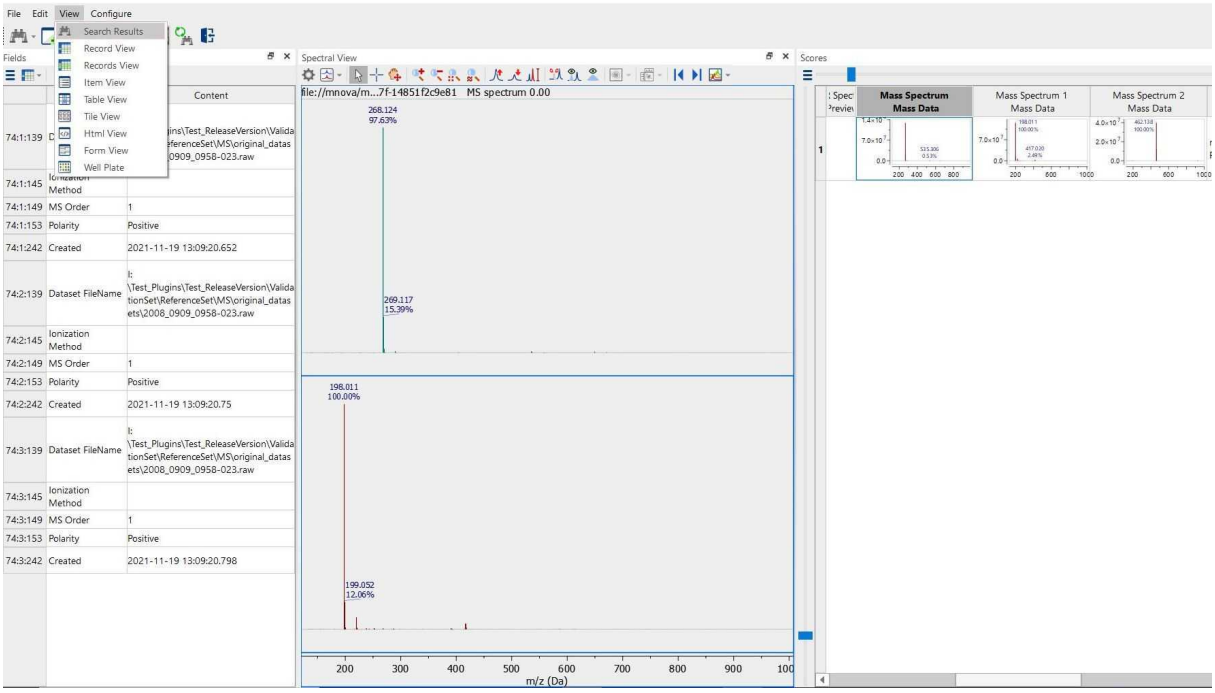


The default template is called 'Search Result', but you will find additional templates by following the 'View' menu (or by clicking on the 'binoculars' scroll down menu):

© 2024 MESTRELAB RESEARCH

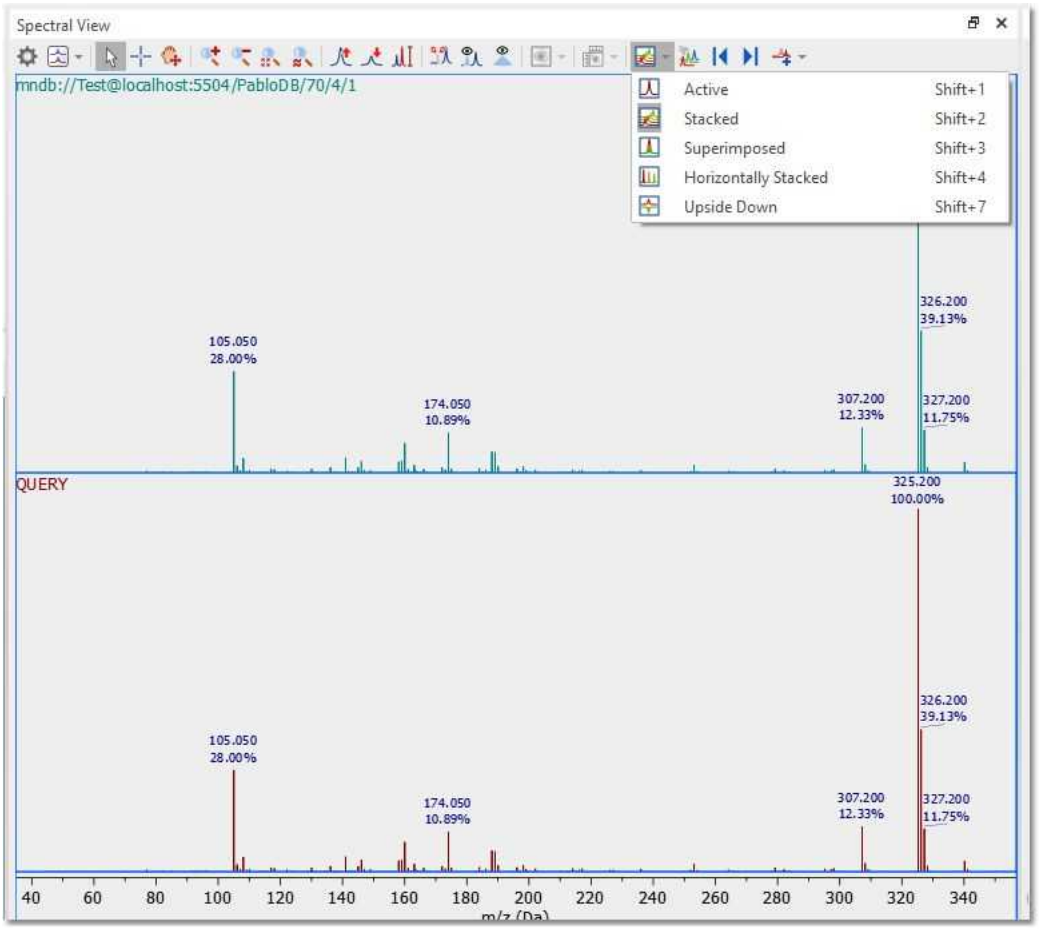


For MS searches, we recommend to use the Search Result view, which will allow you to compare the Query and the Match and easily apply zoom in, zoom out, keep the scale or synchronize the retention time ranges by using the toolbars:

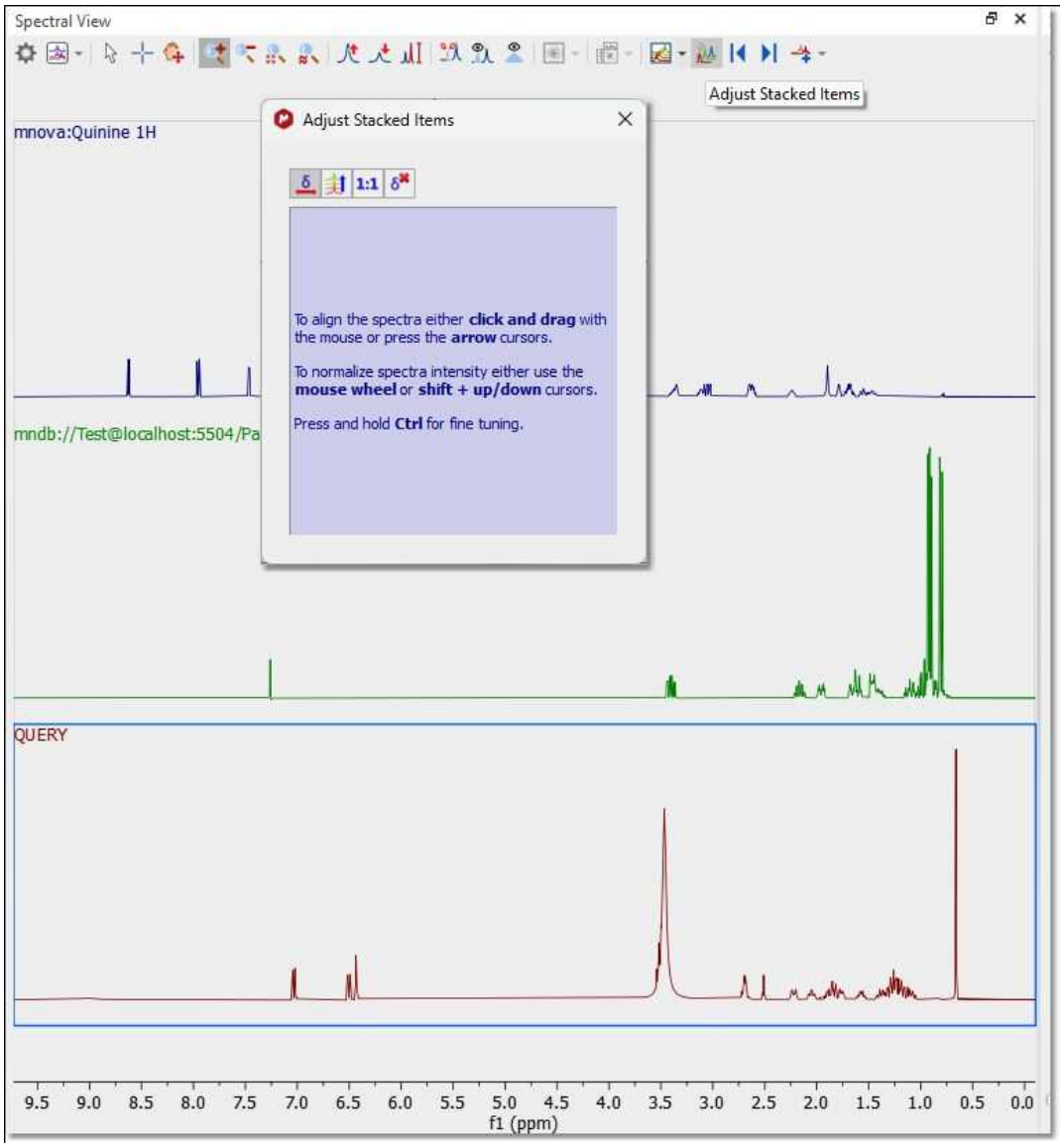


From the spectral viewer, you can also change the view and get the query and the hit in stacked or superimposed modes:

© 2024 MESTRELAB RESEARCH

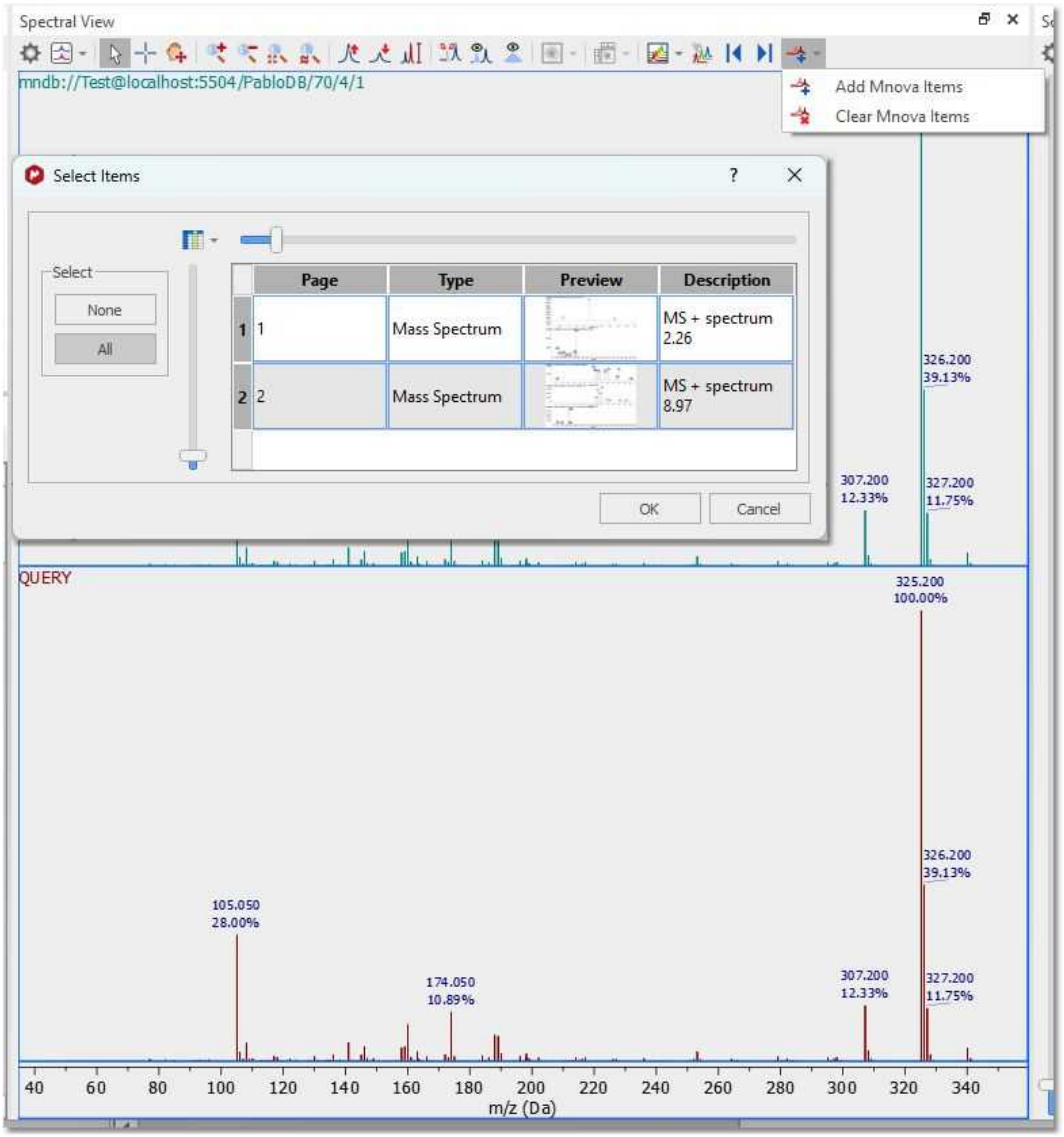


Selecting 'Adjust Stacked Items' option will allow you to align the spectra and normalize their intensities:

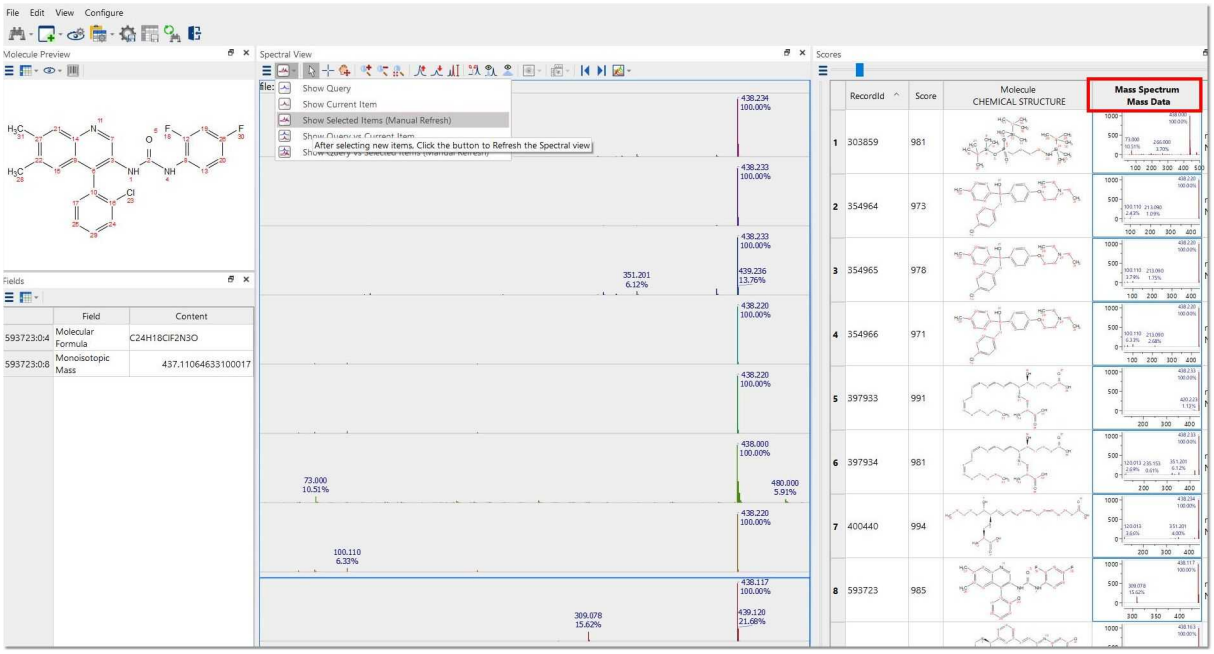


Clicking on the 'Add Mnova Items' button will allow you to load any loaded spectrum from your Mnova document to add them to the stacked plot:

© 2024 MESTRELAB RESEARCH

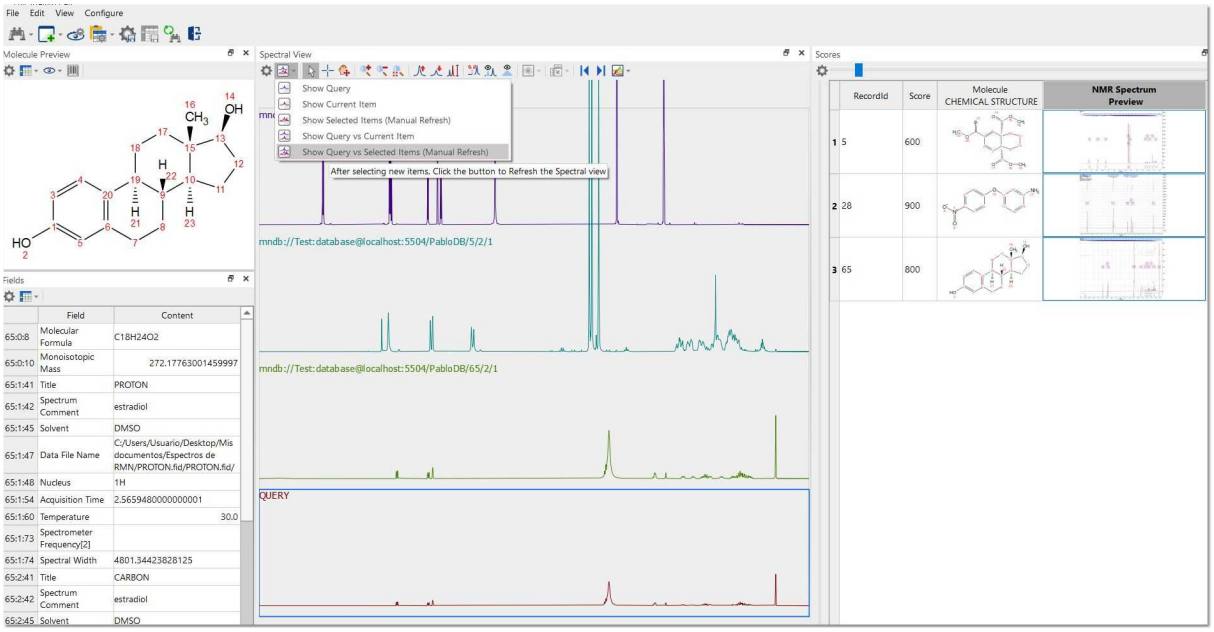


You can also select several previews from the 'Score' widget and stack them with the query by selecting the appropriate option:



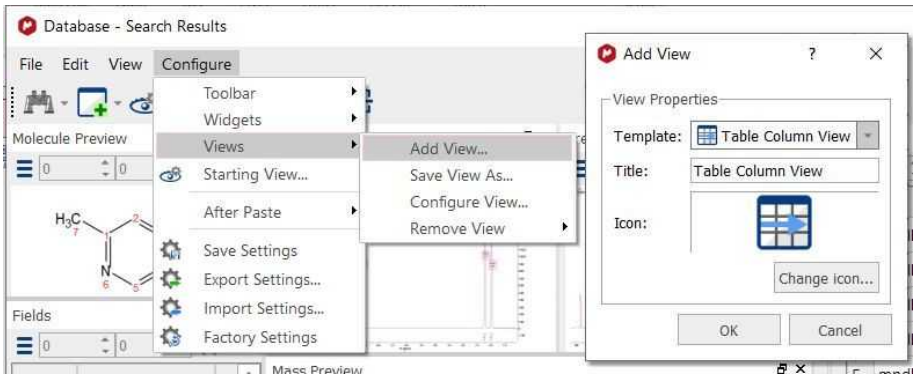
-Show Query: displays an interactive spectrum of the Query, if the query exists.
-Show Current Item: shows an interactive spectrum of the current selected item.
-Show Selected Items: shows an stacked spectra of all selected items of the same type.
-Show Query vs Current Item: shows an stacked spectra of the current selected item and the query .
-Show Query vs Selected Item: shows an stacked spectra of all selected items of the same type and the query.

This feature can also be used for chromatograms, NMR and ElViS spectra:

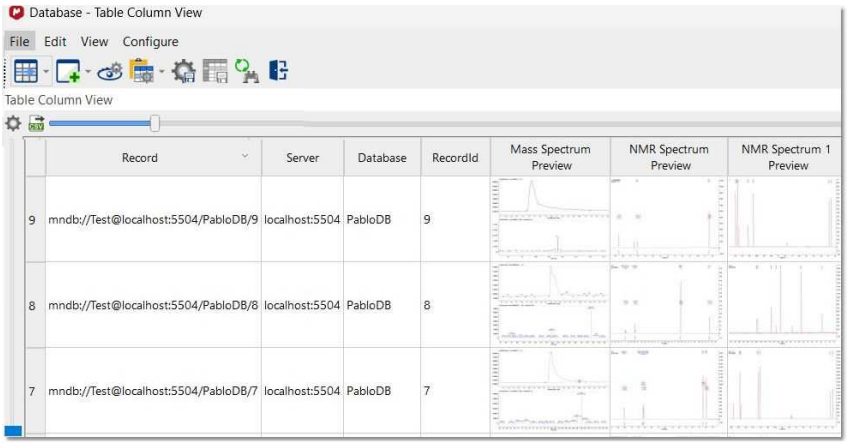


You can create new views by following the menu 'Configure/Views/Add View'

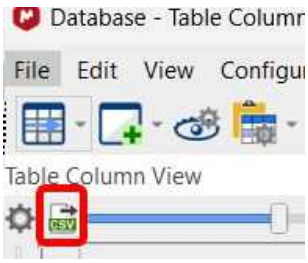
© 2024 MESTRELAB RESEARCH



The 'Table Column view' will display only one row per Db Record. The user will be allowed to sort and customize the columns. If a record has several items of the same type, the table will display one column per item (i.e NMR1, NMR2, NMR3, .. MS1, MS2...).

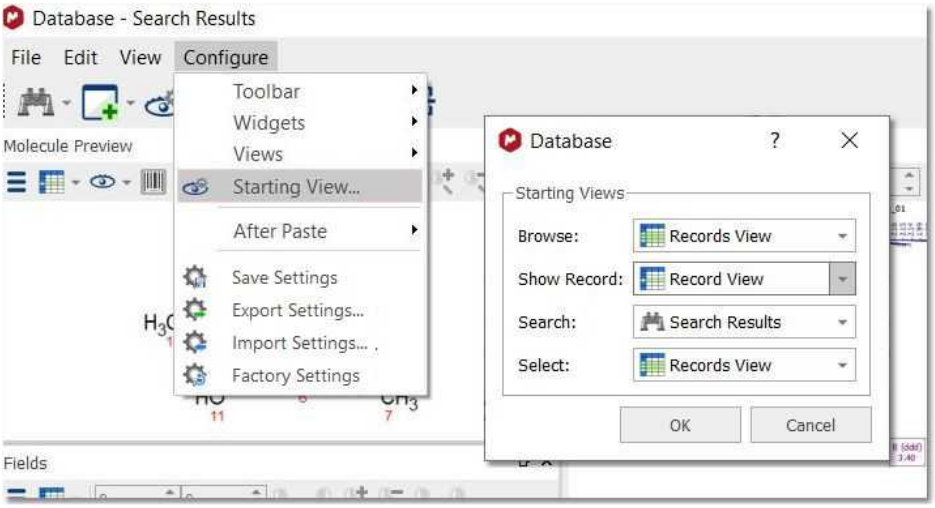


Click on the 'Export to CSV' button to export the table as a CSV file:

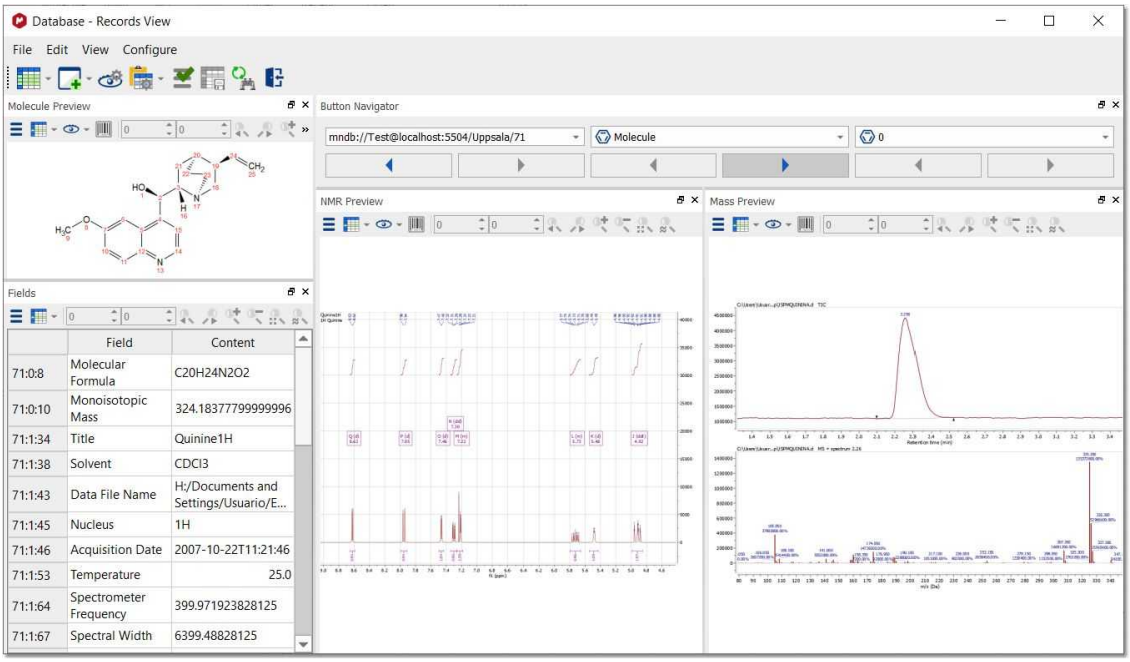


In the Configure menu, you will find an option to select what template you want to use to display your results when you are browsing, showing records, searching or selecting:

© 2024 MESTRELAB RESEARCH

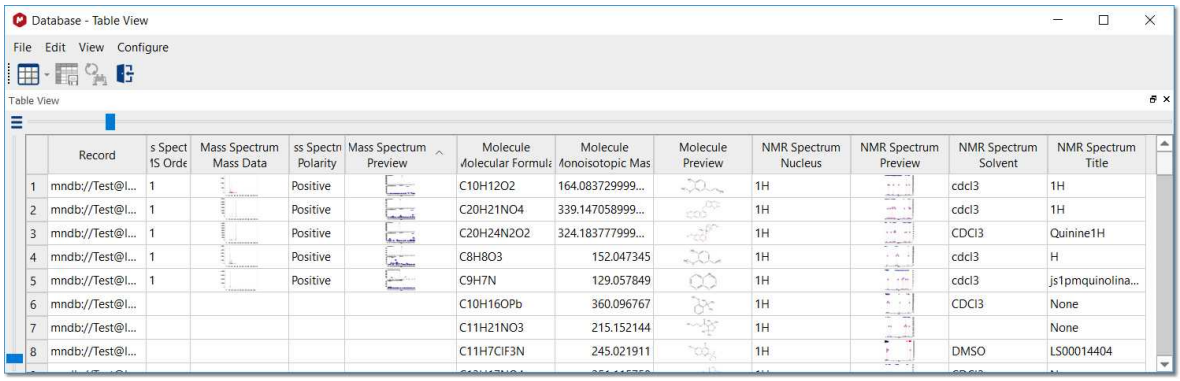


Here you can see for example the 'Records view', with information about molecular structure, a table with several fields, a spectral view and a button navigator to change the compound or the spectrum of the preview.

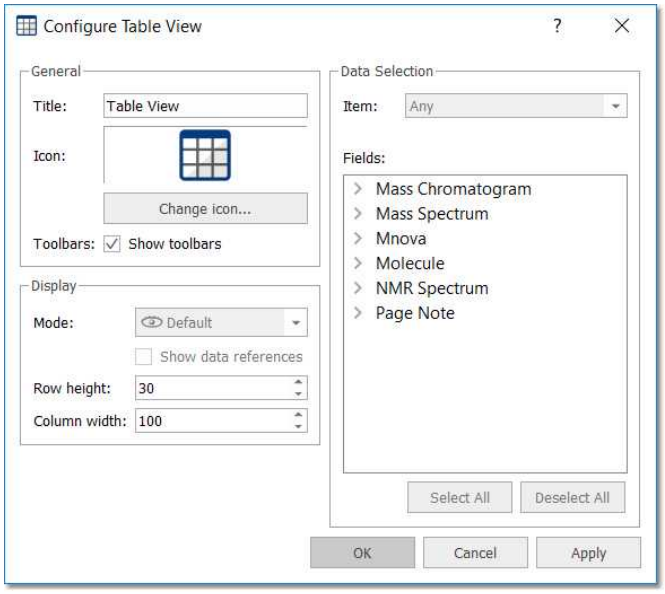


Here you can see the Table view, with information about the compound ID, molecular Formula, Molecular Preview, Title, Solvent, Nucleus, Frequency and Spectrum Preview. You can sort the columns just by clicking and dragging the headers.

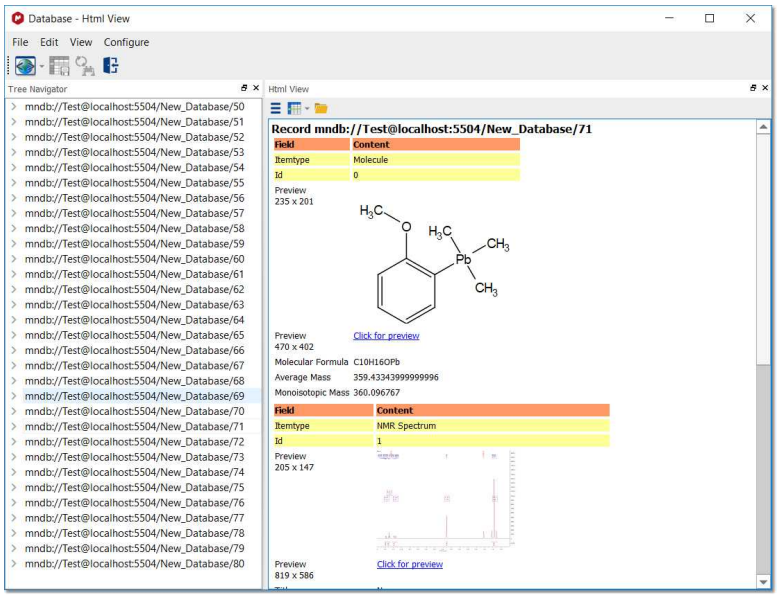
© 2024 MESTRELAB RESEARCH



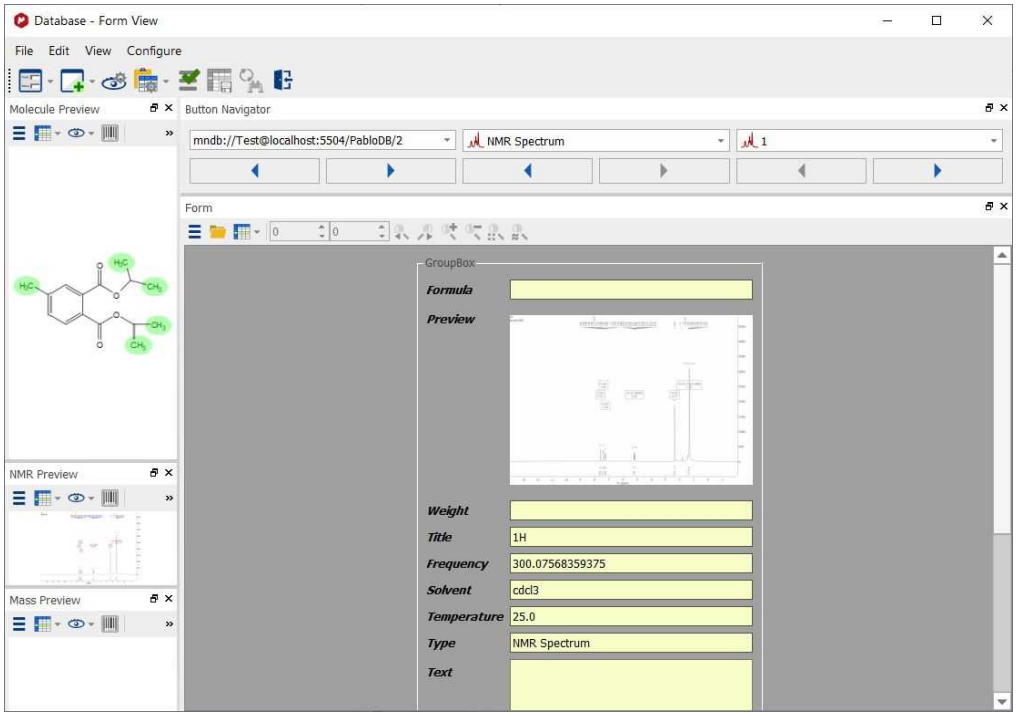
Of course you can add additional information to the table view just by following the menu 'Configure/Configure Widget/Table View' and checking the applicable fields from the list:



You will find below the HTML view:

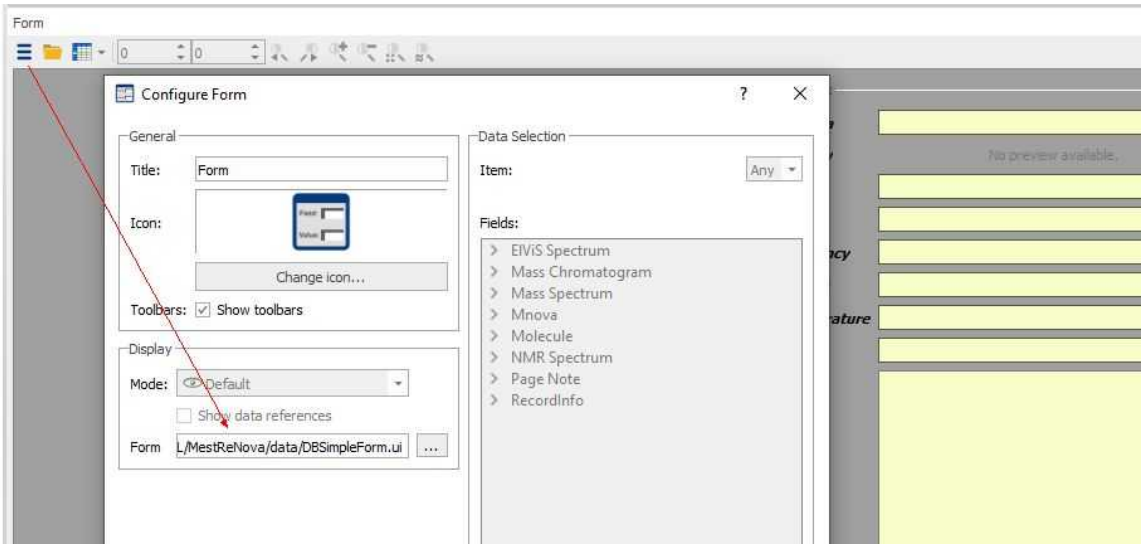


There is also a 'Form View':



This view can be customized by creating a new.ui file and assign this file as the form layout file in the form widget (from the 'Configure Form' dialog):

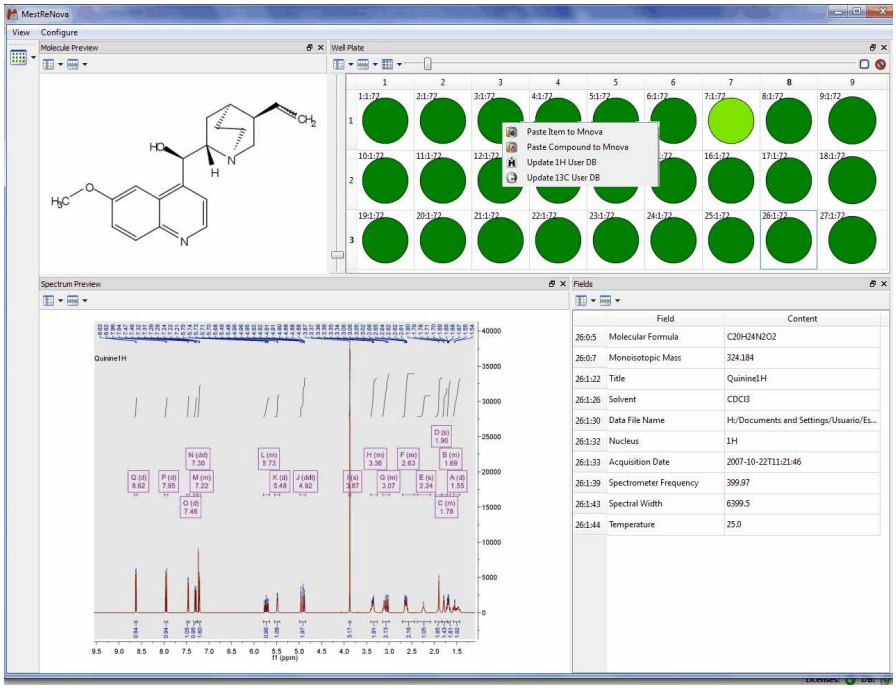
© 2024 MESTRELAB RESEARCH



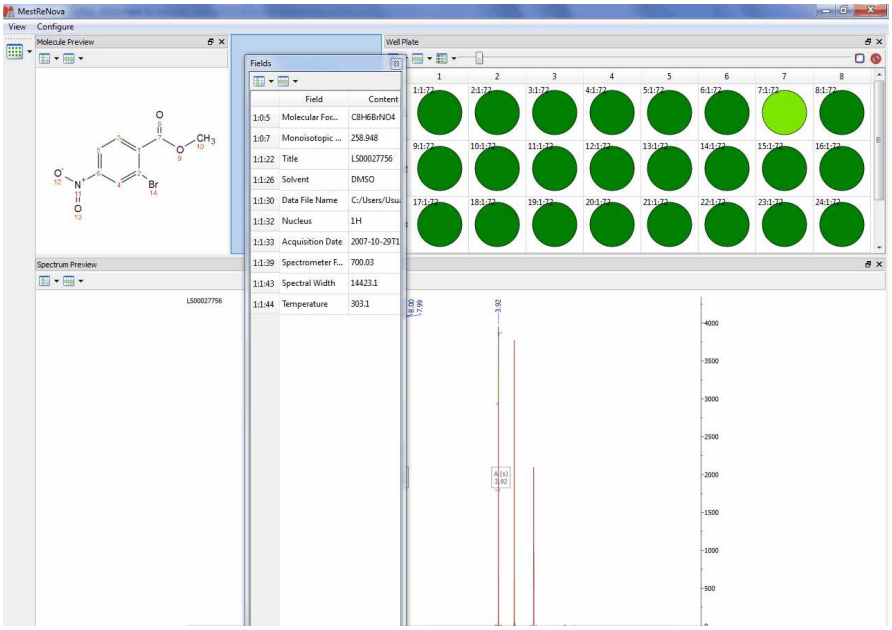
Fields, positions, and sizes are specified in the .ui file. DBSimpleForm.ui is provided as an example in the Mnova installations folder.

If you have used the Verification plugin to build your database, then you will find useful the 'Well Plate View' which will contain information about the Verification results (by using the typical color code for the plates; green for a good verification result, yellow for a doubt and the red for a bad result in the verification).

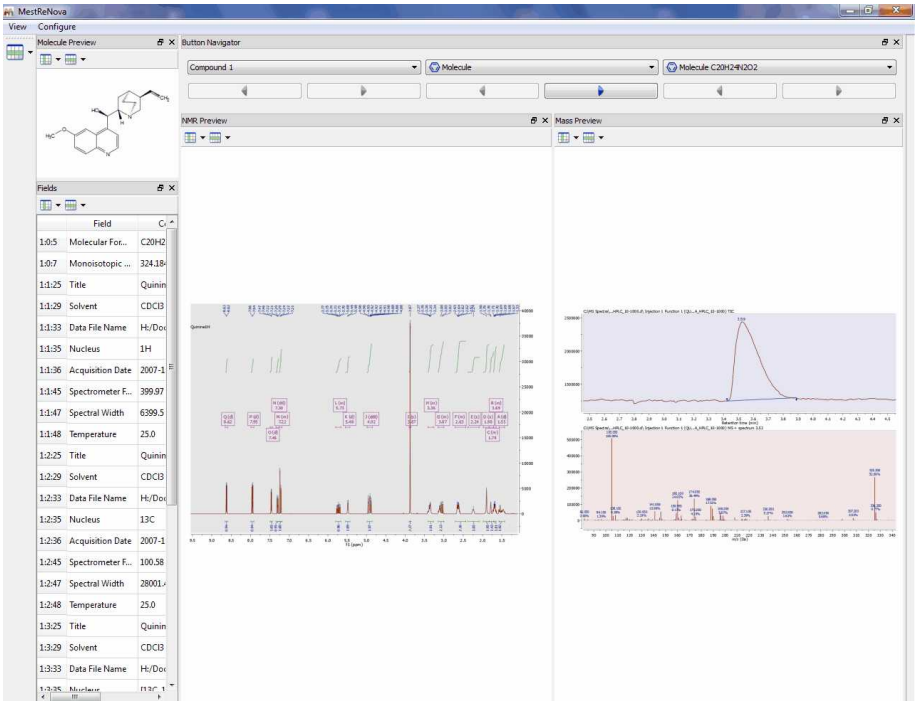
Clicking on any of the plates, will show the corresponding molecule, spectrum and table of fields. Double clicking on the plate will copy all the information to Mnova as a new document. Right clicking on the well plate (or on several) will allow you to 'Paste the item(s) or the compound(s) to Mnova' or to 'Update the 1H or 13C Prediction Database':



You will be able to freely customize any of the templates or even create your own ones. All the objects are linkable and the user will be able to change its position by just clicking&dragging. In the example below, we are changing the location of the 'Fields' table:



In the example below, we are showing a template with 1H and MS datasets.

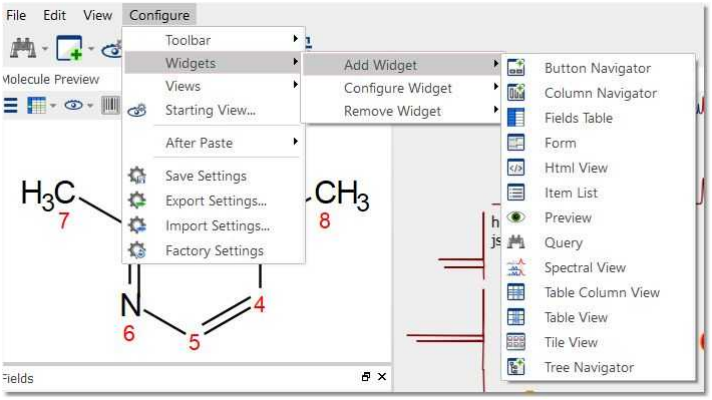


To create it, we have used the default 'Record View' template and added the MS preview by following the menu 'Configure/Add Widgets/Preview'. Once the new 'Preview' has been created, we have selected 'Mass Spectrum/Preview' after having clicked on 'Configure' button.

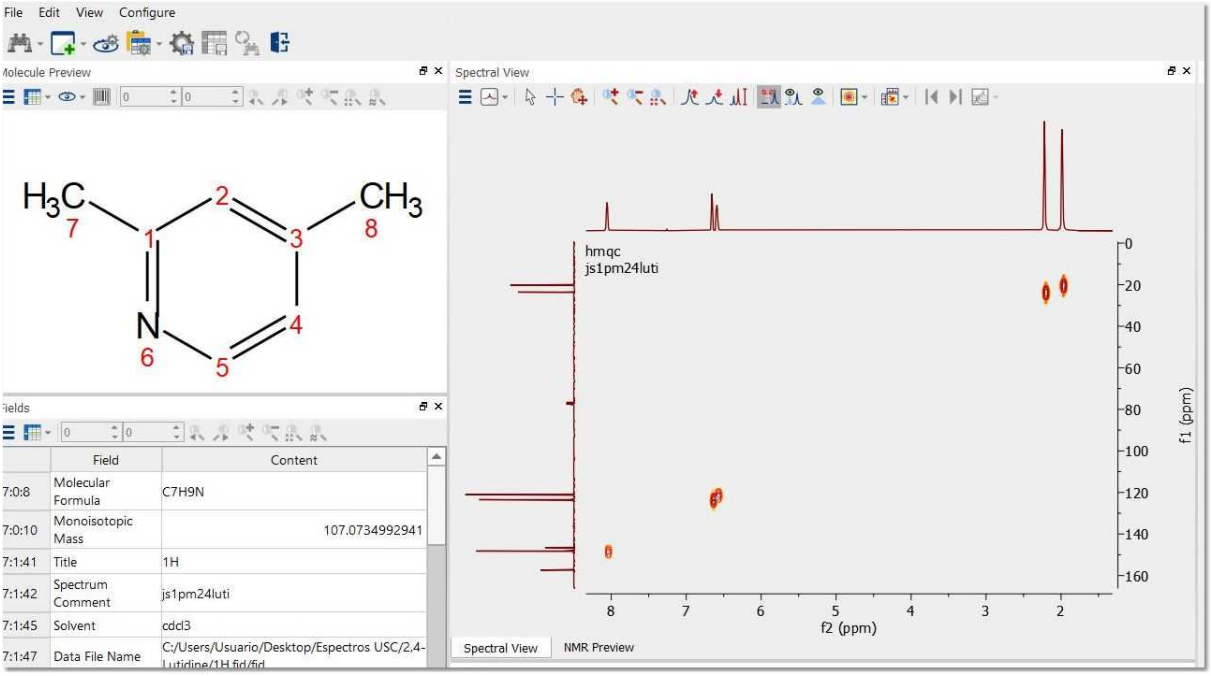
© 2024 MESTRELAB RESEARCH

In addition the user will be able to customize the 'Views' by adding/changing information of the 'Previews' or 'Tables' boxes.

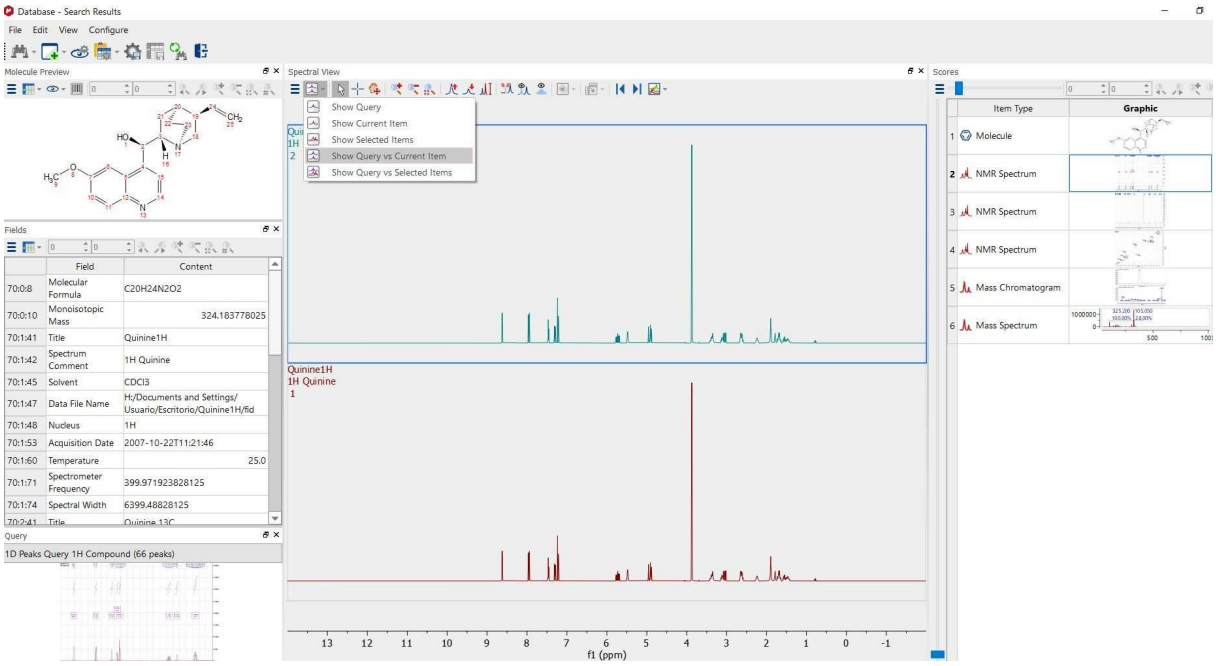
From the Configure menu, the user will be able to add, configure and remove widgets (such as 'button, column or tree' navigators, Fields Tables, Previews, etc...).



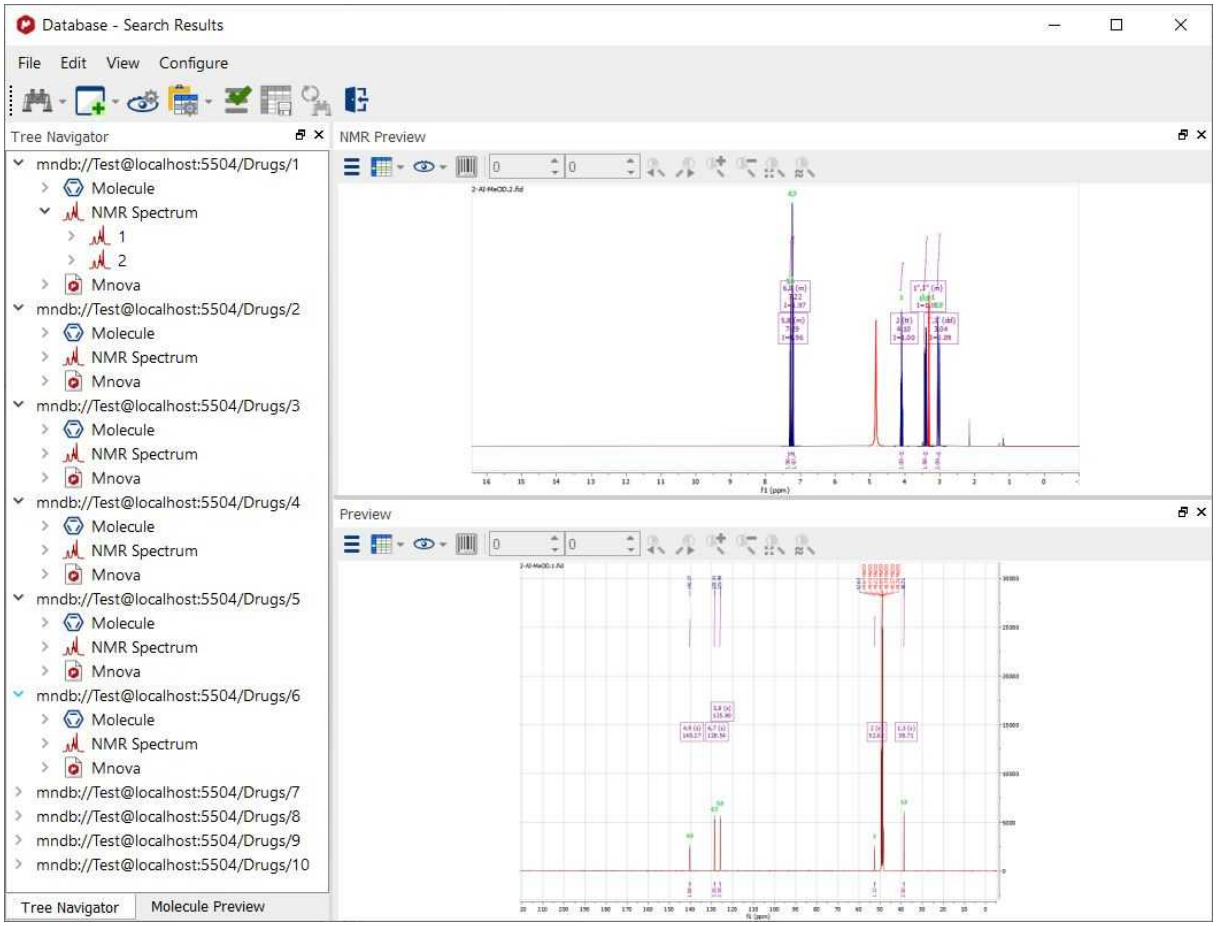
The '*Spectral View*' widget will be very useful to interact with your 1D, 2D and stacked NMR spectra. You will be able to use the crosshair, pan, zoom, intensity, cuts, show 2D traces, etc:



If you have run a search, you could compare the query and the hits in a stacked plot:



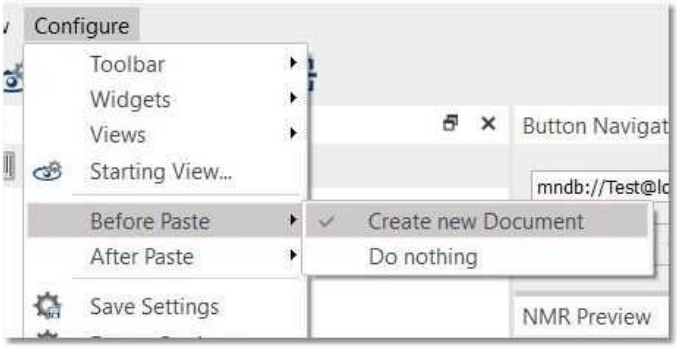
The Tree Navigator could be really useful to view all the records in a shot:



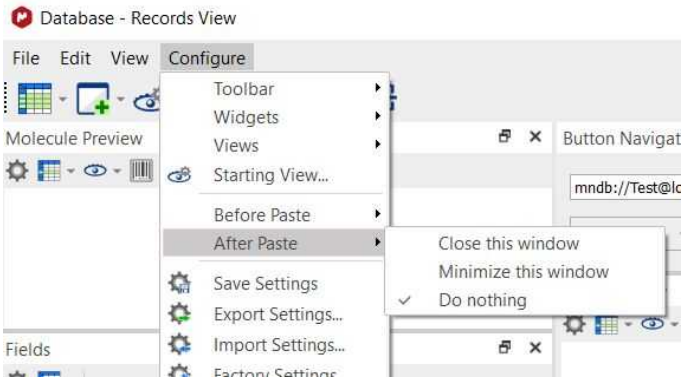
© 2024 MESTRELAB RESEARCH

If you want to show the original spectrum or molecule that you used for the search, you will need to add the 'Query' widget and you will be able to compare the query spectrum with the hits from the database.

The 'Before Paste' option will allow you to create a new document in Mnova before pasting an item or a record into Mnova:



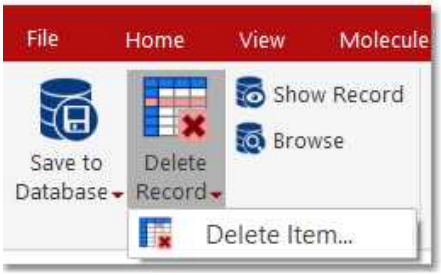
The 'After Paste' scroll down menu will allow you to decide if you want to close or minimize the DB Browser after having pasted an item or a record to Mnova.



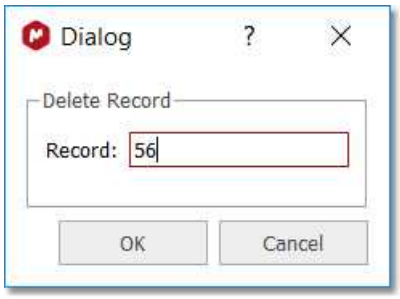
From the Configure menu, you can also Add//Configure or remove views or to load/save the settings of your templates.

16.6 Deleting items/Records

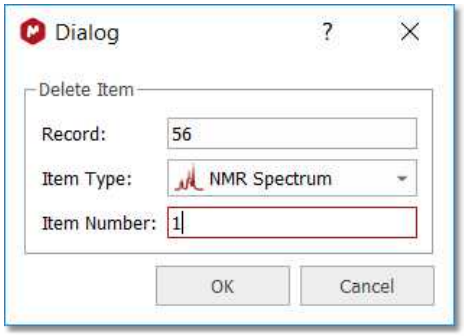
The user will be able to delete items or records by using these options of the menu:



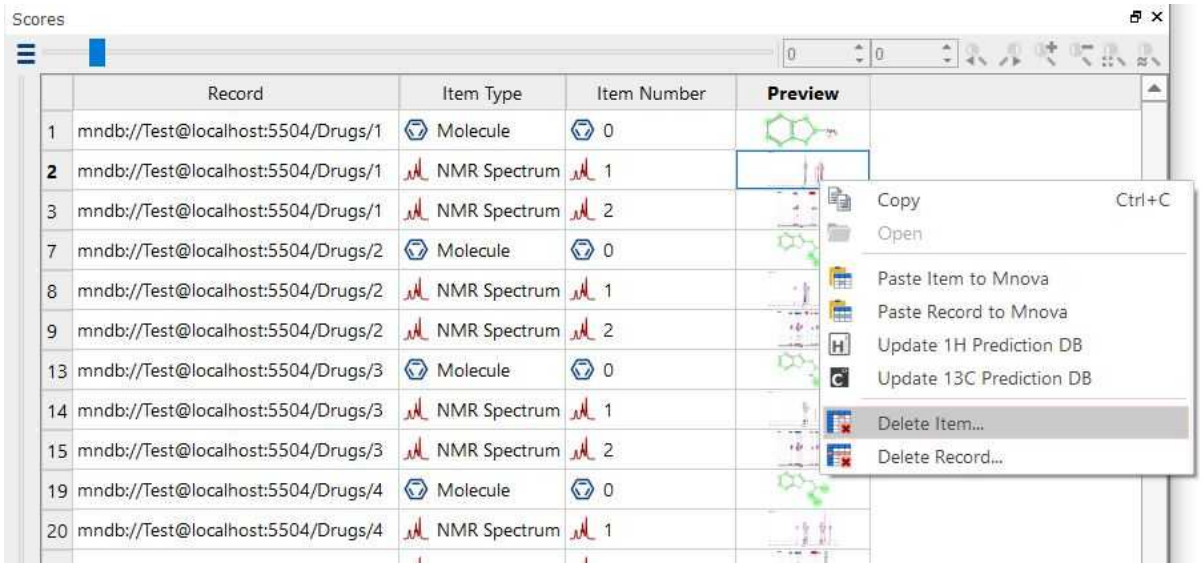
To permanently delete a record, just click on the 'Delete Record' button:



Clicking on the 'Delete Item' option, will display a new window with some edit boxes to fill with the information (Compound, Type and number) about the item that the user wants to delete. In the example below, it is shown how to delete the NMR Spectrum 1 of the record 56:



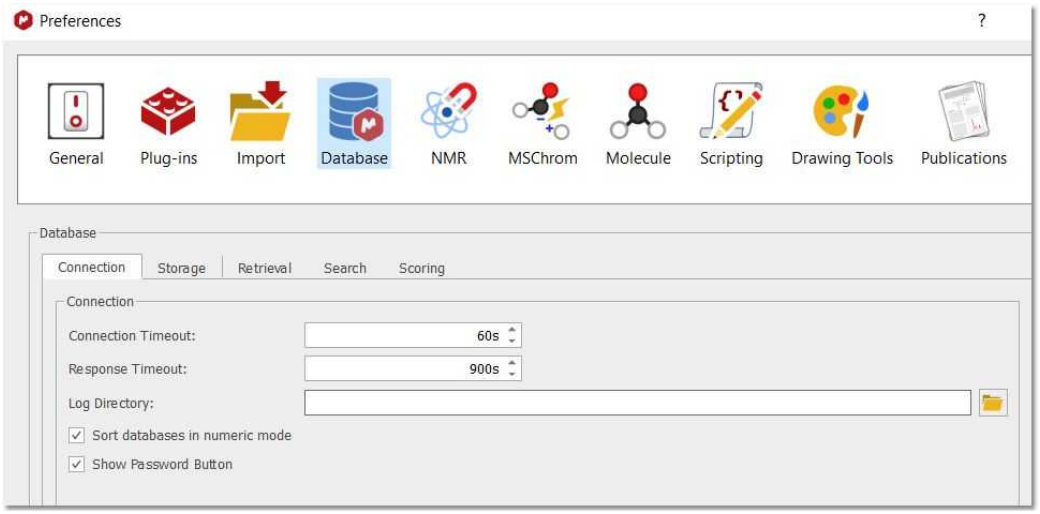
You can also delete records or items from the DB Browser (Item list, Table, Table Column, Tree Navigator and Search Results views), just by right clicking on it and selecting the applicable option from the context menu:



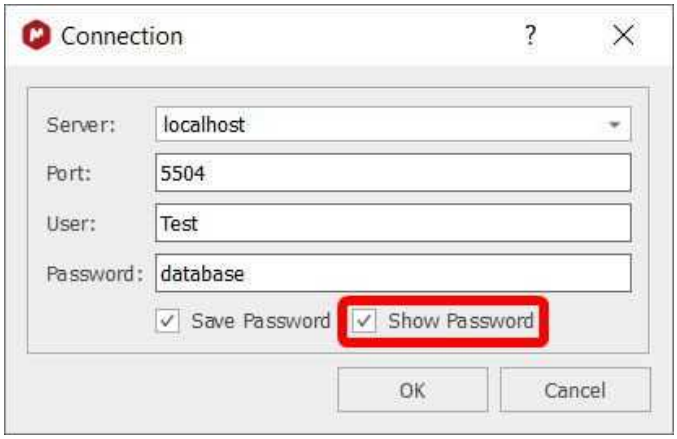
16.7 User Preferences

It is possible to select set up the preferences for the DB plugin, just by following the menu 'File/Preferences' and selecting DB:

© 2024 MESTRELAB RESEARCH

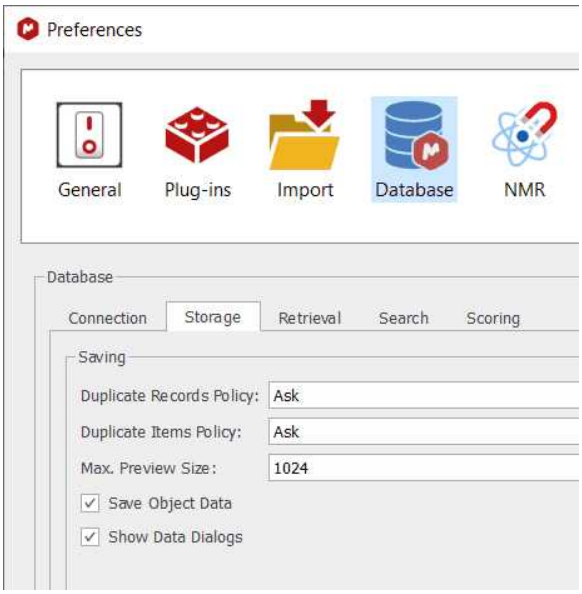


From here, you can select the number of seconds to wait for a connection or response with the server or the path to store the packet log file. You can sort the databases in numeric order and you can decide if you want to display the 'show password' checkbox button in the 'connection' dialog box:

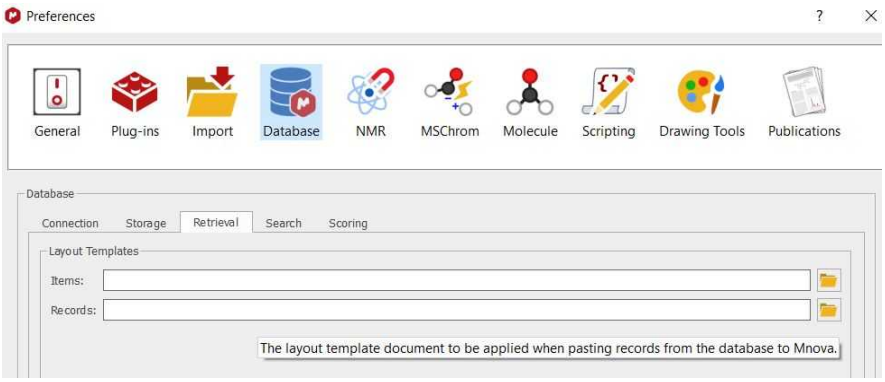


Selecting the Storage tab, will allow you to select the resolution of the 'Previews' and to set the duplicate records and Items policy (Ask or Add). Unchecking the 'Show Data Dialogs' will disable the pop-up windows (to introduce additional information) which appear when you are uploading MS datasets to the database. If you check the 'Save Object Data' box, the MS dataset will be fully saved to the DB:

© 2024 MESTRELAB RESEARCH

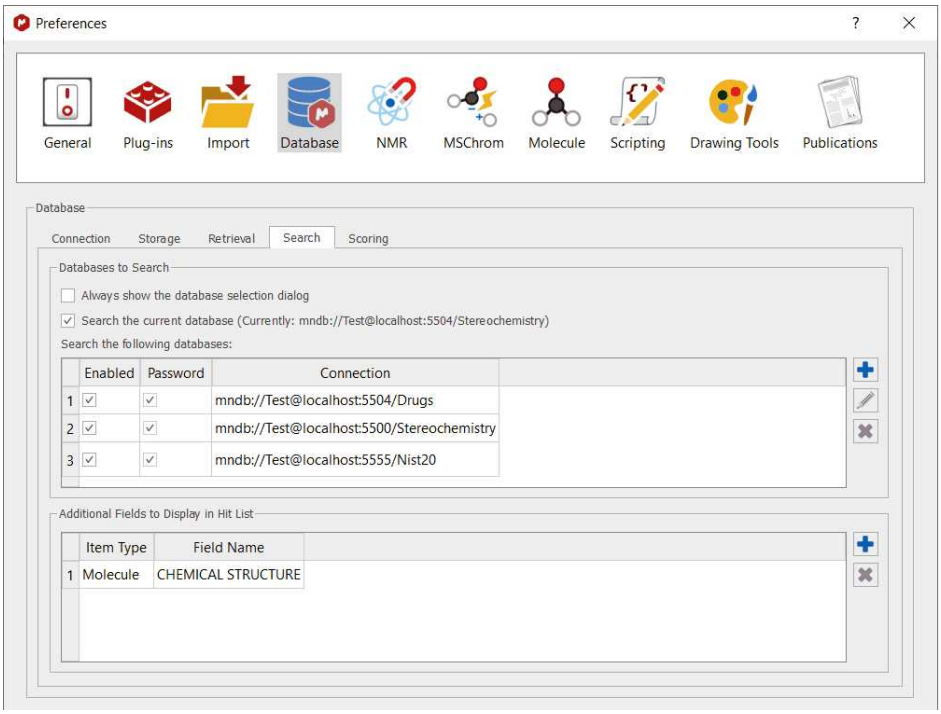


The 'Retrieval' tab will be used to load layout templates (which will be applied when you paste items or records from the DB to Mnova).

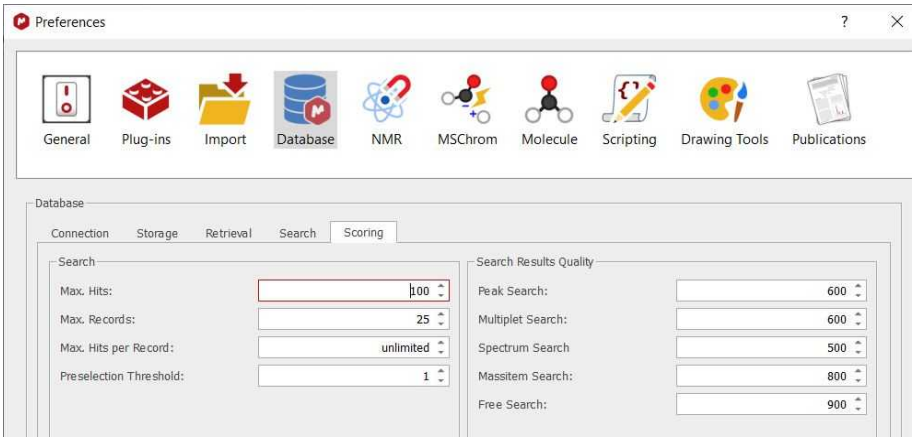


From the Search tab, your can to set different databases to be searched in or add additional fields to be displayed in the 'Hit List':

© 2024 MESTRELAB RESEARCH



From the 'Scoring' tab, the user will be able to select the maximum number of hits or records returned by a search, the 'Maximum number of hits per record', or the 'Preselection Threshold' (the minimum number of search hits for preselection dialog to be shown).



You can select the minimum score for Peaks, multiplets or free search hits to be included in the results. If for example you are searching for 3 peaks and Mnova DB finds spectra with 2 of the 3 peaks, then the score will be 666.

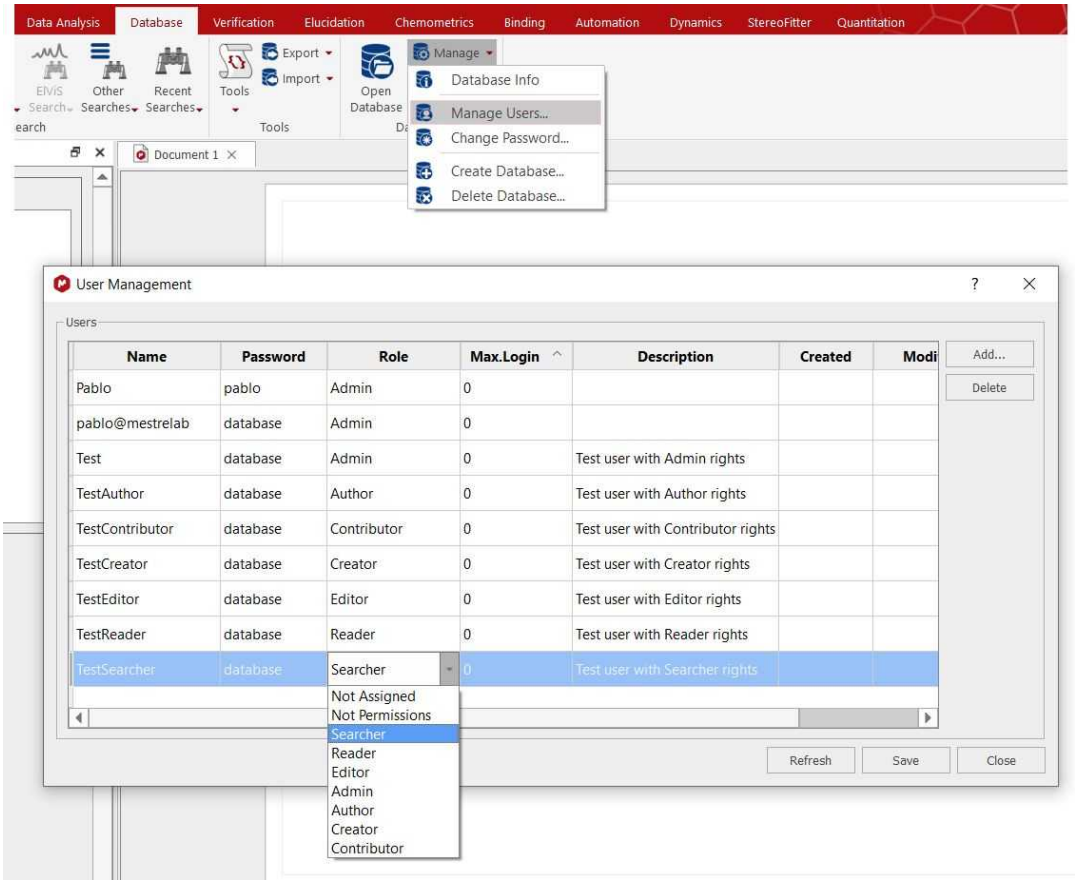
16.8 Managing Databases

You can manage the Databases from the menu:

© 2024 MESTRELAB RESEARCH



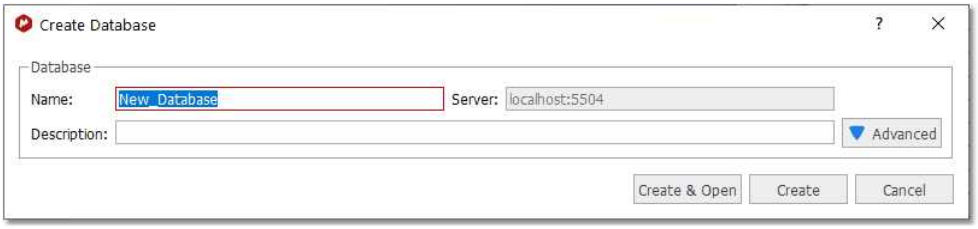
Users with admin role will be able to manage users (creating/editing/deleting users, changing passwords, user roles, descriptions, etc):



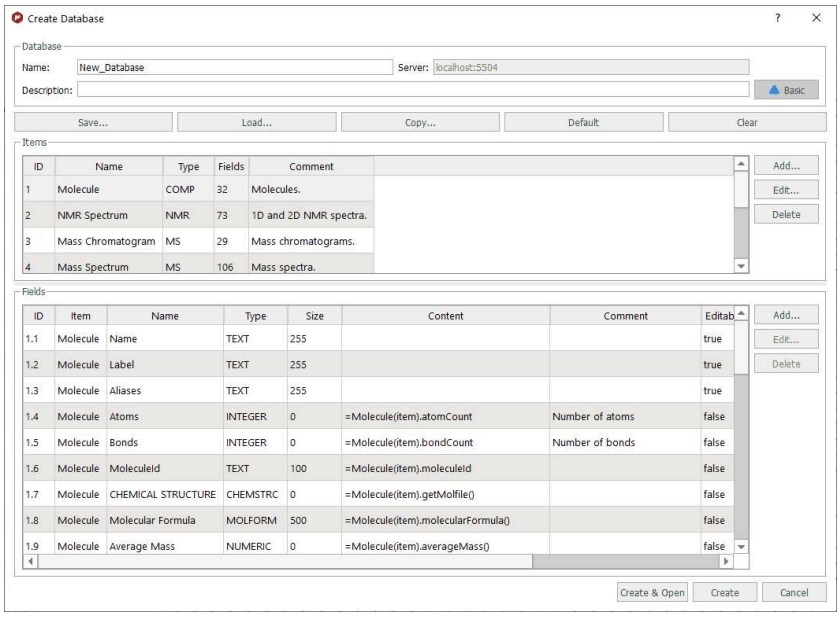
Users with admin, contributor, editor or author roles can change his own password from the menu: "Database/Manage/Change Password".

You can create a new database just by following the menu 'Database/Manage/Create Database' to display the dialog box below:

© 2024 MESTRELAB RESEARCH



Click on the 'Advanced' button to customize the options of your Database:



From here, you can define several fields for each object just by using small scripts (after having clicked on the 'Add...' button of the Field menu). For example in the example above, you can see how we have defined 40 fields for the object 'Spectrum', such as 'Peaks1D', 'Peaks2d', 'Multiplets1D, 'Title', etc. You can also get the information of the Database (showed in the picture above) just by following the menu 'Database/Database Info'.

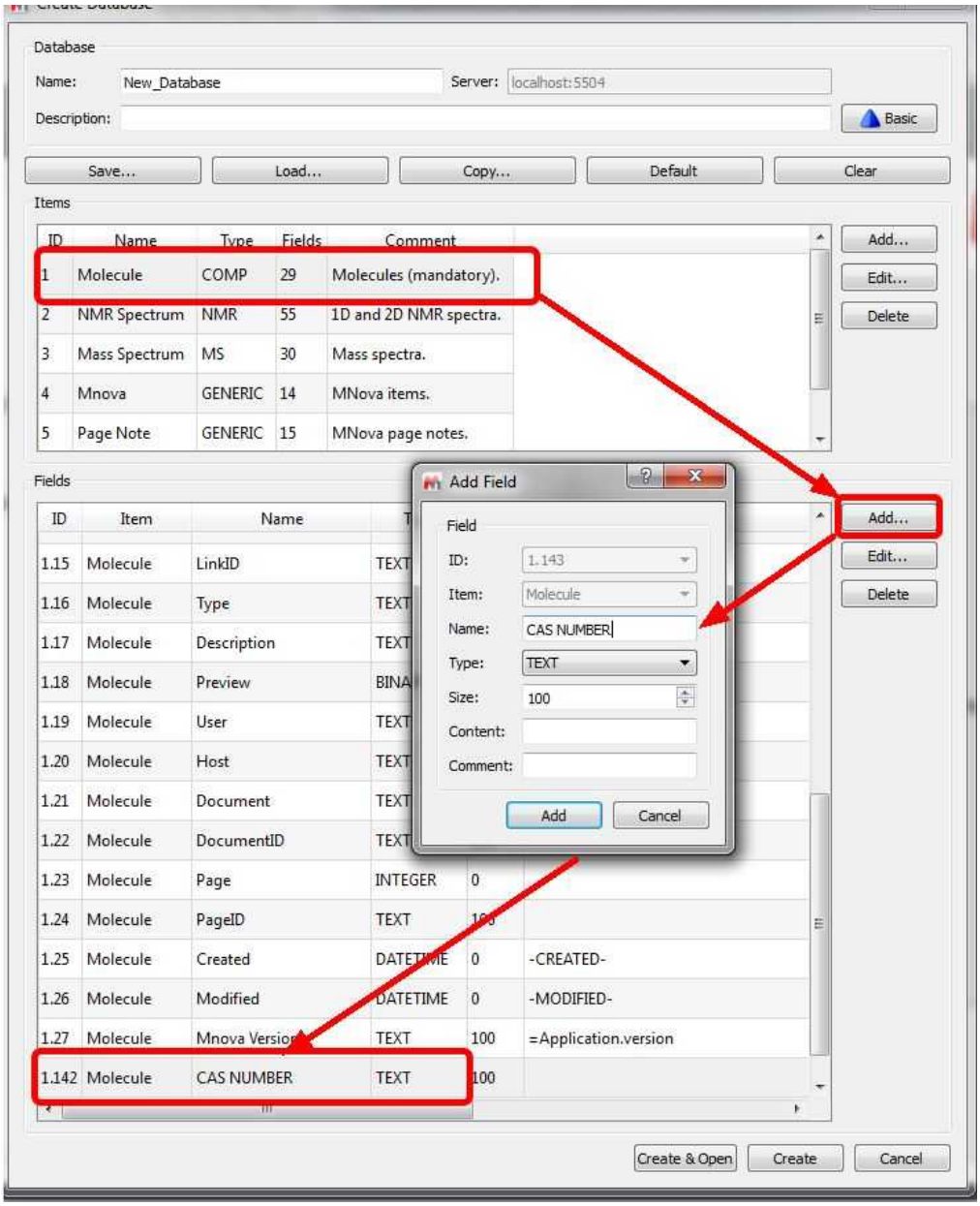
You can set a flag as 'Editable', 'Required' or 'Unique' for each item:

Editable: a field set as Editable will be editable (from the DB Browser) after being stored in the DB.
Required: a field set as Required will need to have a value when saved to a DB.
Unique: a field set as Unique will be used to check for duplicated records when saving.

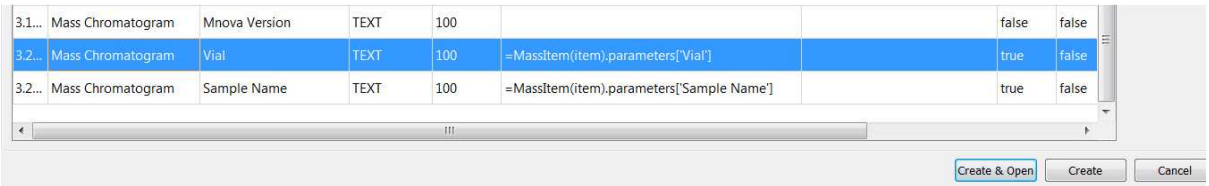
You can add new items, edit the existing ones or delete them by using the applicable buttons.

For example, if you want to add a new field, you will need to click on the ‘Add’ button to add a new field. For example, let´s add a ‘text’ field called ‘CAS NUMBER’ in a molecule:

© 2024 MESTRELAB RESEARCH



Here you can see an example to add a couple of parameters as new fields into the 'Mass Chromatogram' item; by typing ***=MassItem(item).parameters['Sample Name']*** under the 'Content' field':

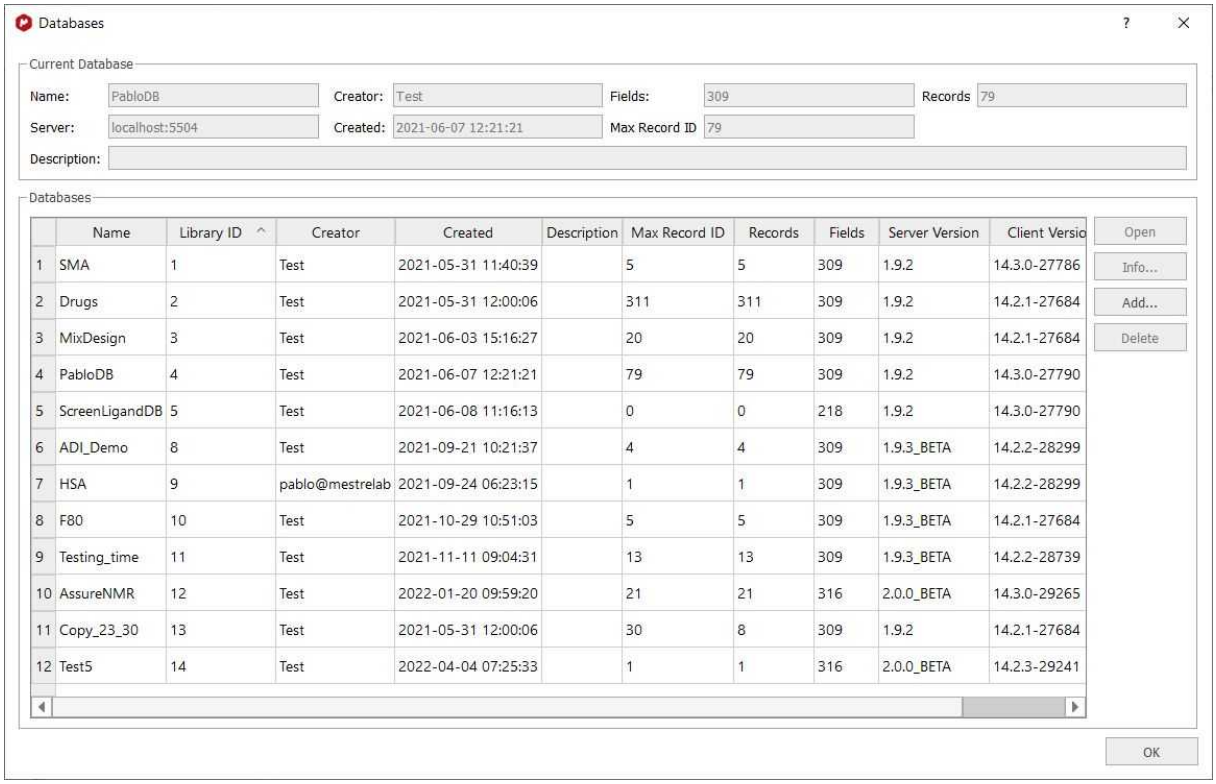


Once you have selected the desired items, click on the 'Create&Open button.

© 2024 MESTRELAB RESEARCH



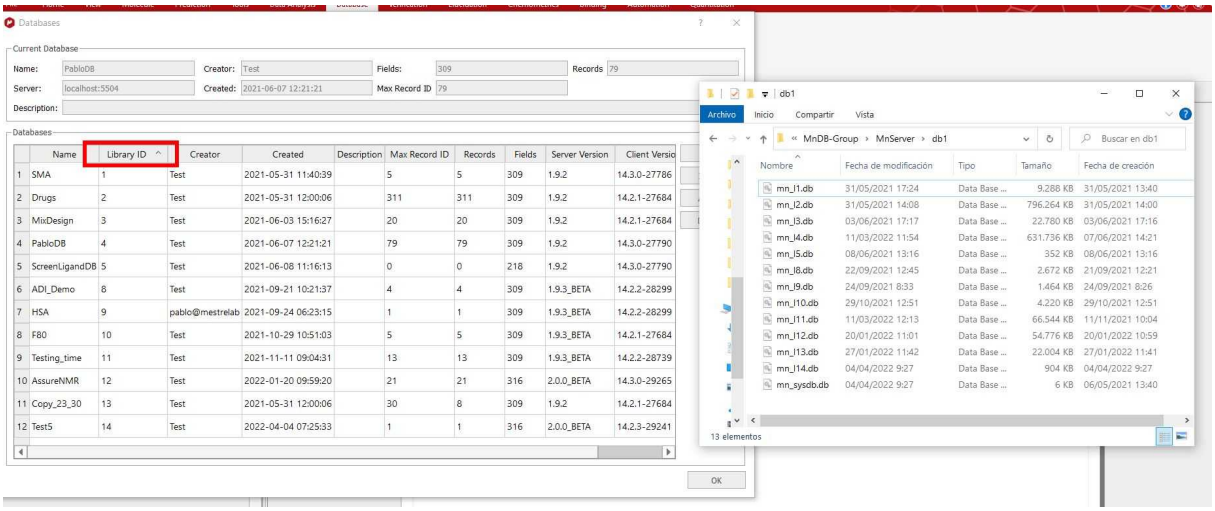
Clicking on the Manage button (of the Database ribbon) will display some information about the Databases that you have created:



	Name	Library ID	Creator	Created	Description	Max Record ID	Records	Fields	Server Version	Client Versio
1	SMA	1	Test	2021-05-31 11:40:39		5	5	309	1.9.2	14.3.0-27786
2	Drugs	2	Test	2021-05-31 12:00:06		311	311	309	1.9.2	14.2.1-27684
3	MixDesign	3	Test	2021-06-03 15:16:27		20	20	309	1.9.2	14.2.1-27684
4	PabloDB	4	Test	2021-06-07 12:21:21		79	79	309	1.9.2	14.3.0-27790
5	ScreenLigandDB	5	Test	2021-06-08 11:16:13		0	0	218	1.9.2	14.3.0-27790
6	ADI_Demo	8	Test	2021-09-21 10:21:37		4	4	309	1.9.3_BETA	14.2.2-28299
7	HSA	9	pablo@mestrelab	2021-09-24 06:23:15		1	1	309	1.9.3_BETA	14.2.2-28299
8	F80	10	Test	2021-10-29 10:51:03		5	5	309	1.9.3_BETA	14.2.1-27684
9	Testing_time	11	Test	2021-11-11 09:04:31		13	13	309	1.9.3_BETA	14.2.2-28739
10	AssureNMR	12	Test	2022-01-20 09:59:20		21	21	316	2.0.0_BETA	14.3.0-29265
11	Copy_23_30	13	Test	2021-05-31 12:00:06		30	8	309	1.9.2	14.2.1-27684
12	Test5	14	Test	2022-04-04 07:25:33		1	1	316	2.0.0_BETA	14.2.3-29241

The library ID will be very useful to identify the file in your disk; as the filename will contain the applicable libraryID number:

© 2024 MESTRELAB RESEARCH



If you want to delete any existing database, just follow the menu 'Database/Manage/Delete Database' and select the undesired database in the window displayed:

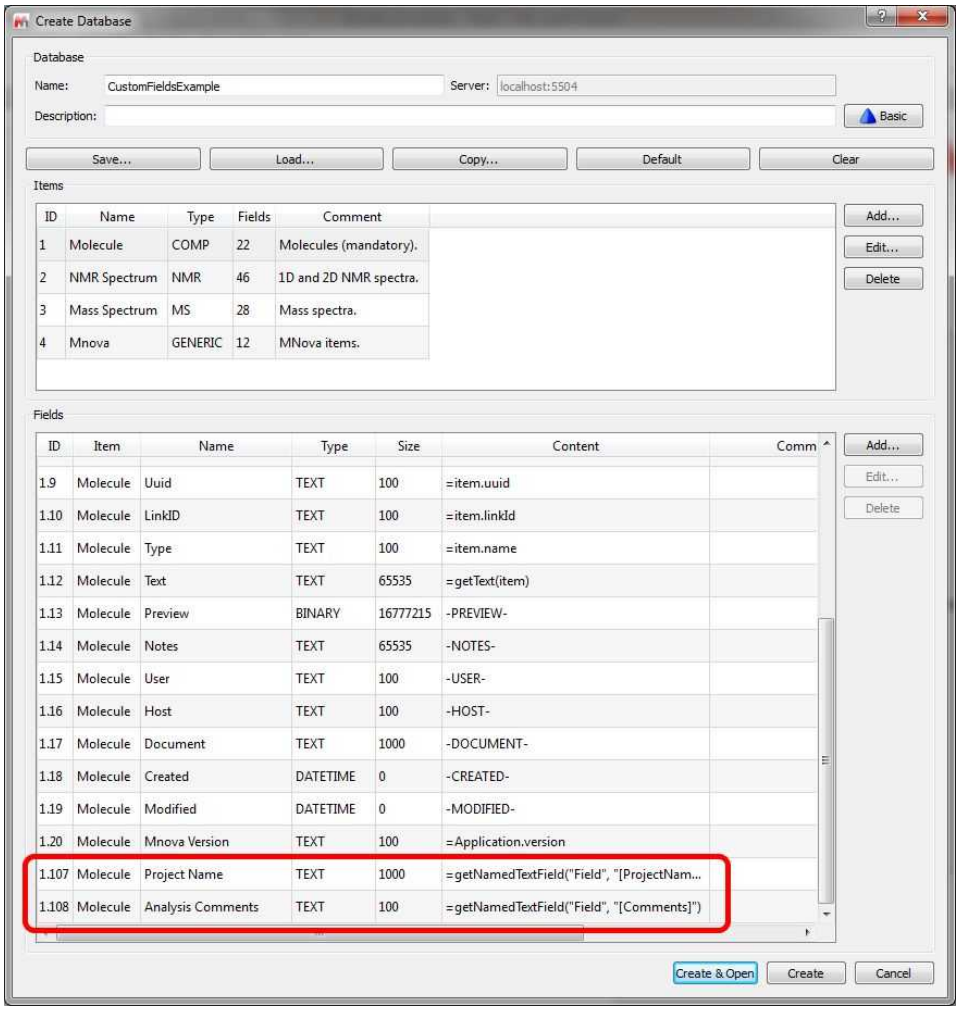


16.8.1 Create custom fields

It is possible create custom text fields by using a layout template. Let´s see an example which you can download from this link

First of all, you will need to create a new Database with the new text fields. You can do it by following the menu 'Database/Create Database/Advanced/Load' and select the XML which is on the file that you downloaded above (or you can add the new text fields that you want). This will add two new text files ('Project Name' and 'Analysis Comments') to the Database. Click on the 'Create&open' button to generate the new Database:

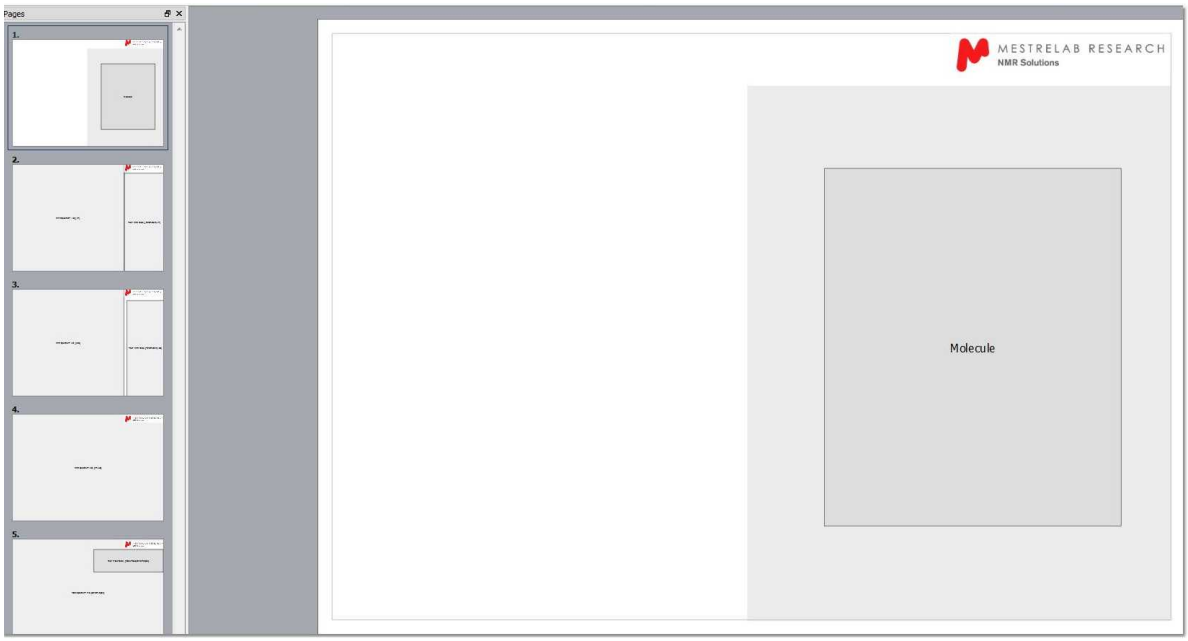
© 2024 MESTRELAB RESEARCH



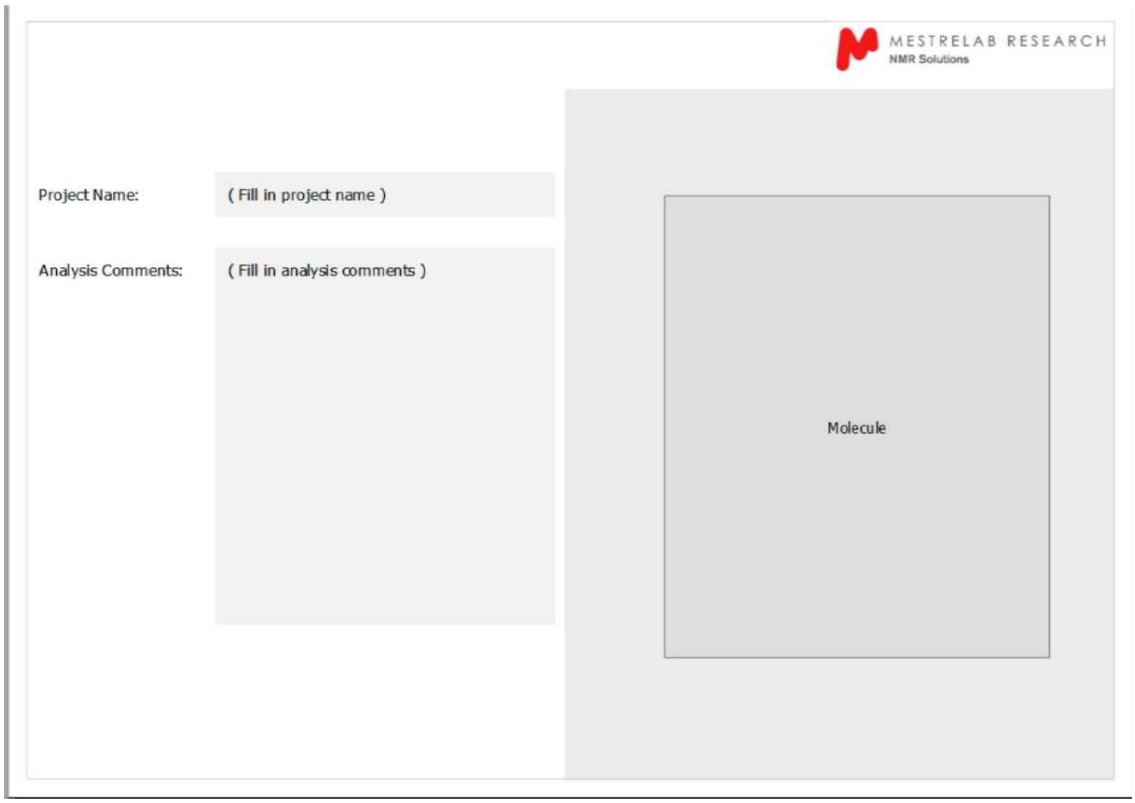
Then, you will need to create a layout template (in the example the template is called "LayoutOrig.mnova".

Here you can see a layout template with 5 pages (molecule, 1H-NMR, 13C-NMR, 2D-NMR and MS):

© 2024 MESTRELAB RESEARCH

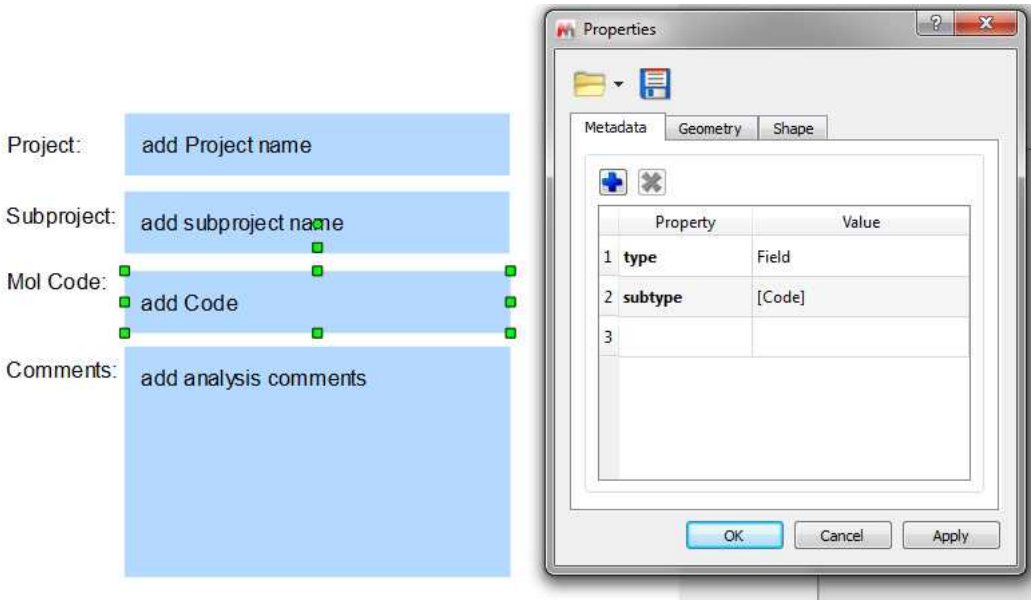


Then, run the script called "addNamedFields.qs". This will add two named text fields [ProjectName] and [Comments] to the layout:



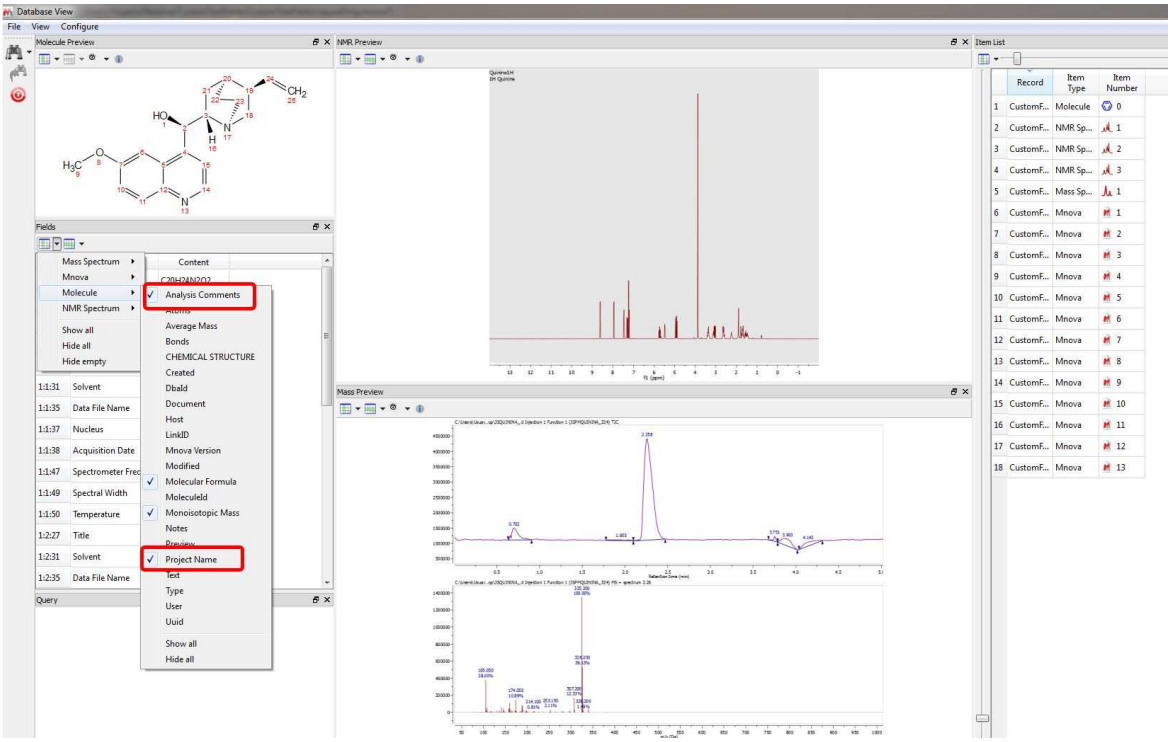
Then save the results as "LayoutWithFields.mnova" by following the menu 'File/Save As'. Those text fields will have its own Properties, even you could add new text properties to the page items and edit them by selecting the 'Metadata' tab:

© 2024 MESTRELAB RESEARCH



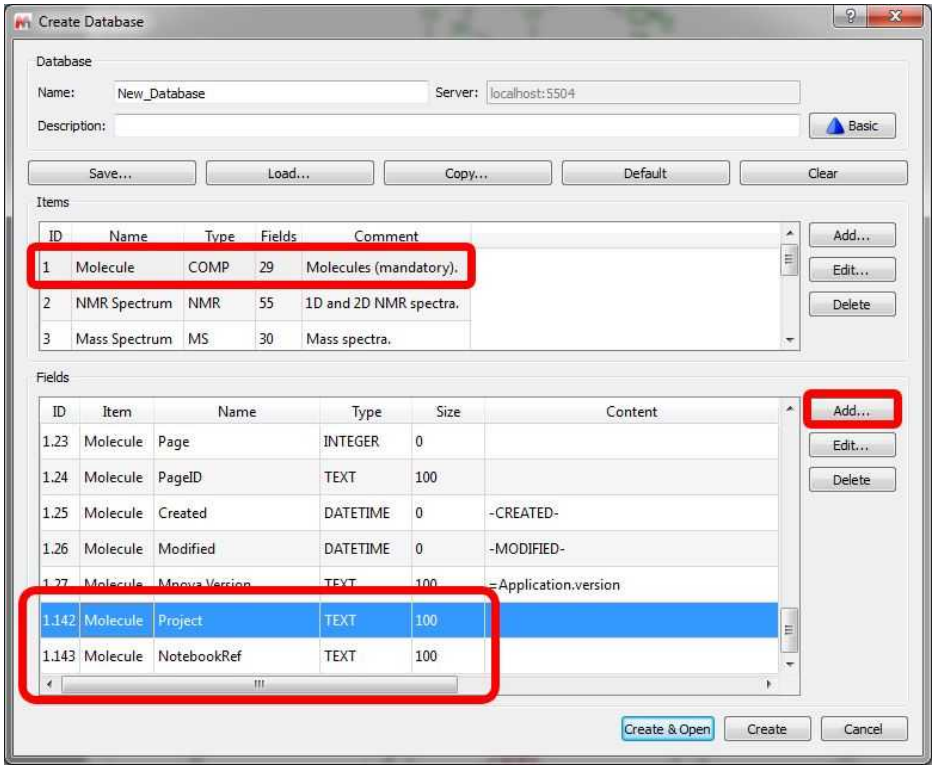
Finally apply the layout template over your datasets, fill the text fields manually and save the documents to the Database (by following the menu 'Database/Save').

To display the new custom fields after a search, go to the DBBrowser and add the new fields to views as desired and save the configuration by following the menu "Configure/Save Settings As":



You can create **mandatory fields** in order to save them to the Database, You will find an example script called "dbMandatoryFieldsExample1.qs" into the examples installation folder of Mnova. Save this script into one of your 'Scripts directories' (File/Preferences/Scripting), restart Mnova and then create a new

database with two added custom fields "Project" and "NotebookRef" in the Molecule item type:

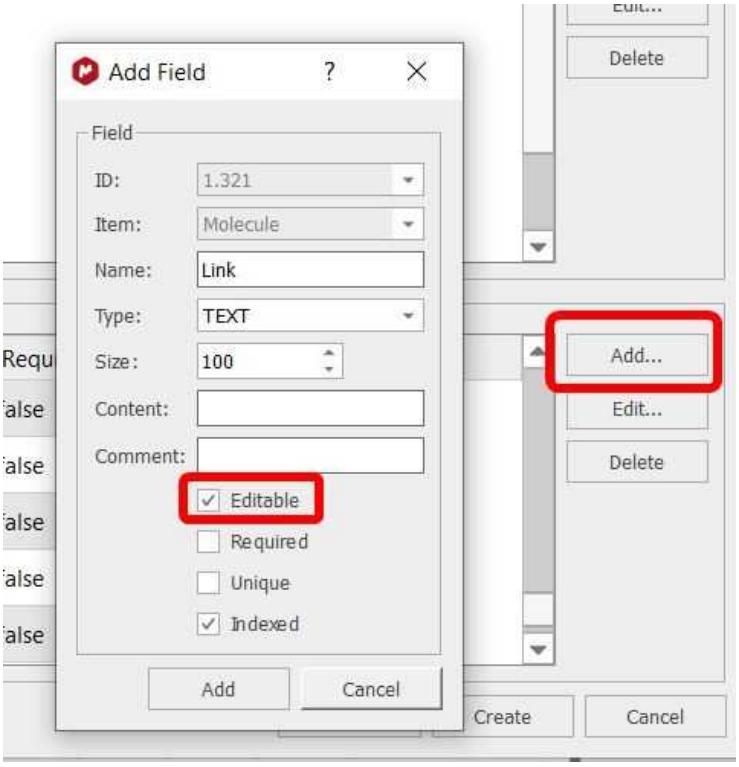


Next, save any dataset to the new database and a dialog asking for the content of the mandatory fields will be shown with each save operation:

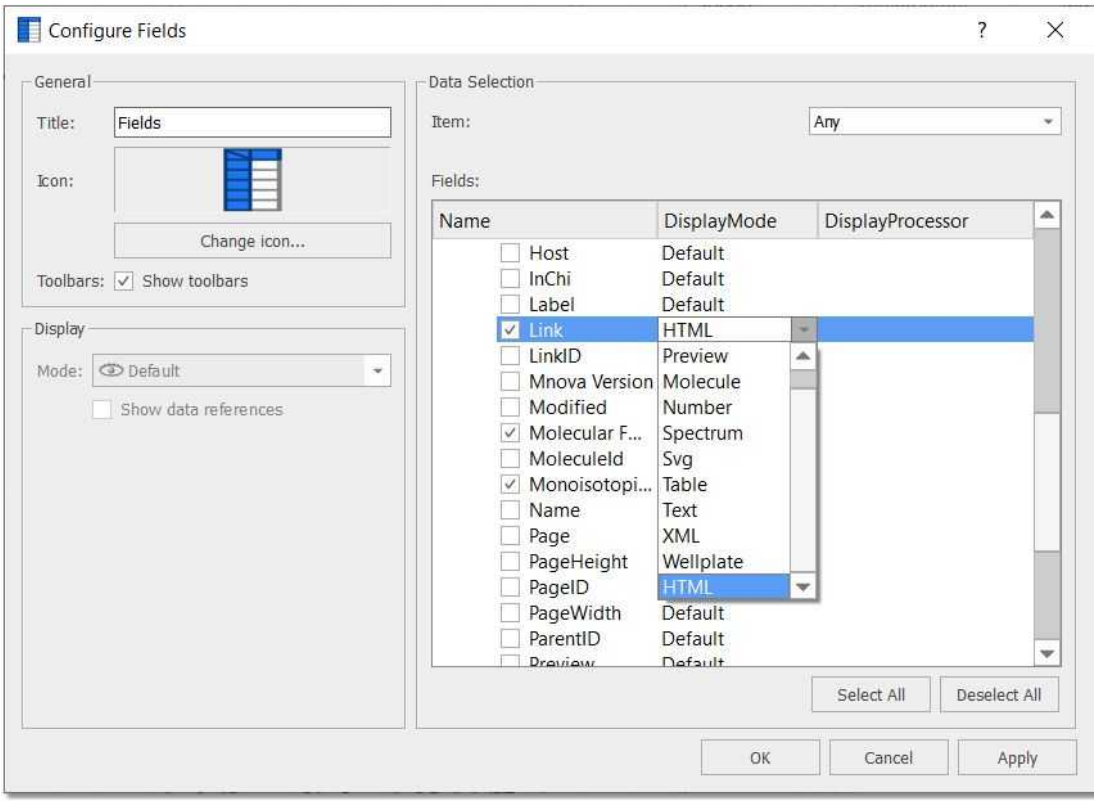


You can also include fields in the DB Browser with hyperlinks. To do it, make sure that you create a database with an editable TEXT field; for example, the "Link" field in the Molecule item.

© 2024 MESTRELAB RESEARCH



Next, save some records in the database and open the DBBrowser to configure a widget to show the new column with the display mode as HTML:

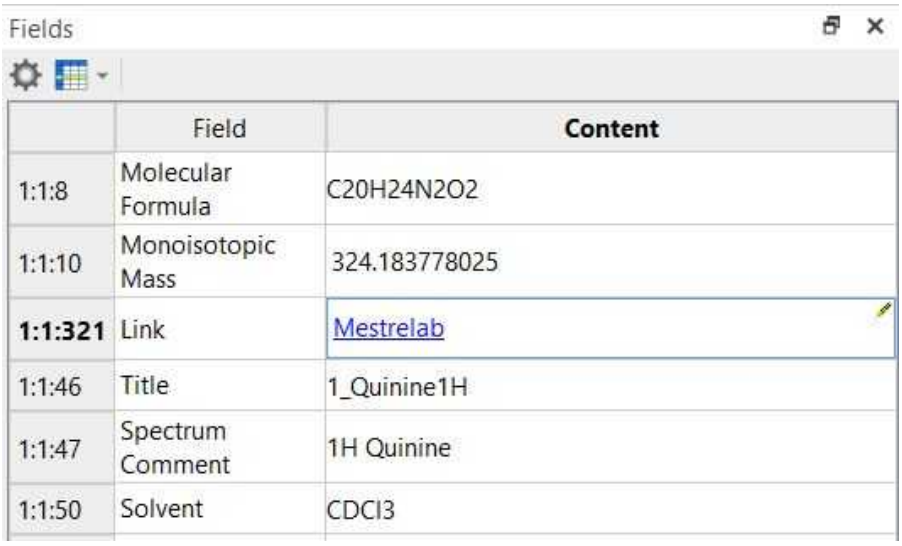


© 2024 MESTRELAB RESEARCH

Then, write the HTML hyperlink tag:



and you will get the expected hyperlink:



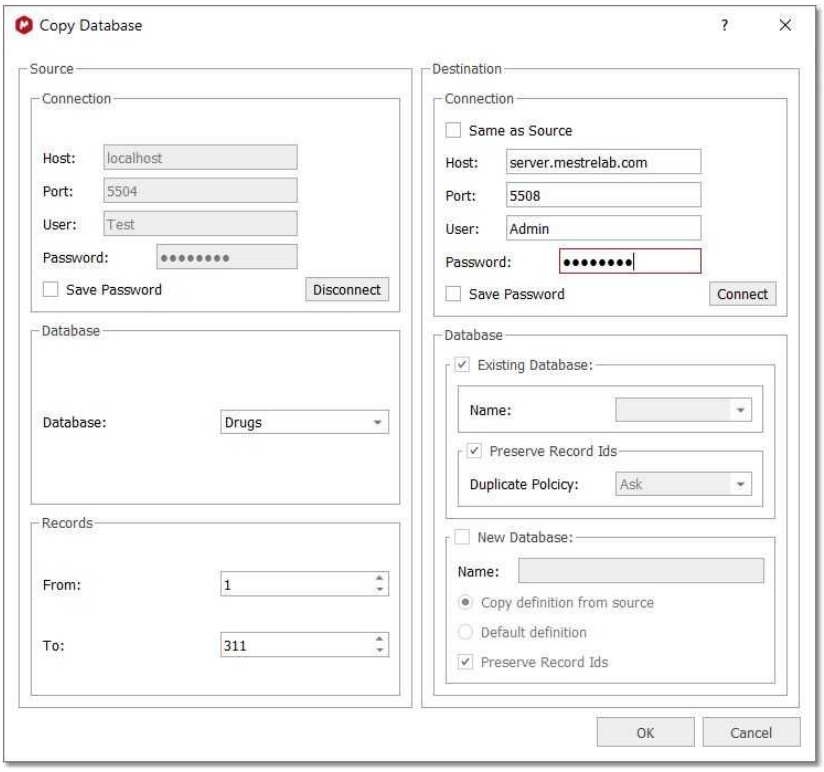
16.9 Mnova DB scripts

The user will find in the Tools menu some useful scripts for the Database plugin:

© 2024 MESTRELAB RESEARCH



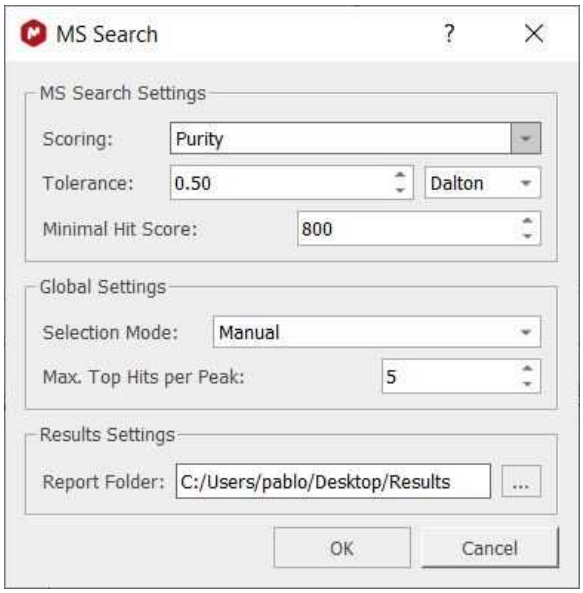
The **Copy Database** script will allow you to export databases to any destination:



You will have the capability to copy the entire database or just a few records.

The **Search MS in chromatogram** script will be useful to search for the MS spectra sitting under the major peaks of a chromatogram. You can select the scoring and tolerance settings and and also the selection mode and the maximum number of top hits per peak:

© 2024 MESTRELAB RESEARCH



The "**SDFile to definition**" script takes an SDFile and outputs a database definition .xml file. The Database definition .xml file can then be used to create new databases into which the SDFile which can be imported by using Scripts/Database/Import SDFile, preserving 'all tags' and data. So, if you want to import all the tags of your SDF file into a Database, you will need to use this script.

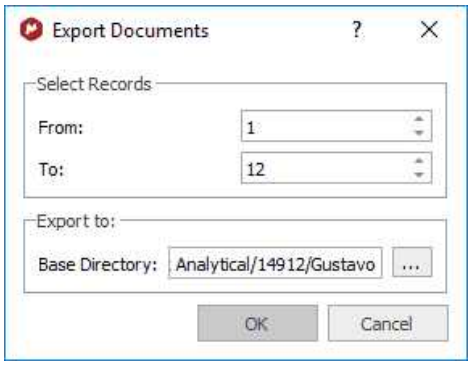
Mnsr files can be played to the MnDB server by using the **Play Mnsr File script**.

From the **Export** section, you will also find some several scripts:



The **'Export CSV'** script will "dump" all the metadata information from a selected database into a text file format.

The **Export Documents** scripts will be very useful to export all the selected compounds of the Dabatase as Mnova documents into the desired directory:

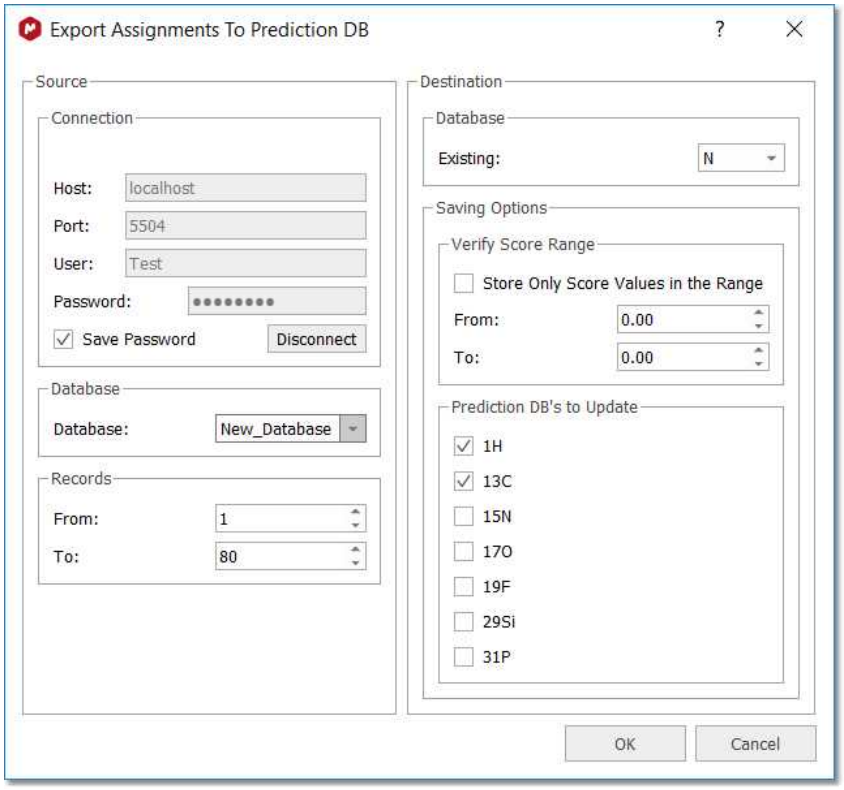


© 2024 MESTRELAB RESEARCH

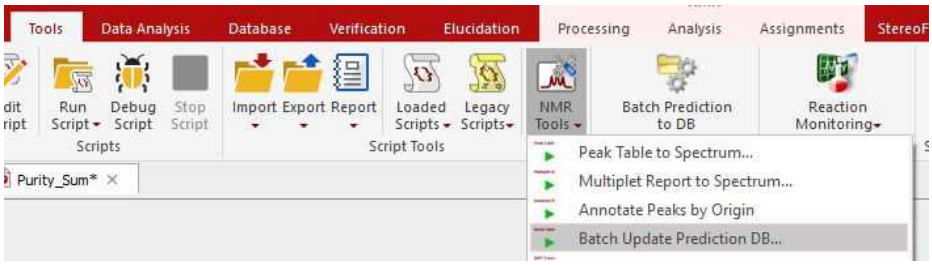
Export Assignments to Prediction Database

This script will be useful for example to export a third party prediction DB into the Mnova Prediction Database. For example, let´s import a SDF file containing all the assignments (you can see those assignments by loading the SDF file and following the menu 'View/Table Assignments'. The molecules and the assignments contained in the SDF file can be stored in a Database by following the steps below:

1. Create a DB definition from SDFile with "SDFile to Definition" script (under the 'Database/Scripts' menu).
2. Create the database with the desired definition by following the menu 'Database/Create/Advanced/Load' and selecting the xml file created in the step 1
3. Import the SDFile to the DB with "Import SDFile" script (under the 'Database/Scripts' menu)
4. Finally the assignments can be exported to a Prediction DB using the script "Export Assignments to Prediction DB":

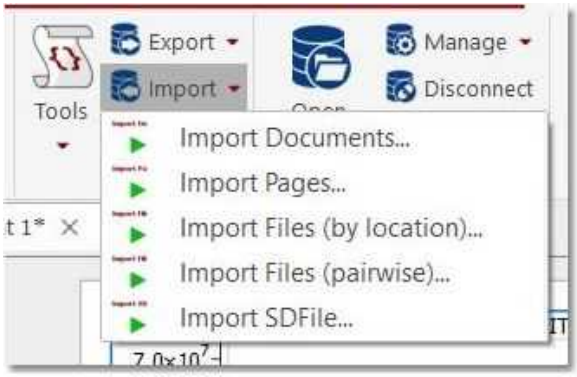


You can also load the SDF file into Mnova containing several molecules with assignments and update the prediction Database by following the menu 'Tools/NMR Tools/Batch Update Prediction DB':

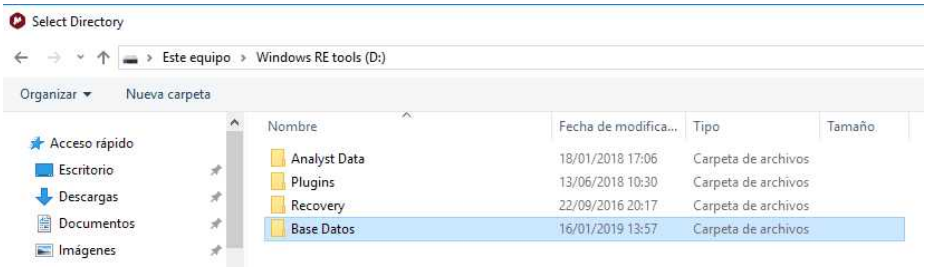


From the Import section, you will find useful script to import files

© 2024 MESTRELAB RESEARCH



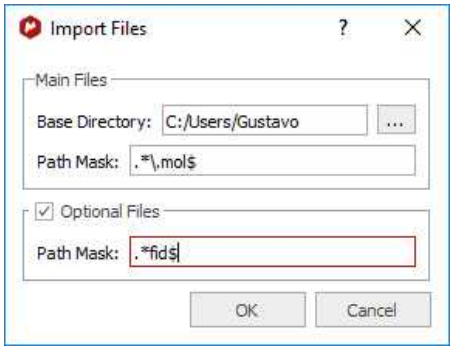
The **Import Documents** script will allow you to save to the Database Mnova documents from a selected directory. This script looks for Mnova documents on disk, everything in the same document goes into the same database record:



The **Import Pages** script will save all the pages in the current document to the current Mnova Database.

This script goes through the currently open Mnova document, creates a new record for each page, everything on the same page goes into the same database record.

The **Import Files (by Location)** will save to the database the desired type of files from a selected directory:



For the 'Path Mask'; you can use wildcards, so this could be, for example, .*\.mol$; or just .*fid$ and click 'OK'. The $ symbol means in '.*fid$' that it will search for the FIDs that are stored at the end of the folders structure (valid for those cases where all the spectra of a given molecule are grouped in the same folder).

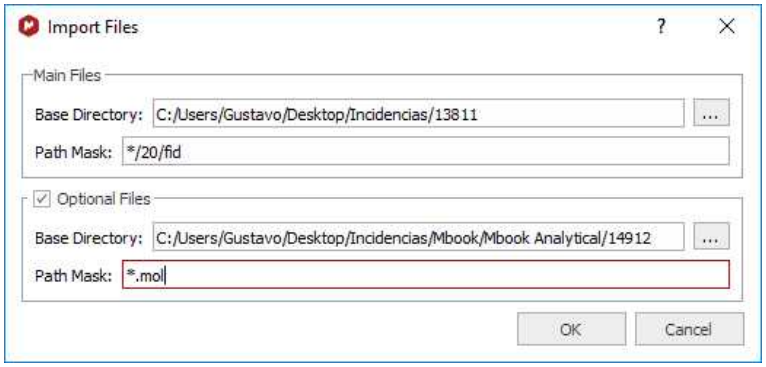
This script looks for main files matching the given path mask in the specified folder. Then, for each main file found, it looks for optional files according to the second path mask *relative to each main file location*. Depending on your situation, main files could be molfiles and optional files spectra, or vice versa. E.g. You have many spectra (main files), and for some of them you have a molfile (optional files).

© 2024 MESTRELAB RESEARCH

Or, you have molfiles (main files), and for each molfile you have zero, one, or several spectra (optional files).

E.g:
\Data\ABC123\ABC123.mol
\Data\ABC123\NMR\XBlah.fid
\Data\ABC124\Molecule.mol
\Data\ABC124\NMR\124.fid
\Data\ABC125\Whatever.mol
\Data\ABC125\NMR\Any.fid

The **Import Files (pairwise)** will import pairs of molecules and spectra (first spectrum with the first molecule in the applicable folder; second spectrum with second molecule and so on...) and will save them into the Database. Please bear in mind that you must have the same number of molecules and spectra.



You can use wildcards for the 'Path Mask', so this could be, for example, */10/pdata/1r, or just */fid, or */ser. Finally click 'OK' and all the selected spectra and molecules will be saved in the database.

It works exactly as the Multi-Open script: You give it two path masks and it will look for files of each type according to the corresponding path mask. Files are associated to each other by the order in which they are found. So there needs to be a 1-to-1 correspondence between spectra and molecule files. The order in which they are found is determined by the file names. You would use is this script if you have two folders (one for molecules and another for spectra) and the files can be associated witch each other via the file name.

E.g:
\Data\Molecules\ABC123.mol
\Data\Molecules\ABC124.mol
\Data\Molecules\ABC125.mol
\Data\Spectra\NMR\ABC123.fid
\Data\Spectra\NMR\ABC124.fid
\Data\Spectra\NMR\ABC125.fid

The **'ImportSD File'** script will allow you to import to your Database the molecules contained in a SDF file.

© 2024 MESTRELAB RESEARCH

Part XVI

17 Scripting with Mnova

Benefits and uses

The availability of scripting in Mnova provides opportunities and benefits both for skilled NMR and casual, non-expert users:

- Users are less dependent on the application developers for new features since scripting allows them to implement minor features themselves which can be deployed to less expert users.
- NMR administrators can write scripts to customize applications before deploying them to a group of users. For example, an NMR manager could create a script to automate some particular processing which he can distribute among his users in such a way that they will simply have to click one button (e.g. toolbar button) to apply that processing.
- Mnova developers can use scripting to prototype new features that can later be incorporated into the next version of the application. This is ideal when a client requires some processing/analysis feature not available in the application and cannot wait until a new version of Mnova is released.
- Mnova scripting language is based on an ECMAScript-based language making it easier for existing Web developers to customize and extend Mnova.

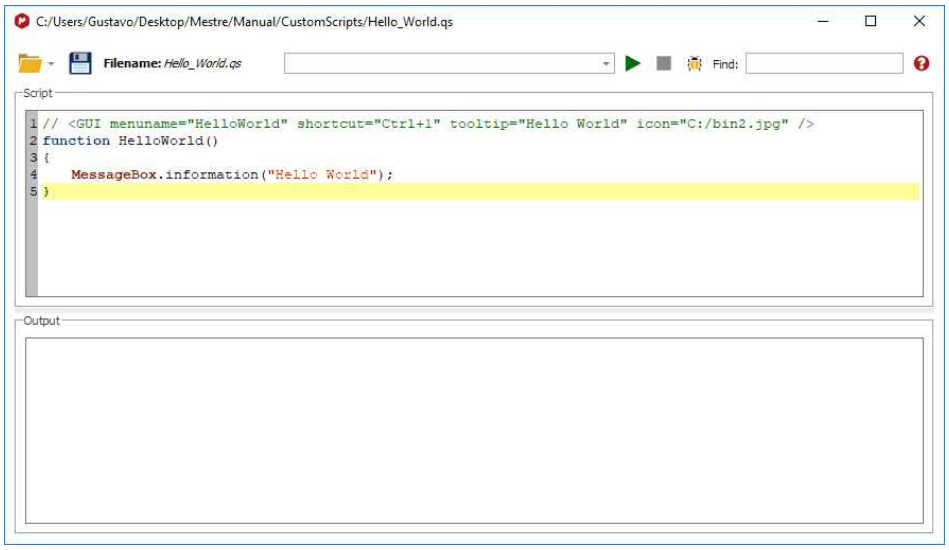
In essence, nearly everything that can be done with the Mnova graphical interface (UI) can be reproduced with Mnova's scripting module, making it very easy to automate repetitive tasks. For example, you may have to routinely process 1H NMR spectra using the same processing operations. You can create a script which wraps up all these processing functions in such a way that by simply clicking a toolbar button or pressing a keyboard shortcut, all of your 1H NMR spectra will be processed accordingly.
Furthermore, if some particular processing function is not available in Mnova, you can create a script which modifies the spectral data points according to your requirements.

For further information about the scripting documentation, please check the Mnova installation folder at:
C:\Program Files\Mestrelab Research S.L\MestReNova\documents\scripts

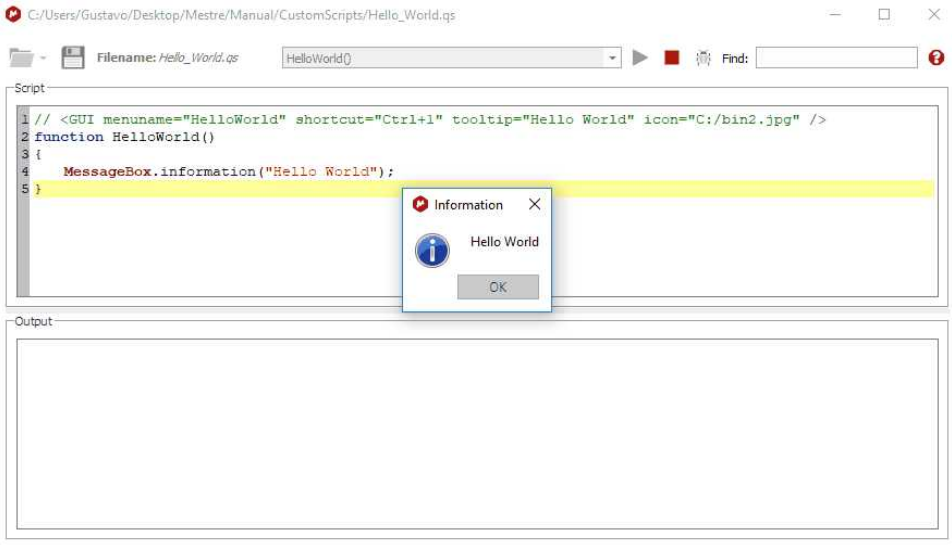
Introducing Mnova Script – Hello World

A "hello world" program has become the traditional first program that many books use to introduce a programming language. It will act as a cheatsheet for you until you familiarize yourself with the language. The point of this application is to use all the most commonly used elements of Mnova Script language and putting it in a single place so that you can refer to it later. We will start showing the basic elements of an Mnova script and from that point some more advanced methods will progressively be added.
Mnova scripts can be created with any text editor, although Mnova includes a specialized script editor with syntax highlighting and debugging output capabilities. This text editor is available from the Tools/Edit Script menu command. Issue this command and type in the following text:

© 2024 MESTRELAB RESEARCH

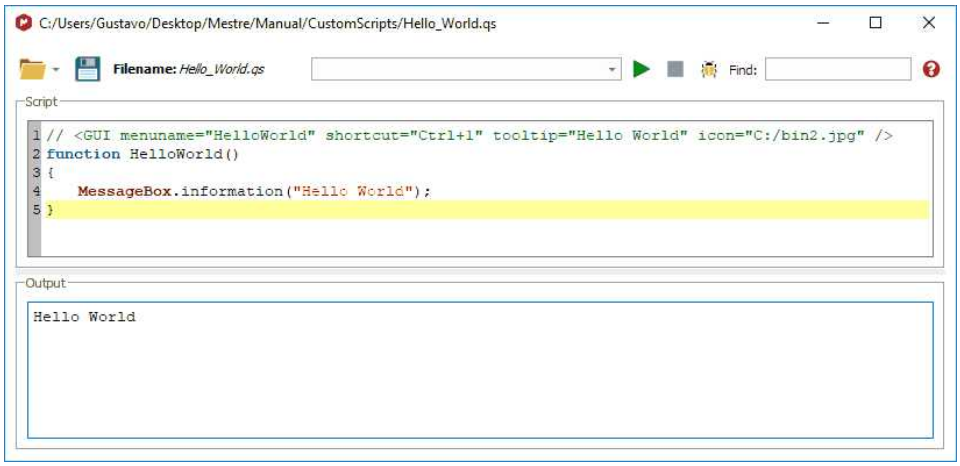


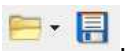
In this example we have defined a function called **helloWorld()** which will simply display a message box with the text 'Hello World'. Note that **MessageBox** is in bold, meaning that Mnova script editor recognizes it as a valid function.
Next, go to the edit control at the top of the script editor and type **helloWorld()**. Do not forget the brackets as they are required by the scripting framework in order to properly interpret the function. Once you have typed it, click on the green arrow at the right side of the edit control. You should get the pop up message box.

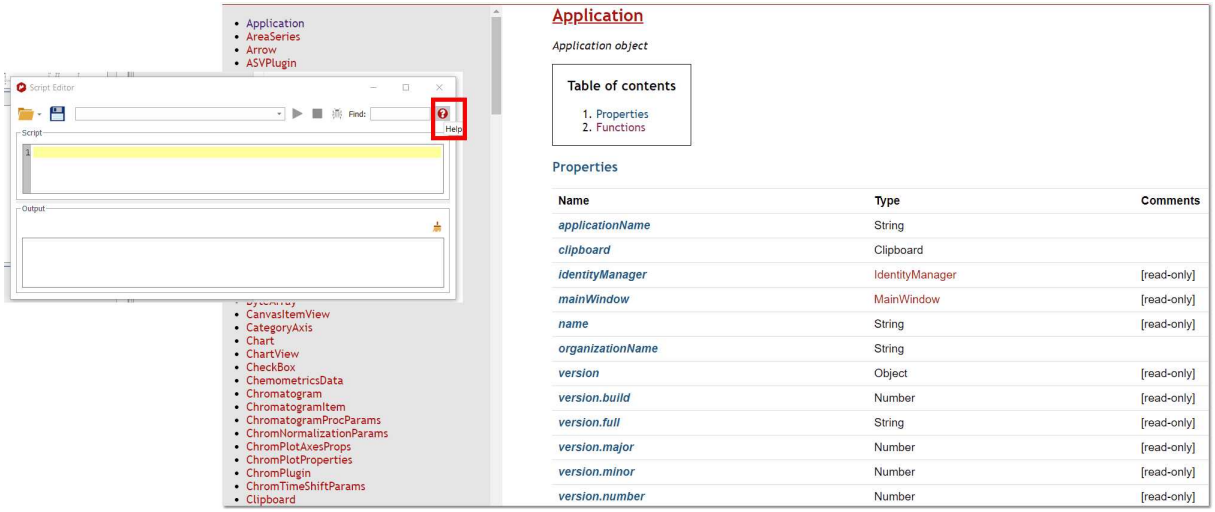


Use the **print** command to write information directly on the **output** control in the script dialog. This is very useful when debugging scripts.

© 2024 MESTRELAB RESEARCH

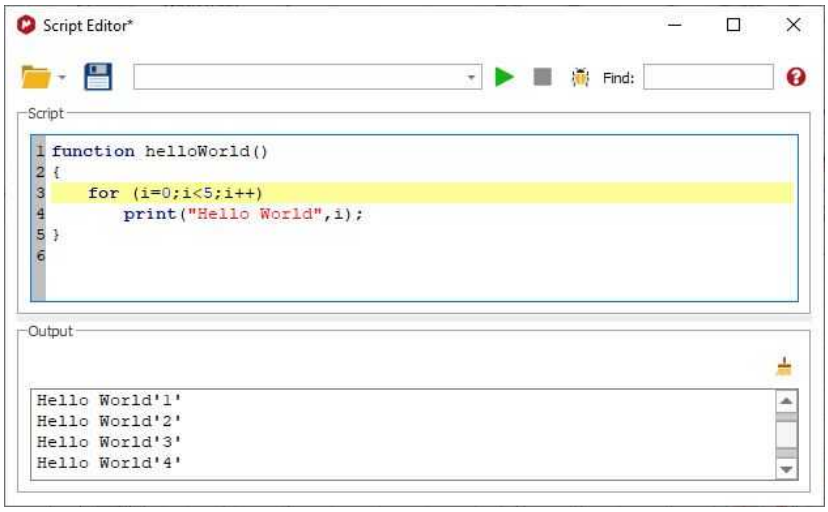


Please note that you will be able to load/save any script by using the applicable buttons . The play button will be used to run a script by typing the function on the edit box, whilst the stop button will stop a script in action. Clicking on the 'Help' button or pressing F1 will show you the 'Scripting help' window:



As Mnova scripting is an ECMAScript-based language, you can use any of the many functions and statements supported by this standard such as loops, conditions, etc. Remember that JavaScript is ECMAScript based too so if you are familiar with it or with C/C++, writing Mnova scripts should be very easy for you. If you are not familiar with the ECMAScript language, there are several existing tutorials and books that cover this subject, such as JavaScript: The Definitive Guide.

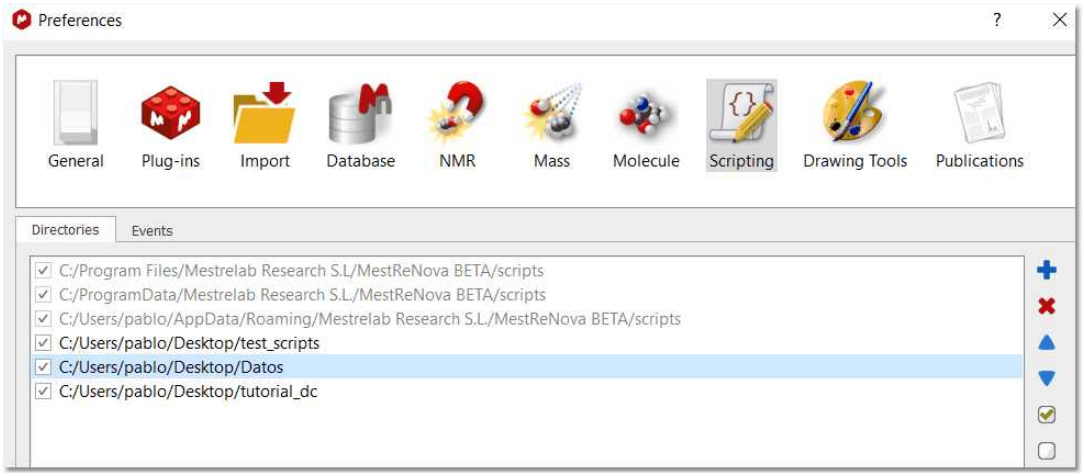
© 2024 MESTRELAB RESEARCH



You can also run scripts by following the menu 'Tools/Run Script' (shortcut: Ctrl+Shift+R) and loading the applicable .qs file.

Where should the scripts be saved?

In principle you could save your scripts anywhere in your computer. However, it's strongly recommended to save them in a custom **scripts** folder, that you can create from the 'File/Preferences/Scripts/Directories' menu (after having clicked on the blue plus button):



Use the red cross button to delete any added directory and the blue arrow buttons to sort the folders.

This is very important to allow Mnova to find these scripts when starting in such a way that they can be added to the GUI of Mnova (e.g. Toolbars, Menus, etc).

For example, type the following script:

```
//  <GUI  menuname="HelloWorld"  shortcut="Ctrl+1"  tooltip="Hello
World" icon="C:/bin2.jpg" />
function HelloWorld()
{
      MessageBox.information("Hello World");
```


© 2024 MESTRELAB RESEARCH

```
}
```

And save it in the scripts directory (program data …). Now close Mnova and open it again. The new script will appear under the 'Tools/Legacy Scripts' button:



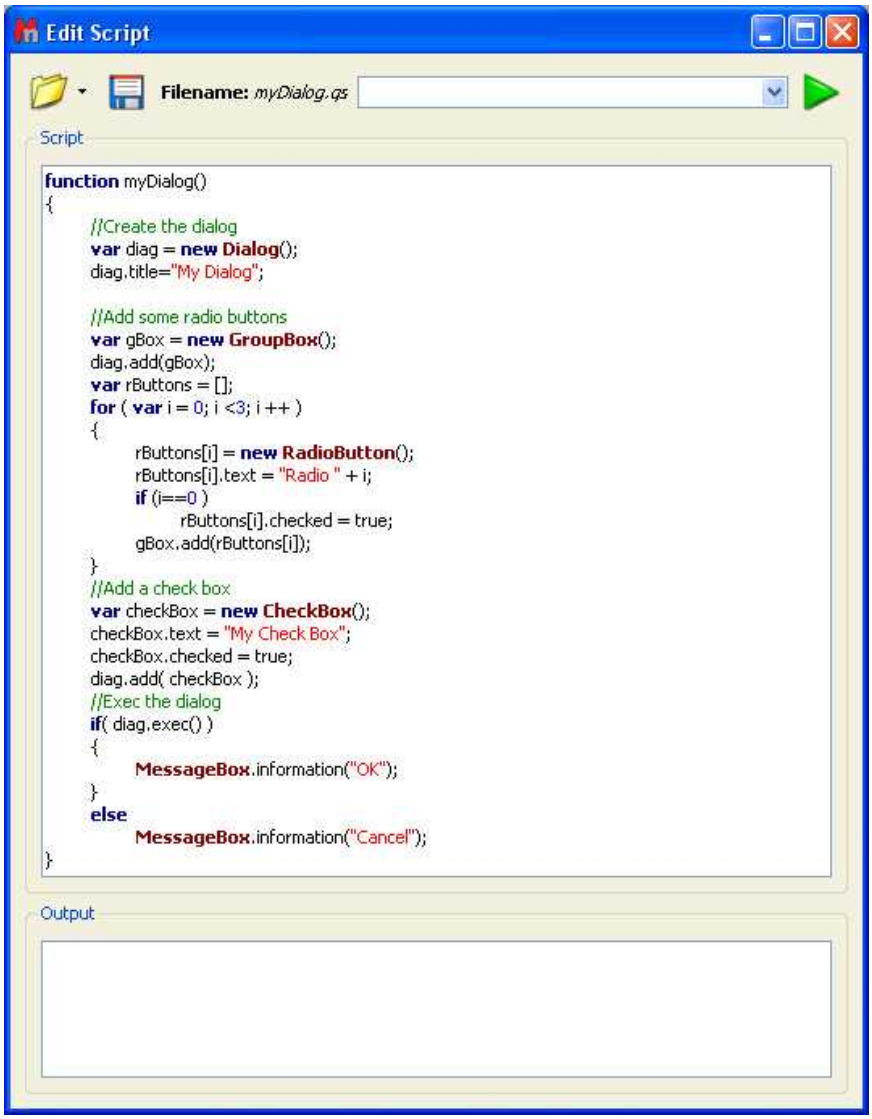
You will appreciate that the script is available in the menu bar. The key to get this working lies in the first line of the script (and of course, in the fact that Mnova can locate the script as it has been saved in the appropriate folder):

```
//     <GUI     menuname="HelloWorld"     shortcut="Ctrl+1"
tooltip="Hello World" icon="C:/bin2.jpg" />
```

This line is used to instruct Mnova about the name to be displayed in the scripts menu along with the keyboard shortcut and the text used as a tooltip. For a full description on the usage of this line, see connecting scripts and Mnova GUI section below.

You can create dialog boxes with controls. The script below shows a simple example.

© 2024 MESTRELAB RESEARCH



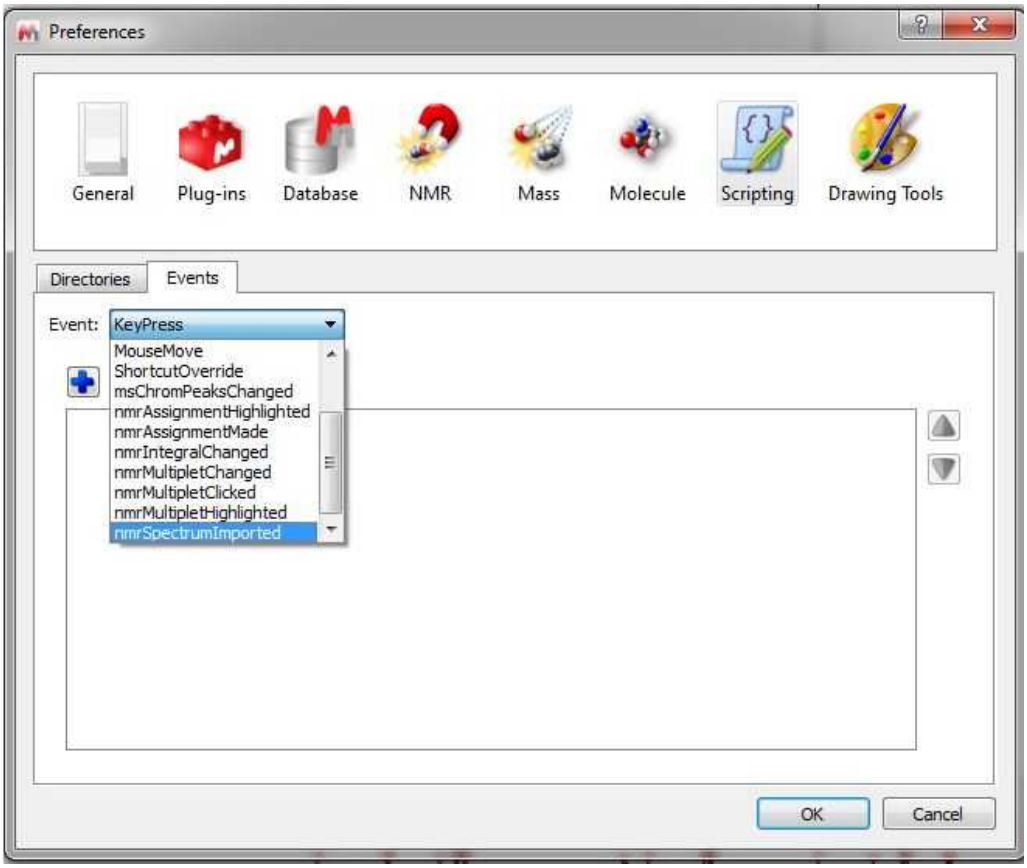
Execution of this script results in the following dialog box:



Scripting Events

Following the menu "File/Preferences/Scripts/Directories', you will find an 'Events' label; which will allow you to add any event (which will run by default in Mnova). You will find an example script about it (eventHandlerExample.qs) in the MestReNova examples script installation folder.

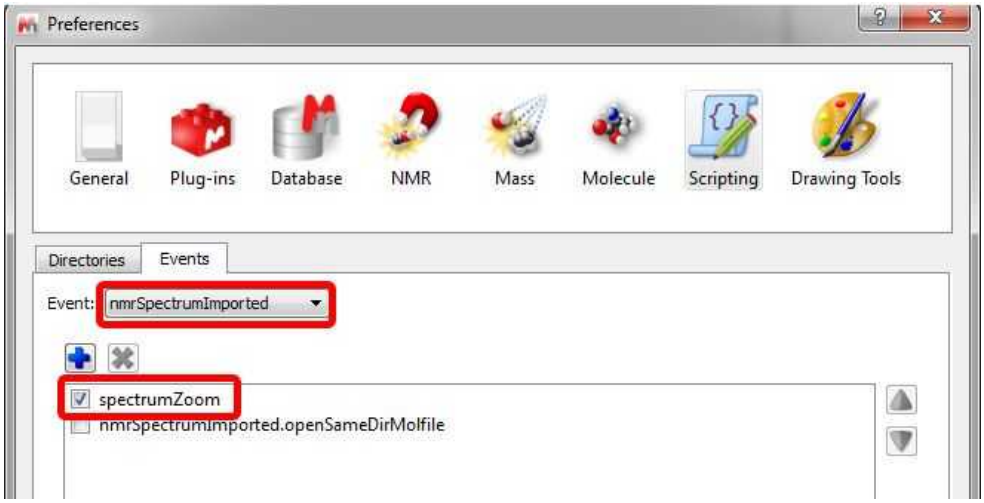
© 2024 MESTRELAB RESEARCH



For example, imagine that you always want to open your 1D datasets with a specific zoom area. You can create an event to do it. To do it, just save this script in your Scripts directories:

```
function spectrumZoom(aJSONString)
{
	var info = JSON.parse(aJSONString)
	var actDoc = mainWindow.activeDocument
	var spc = new NMRSpectrum(actDoc.getItem(info.itemId));
	if( !spc.isValid() )
		return false;
	spc.horzZoom(1.0, 5.0);
	spc.vertZoom(-100.0,8000.0);
	/*if( spc.dimCount == 1 )
		spc.zoom(3.0, 4.5);
	else
		spc.zoom(1.0, 5.0, 1.0, 5.0);*/
	//spc.zoom();
	spc.update();
	mainWindow.activeWindow().update();
	return true
}
Application.mainWindow.installEventHandler("nmrSpectrumImported",
"spectrumZoom")
```

Then restart Mnova and select it (spectrum zoom) as an event in the 'File/Preferences/Scripts/Events/nmrSpectrumImported' menu. this will open your 1D-NMR spectra with a spectral window from 1 to 5 ppm



17.1 Connecting scripts with Mnova objects

Simple access to an NMR spectrum

The examples we have shown so far are certainly not very useful. The good thing about scripting in Mnova is that it can be used to manipulate objects (e.g. spectra, molecules, pictures, etc) in Mnova and automated repetitive tasks. This section will explain how to access an NMR spectra from within the scripting framework.

Consider the following code snippet:

```
/*globals Application, File, TextStream*/
/*jslint indent: 4, plusplus: true*/

function dumpSpectrum() {
      "use strict";
      //To get the active spectrum
      var d, dumpStream, colCount, rowCount, r, c,
            spec = Application.nmr.activeSpectrum(),
            dumpFile = new File("C:/mnova/dump.txt");

      //The function isValid informs about if the spectrum obtained is correct
      if (spec.isValid()) {
            if (dumpFile.open(File.WriteOnly)) {
                  dumpStream = new TextStream(dumpFile);
                  try {
                        //To dump the spectrum information
                        dumpStream.writeln("Solvent:\t", spec.solvent);
                        //To dump information about each dimension
                        for (d = 1; d <= spec.dimCount; d++) {
                              dumpStream.writeln("Dimension:\t", d);
                              dumpStream.writeln("Frequency:\t",
spec.frequency());
                              dumpStream.writeln("Lowest Frequency:\t",
spec.hz());
```


© 2024 MESTRELAB RESEARCH

```
                                    dumpStream.writeln("Nucleus:\t",
spec.nucleus(d));
                                    dumpStream.writeln("Spectral Size:\t",
spec.count(d));
                             }
                             //To dump the values of the spectral points
                             if (spec.dimCount === 1) {
                                    rowCount = spec.count();
                                    for (r = 0; r < rowCount; r++) {
                                           dumpStream.writeln(spec.real(r), "\t",
spec.imag(r));
                                    }
                             } else if (spec.dimCount === 2) {
                                    colCount = spec.count(1);
                                    rowCount = spec.count(2);
                                    for (r = 0; r < rowCount; r++) {
                                           for (c = 0; c < colCount; c++) {
                                                  dumpStream.write(spec.real(r, c));
                                                  if (c < colCount - 1) {
                                                         dumpStream.write("\t");
                                                  }
                                           }
                                           dumpStream.writeln();
                                    }
                             }
                      } finally {
                             dumpFile.close();
                      }
               }
        }
}
```

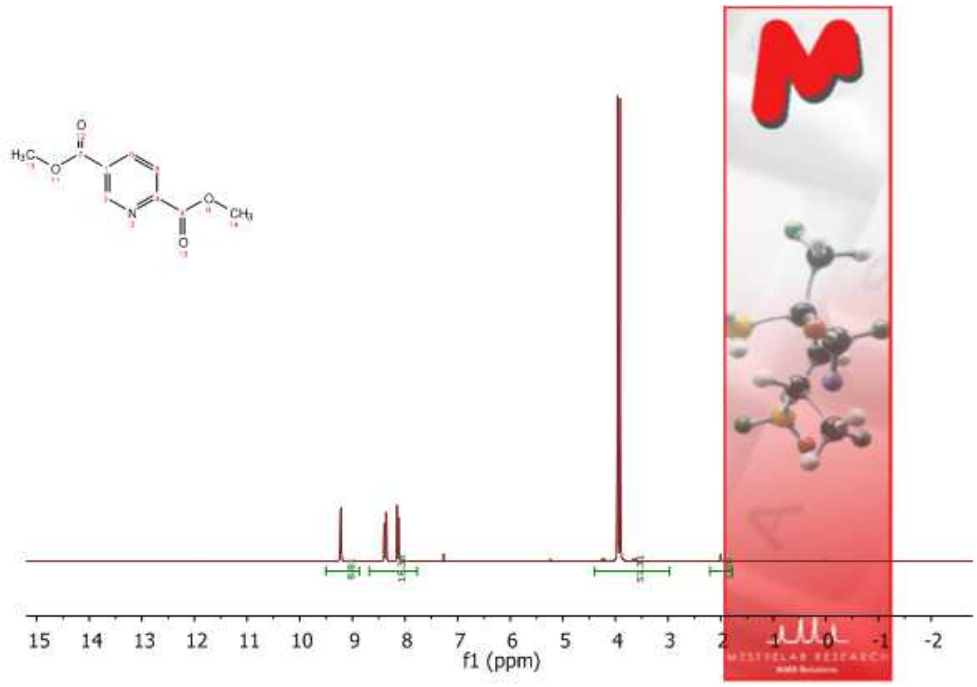
The first thing to note is the **nmr** object. This object gives access to the NMR plug-in framework and as such it allows you to get access to the NMR spectra in Mnova. For example, `spec = Application.nmr.activeSpectrum()`function makes it possible to select current active spectrum in Mnova. From that point, you can read relevant information of the NMR spectrum such as the number of points, spectral width, etc.

Let's now move to a more comprehensive example which will illustrate, step by step, how to access all objects within an Mnova page.

Having access to multiple objects: the layout script

As an illustrative example, we will build a script which will make it possible to organize different objects contained within a page. This script will be called **layout** for obvious reasons.
To start off with, open a spectrum, a molecule and a picture (which could be, for example, your company logo). The following picture shows the initial conditions for this example:

© 2024 MESTRELAB RESEARCH



The goal here will be to create a script which will arrange these 3 objects in such a way that the picture will be located at the left side of the page whilst the spectrum and the molecule will be moved next to the picture.

```
// <GUI menuname="Layout"shortcut="Ctrl+2" tooltip="Layout" />
function layout() {

	var i, item, width, height,
		w = mainWindow.activeWindow(),
		p = w.curPage(),
		n = p.itemCount();

	for(i = 0; i < n; i++) {

		item = p.item(i);
		width = item.width;
		height = item.height;

		switch(item.name) {
			case "NMR Spectrum":
				item.left = p.left + 50;
				item.right = p.right - 10;
				item.top = p.top + 20;
				item.bottom = p.bottom;
			break;

			case "Molecule":
				item.left = p.left + 50;
				item.right = item.left + width;
				item.top = p.top;
				item.bottom = p.top + height;
			break;
```


© 2024 MESTRELAB RESEARCH

```
                    case "Image":
                          item.left = p.left;
                          item.right = item.left + width;
                          item.top = p.top;
                          item.bottom = item.top + height;
                    break;
              }
        }
        w.update();
}
```

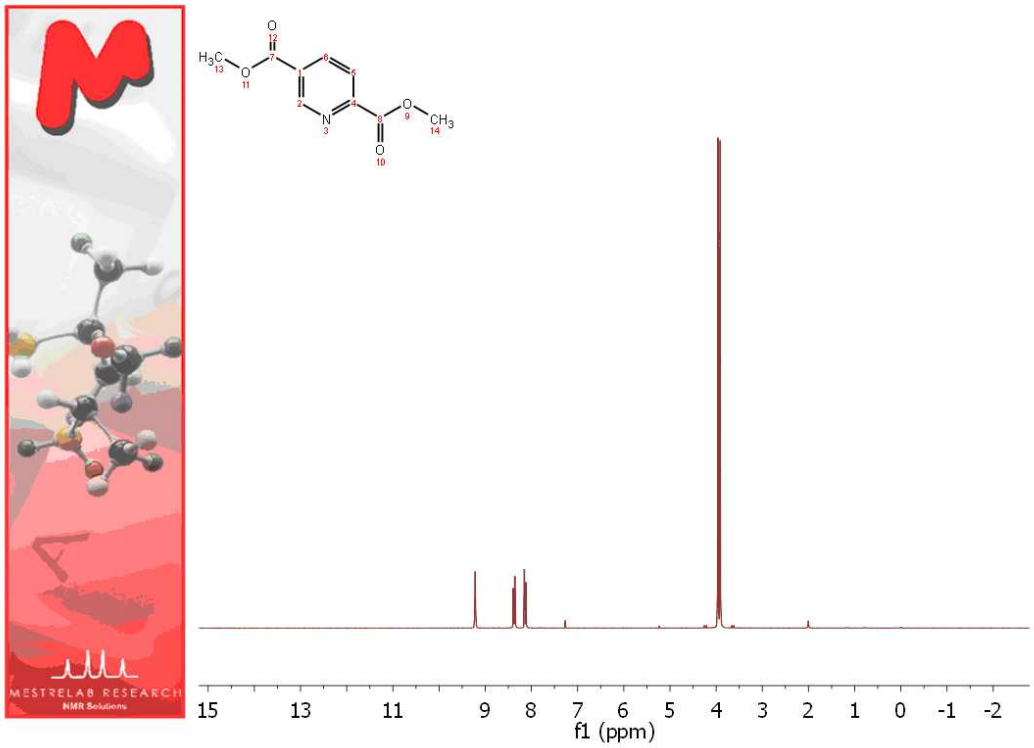
Every object in Mnova is contained in a page which in turn is contained in a document. So the first thing we should get access to is the document. This is done in this line:

```
w = mainWindow.activeWindow(),
```

Basically we access to the document window via the **Application** object.
Once we have the document, we can easily access to the currently active page through Page object like this:

```
p = w.curPage(),
```

Now we are ready to navigate through all the objects contained in this page. For every object, the exact type is checked within the switch command and then the objects are repositioned accordingly. This is the result for this example.



Tuning up the layout script

We will now modify the layout script in order to achieve the following two objectives:

1. Load the image from a file

2. Show the spectrum title automatically on the page when the script is executed

First off, we will show how to load an image from the disk. To do that, you have to use the serialization object. For example, assuming that the image is located in `c:/mnova/logo.png`, you can use this code:

```
serialization.open("c:/mnova/logo.png");
```

Next thing is how to show some parameters (Spectral Width, Solvent, Nucleus) on the page. In order to do that, it will be necessary to create a specialized NMR spectrum object rather than using the default properties available in every item object. See the following code snippet:

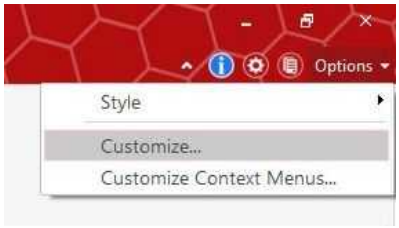
```
"<b>Frequency (MHz): </b> {0}<br/>\
                          <b>Spectral Width (Hz): </b> {1}<br/>\
                          <b>Solvent: </b> {2}<br/>\
                          <b>Nucleus: </b>
{3}".format(specItem.frequency().toFixed(4),

specItem.scaleWidth().toFixed(2),

specItem.solvent,

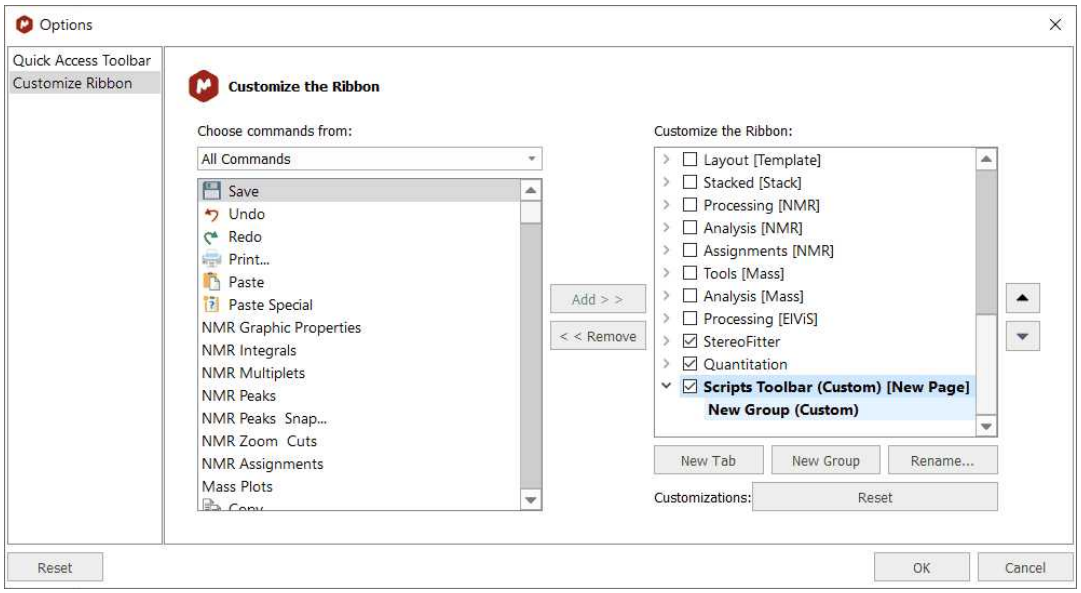
specItem.nucleus()),
                          true
                    );
```

Once you have the script saved (please bear in mind that the script file will need to have the same name as the function which you want to call, in this case the name of the script must be ‘layout2.qs’) in the Mnova scripts folder (and then have restarted the program), you will be able to add it to the toolbar just by following the menu: 'Options/Customize'.



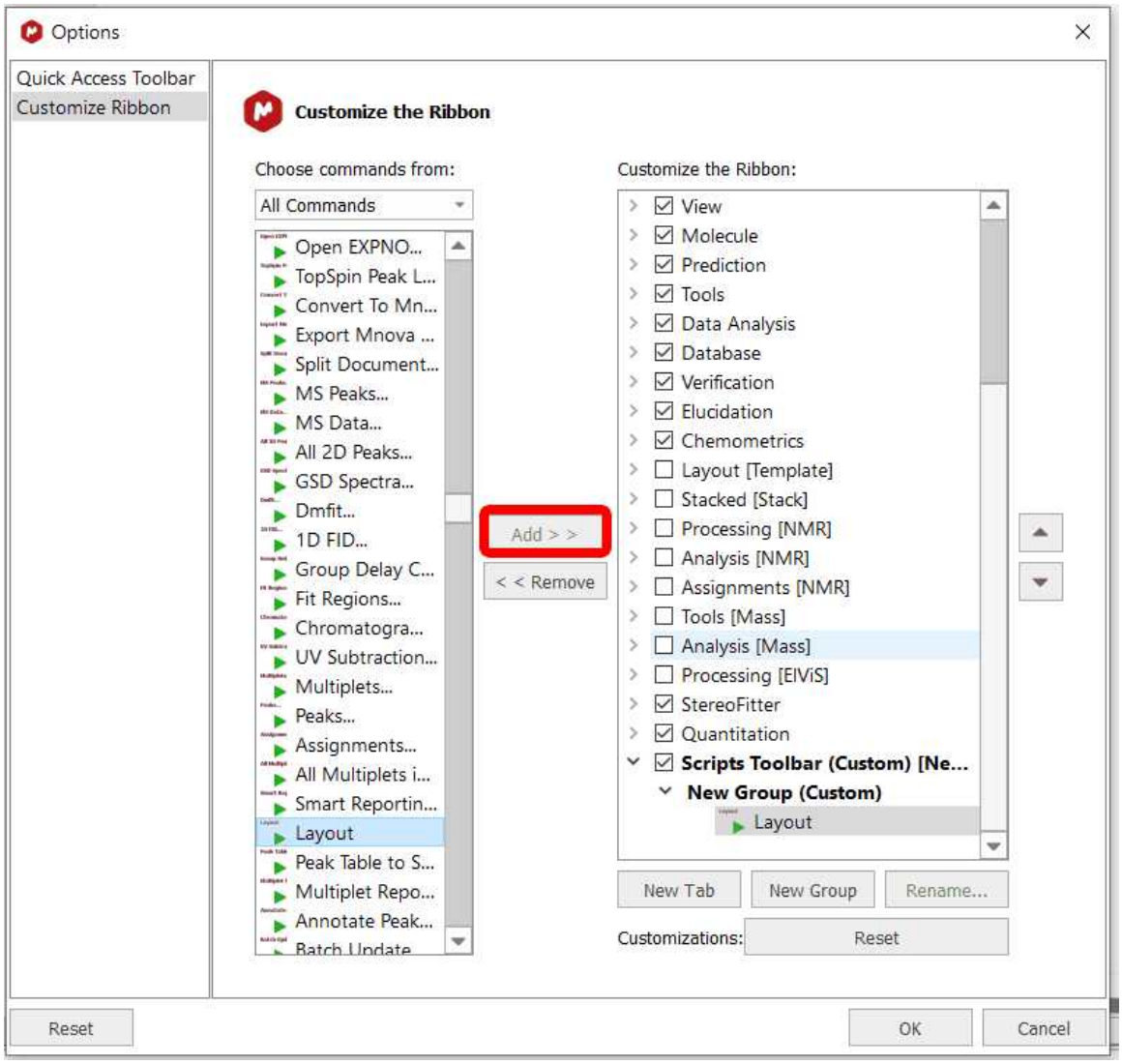
This will display the 'Customize Toolbars' dialog box. Then, click on the ‘New Tab’ button to create a new toolbar and change its name (Scripts Toolbar) by clicking on the ‘Rename’ button.

© 2024 MESTRELAB RESEARCH



Next, navigate to the 'Script' icon (Layout) in the 'Commands' menu (at the left-hand side of the window) and Click on the 'Add' button to add the 'Layout' script to the 'New Group' of the Scripts Toolbar.

© 2024 MESTRELAB RESEARCH



Finally, click on OK and you will be able to see the 'Layout' icon in the 'New toolbar'.



You will also be able to introduce the script in any existing contextual menu by following a similar procedure in the 'Customize Context menus' dialog box.

Custom NMR Processing

There are two ways to process NMR spectra via scripting:

1. By means of Processing Templates
2. By using the Processing interface

© 2024 MESTRELAB RESEARCH

1 Processing templates

Mnova is able to process a 1D or 2D NMR spectrum via scripting by using the processing templates (generated with the Full Processing feature). As you can see in the below example, you can process (with an easy script) any kind of spectrum (1H, 13C, 1D or 2D). You only need to create the processing template for each experiment. In the example below we have created 3 different processing templates, one for the processing of 1H-NMR spectra (1H.mnp), the second for the processing of 13C-NMR spectra (13C.mnp) and the last one for the processing of COSY spectra (COSY.mnp). As you can see in the scripts, the three the templates were saved at c:/mnova/ (of course you can change this path and also the name of the template files).

```
/*globals Application*/
/*jslint indent: 4*/

function procTemplate() {
	"use strict";
	var procFileName,
		spec = Application.nmr.activeSpectrum();

	if (!spec.isValid()) {
		return;
	}
	if (spec.dimCount === 1) {
		if (spec.nucleus() === "1H") {
			procFileName = "c:/mnova/1H.mnp";
		} else if (spec.nucleus() === "13C") {
			procFileName = "c:/mnova/13C.mnp";
		}
	} else if (spec.dimCount === 2) {
		if (spec.nucleus(1) === "1H" && spec.nucleus(2) === "1H") {
			procFileName = "c:/mnova/COSY.mnp";
		}
	}
	if (procFileName) {
		Application.nmr.processSpectrum(spec, procFileName);
	}
}
```

2 Processing interface

The Processing interface makes it possible to change or apply any processing operation available in Mnova.
For example, let's make a simple script which will set an exponential weighting function:

```
/*globals Application*/
/*jslint indent: 4*/

function myProcess() {
	"use strict";
	var spec = Application.nmr.activeSpectrum(),
		p = spec.proc;
```

© 2024 MESTRELAB RESEARCH

```
        p.setParameter("Apodization[1].Apply", true);
        p.setParameter("Apodization[1].Exp.Apply", true);
        p.setParameter("Apodization[1].Exp.Value", 0.5);
        spec.process(p);
        Application.mainWindow.activeDocument.update();
}
```

After creating a spectrum object which represents current active spectrum, we create a processing variable (p) which represents the processing operations for that particular spectrum. Once this new object is created, we can read or change its values using getParameter and setParameter functions. For instance, in this example we first set to true the Apodization property (otherwise no apodization will be applied) and then, after setting to true the exponential function, we set the line broadening to 0.5 Hz. Finally, when we are done, we assign this processing object to the spectrum object (`p = spec.proc;`) and we call `spec.process();` to actually apply all the processing operations.

NOTE: For further information about the scripting documentation, please check the Mnova installation folder at:
C:\Program Files\Mestrelab Research S.L\MestReNova\documents\scripts

17.2 Connecting scripts with Mnova GUI

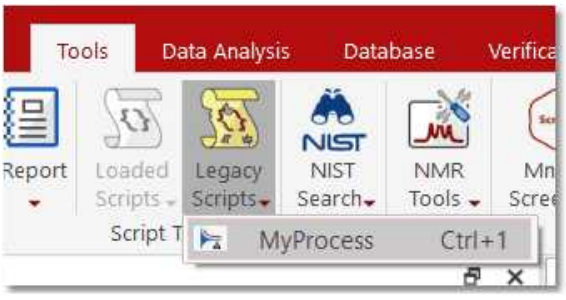
As you have seen in the examples above, you can insert any script in the Mnova Scripts menu, contextual menus or toolbars.

The key to get this working lies in the first line of the script (and of course, in the fact that Mnova can locate the script as it has been saved in the appropriate folder):

```
// <GUI menuname= "MyProcess" shortcut="Ctrl+1" tooltip="Exponential
0.5" icon="C:/bin2.jpg" />
```

This line is used to instruct Mnova about the name to be displayed in the scripts menu along with the keyboard shortcut and the text used as a tooltip. Please bear in mind that you can use any created image file as icon. In this case, the name of the script is *MyProcess*, the shortcut is *Ctrl+1*, the tooltip is *Exponential 0.5* and the icon used was saved at *C:/bin2.jpg*.

You can see in the picture below, that we added the script ‘MyProcess’ to the ‘Legacy Scripts’ menu with the corresponding shortcut (Ctrl+1):



Please bear in mind that the script file will need to have the same name that the function which you want to call, in this case the name of the script must be ‘MyProcess.qs’, because the name of the function is: MyProcess().

© 2024 MESTRELAB RESEARCH

17.3 Mnova processing scripts

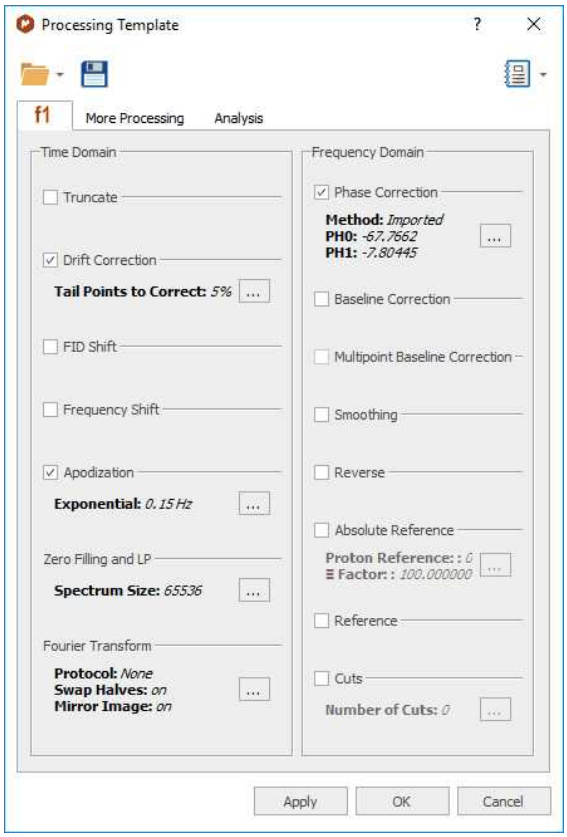
This reference contains a list of objects, functions and properties supported by Mnova script. For a complete ECMAScript Reference, consult this link.

Example scripts are present in the examples/scripts installation directory.

You can find the Mnova script reference documentation in the Mnova installation folder ('documents/scripts'), or by clicking on the 'Help' button in the scripts editor (or pressing F1 key).

NMRProcessing. Describing Processing parameters (Argument Strings).

You will find in the Mnova installation folder: 'documents/scripts' a list of the argument strings (which have the form ***function1.function2...functionN.parameter***) that you will be able to use in your processing scripts. These parameters appear in the graphical interface of the software (in the 'Processing / Full Processing' menu) and are described in depth in the manual of Mnova.

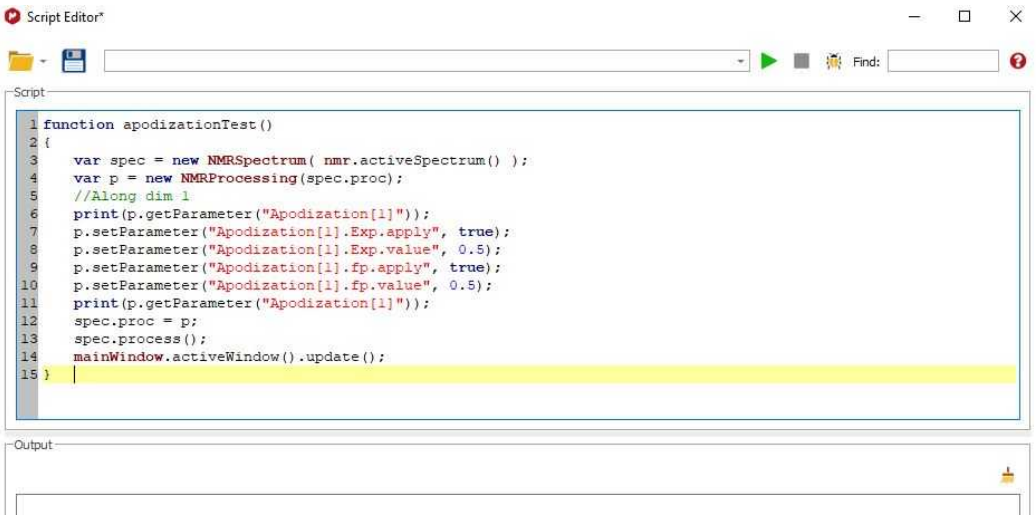


You can load a saved processing template by running this easy script:

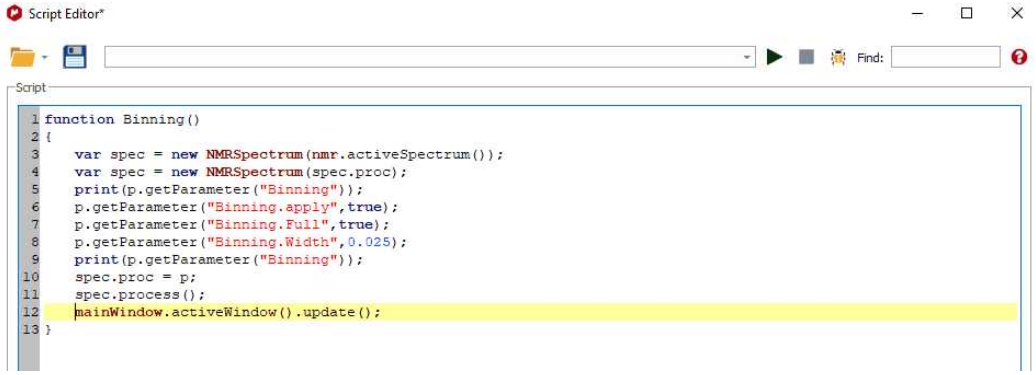
```
//<GUI menuname="ApplyProcessingTemplate"shortcut="Ctrl+2"tooltip="Apply a saved processing tem
function ApplyProcessingTemplate()
{
var spec = new NMRSpectrum( nmr.activeSpectrum() );
var processing = new String;
if (!spec.isValid())
return;
```

```
processing = "C:\\ProcessingTemplate.mnp";
nmr.processSpectrum(spec, processing);
spec.process();
mainWindow.activeWindow().update();
}
```

Here you can see additional examples. This is a script to apply an Exponential apodization function of 0.5 Hz:

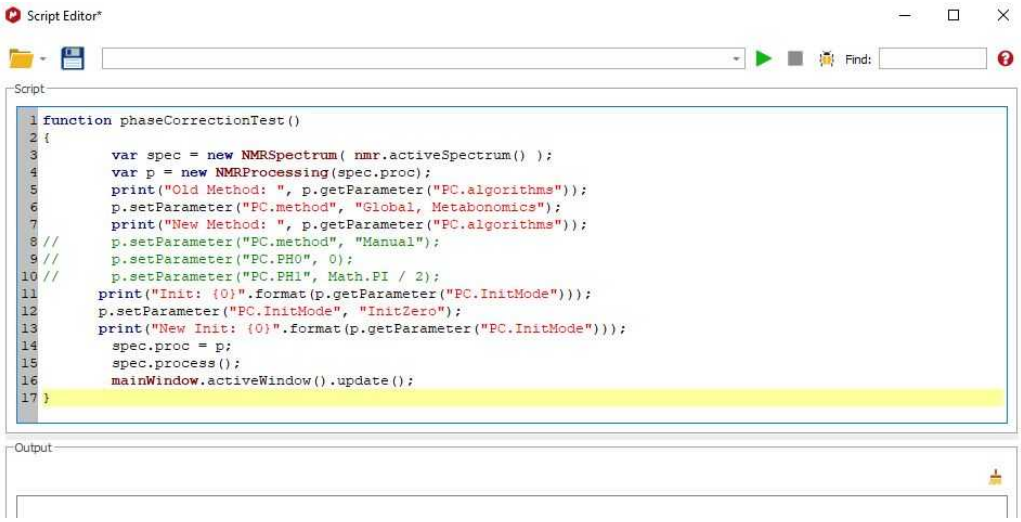


Here you can see an example to Bin your spectra with 0.025 ppm:

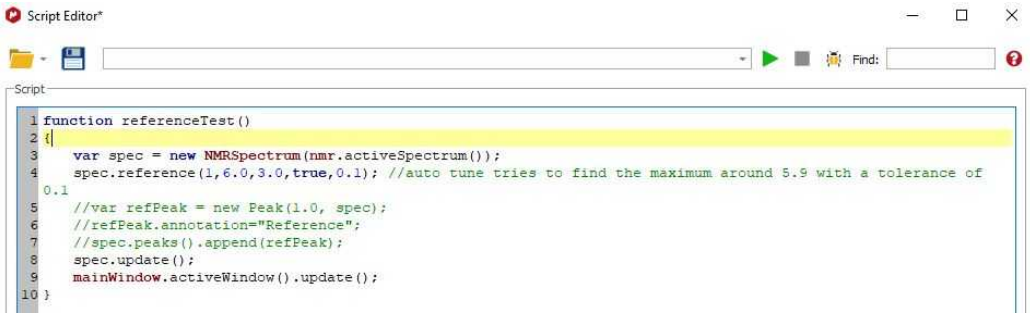


This is an example to apply a phase correction:

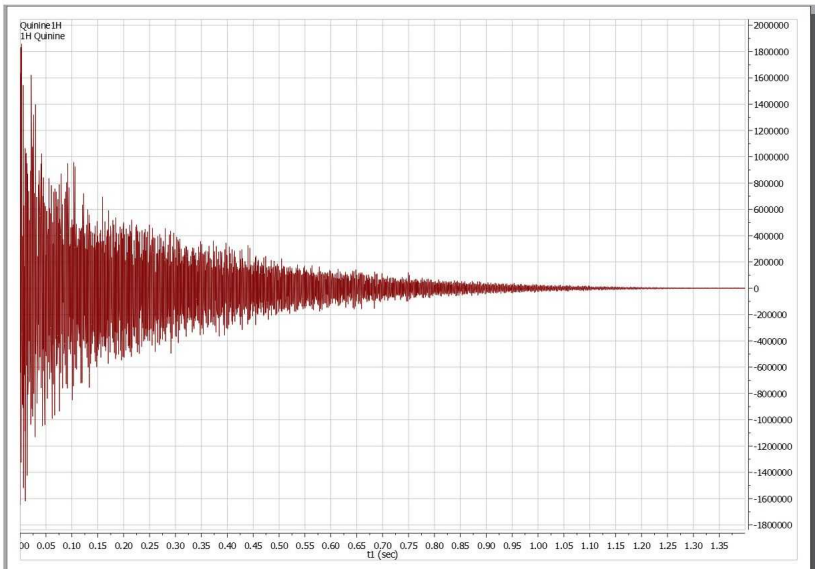
© 2024 MESTRELAB RESEARCH



And this script can be used to reference your spectra:



You will find in the example/scripts folder a script called "customImportProcessing.qs", which will allow you to import your NMR spectra without applying any processing. If you add this script to a 'Script directory' you will obtain just the FID of your datasets.
You can also edit the "customImportProcessing.qs" script to always apply a baseline correction when you import a dataset.

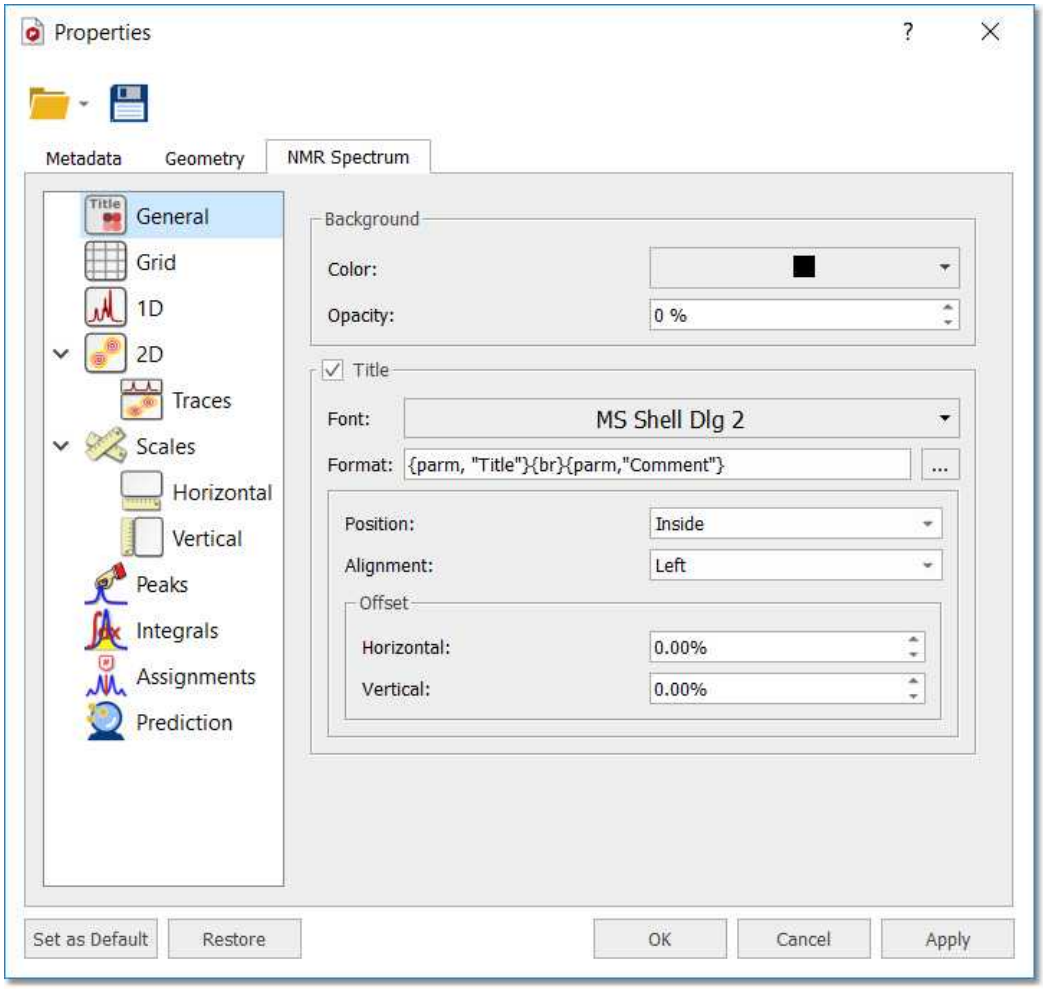


© 2024 MESTRELAB RESEARCH

17.4 Mnova properties scripts

NMRSpectrum.getProperty/setProperty Arguments

You will find in the Mnova installation folder ('documents/scripts') the spectrum properties (***aParamName***) that you can manage via script, by using the functions 'SetProperty' and 'GetProperty'. You will find an example script in the examples/scripts installation directory and also in the 'Script Samples' chapter. All of these properties can also be selected by using the GUI:



You will find below an example of a script to change some properties of your spectra. In this case, the colour of the spectrum will be changed to red, the peaks font to Arial and the integral curves will be showed.

```
function properties()
{
	var spec = new NMRSpectrum(nmr.activeSpectrum());

	spec.setProperty("curve.color","red");
	spec.setProperty("peaks.font","Arial");
	spec.setProperty("integrals.curve.show", true);
	spec.update();
	mainWindow.activeWindow().update();
```


© 2024 MESTRELAB RESEARCH

```
}
```

NOTE: For further information about the scripting documentation, please check the Mnova installation folder at:
C:\Program Files\Mestrelab Research S.L\MestReNova\documents\scripts

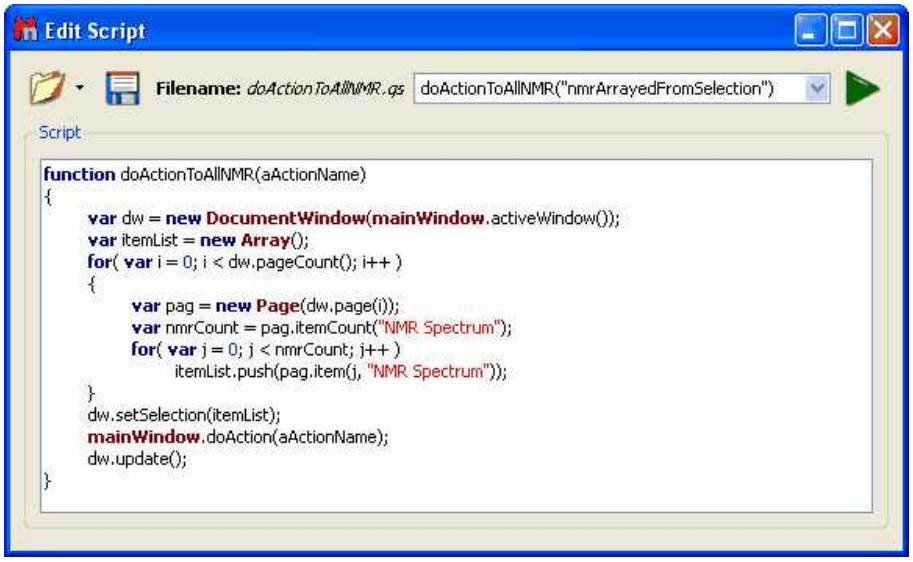
17.5 GUI Actions

You will find in the Mnova installation folder ('documents\scripts') the GUI actions that you will be easily able to run with an easy script. The script below will apply an "Auto Peak Picking" analysis:

```
function doActionTest()
{
mainWindow.doAction("nmrAutoPeakPicking
");
}
```

If you want to run any other action just replace the red action with any action of the the list below. Of course, you can add to the script as many actions as you want.

Here you can see an example of this tool to apply several actions to all the spectra opened in the same document:



This script will generate a stack plot of all the opened spectra. You can use this script to run whatever doAction you de

17.6 Running Scripts from the command line

You can run scripts from the command line, just by typing the paths where the Mnova .exe file and the script are located and -sf "name of the script function": **"mestrenovaPathname" "scriptPathname" -sf "scriptFunctionToRun"**

For example:

1. **mestrenova scriptPath**
For example: mestrenova c:\temp\multiOpen.qs will run the function multiOpen() from multiOpen.qs file, i.e. it runs a function with the same file name.

2. **mestrenova scriptPath -sf functionName,arg1,arg2,...,argN**
This runs functionName(arg1, arg2, ... argN) from the scriptPath file.

The below command line is doing the same as the above one:
mestrenova -sf functionName,arg1,arg2,...,argN scriptPath

Examples:
You can also use: **"mestrenovaPath" "scriptPath" -sf "scriptFunctionToRun"**

"C:\Program Files\Mestrelab Research S.L\MestReNova\MestReNova.exe" "C:\Program Files\Mestrelab Research S.L\MestReNova\scripts\myScript.qs" -sf "myFunction"
It is also possible to specify arguments:

"C:\Program Files\Mestrelab Research S.L\MestReNova\MestReNova.exe" "C:\Program Files\Mestrelab Research S.L\MestReNova\scripts\myScript.qs" -sf "myFunction",0.1,10,true,off

In this case, MNova will run myFunction(0.1,10,true,"off") from myScript.qs with the arguments specified. No spaces after commas!

3. **mestrenova -sf functionName,arg1,arg2,...argN**

In this case it is supposed that the function functionName is taken from one of the script directories, either MNova standard or user defined.

Note: Command line arguments are separated with white-spaces. This is why it is important not to use white-spaces after commas. If script arguments contain white-spaces then use quotes: "C:\Program Files\Mestrelab Research S.L\MestReNova\MestReNova.exe" -sf openSpecWithMols,"C:\1H\fid".

This doesn't need quotes: "C:\Program Files\Mestrelab Research S.L\MestReNova\MestReNova.exe" -sf openSpecWithMols,C:\1H\fid

Complex case example:
mestrenova specPath1 specPath2 -sf functionName,arg1,arg2 specPath3 specPath4

This will first open the spectra specPath1, specPath2, specPath3, specPath4. Next, apply the script: functionName(arg1, arg2)

There is also a single command line parameter **-w** (w stands for wait). If this parameter is present in the command line and there is no Mnova already running, then Mnova will open, load any files and run any scripts passed in the command line and, finally, it will close.

If this parameter is present but Mnova is already running, then the second Mnova instance will pass the command line information to the first running instance, the first running will load/run files/scripts and finally it will notify to the second instance that it has finished, so the second instance will close.

Some examples:

MestReNova.exe -w
Mnova not running: Mnova opens and closes immediately.
Mnova running: The second Mnova instance will return immediately.

MestReNova.exe myscript.qs -w
Mnova not running: Mnova opens run the myscript function in the myscript.qs file and closes when it finishes.

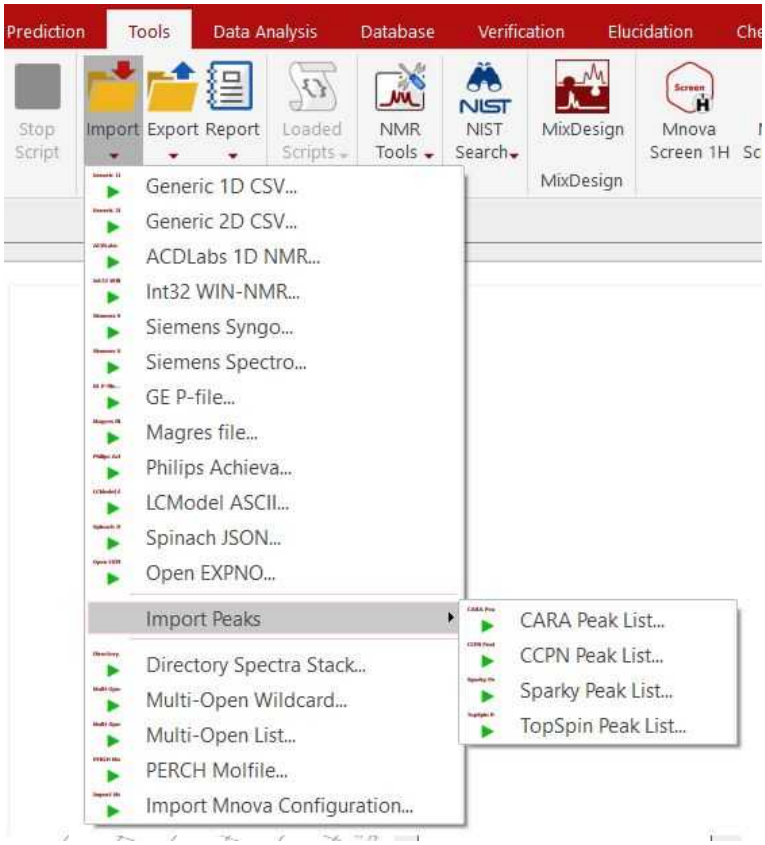
© 2024 MESTRELAB RESEARCH

Mnova running: A second Mnova instance is run and passes the parameters to the already running one. The myscript function from the myscript.qs file is run in the already running Mnova instance and when it finishes, notifies the second one and the second one Mnova instance (the one you have started from the command line) it closes.

17.7 Import

There are some specific spectral formats (such as ACD Labs, LC Model ASCII, Philips Achieva, GE-P, Siemens Syngo, Siemens Spectro, Magres...) which can only be imported in Mnova by using the scripting engine.

To import these kinds of spectra, just run Mnova, follow the menu 'Tools/Import' and select the desired format

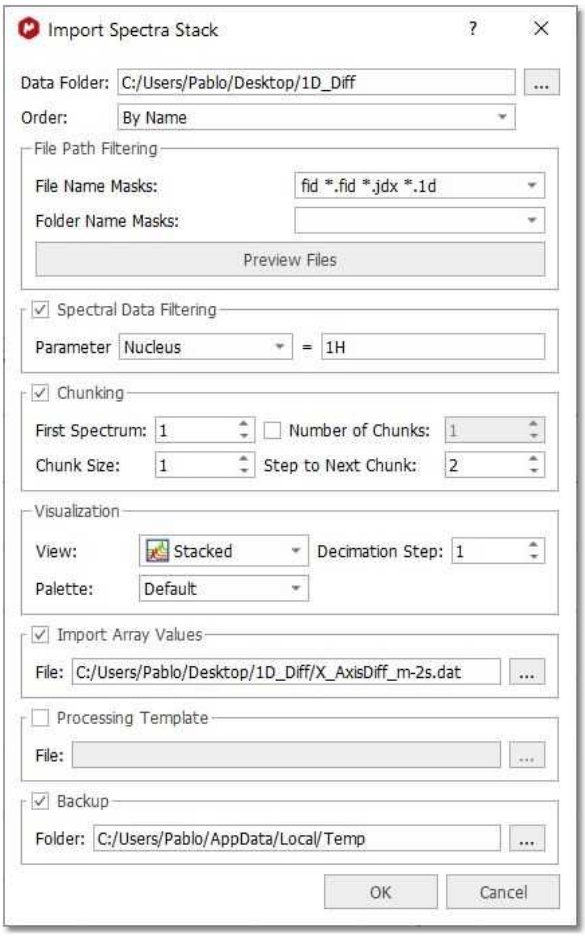


The user can use these scripts as a starting point to import any other spectral format. If you need help about it, just contact us.

Mnova includes a powerful script which makes it possible to import all spectra (1D, 2D, mnova documents, UV, IR, Raman, etc) contained in a folder and automatically stack them for forthcoming R-M analysis. This script is under the "Tools/Import' menu and it is called '**Directory Spectra Stack**'. It will allow the user to select the directory where the spectra are contained. Then Mnova will open and process all those spectra (FIDs) automatically in bunches of 20 spectra and finally will stack all of them.

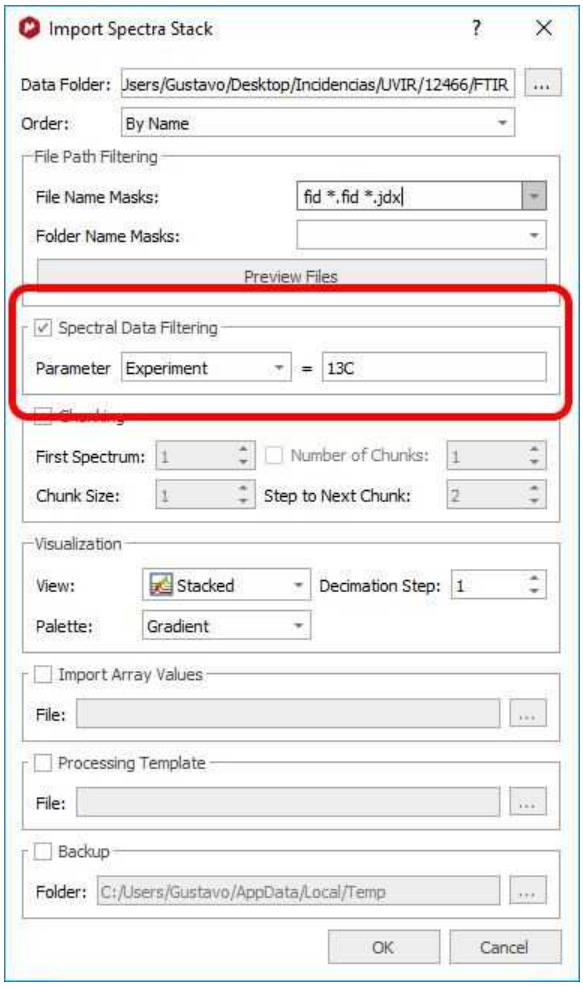
Note: This script has also the option to decimate the spectra while loading (i.e. the program can skip every 10 spectra in the data set) and to apply a processing template (or to load the 1r processed data):

© 2024 MESTRELAB RESEARCH



The script will firstly apply the **spectral data filtering** and then the data chunking. You can filter by any parameter displayed in the Parameters Table; you can find below an example to filter by experiment (13C):

© 2024 MESTRELAB RESEARCH



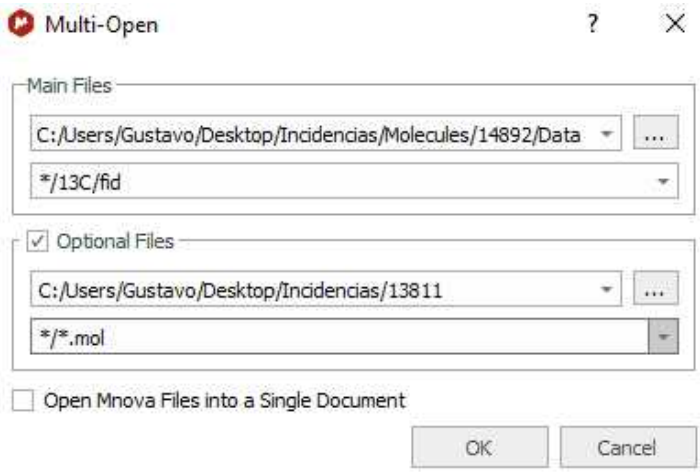
The 'Preview Files' button will preview inside the File Path Filtering in order to list all files which will be processed.

- Chunking: the check box will disable/enable the feature, allowing the user to define data intervals. If the 'Number of chunks' checkbox is disabled, it will take all chunks since it found the first spectrum (going up to the end). For example, the settings above could be used to get a stack of just the first 96 spectra. The script will not go on importing the rest of the spectra to end of the directory.

You can change the 'default import' options for your datasets, just by using the 'ImportProcessing' script. You will find an example of that script called "customImportProcessing.qs" in your Mnova installation folder. You can freely edit this script to make your own automatic import processing options or even you can create 'Processing Templates' that will be applied automatically over your spectra after having loaded them in Mnova.

MultiOpen script: Mnova incorporates a script named 'MultiOpen Wildcard' (under the 'Tools/Import' menu). This tool is very useful if, for example, you have to routinely handle a large number of datasets. The script allows to open simultaneously a collection of several spectra. Make sure that all spectra are located in the same folder:

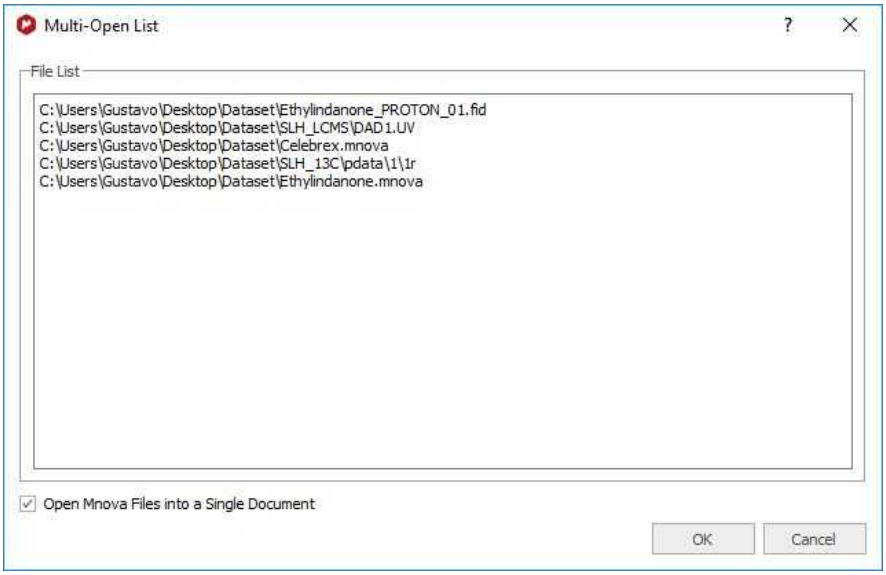
© 2024 MESTRELAB RESEARCH



Finally, write the 'Path Mask' name (you can use wildcards, so this could be, for example, */10/pdata/1r, or just */fid, or */ser), and click 'OK'. All selected spectra will open simultaneously.

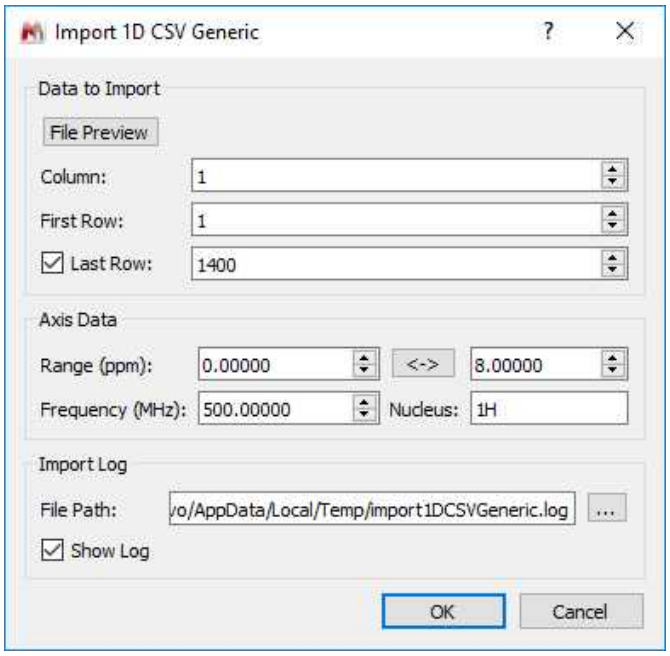
The user can also use the 'Full Processing' command to automate the processing of both 1D & 2D NMR data sets. (You can find a full description of this tool further on in the Automated Processing chapter.)

The 'Multi-Open List' script will show a dialog with a multi-line text edit box, into which you can either type or paste your lists of files to open.



The **'Generic 1D CSV'** script allows to create a NMR spectrum by importing a 1D .csv or .txt file:

© 2024 MESTRELAB RESEARCH

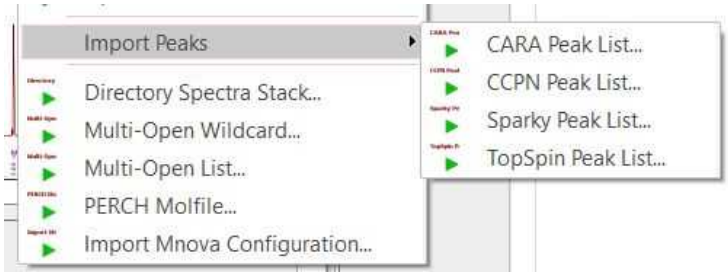


You would need to select the number of columns and rows you want to import from the files, also the spectral range(in ppm) as well the frequency and nucleus.

The 'Generic 2D CSV' script will allow you to import a 2D-NMR dataset from a .csv or .txt file.

The script to 'import Mnova configuration' will allow you to load an .ini file created with the script 'Export Mnova configuration' which includes all the Mnova preferences and settings (including tables, toolbars, properties, etc)

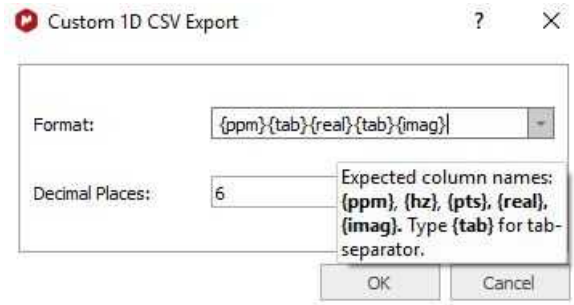
The Import Peaks scripts will allow you to import the peak lists from files generated in CARA, CCPN, Sparky and TopSpin:



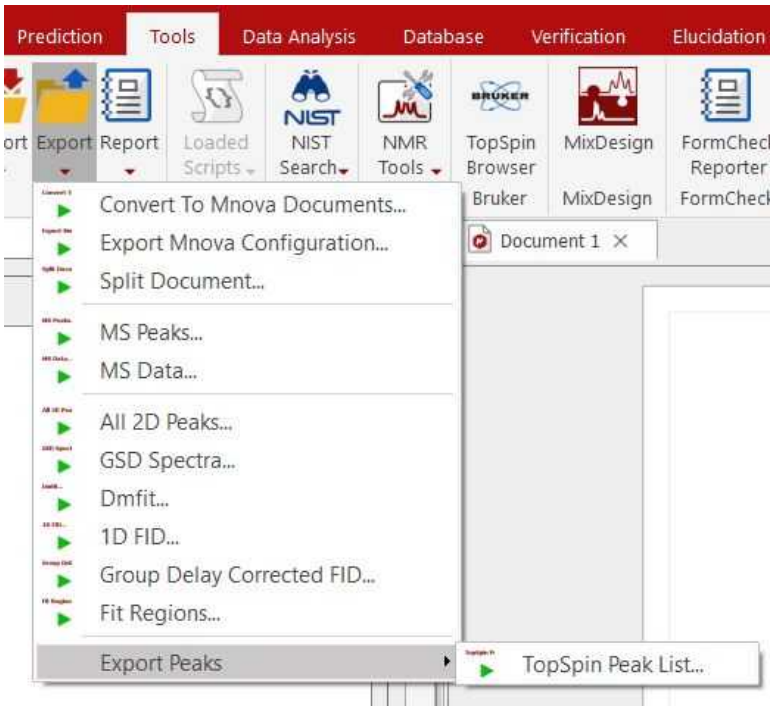
17.8 Export

You can export NMR spectra via 'File/Save As/NMR 1D Custom CSV File' with options to export PPM+Re+Im, Hz+Re, PPM+Re or Hz+Re+Im, either from right to left or from left to right.

© 2024 MESTRELAB RESEARCH

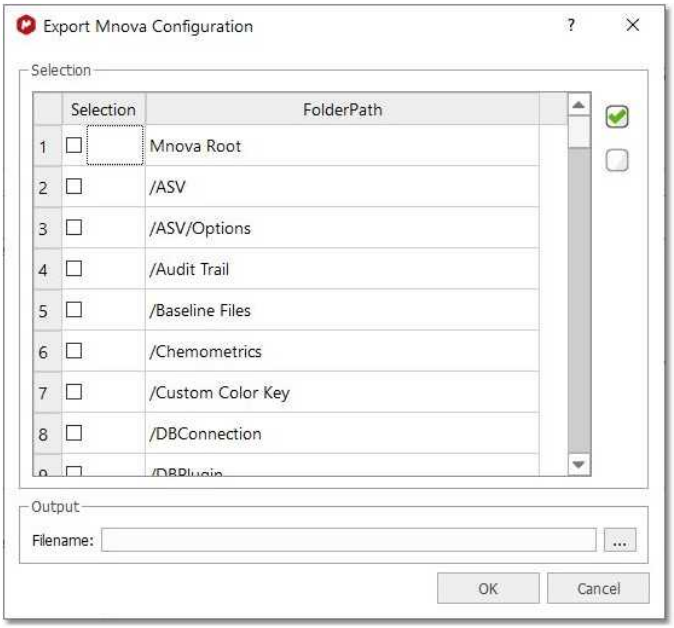


You can use some scripts from the 'Tools/Export' menu:



The script to **'Export Mnova configuration'** will allow you to export all the desired Mnova preferences and settings (including tables, properties, toolbars, etc) as an .ini file, to be imported from the script located under the 'Tools/Import/Import Mnova Configuration'.

© 2024 MESTRELAB RESEARCH



The "Split Document" script will allow you to save pages and individual Mnova documents.

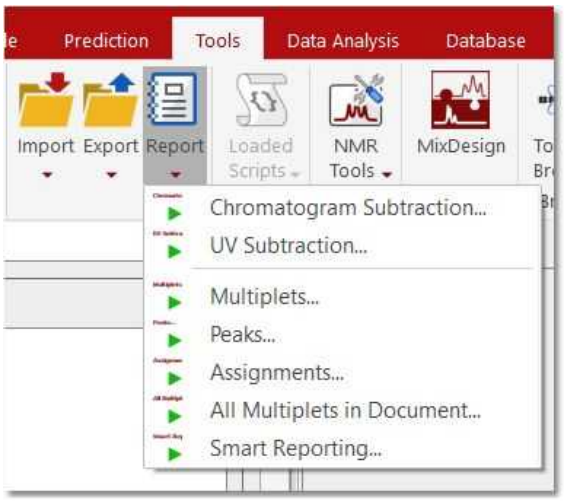
The 'MS Peaks' and 'MS Data' scripts will export the peaks of your MS spectra and chromatograms as ASCII files.

The script called **'All 2D Peaks'** will export all the peaks of 2D datasets in the current document. The '**DmFit**' will export 1D-NMR spectra to Dmfit ASCII format. The **'FID'** script can be used to extract a XY text file for the FID. This script saves original FID independently on what is shown on the screen (FID, or FID', or FT Spectrum). The **'Fit Regions'** script will export the fit peaks to a text file. The '**GSD Spectra'** script will generate an ASCII file with the GSD peaks.

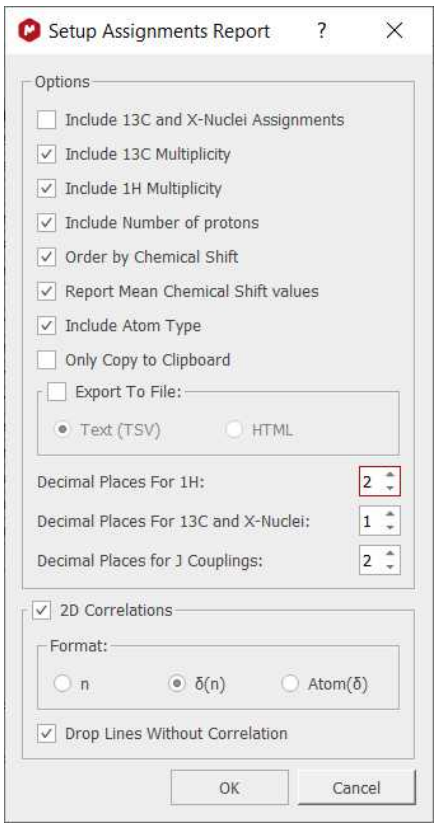
The "Export Peaks/Top Spin Peak List" script will export your peak picking as a .xml file with the TopSpin format.

17.9 Report

We have some scripts to report your multiplets, peaks list, assignments, etc under the 'Tools/Report' menu:

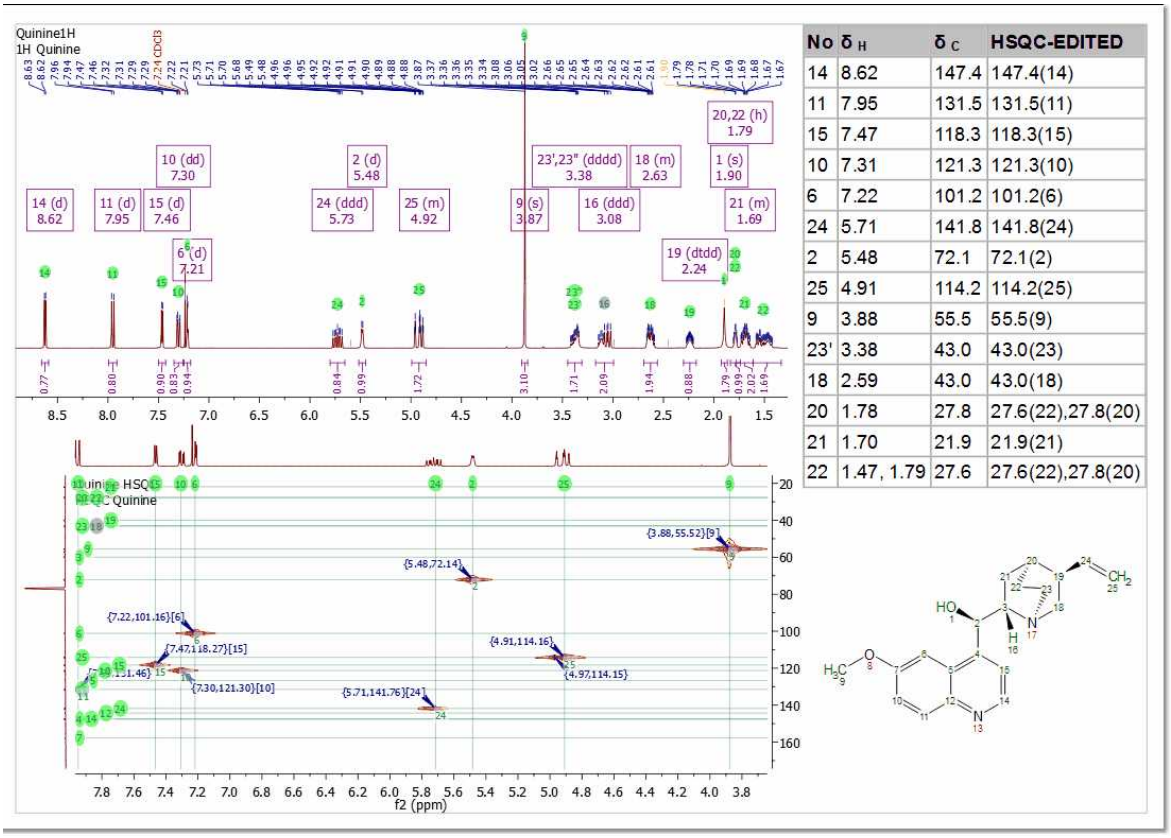


It is possible to export your **assignments** in a table by following the menu 'Tools/Report/Assignments'. Then, a dialog will appear, from where you can select the correlations to be shown, order by chemical shift, report ranges or mean chemical shift values, show the atom types and copy the report assignments to clipboard:



Finally, you will get the report of the assignments pasted on the spectral window:

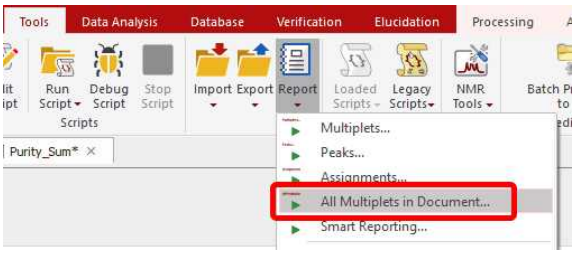
© 2024 MESTRELAB RESEARCH



No	δ_H	δ_C	HSQC-EDITED
14	8.62	147.4	147.4(14)
11	7.95	131.5	131.5(11)
15	7.47	118.3	118.3(15)
10	7.31	121.3	121.3(10)
6	7.22	101.2	101.2(6)
24	5.71	141.8	141.8(24)
2	5.48	72.1	72.1(2)
25	4.91	114.2	114.2(25)
9	3.88	55.5	55.5(9)
23'	3.38	43.0	43.0(23)
18	2.59	43.0	43.0(18)
20	1.78	27.8	27.6(22),27.8(20)
21	1.70	21.9	21.9(21)
22	1.47, 1.79	27.6	27.6(22),27.8(20)

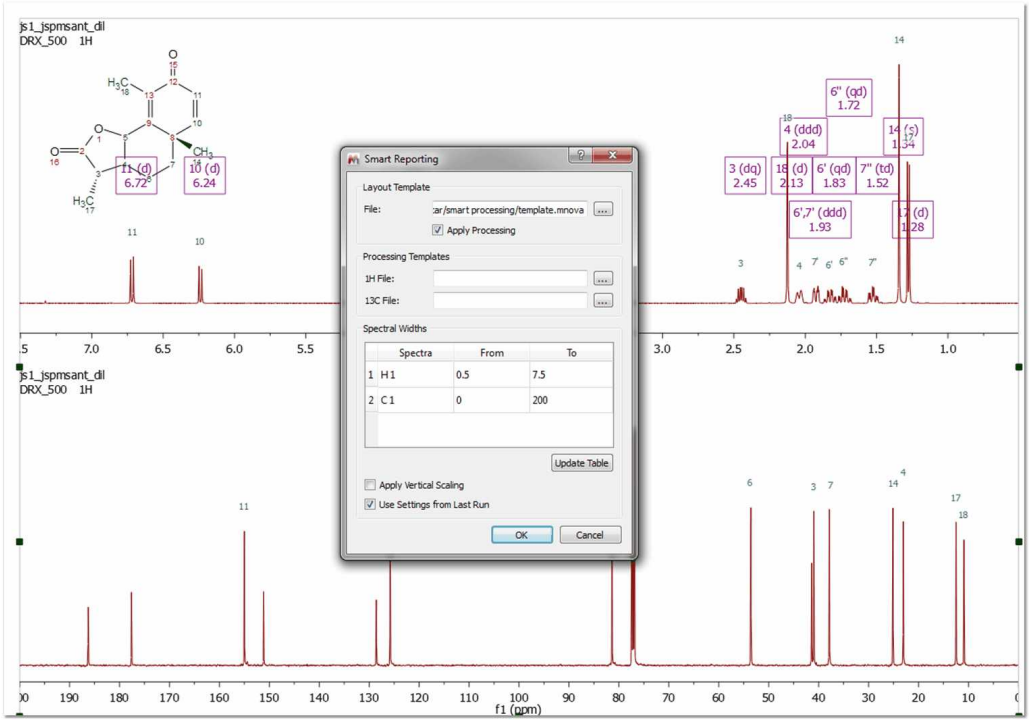
All Multiplets in Document

Follow the menu 'Tools/Report/All Multiplets in Document' to generate multiplet reports in each spectral page of a document:



Smart Reporting

This script will allow you to load a layout template, with the option to include the applicable processing template embedded in the layout or to load other processing templates. The script will allow you also to select 'Spectral Widths' for each nuclei:

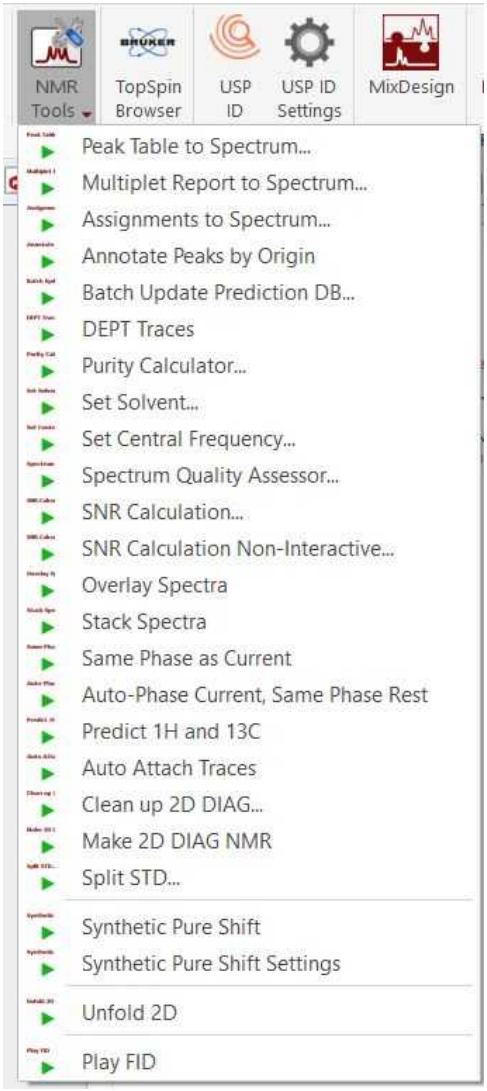


See also 'Background and UV subtraction'

17.10 NMR Tools

You will find several scripts under the 'Tools/NMR Tools' menu:

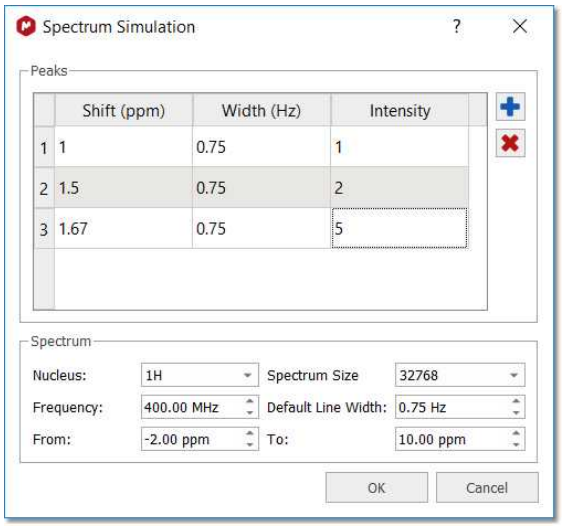
© 2024 MESTRELAB RESEARCH



Peak Table to Spectrum

This script will allow you to synthesize a NMR spectrum from a peak table:

© 2024 MESTRELAB RESEARCH

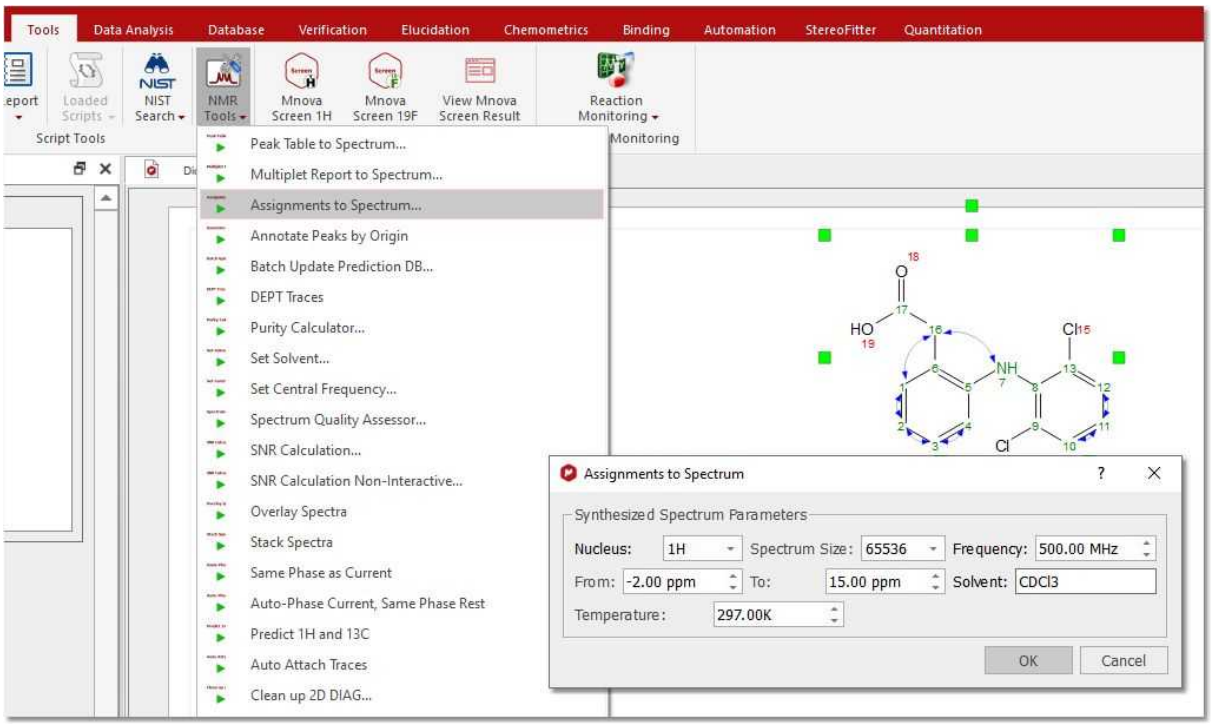


Multiplet Report to Spectrum

To synthesize a spectrum from a multiplet report copied from a paper. See this chapter for further information.

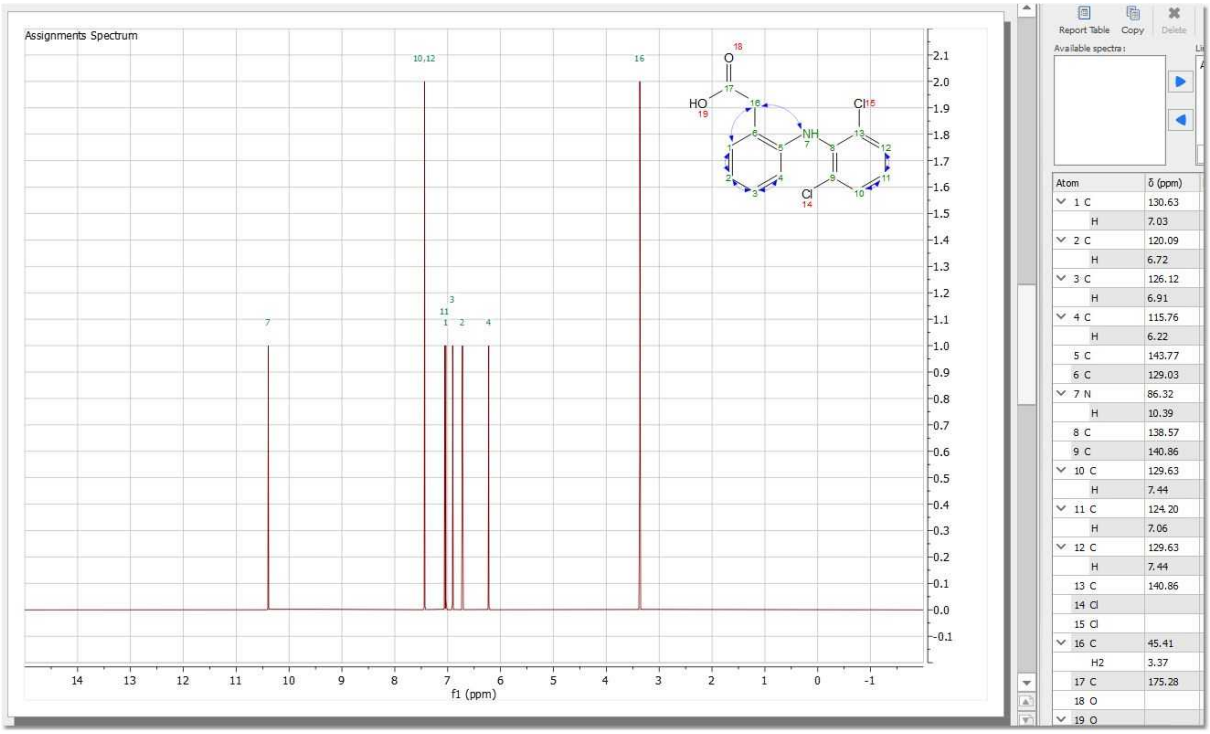
Assignments to Spectrum

To synthesize a spectrum from the molecular assignments:



Once you have selected the desired parameters, you will get your synthetic spectrum:

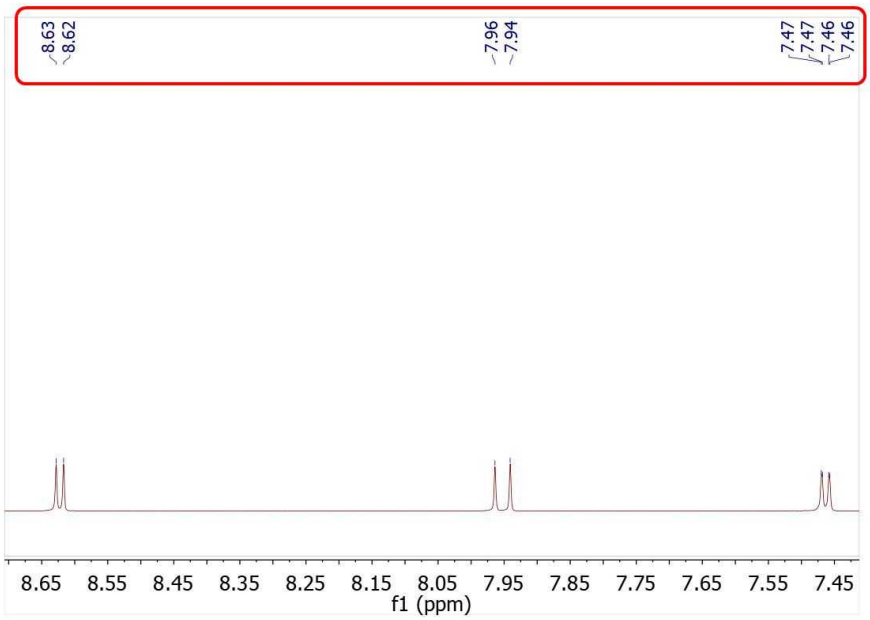
© 2024 MESTRELAB RESEARCH



Annotate Peaks by Origin

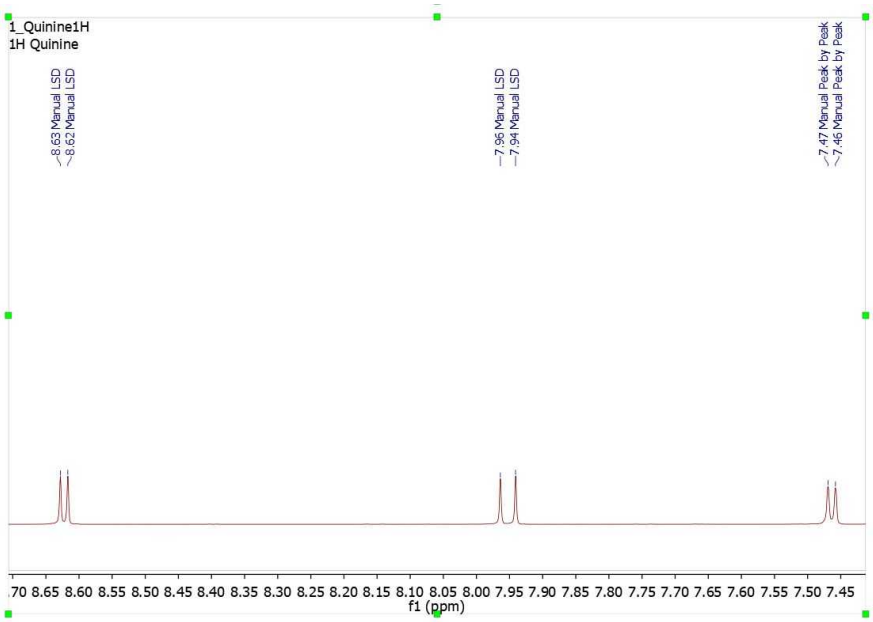
This script can be used to know the peak picking method which was used in any processed NMR spectra:

Now let´s see how it works. First of all load an spectrum file and apply a peak picking method. Mnova will show you a pick list with labels on the top of the spectra (red square in the image below):



Then, apply the '**Annotate Peaks by Origin**' script by following the 'Tools/NMR Tools' menu. After that, Mnova will include the peaks origin on each applicable peak label (very useful when if the spectra have

two or more differents peak picking methods). You can see the result of use this script in the image below:



The annotations that Mnova shows in the peak labels are the following:

Manual Threshold (GSD): "Manual LSD"
Manual Peak by Peak (GSD): "Manual"
Manual Threshold (Standard): "Manual Standard"
Manual Peak by Peak (Standard): "Manual"
Auto Peak Picking (GSD): "Auto GSD"
Auto Peak Picking (Standard): "Auto Standard"
CRAFT peak picking via loading a table: "Auto CRAFT"

See also:
Peak Picking

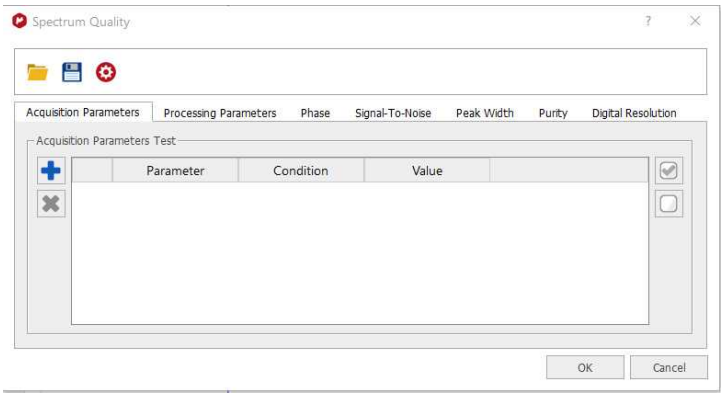
DEPT traces. See this tutorial to process Varian DEPT spectra

Purity Calculator. See this chapter

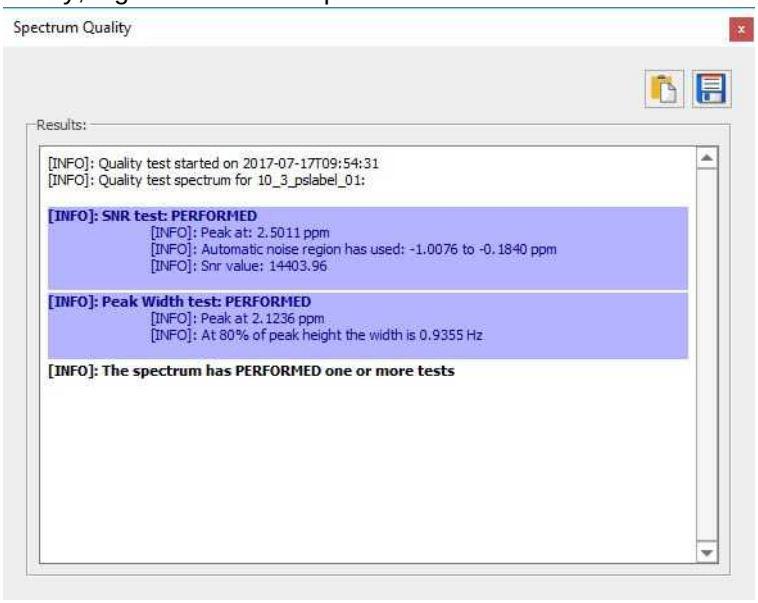
Set Central Frequency. It allows to set the center value of a spectrum by entering a value (in Hz) for f1 (in case of 1D spectra) or f1 and f2 (in case of 2D spectra). The last f1/f2 value used is remembered the following time you open the dialog (the first time the dialog shows the center value of the current spectrum). If several spectra are selected in the page navigator, the entered value is applied to all the selected spectra with equal dimensions.

Spectrum Quality Assessor. There are many instances in automation where it is useful to be able to automatically check the spectrum quality before proceeding with automated analysis such as Verify, qNMR, SMA. This script call this "quality test" functionality.
The script takes as input the chemical shift of the peak to be used for the tests (acquisition and processing parameters, phase error, half height peak width, signal to noise ratio, purity and digital resolution). If no peak is specified then the biggest compound peak in the spectrum should be used (respecting blind regions).

© 2024 MESTRELAB RESEARCH



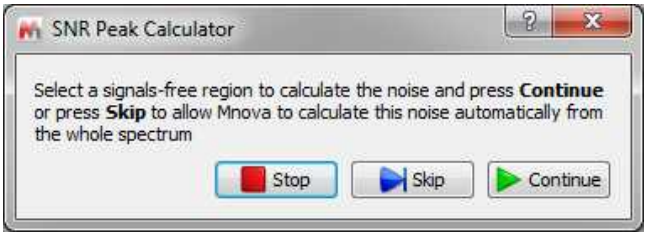
Finally, it generates and output with the results of each test:



Signal to Noise Ratio Calculation

To calculate the SNR. The script will get the noise level and then calculate the SNR by measuring the height of a peak and dividing this value by the noise level.

After running the script, it's necessary to select a signals-free region to calculate the noise and press 'Continue' (if you have not applied apodization, you could skip this step):



Next, select the region of the spectrum (or just a single peak) where you want to calculate the Signal to Noise Ratio. Once this is done, the program will populate the 'comment' section in the Peak List table with the SNR values.

The script to calculate the SNR needs to have two parameters:

1) The noise value
2) The peak intensity

Then it calculates the SNR using a ratio between those parameters: SNR = Y / noise;

Where Y is the amplitude of the peak and noise is the root-mean-square deviation calculated in a signal free area of the spectrum.

The noise value can be calculated either manually or fully automatically, as follows:

a) Manually. The user needs to select a 'signals-free' region' in the spectrum where the noise will be calculated according to this formula:

$$\sqrt{\sum (Y_i - Y_m)^2 / (N-1)}$$

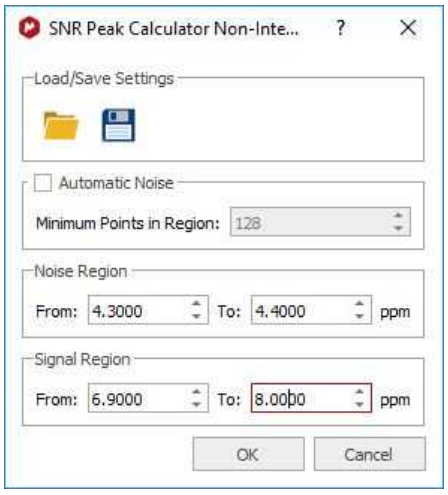
where:
N = number of points of the signal-free region
Y_i = value of each digital point in the spectrum (real part)
Y_m = average of the digital points (real part) in that region

b) Fully automatic (which is different with Bruker); the user does not need to select any region of the spectrum.

With respect to the peak intensity, the script uses the peaks in this way:
1. If the user did a manual peak picking (or Peak by Peak or an automatic PP), the program will use those peaks in the zoomed region.
2. If the user did not applied any PP, the script will apply it for the user over the selected region using the automatic PP options.

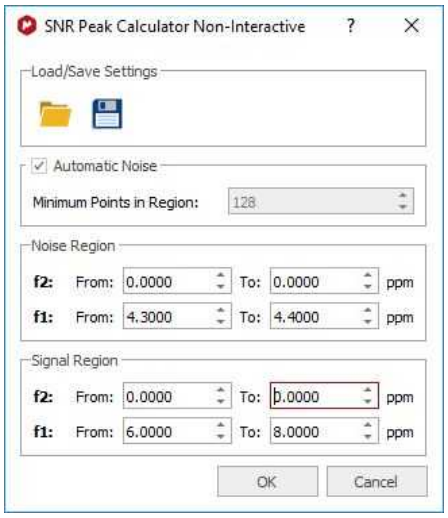
SNR Peak Calculator "Non interactive"

This script will allow the user to select the noise region and the region of interest in order to get the applicable 'signal to noise ratio':



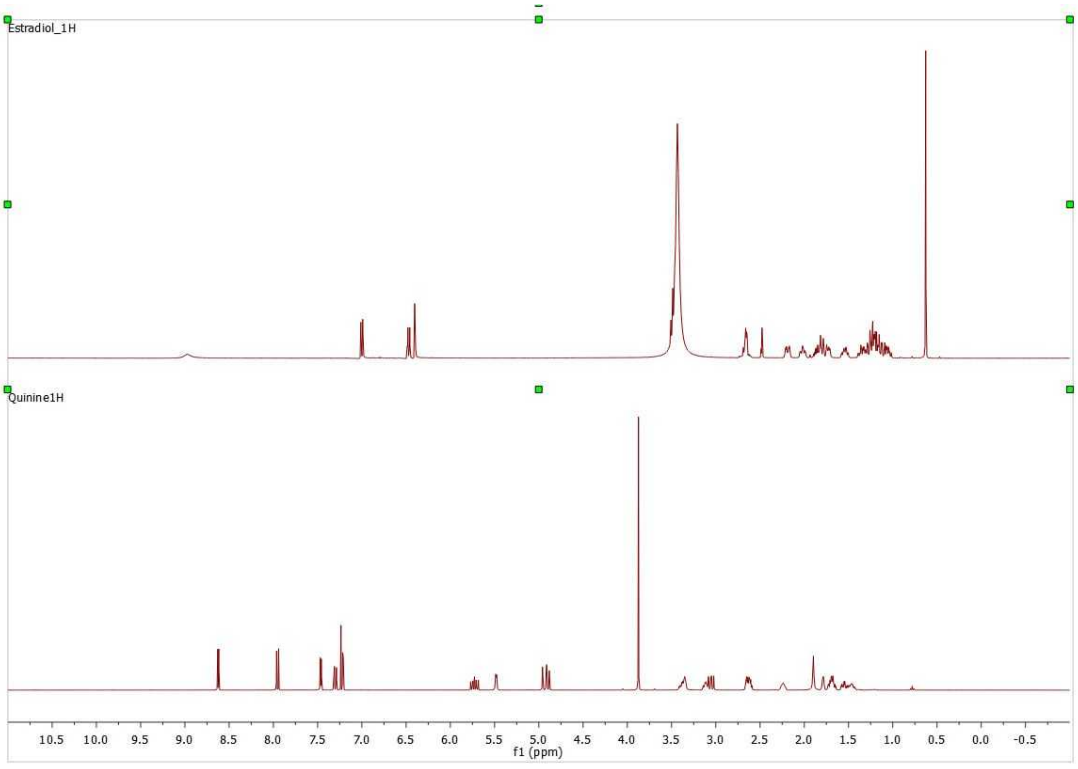
It can be used also for 2D:

© 2024 MESTRELAB RESEARCH



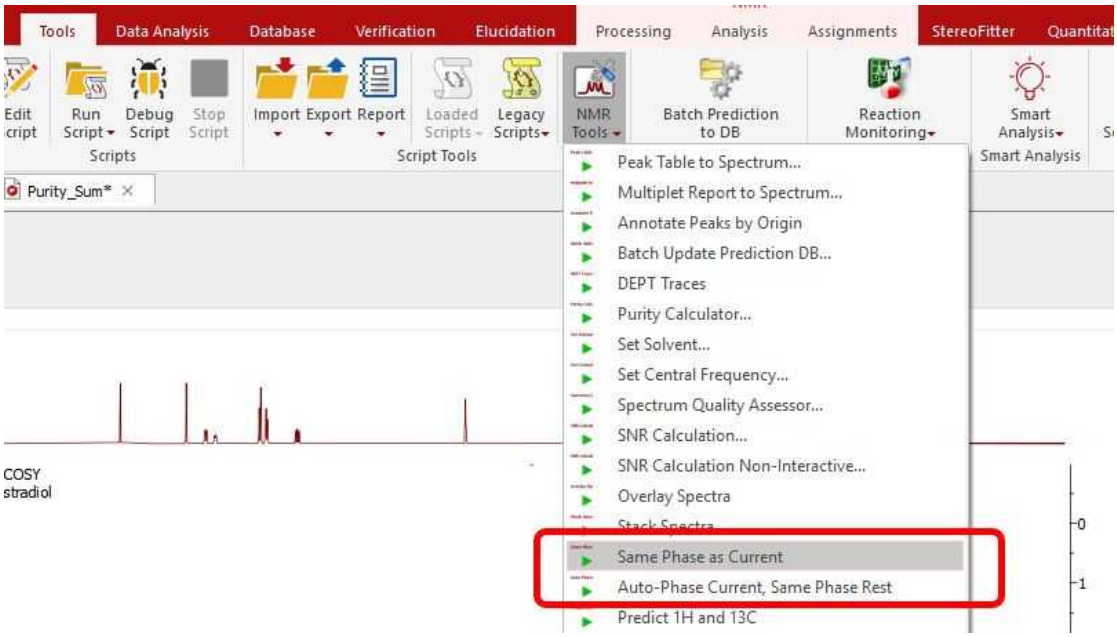
Overlay/Stack Spectra

You can 'stack or overlay' the selected spectra **plots** by clicking on the options "Overlay Spectra" and "Stack Spectra" available in 'Tools/NMR Tools' menu. The result will not be a real stacked/overlaid spectrum, instead of that, you will get the spectral windows stacked or overlaid, allowing you to freely move them up&down, as each spectrum will be an independent item:



Scripts to autophase importing autophase stacked spectra when importing.

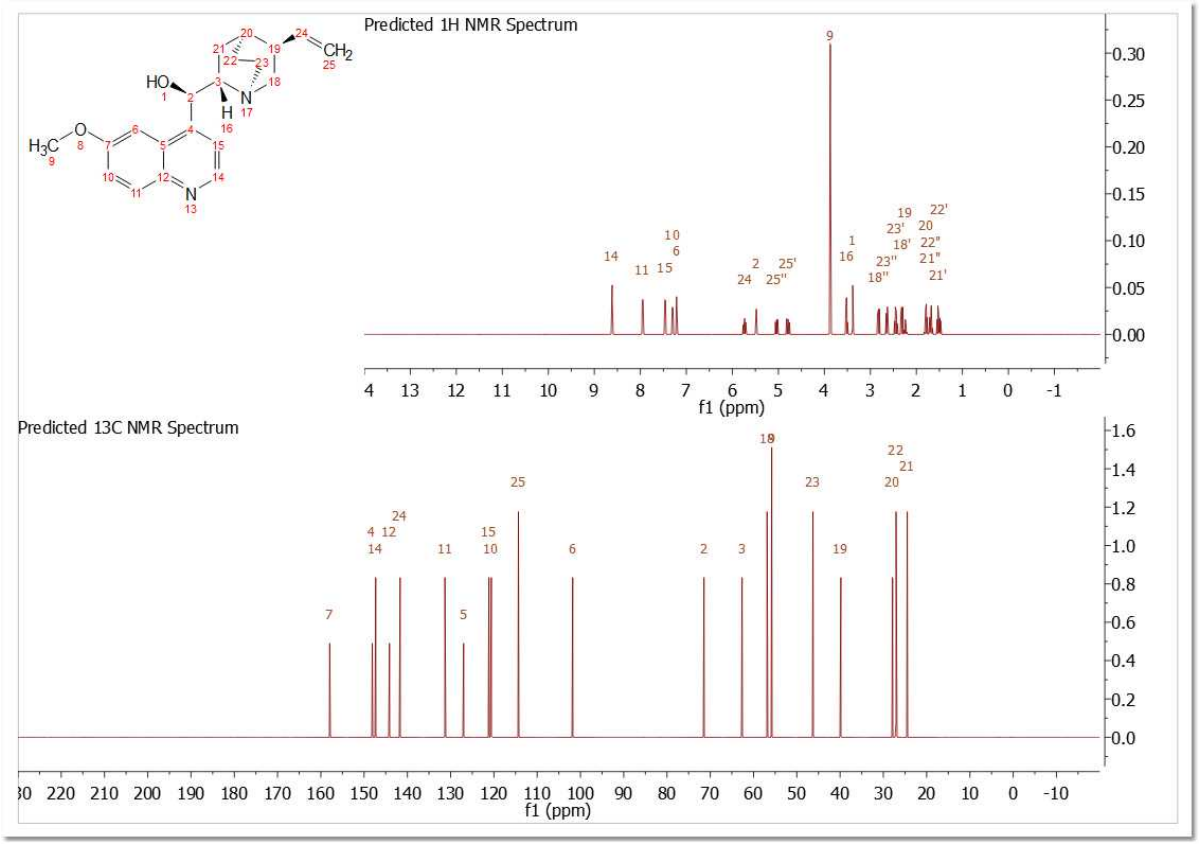
Following the menu 'Tools/NMR Tools', you will find a couple of scripts to apply automatic phase correction to a stacked plot:



- **Auto-Phase Current, Same Phase Rest**: the active spectrum is automatically phase corrected, and the resulting values of PH0 and PH1 are applied to the other spectra in the stacked.
- **Same Phase as Current**: PH0 and PH1 values of the active spectrum are applied to the other spectra in the stacked.
- **Set Solvent:** it can be used to change the 'solvent' in the Parameters table in the case that the solvent is wrong or missing. You can select several pages in the same document and change the solvent to all of them by using this script.

The script 'Predict 1H and and 13C' will generate a plot with both predictions from a given molecular structure (a license of NMRPredict Desktop is needed):

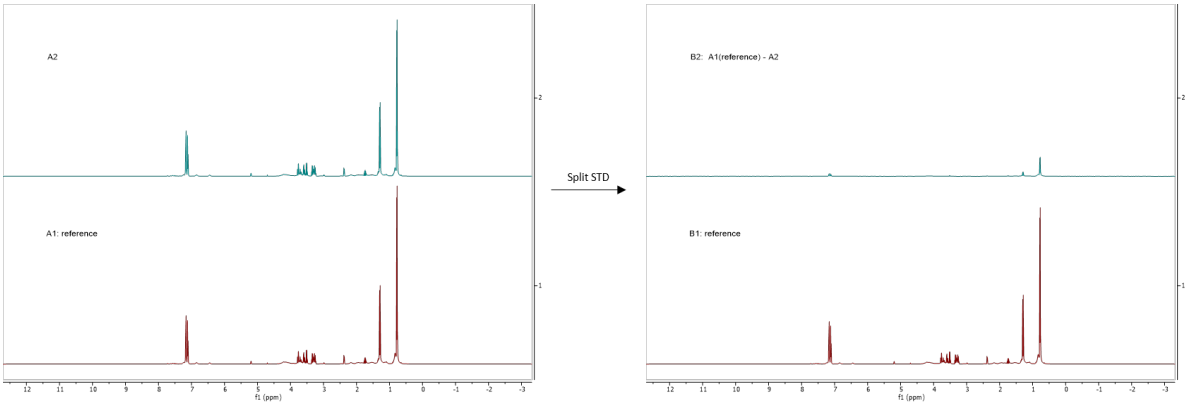
© 2024 MESTRELAB RESEARCH



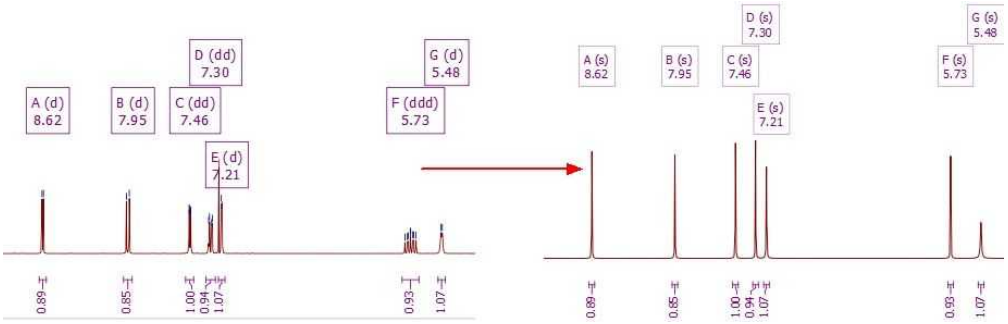
The script **'Auto Attach Traces'** will automatically setup the 1D spectra as external traces of a 2D (all the spectra must be in the same document).

The script **'Make 2D DIAG NMR'** will synthesize a 2D spectrum from a 1D experiment so that the latter will be the diagonal part of the 2D spectrum. It could be useful to obtain 2D pure shift spectra by using Indirect Covariance NMR. See this paper for further information.

The script **'Split STD'** will divide the Bruker pseudo 2D files (special kind of 2D with just 2 increments files) that their STD parameter set generates When running the script , you will be asked to select the reference experiment. The resulting stack will show the reference spectrum at the bottom and the difference between the reference and the other spectra in the stack:



The "**Synthetic Pure Shift'** script will convert all the multiplets (with GSD Peak Picking) into single peaks (keeping the chemical shift and area constant).



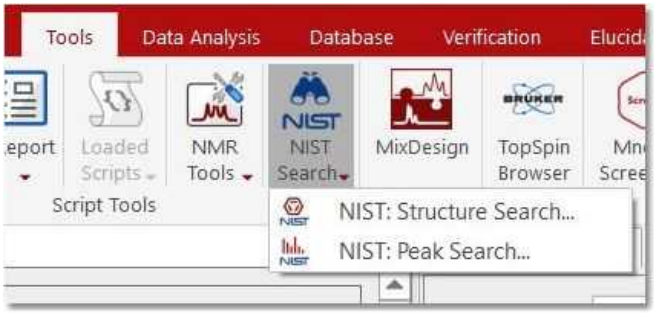
The "**Unfold 2D'** script will be useful to assign an aliased/folded peak in a 2D spectrum.

The '**PlayFID**' script will allow the acoustic reproduction of 1D (or stacked) NMR processed FID. You will have options to play/pause/export the FID (or NMR multiplets) as a WAV file.

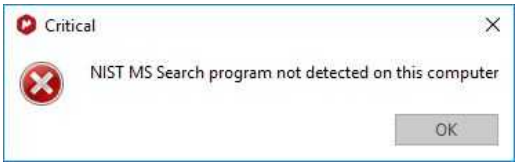


See the script in action here: https://youtu.be/Uges1EK3GnQ

17.11 NIST Search

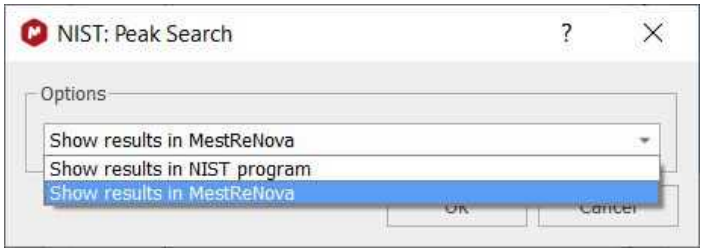


The "NIST" scripts (only for Windows) will allow you to search for **molecular structures and peaks** in you previous installed NIST Database. If you do not have it installed, you will get an error message:

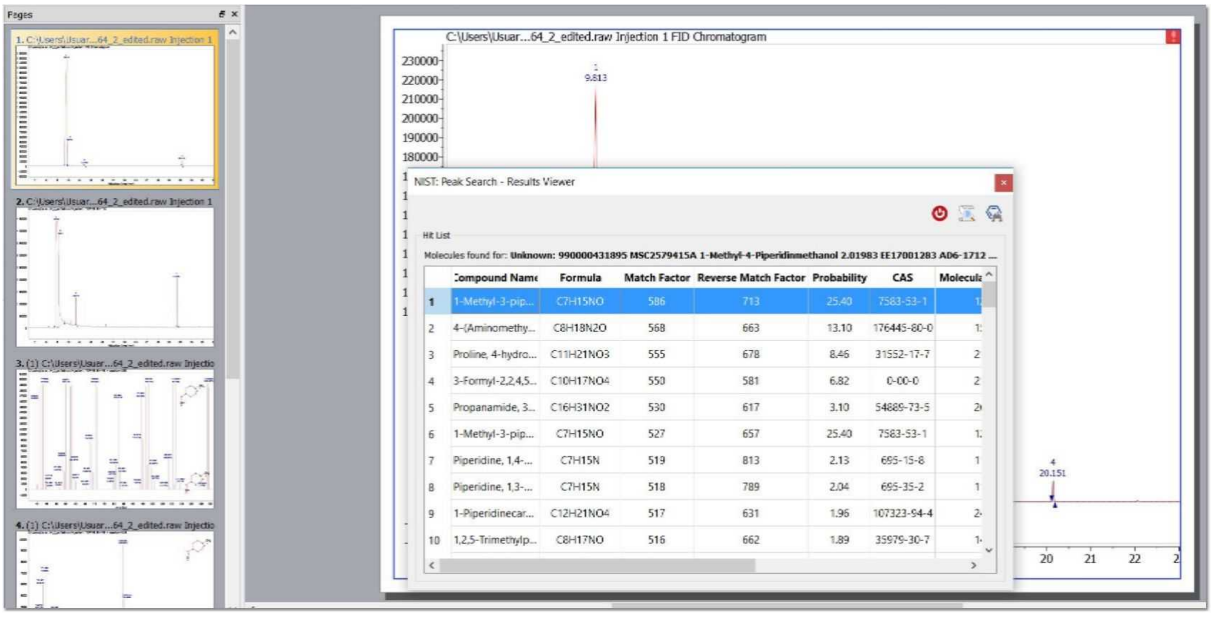


If you have NIST installed on your computer, you will be allowed to run peak searches:

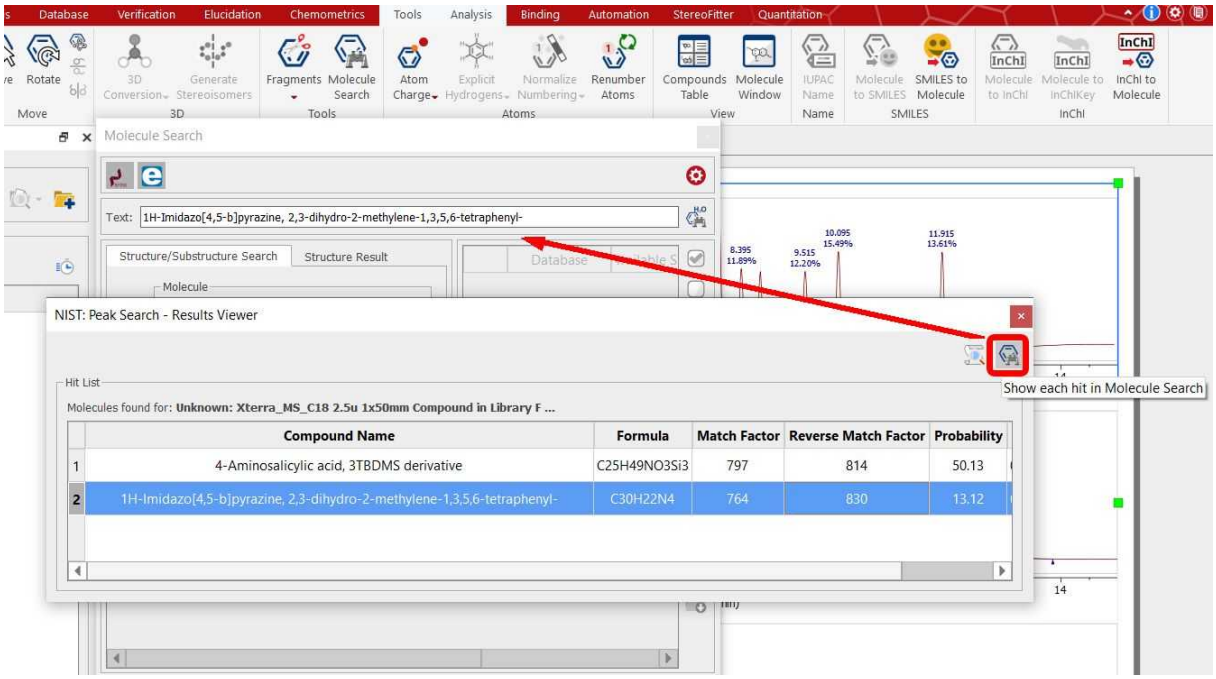
© 2024 MESTRELAB RESEARCH



The results can be shown in Mnova or directly on NIST program:



Once you have got the results, you will be able to search for the compounds into the BRMB and eMolecules databases (with the Molecule Search feature):



17.12 Scripts Samples

All the scripts are present in the examples/scripts installation directory. Some of them will be described in this chapter. Please bear in mind that you can run scripts from the command line, just by typing the path where the Mnova .exe file and the script are located and -sf "name of the script function": **"mestrenovaPathname" "scriptPathname" -sf "scriptFunctionToRun"**

For example:

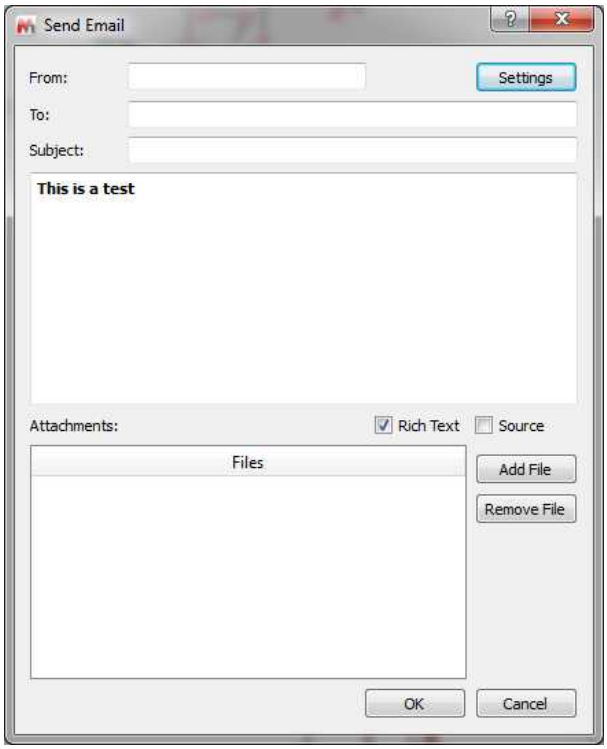
"C:\Program Files\Mestrelab Research S.L\MestReNova\MestReNova.exe" "C:\Program Files\Mestrelab Research S.L\MestReNova\scripts\myScript.qs" -sf "myFunction"
It is also possible to specify arguments:

"C:\Program Files\Mestrelab Research S.L\MestReNova\MestReNova.exe" "C:\Program Files\Mestrelab Research S.L\MestReNova\scripts\myScript.qs" -sf "myFunction",0.1,10,true,off

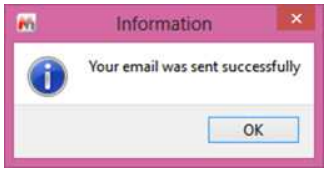
In this case, MNova will run myFunction(0.1,10,true,"off") from myScript.qs with the arguments specified. No spaces after commas!

SendEmail: It is possible to send an email containing attachments (it needs Python installed in the same computer).

© 2024 MESTRELAB RESEARCH



When the email is sent a message is shown:



testFilePermissions: this script will allow you to generate 'read-only' documents.

customImportProcessing: This script can be used to change the way of Mnova imports your NMR spectra. For example to stop the processing in the FID. Please follow the procedure below to set this script by default:
1: Go to 'Edit/Preference/Scripts' and add a new folder to sample scripts by clicking on the 'blue plus button'
2: Save this script as a .qs file to the folder above.
3: Restart Mnova and load a 1H-NMR dataset. You will get your spectrum without any processing (just the FID).

Open and Save Document Script: Let´s take a look at an easy script which you can use to open a .mnova file and export it as a PDF file. In this case, we will open a .mnova file named 'Dimethoxy':

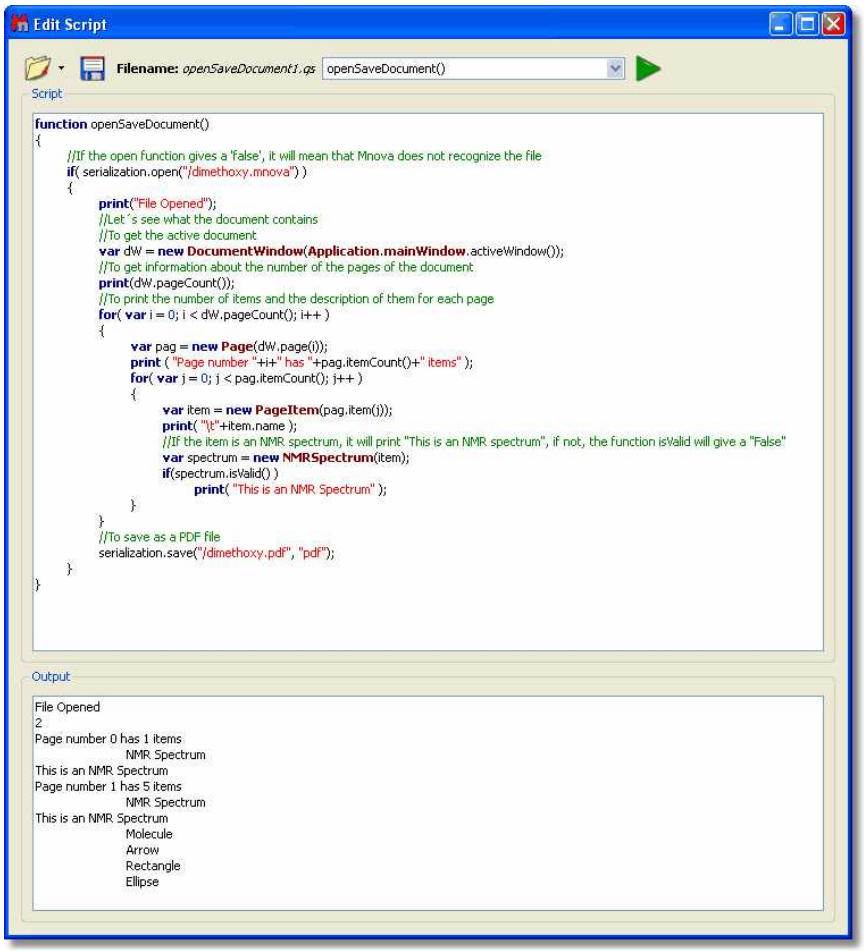
```
function openSaveDocument()
{
	//If the open function gives a 'false', it will mean that Mnova does not recognize the file
	if( serialization.open("/dimethoxy.mnova") )
	{
		print("File Opened");
		//Let´s see what the document contains
		//To get the active document
```

```
        var dW = new DocumentWindow(Application.mainWindow.activeWindow());
        //To get information about the number of the pages of the document
        print(dW.pageCount());
        //To print the number of items and the description of them for each page
        for( var i = 0; i < dW.pageCount(); i++ )
        {
            var pag = new Page(dW.page(i));
            print ( "Page number "+i+" has "+pag.itemCount()+" items" );
            for( var j = 0; j < pag.itemCount(); j++ )
            {
                var item = new PageItem(pag.item(j));
                print( "\t"+item.name );
                //If the item is an NMR spectrum, it will print "This is an NMR
spectrum", if not, the function isValid will give a "False"
                var spectrum = new NMRSpectrum(item);
                if(spectrum.isValid() )
                    print( "This is an NMR Spectrum" );
            }
        }
        //To save as a PDF file
        serialization.save("/dimethoxy.pdf", "pdf");
    }
}
```

To run this script, just copy it to the 'Edit Script' dialog box and type "openSaveDocument()" in the combobox. Please bear in mind that, you will need to write the corresponding path of the saved Mnova file in the line 4 of the script (in this case the dimethoxy.mnova file was saved in C:\ ).
As you can see in the script, if Mnova does not recognize the spectrum, you will obtain a 'False' message in the output due to the statement:
`if( serialization.open("/dimethoxy.mnova") )`

The line `print("File Opened");` will give the corresponding message in the output, as you can see in the picture below:

© 2024 MESTRELAB RESEARCH



The object 'Application' of the variable **var** dW = new **DocumentWindow**(**Application.mainWindow**.activeWindow()); gives us the 'Active Window' of the 'Main Window', that is to say, the document which we can see in the screen. The function **print**(dW.pageCount()); is used to know the number of the pages of the document.

The following lines of the script are used to obtain information about the items included on the document. For example the function pageCount is used to know the number of pages of the document. The variable **var** pag = **new** **Page**(dW.page(i)); will analyze page by page to find the information. The line **print** ("Page number "+i+" has "+pag.itemCount()+" items"); will print the corresponding phrase in the output, following the number of the page (+i+) and the number of the items included in the page **for**(**var** j = 0; j < pag.itemCount(); j++). The variable **var** item = **new** **PageItem**(pag.item(j)); will inform about the type of the item, and the function **print**("\t"+item.name); will print the name of the item. In this example (as we can see in the output of the picture below), the document has 2 pages; page 0 has 1 item, which is an NMR spectrum and page 1, has 5 items (NMR Spectrum, molecule, arrow, rectangle and ellipse).

The variable **var** spectrum = **new** **NMRSpectrum**(item); will rebuild the active spectrum, and if the item is an NMR spectrum, it will print "This is an NMR spectrum", if not, the function isValid will give a "False" due to the lines: **if**(spectrum.isValid()) // **print**("This is an NMR Spectrum");.

Finally, the plugin serialization.save("/dimethoxy.pdf", "pdf"); will save the document as a PDF file in the specified location of the hard-disk (in this case, the PDF will be saved in C:\).

Open a spectrum with molecules:

© 2024 MESTRELAB RESEARCH

It is possible to open spectra with molecules by using the openSpecWithMols.qs example script. You will need to add the directory where you have the script to the 'Scripts directories' (by using the ''File/Preferences/Scripting/Scripts Directories' menu). Then restart Mnova and you will be able to run the script from the command line by typing:
"MestReNova path" –sf openSpecWithMols,"Spectrum path"

For example:

"C:\Program Files\Mestrelab Research S.L\MestReNova\MestReNova.exe" -sf openSpecWithMols,"C:\1H\fid"

Where, the yellow is the path where you have installed Mnova and the red is the path of the spectrum that you want to open.

```
// Opens a spectrum at aSpecPath and all the molfiles located in the same directory
function openSpecWithMols(aSpecPath)
{
var dirPath = File.absDirPath(aSpecPath);
var dir = Dir(dirPath);
dir.cdUp();
var files = getMaskFiles(dirPath, dir.absPath, "*.mol", "*", false);
files.push(dirPath);
serialization.open(files);
}
```

Import_Process_Save_Loop Script: This script can be used to apply a previously existing 'full processing' template (in this case, it is a template saved at C:/temp/proc.mnp) to several spectra (saved at C:/spectra/ and inside sub-folders with the extension ".fid", for example: "C:/spectra/1H.fid") and to save the result in different files formats (.mnova, .pdf and .txt). This script is only valid for these spectra containing a FID file.

```
/**************************************************************************************
******************
Copyright (C) 2007 Mestrelab Research S.L. All rights reserved.

This file is part of the MNova scripting toolkit.

Authorized users of MNova Software may use this file freely, but this file is
provided AS IS
with NO WARRANTY OF ANY KIND, INCLUDING THE WARRANTY OF DESIGN, MERCHANTABILITY AND
FITNESS
FOR A PARTICULAR PURPOSE.
**************************************************************************************
****************/
// This example function demonstrates how to apply some processing to a set of
spectral files and to save the result in the different file formats.
function import_process_save_loop()
{
      const dirName = "c:/spectra"; // Source directory with spectra to be
processed.
      const resultDirName = dirName; // Destination directory where processed
spectra should be put.
      const procName = "c:/temp/proc.mnp"; // Processing file. It must be created
in the Full Processing dialog of MestReNova.
      const fileMask = "fid"; // This mask specifies files to be processed.
      const dirMask = "*.fid"; // This mask specifies directories to be scanned for
spectra.
```


© 2024 MESTRELAB RESEARCH

```
	var dir = new Dir(dirName);
	if (!dir.exists)
	{
		MessageBox.critical("Directory " + dirName + " does not
exist.",MessageBox.Ok);
		return;
	}
	dir.cdUp();

	// The below function getMaskFiles is located in the files.qs script.
	// If you run import_process_save_loop from QSA Workbench then it is
necessary to introduce getMaskFiles to the interpreter by importing files.qs.
	var files = getMaskFiles(dirName, dir.absPath, fileMask, dirMask, true);
	for(var i = 0; i < files.length; i++)
	{
		var dw = new DocumentWindow(Application.mainWindow.newWindow()); //
Create new document window
		if (serialization.open(files[i]))
		{
			nmr.process(procName);
			const toReplace = new RegExp("[/|:]", "g");
			fileName = resultDirName + "/" + files[i].replace(toReplace,
"_"); // Generate a filename for processed spectrum.
			serialization.save(fileName+".mnova", "mnova");	//
MestReNova file format
			serialization.save(fileName+".pdf", "pdf"); // Adobe PDF file
format
			serialization.save(fileName+".txt", "ascii"); // ASCII
			print(fileName); // Prints file name in the debug window
		}
		dw.close(); // Close document window of processed spectrum
	}
}
```

Parameters Script: This script can be used to obtain the desired parameters on your spectrum. You will be able to add or remove any parameter and to change the font by just using HTML code.

```
function spectrumParams()
{
	var spec = new NMRSpectrum( nmr.activeSpectrum() );
	var htmlText = "<b>Nucleus</b>: %1 <br/> <b>Frequency: </b> %2 <br/> <b>Pulse
Sequence: </b> %3 <br/> <b>psLabel: </b> %4 <br/> <b>Solvent: </b> %5 <br/>
<b>Title: </b> %6";
	var nuc = spec.getParam("Nucleus[1]");
	var freq = spec.getParam("Spectrometer Frequency");
	var seqfill = spec.getParam("Pulse Sequence");
	var psLabel = spec.getParam("psLabel");
	var Solvent = spec.getParam("Solvent");
	var Title = spec.getParam("Title");
	print(spec.getParam("Nucleus[1]"));
	print(spec.getParam("Spectrometer Frequency"));
	print(spec.getParam("Pulse Sequence"));
	print(spec.getParam("psLabel"));
```

© 2024 MESTRELAB RESEARCH

```
	print(spec.getParam("Solvent"));
	print(spec.getParam("Title"));

htmlText=htmlText.arg(nuc).arg(freq).arg(seqfill).arg(psLabel).arg(Solvent).arg(Titl
e);
		draw.text(htmlText, true);
}
```

In this case, the script will print the parameters Nucleus, Spectrum Frequency, Pulse Sequence, psLabel, Solvent and Title. Please bear in mind that getParam function returns only parameters which exist in the table of parameters (including invisible ones). Please make sure that you have these parameters included on the table of parameters (via customization dialog).

Fid Test Script: you will get information about the real and the imaginary part of the FID by running this script:

```
function fidTest()
{
	var spec = new NMRSpectrum( nmr.activeSpectrum() );
	print(spec.fidImag(0));
	if( spec.fidSetImag(0,0) )
		print(spec.fidImag(0));
		print(spec.fidReal(0));
	if(spec.fidSetReal( 0, 0 ) )
		print(spec.fidReal(0));
	spec.process();
	mainWindow.activeWindow().update();
}
```

The command **print**(spec.fidImag(0)) will return the value of the imaginary part of the FID at the *aIndex* point (in this case; 0. You can type any number from zero to the number of points of your spectrum), whilst the line **print**(spec.fidReal(0)); will give you the value of the real component. The object *fidsetImag;* sets the imaginary value of the *aIndex* point of the spectrum fid.

Integral Test Script: This script can be used to get information about the integrals of the spectrum:

```
function integralTest()
{
	var spec = new NMRSpectrum( nmr.activeSpectrum() );
	var intList = new Integrals(spec.integrals());
	print(intList);
	print(intList.count);
	print(intList.normValue);

	var myInt = new Integral(intList.at(0));
	print(myInt);
	print(myInt.rangeMax(1));
	print(myInt.rangeMin(1));
	if( spec.dimCount > 1 )
	{
		print(myInt.rangeMax(2));
		print(myInt.rangeMin(2));
	}

	print("Integral Value "+myInt.integralValue());
```


© 2024 MESTRELAB RESEARCH

```
        print("Normalized value "+myInt.integralValue(intList.normValue));

        var sReg = new SpectrumRegion(7.2, 7.5);
        var newInt = new Integral( spec, sReg, false );
        print(newInt);
        print(newInt.integralValue());
        spec.integrals().append(newInt);
        spec.process();
}
```

The first part of the script is used to count the integrals and to report the normalized value applied to the integrals (in the list 'intList'). With the second part of the script, the user is able to select any integral of the list (in this case the integral which appears in the first position, if we wanted to select the second integral, we should have to type `var myInt = new Integral(intList.at(1));`). Then the scripts returns the absolute value and the limits of the selected integral. The third part of the script returns the absolute and the normalized value of the integral.

The last part of the script can be used to add a new integral to the list (in this case an integral with the spectral region from 7.2 to 7.5 ppm). the script reports the absolute value of this integral.

Multiplet Test Script: This script is valid to obtain information about the multiplets of the spectrum and also to apply a 'manual multiplet analysis':

```
function multipletTest()
{
        var spec = new NMRSpectrum( nmr.activeSpectrum() );
        print(spec.multiplets());
        var sMult = new Multiplets(spec.multiplets());
        print(sMult.count);
        print(sMult.at(2));

        var sReg = new SpectrumRegion(7.0, 7.5);
        var mult = new Multiplet( spec, sReg );
        var myMultiplets = new Multiplets();
        myMultiplets.append( mult );

        var mult2 = new Multiplet( spec, new SpectrumRegion(3.80,3.90) );
        myMultiplets.append( mult2 );

        var nM = new Multiplet(new SpectrumRegion(3.65,3.75), "t");
        nM.name="My mulltiplet";
        myMultiplets.append(nM);

        print(myMultiplets);
        spec.setMultiplets(myMultiplets);
        print(spec.multiplets());
        spec.process();
}
```

The first part of the script creates a list of the multiplets which appear on the spectrum and prints some information about the selected multiplet (in this case, the multiplet number 2, but of course you can select any other multiplet). The second part of the script creates a multiplet in the selected region 'sReg' (in this case the region is 7.0-7.5 ppm. It works as a manual multiplet analysis). Then, it creates a new empty list of multiplets which is added.

© 2024 MESTRELAB RESEARCH

The third part of the script consists in a new region (from 3.80 to 3.90 ppm) to apply the multiplet analysis. The following part of the script is used to add a multiplet manually to the multiplet list (the user will be able to remove this part of this script without any problem). In this case, we wanted to add a triplet, which appears in the region from 3.65 and 3.75 ppm, with the name 'My multiplet'.

The end of the script is used to replace the original multiplet list for the list which we have just created.

Peak Test Script: A similar script used to obtain information about the chemical shift and the intensity of the peaks of a 1D-NMR spectrum:

```
function peakTest()
{
	var spec = new NMRSpectrum( nmr.activeSpectrum() );
	var sR = new SpectrumRegion( 0, 9.5 );
	var ps = new Peaks(spec, sR); //fill the list with the peaks in sR
	print(ps);
	var p = new Peak(1.265, spec);
	print(p);
	ps.append(p);
	spec.setPeaks(ps);//Set the peaks to ps
	print( spec.peaks());
	spec.process();
}
```

After running this script, we will get the peak picking analysis of a 1D-NMR spectrum from 0 to 9.5 ppm (of course, we will be able to change this range). Then, a new peak at 1.265 ppm will be added to the peak list (again, you can add any other peak, if you want), reporting the intensity value of this new peak. Finally, the spectrum will be re-analyzed to obtain the new number of peaks.

A similar script can be used for a 2D-NMR spectrum, but in this case we have to type the corresponding spectrum region in 2D mode:

```
function peakTest()
{
	var spec = new NMRSpectrum( nmr.activeSpectrum() );
	var sR = new SpectrumRegion(4.0, 3.5, 75, 65 );
	var ps = new Peaks(spec, sR); //fill the list with the peaks in sR
	print(ps);
	var p = new Peak(72.73, 3.17, spec);
	print(p);
	ps.append(p);
	spec.setPeaks(ps);//Set the peaks to ps
	print( spec.peaks());
	spec.process();
}
```

Multiplet Reporter script: Let´s take a look now at a very important part of the 'Multiplet Reporter_JACS' script:

```
// This function defines JACS-like multiplet report.
// To customize report, edit this class or implement another one.
function JACSMultipletReporter()
{
MultipletReporter.call(this);
```


© 2024 MESTRELAB RESEARCH

```
this.onlyElementName=false;
// Define font size and font family
this.font = "<font style=\"font-size: 10pt; font-family: Times New Roman\">";

this.nucleusTemplate="%1";
// Report header. %1 will be replaced with nucleusString, %2  with frequency,
%3 with solvent
this.header = "%1 NMR (%2 MHz, %3) &delta; ";

// Multiplet templates. %1 - delta, %2 - category, %3 - nH
this.reportRange = true; // set to true to get multiplet range instead of
delta.
this.withoutJsTemplate = " %1 (%2, %3H)"; // multiplet template without J's
this.withJsTemplate = " %1 (%2, %4, %3H)"; //  multiplet template with J's
this.rangeTemplate = "%1 &ndash; %2";

// J's list template. %1 - list of J's
this.jListTemplate = "<i>J</i> = %1";

this.jPrecision = 1; // J's precision
this.deltaPrecision = 2; // delta precision
this.mSeparator = ", "; // multiplet separator
this.jSeparator = ", "; // J's separator

this.start = this.font;
this.end = ".</font>";
}

JACSMultipletReporter.prototype = new MultipletReporter();
JACSMultipletReporter.prototype.toString     =     function()     {     return
"JACSMultipletReporter()"; }

(...)
```

The user will be able to change the multiplet report template to obtain the desired multiplet report; for example:

this.onlyElementName=false; changing false with true, we will obtain only the element name without the atomic mass (For example: H, C instead of ^{1}H, ^{13}C).

The function: this.font = "<font style=\"font-size: 10pt; font-family: Times New Roman\">"; will define the font size and the font family of the multiplet report.

The line: this.header = "%1 NMR (%2 MHz, %3) δ "; is used to print the header of the report, where %1 will be the nucleus (H or C), %2 the frequency of the spectrometer (in MHz), and %3 the solvent, followed by a delta symbol (δ). For example: "^{1}H NMR (500 MHz, CDCl3) δ".

The sentence: this.reportRange = true is used to obtain the multiplet range instead of the chemical shift.

The functions: this.withoutJsTemplate = " %1 (%2, %3H)" and this.withJsTemplate = " %1 (%2, %4, %3H)" are used to customize the appearance of the multiplet report by changing the positions of %1, %2, %3H, or %4 (where, %1 means: chemical shift; %2 means: type of multiplet (s, d, t, etc); %3H means: number of hydrogens and %4 means the coupling constant value). As you can see, the first line shows a multiplet without coupling constants, (while the last line shows a multiplet with coupling constants).

© 2024 MESTRELAB RESEARCH

So, if you need to obtain something like this (Japanese format):

1H NMR (300 MHz, Solvent) δ ppm 6.43-6.22 (1 H, m), 3.17 (1 H, q, J = 7.15 Hz) etc…

You should modify both lines, as you can see below:

```
this.withoutJsTemplate = %1 (%3H, %2)";
this.withJsTemplate = " %1 (%3H, %2, %4)";
```

If you prefer to obtain something like this:

1H NMR (300 MHz, Solvent) δ ppm 1.23 (d, *J* = 1.2 Hz, 3 H), etc…

Just replace the original lines with:

```
this.withoutJsTemplate = %1 (%2, %3H)";
this.withJsTemplate = " %1 (%2, %4, %3H)";
```

To obtain the coupling constant symbol in normal instead of *'italic'*, just modify the script by removing the italic format (J). If you prefer to obtain it in "**bold**" just type:

```
this.jListTemplate =  "<b>J</b> = %1";
```

The following paragraph will be used to customize the appearance of the coupling constants list:

```
this.jPrecision = 1; // J's precision
this.deltaPrecision = 2; // delta precision
this.mSeparator = ", "; // multiplet separator
this.jSeparator = ", "; // J's separator
```

The first line is used for the precision of the coupling constants values, the second will be used for the precision of the chemical shift and the remaining two lines will print the separation between the multiplets and the coupling constants values.

If you need to obtain the coupling constants in descending order, replace 'true' with 'false' in the following line:

```
var jList = new JList(multiplet.jList());
jList.sort(true);
```

If you want to obtain the multiplet chemical shifts in ascending order, just replace the 'false' with 'true' in the script:

```
var multiplets = new Multiplets(spectrum.multiplets()); // get multiplets from spectrum
jList.sort(true);
```

To obtain the multiplet range in ascending order, just replace the rangeMin with rangeMax (and vice versa) in the line below of the script:

```
shiftStr = this.rangeTemplate.argDec(multiplet.rangeMax, 0, 'f',
this.deltaPrecision).argDec(multiplet.rangeMin, 0, 'f', this.deltaPrecision);
```

Title and Solvent: running this script, you will get the title and the solvent on your spectrum

```
// <GUI menuname="title1" shortcut="Ctrl+2"
tooltip="Title_and_Solvent" />
```


© 2024 MESTRELAB RESEARCH

```
function title1()
{
//To get the active spectrum
      var spectrum = nmr.activeSpectrum();
//The function isValid informs about if the spectrum obtained is correct
      print( spectrum.isValid() );
                  var   tit   =   draw.text("<h3>"+   spectrum.title+"
"+spectrum.solvent+"</h3>", true);

}
```

Zoom: This script can be used to apply a zoom into a selected range of a spectrum. In order to select the horizontal range, just type the desired values between the brackets on the line `spc.horZoom(1.0, 5.0);`

If you want to set the vertical zoom, just change the values of the line `spc.vertZoom(-100, 7000)`

```
function spectrumZoom()
{
      var spc = nmr.activeSpectrum();
      if( !spc.isValid() )
            return;
      spc.horZoom(1.0, 5.0);
      spc.vertZoom(-100, 7000);
      spc.update();
      mainWindow.activeWindow().update();
}
```

Properties Script: You will find below a script example to change some properties of your spectra. In this case, the colour of the spectrum will be changed to red, the peaks font to Arial and the integral curves will be showed. You will find all the available properties at the 'Mnova Properties' chapter:

```
function properties()
{
      var spec = new NMRSpectrum(nmr.activeSpectrum());

      spec.setProperty("curve.color","red");
      spec.setProperty("peaks.font","Arial");
      spec.setProperty("integrals.curve.show", true);
      spec.update();
      mainWindow.activeWindow().update();

}
```

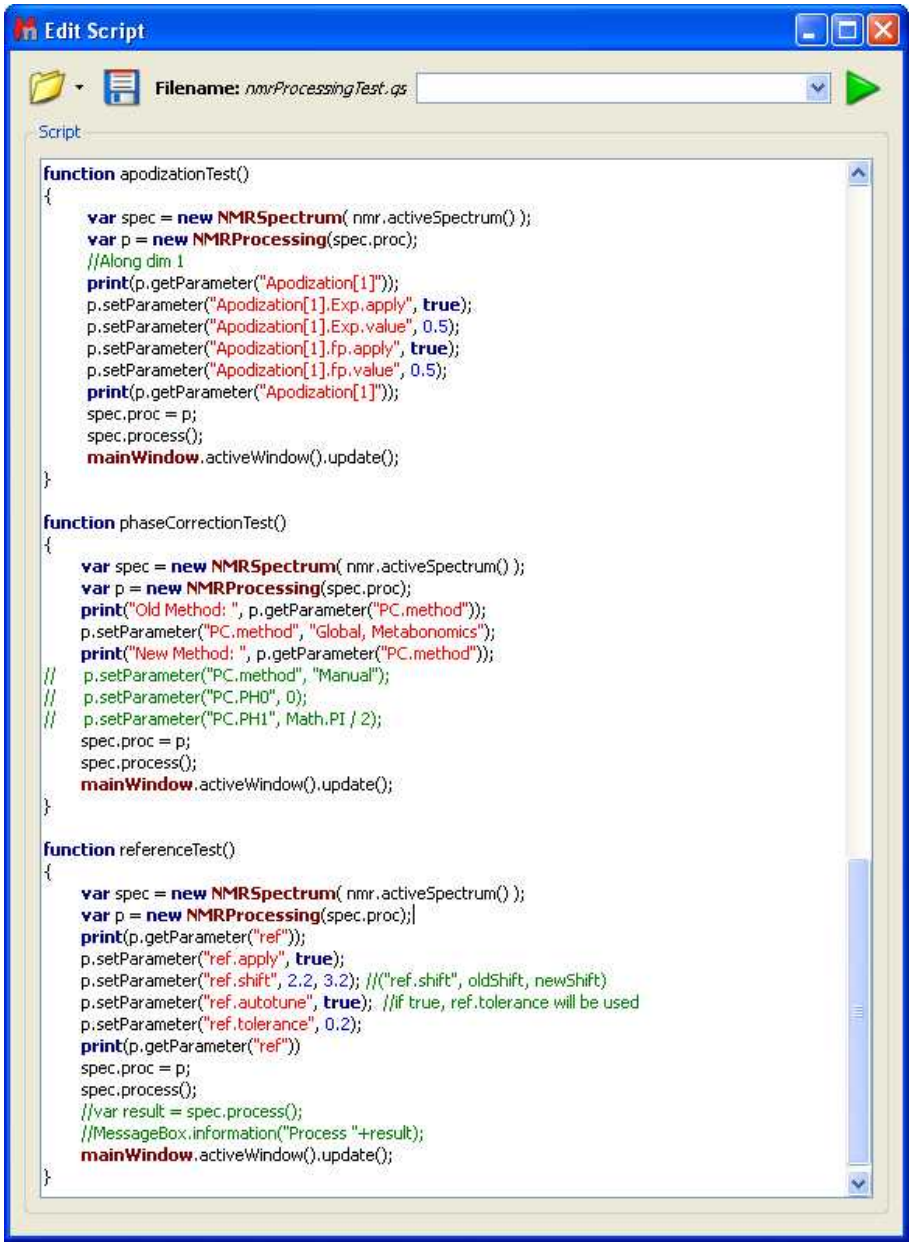
Cutting: This script can be used to apply cuts to the spectra. You will obtain two cuts in your spectrum, between 1.0-3.5 and 7.0-8.0 ppm respectively.

```
function myProcess()
{
var spec = new NMRSpectrum( nmr.activeSpectrum() );
var p = new NMRProcessing(spec.proc);
var regs = new Array(2);
regs[0]  = new SpectrumRegion(1.0, 3.5);
regs[1] = new SpectrumRegion(7.0, 8.0);
```

© 2024 MESTRELAB RESEARCH

```
print(p.getParameter("cuts"));
p.setParameter("cuts.apply", true);
p.setParameter("cuts.list", regs);
spec.proc = p;
spec.process();
mainWindow.activeWindow().update();
}
```

Processing script: Here you can see a part of the 'mnrProcessingTest" script stores under the examples folder:



Magnitude Active Spectrum script: Let´s see another script; in this case this script is used to obtain the 1D-NMR spectra in magnitude. Just open a 1D-NMR spectrum in Mnova, load this script by following

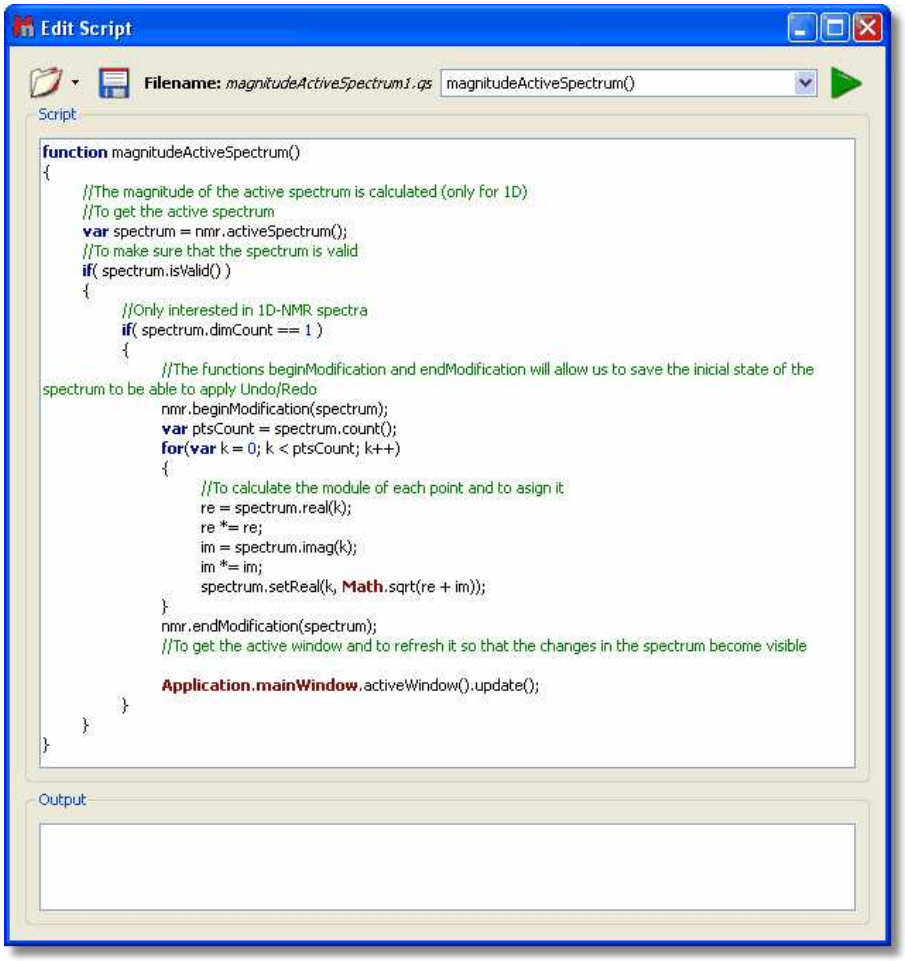
© 2024 MESTRELAB RESEARCH

the menu 'Script/Edit Script', type "magnitudeActiveSpectrum()" in the edit box and click on the green triangle to run the Script. You will obtain automatically your 1D-NMR spectrum in magnitude.

Please bear in mind that it is also possible to run scripts from the command line, just by typing the path where the Mnova .exe file and the script are located and -sf "name of the script function":
"mestrenovaPathname" "scriptPathname" -sf "scriptFunctionToRun"

For example:

"C:\Program Files\Mestrelab Research S.L\MestReNova\MestReNova.exe" "C:\Program Files\Mestrelab Research S.L\MestReNova\scripts\magnitudeActiveSpectrum.qs" -sf "magnitudeActiveSpectrum"



Threshold Script: This script will allow the user to adjust the threshold of the color scheme in 2D-NMR spectra:

```
// <GUI menuname="threshold2D" shortcut="Ctrl+T" tooltip="threshold2D" />
function threshold2D()
{
 var spectrum = nmr.activeSpectrum();
 if(!spectrum.isValid() )
  return

 var th = spectrum.threshold;
```

```
var dlg = new Dialog();
dlg.title = "Threshold";

var edit = new LineEdit();
edit.label = "Threshold";
edit.text = th;
dlg.add(edit);
if (dlg.exec())
{
 spectrum.threshold = edit.text;
}
spectrum.update();

mainWindow.activeWindow().update();
}
```

Running this script will show the 'Threshold' dialog box. From this window, the user will be able to adjust the threshold of the colour scheme by just typing the desired value and clicking on the 'OK' button.



ProcessTest Script: This script can be used to get some data from Mnova, save it as a file, next run some external program in order to make calculations (or whatever you need) and once the external program finishes, Mnova will get the result from another file. In this case the external program is the 'NotePad'.

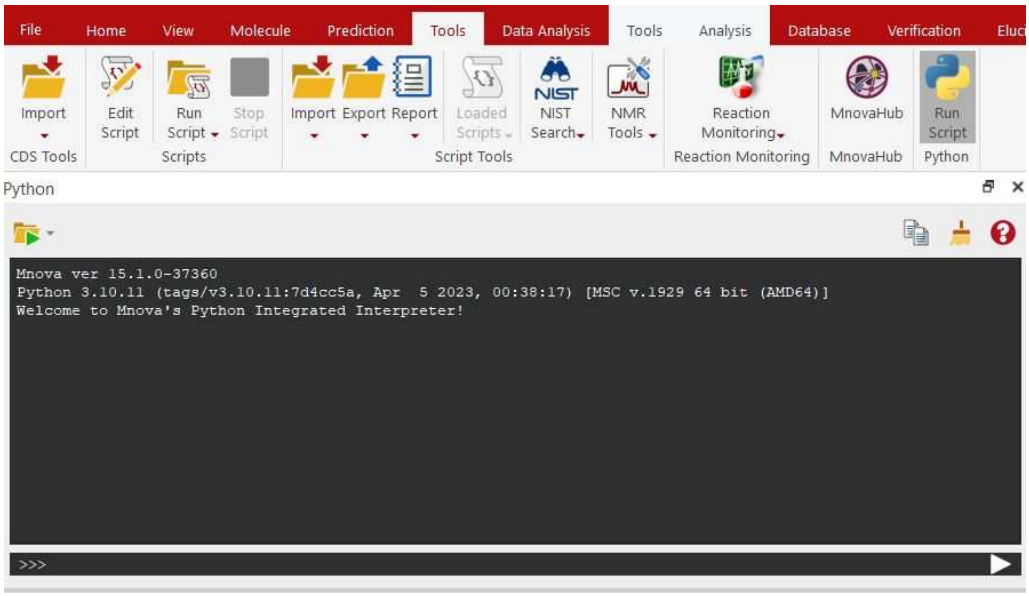
```
function processTest()
{
	fileName  = Dir.temp() + "MNova_Process_Text.txt";
	var f = new File(fileName);
	f.open(File.WriteOnly);
	var s = TextStream(f);
	s.write("Hello Notepad!");
	f.close();
	print(Process.execute("notepad.exe", fileName));

	f.open(File.ReadWrite);
	s.pos = f.size;
	print(s);
	s.write("\r\nHi again!");
	f.close();
	print(Process.startDetached("notepad.exe", fileName));
}
```

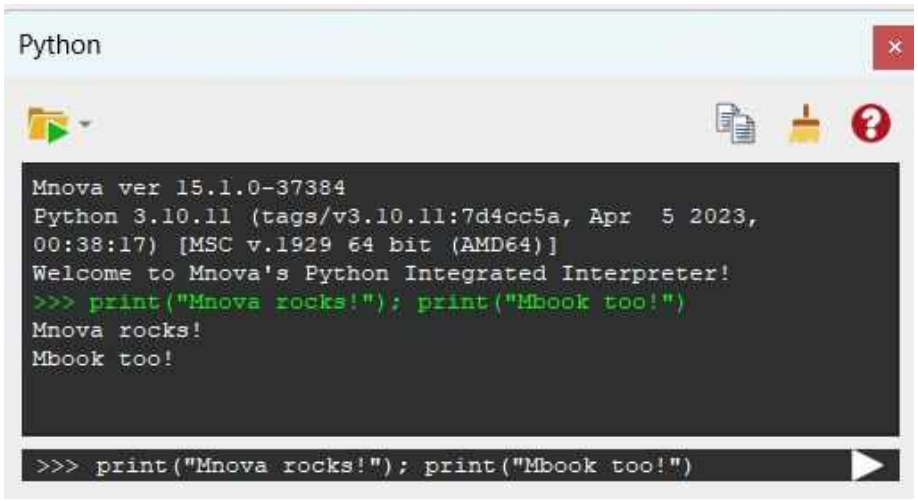
17.13 Python

From Mnova 15.1.0, you can use Python engine to run scripts by following the menu 'Tools/Run Script Python':

© 2024 MESTRELAB RESEARCH



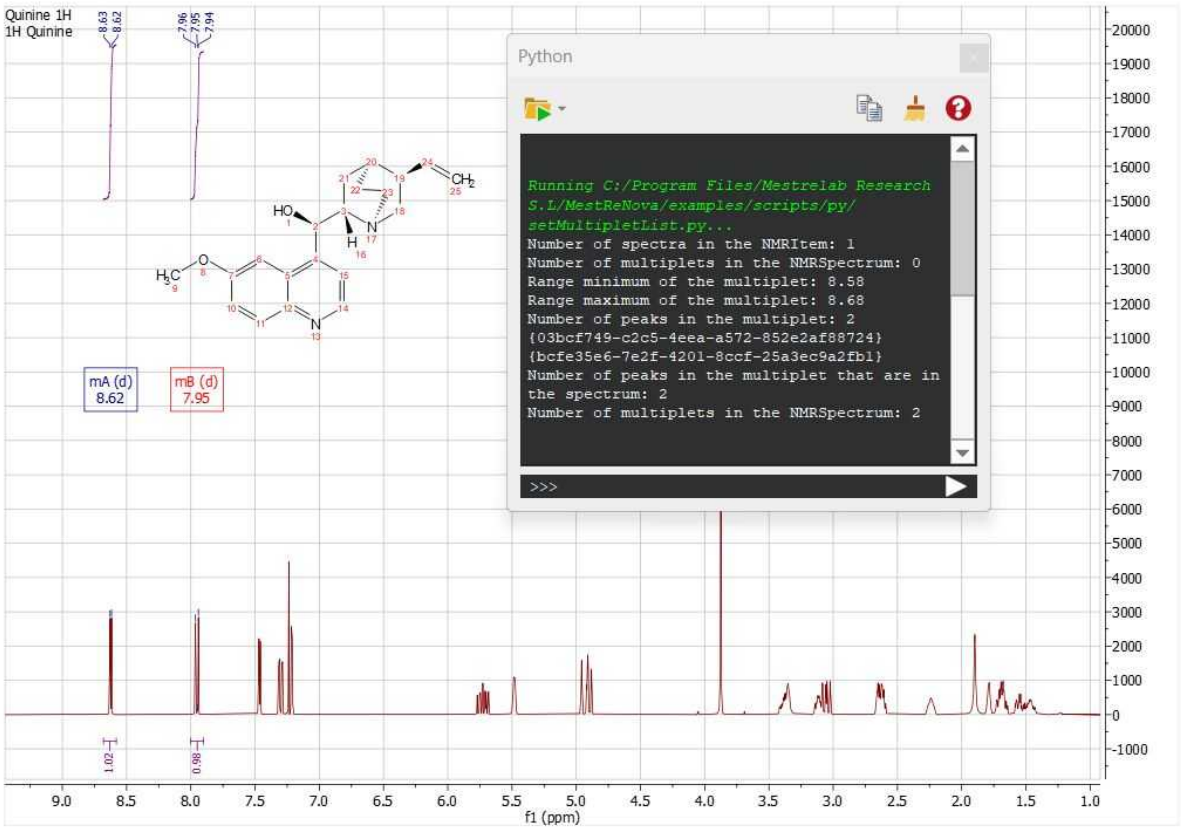
When adding two (or more) commands separated by semicolon, all commands will be executed:



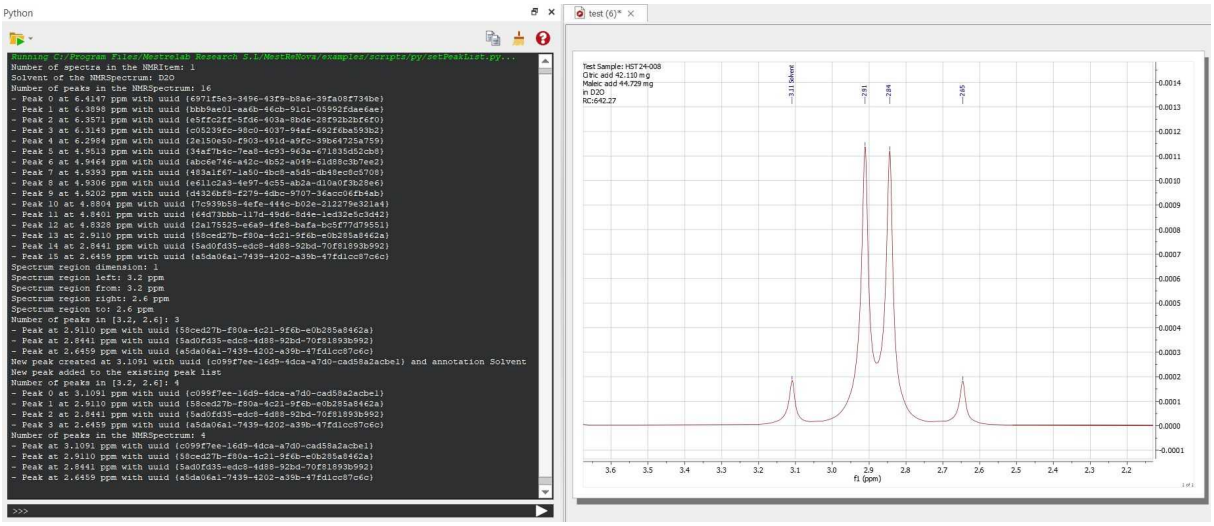
Clicking on the load button will allow you to run scripts with the .py and .pyc extensions. In the Python panel, you will also find buttons to copy the output to the clipboard, to clean the ouput and to launch the scripting documentation.

In the Mnova installation folder, you will find some examples (C:\Program Files\Mestrelab Research S.L\MestReNova\examples\scripts\py). Here you can find the result of loading a 1H-NMR spectrum and running the "setMultipletList.py" script to apply a multiplet analysis in a couple of regions, with the chosen names and colors:

© 2024 MESTRELAB RESEARCH



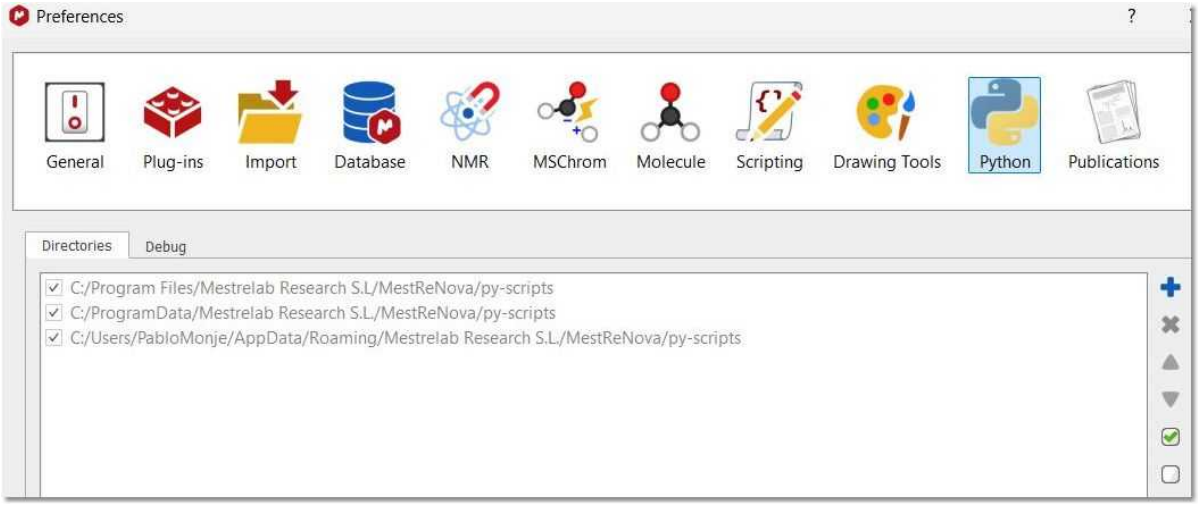
In the same example folder, you will find another script (setPeakList.py) to apply a Peak Picking at 3.11 ppm with a solvent annotation:



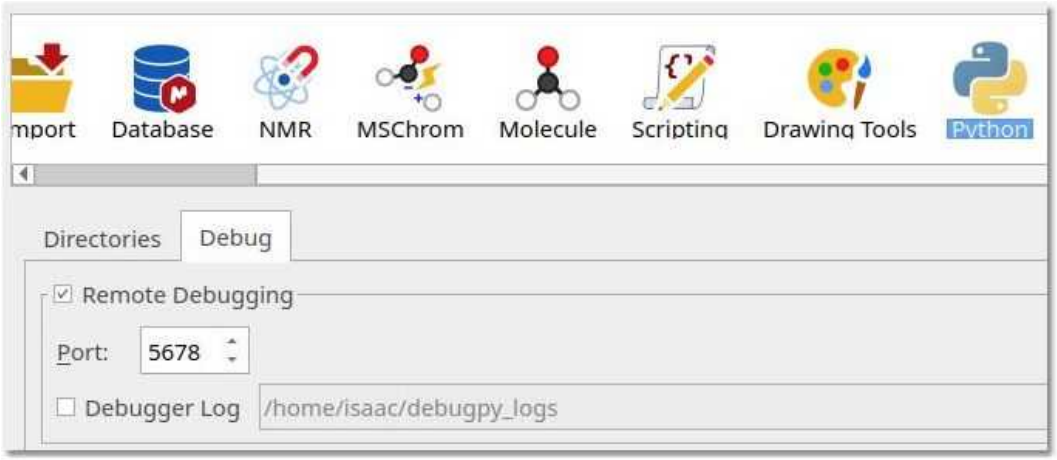
The example script called "createSpectrum.py" can be used to create an Mnova document with four pages. In the first page, you will find a 1D-NMR spectrum with one peak at 1 ppm. In the second page, you will get a 2D-NMR spectrum with one peak at (1.00, 20.00) ppm. In the third page, you will get a 1D-NMR spectrum with 3 multiplets at 8.0.7, 5.88 and 3.92 ppm. Finally, in the last page, you will find a 1D-NMR Spectrum with two peaks at 4.00 and 3.00 ppm.

© 2024 MESTRELAB RESEARCH

You can create scripting directories by following the menu 'File/Preferences/Python'. The scripts located in those folders will be run when launching Mnova:



If you have Visual Studio Code installed with the Python extension pack, you can debug your Python scripts with Mnova:



Part

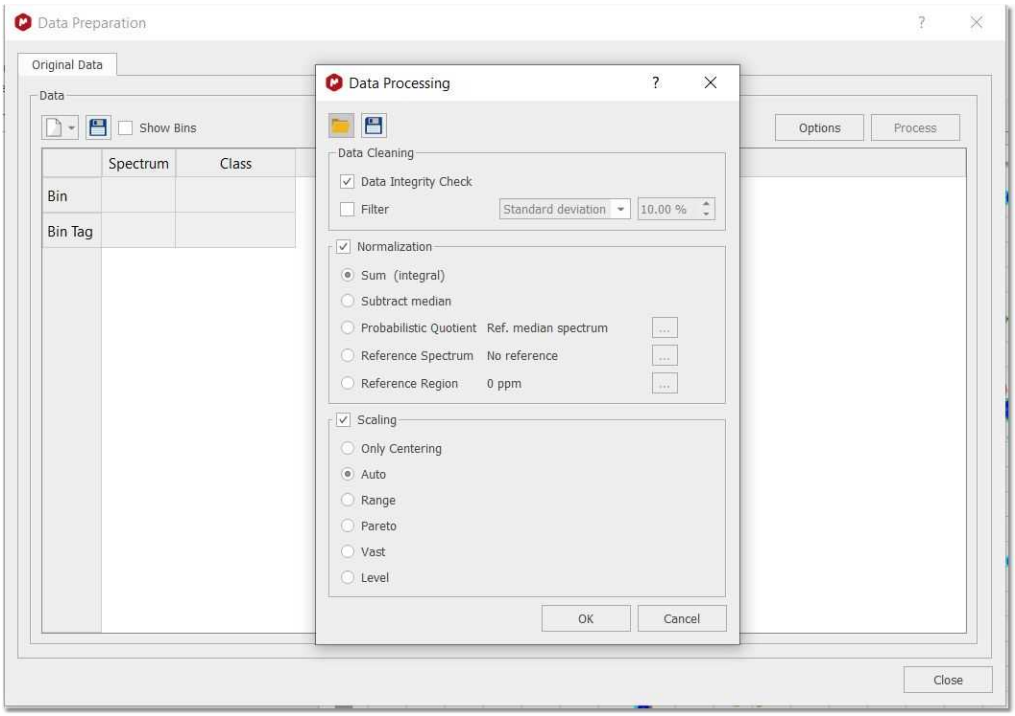


18 Basic Chemometrics - PCA

Principal Component Analysis (PCA) is a procedure which uses orthogonal transformation to convert a set of observations from correlated variables into a set of values of linearly uncorrelated variables (named principal components).

You can use Mnova for your PCA studies if you have a license for the Basic Chemometrics plugin.

You will only need to load your 1D or 2D stacked spectra and follow the menu 'Chemometrics/Data Preparation. Clicking on the options button will display the 'Data Preparation' dialog box:



Data Cleaning: two options are available which could be used simultaneously.

a. *Data Integrity Check* replaces negative bin values (negative integrals) with zeros.

b. *Filter*. Filtering methods are used to remove bins that are null and do not displays any changes among spectra series. By default if a variable (bin) shows zeros among all rows (spectra) it is discarded. Five options are possible. In the first three options Standard Deviation, MAD and IQR a fixed fraction (default 10%) of the bins is discarded (e.g. if the matrix is composed by 100 bins it means that 10 bins are discarded, and the selection is based on the Filter method chosen). In practice Standard Deviation, Median Absolute Deviation and Interquartile Range are calculated for all bins. Furthermore an amount of bins (with the lowest SD, or MAD or IQR values) are discarded with respect a percentage value of the total bins.

In the case of Mean Value or Median Value, user is asked to input a value for the Mean or the Median. By doing, so only bins that display a lower value of the inputted one are discarded.

© 2024 MESTRELAB RESEARCH

Normalization: it is an operation that is performed on the rows of the matrix. Four possible strategies could be selected:

c. *Sum*: every element on a row is divided by the sum of all elements of the same row;

d. *Subtract Median*: every element on a row is reduced by the median value of all the bins that constitute the same row;

e. *Probabilistic Quotient:* Normalization method proposed by Hans Senn et al.. For further information, please check this paper: Anal. Chem. 2006, 78, 4281-4290

f. *Reference Spectrum (or reference series of spectra)*: upon normalization by the sum, every element of a row is divided by the corresponding element of the row of the selected reference spectrum (e.g. when you have a reference spectrum and you wish to compare all the other spectra relative to it). If you select a bundle of spectra (like all spectra belonging to the same class) normalization is performed on the calculated average spectrum.

g. *Reference region (or bin)*: user inputs chemical shift value or range (in ppm) of a reference peak of interest and automatically MNova identify the bin(s) that comprises the selected peak/region. Furthermore every components of a row are divided by the reference component of the corresponding row.

Scaling: it is an operation that is performed on the columns of the matrix. Five different methods are implemented: Autoscaling, Range, Pareto, Vast and Level scaling. Mathematical operations applied are the follows:

Autoscaling	$\tilde{x}_{ij} = \frac{x_{ij} - \overline{x}_i}{s_i}$
Range Scaling	$\tilde{x}_{ij} = \frac{x_{ij} - \overline{x}_i}{x_{i_{max}} - x_{i_{min}}}$
Pareto Scaling	$\tilde{x}_{ij} = \frac{x_{ij} - \overline{x}_i}{\sqrt{s_i}}$
Vast Scaling	$\tilde{x}_{ij} = \frac{x_{ij} - \overline{x}_i}{s_i} \cdot \frac{\overline{x}_i}{s_i}$
Level Scaling	$\tilde{x}_{ij} = \frac{x_{ij} - \overline{x}_i}{\overline{x}_i}$

Where $\overline{x}_i$ is the mean, s_i is the standard deviation and $\tilde{x}$ represent data after scaling.

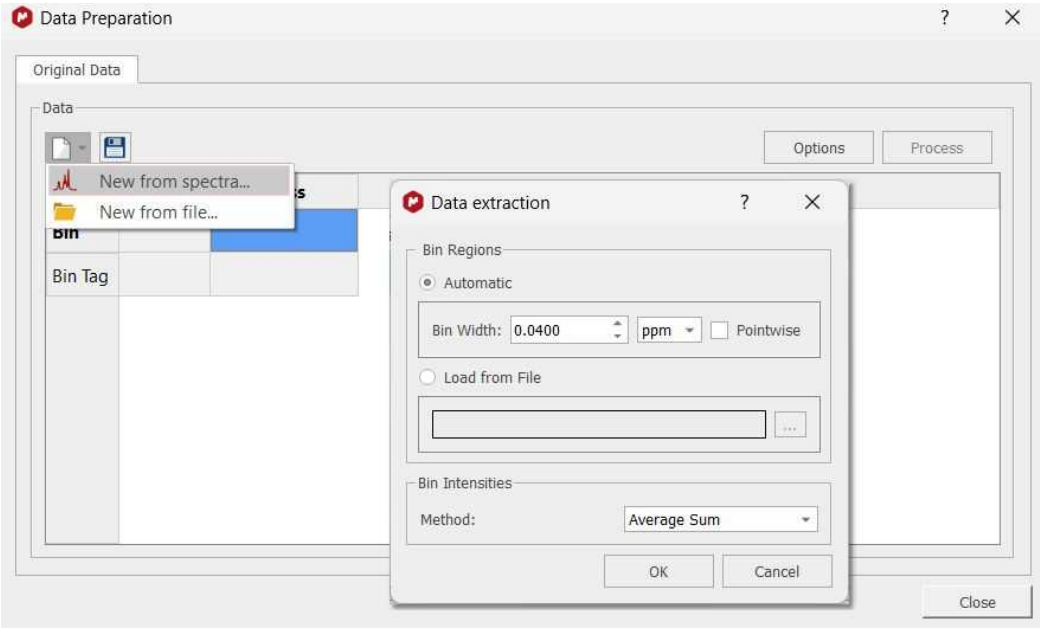
$$\overline{x}_i = \frac{1}{J}\sum_{j=1}^{J} x_{ij}$$

$$s_i = \sqrt{\frac{\sum_{j=1}^{J} (x_{ij} - \overline{x}_i)^2}{J - 1}}$$

For further information about the different scaling methods, please visit this link.

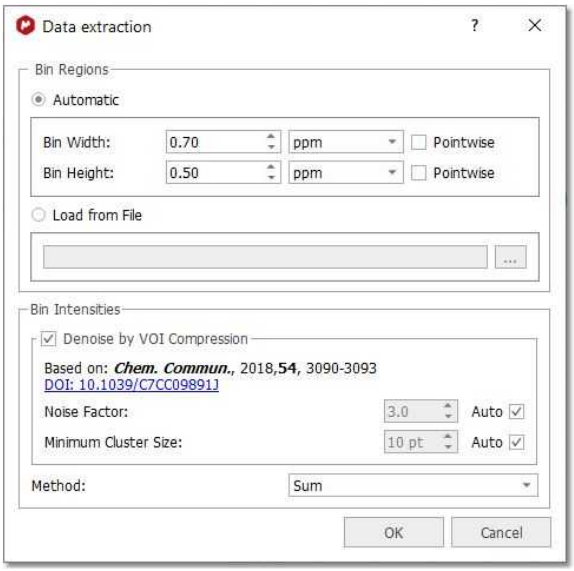
The next step would be to select 'New from from spectrum' (or 'new from file' if you have any saved 'preparation file' from other experiment):

© 2024 MESTRELAB RESEARCH



A new dialog will appear to select the binning options.

If you are working with a 2D stacked dataset, you will get this dialog box:



You can calculate the bin regions automatically or by loading a text file:

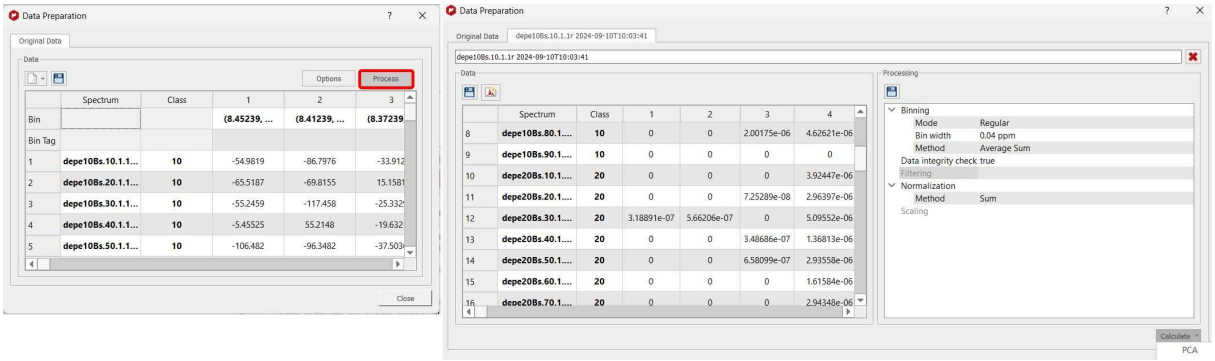
- Automatic: it creates the bin regions using a fixed bin size. You can set the bin width or bin height to *Pointwise* to work with the spectrum resolution in that dimension.
- Load from File: imports the bin regions from any existing 'MestReNova Integral Regions file' (which you can obtain from any integrated spectrum and following the menu "File/Save As/MestReNova Integral Regions (*.txt)")

From here, you can perform a VOI (Variable Of Interest) Compression, to reduce the number of interesting bins. It is recommended to do it for the binning methods: Sum, Average Sum, and Centered.

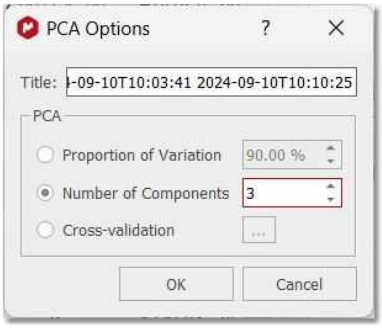
© 2024 MESTRELAB RESEARCH

There are four binning methods are available: Sum, Average Sum, Center and Peak. The 'Sum' method will sum all the points of a bucket, the 'Average Sum' will divide the sum by the number of points in the bin, the 'Center' method will only return the value found in the middle of the bin. For example if a bin has 5 points it will return the value for 3rd point. The 'Peaks' method will perform firstly the GSD (Global Spectral Deconvolution) and then will apply binning over the peak table. For large dataset (more than 50 stacked spectra, it advisable to perform GSD before executing PCA module).

After having clicked on 'Process' button, you will get a new dialog summarizing the processing and allowing you to save the 'Data preparation file' or the 'Processing' file to be used in the future:

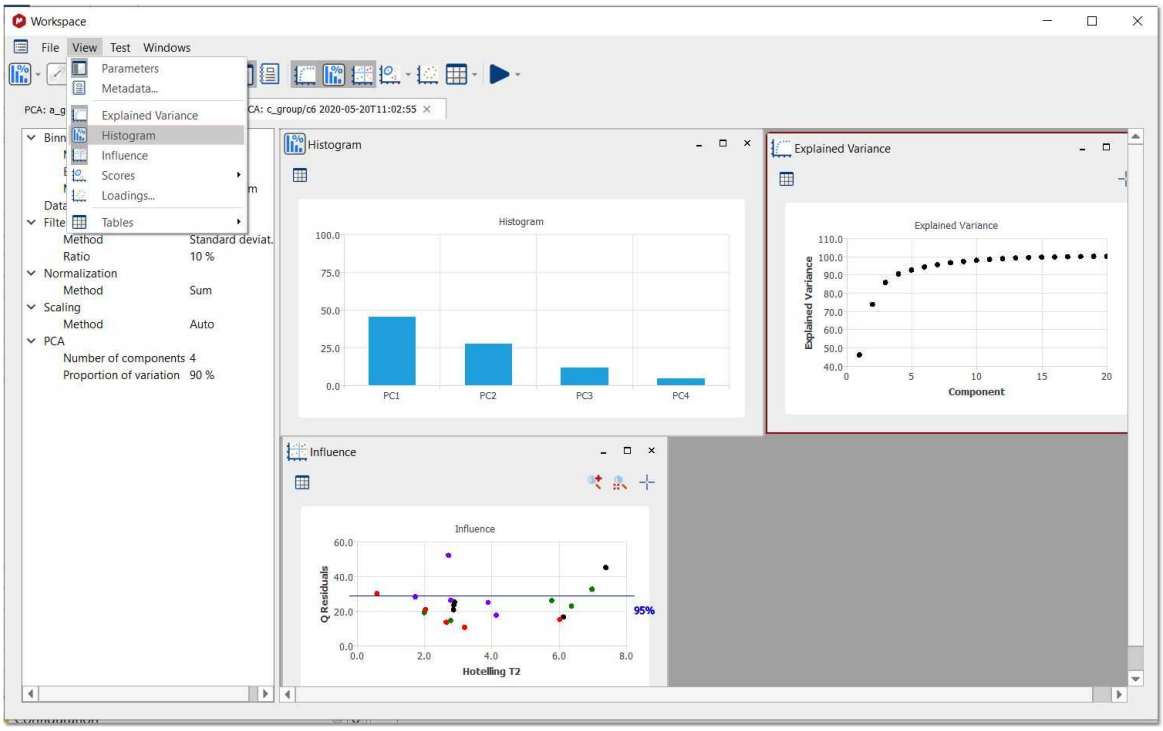


Finally, click on the 'Calculate PCA button' to select proportion of Variation, or the number of principal components. From here you can also change the title or run a cross validation

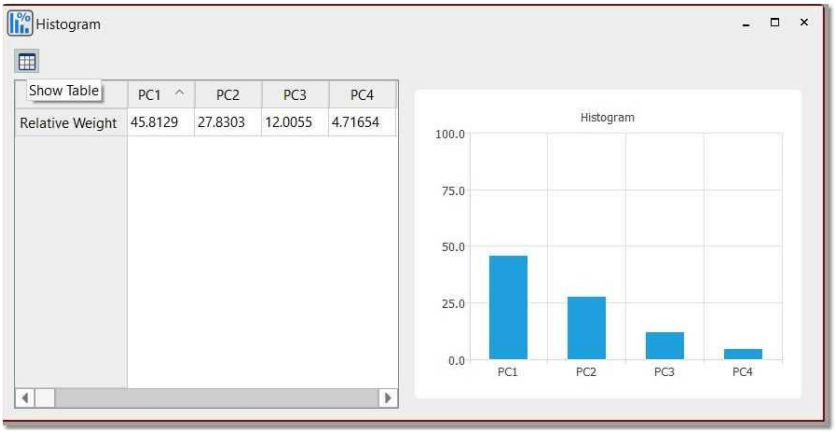


After having clicked on the 'OK' button; the Workspace panel' will be displayed. From the View menu, you will be allowed to display the 'Explained Variance', 'Histogram' and 'Influence' graphs. Right clicking (or Ctrl/Cmd+C) on any of the plots, will allow you to copy to clipboard, report or save the image:

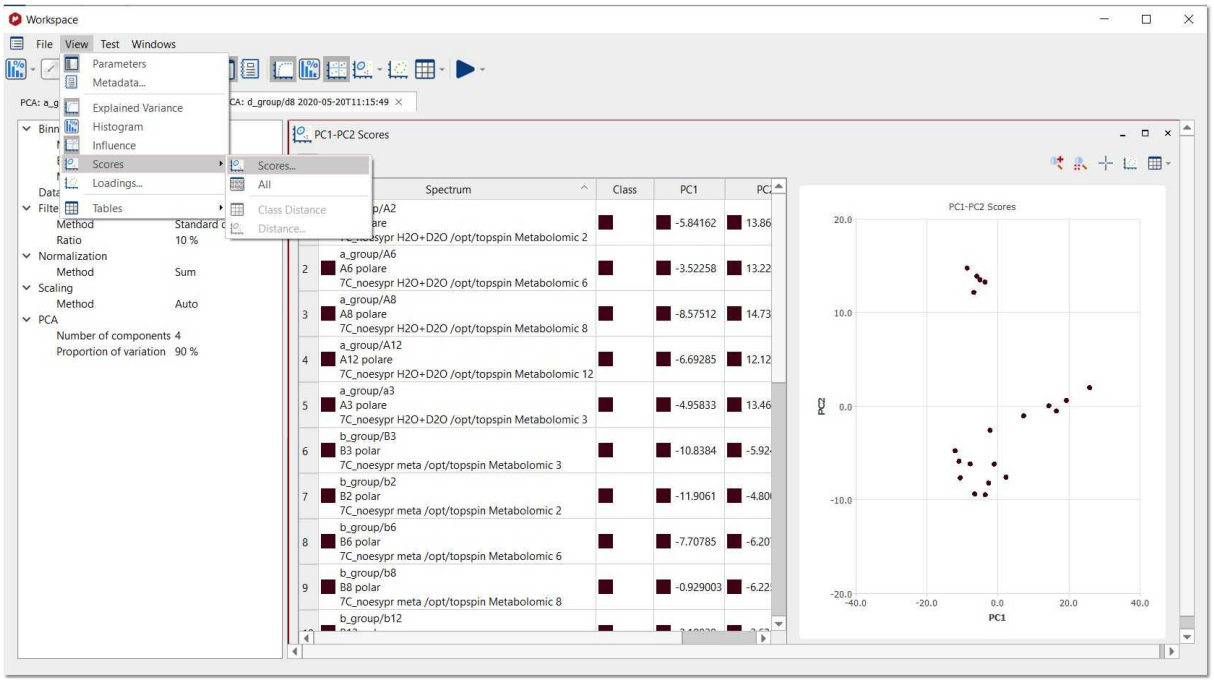
© 2024 MESTRELAB RESEARCH



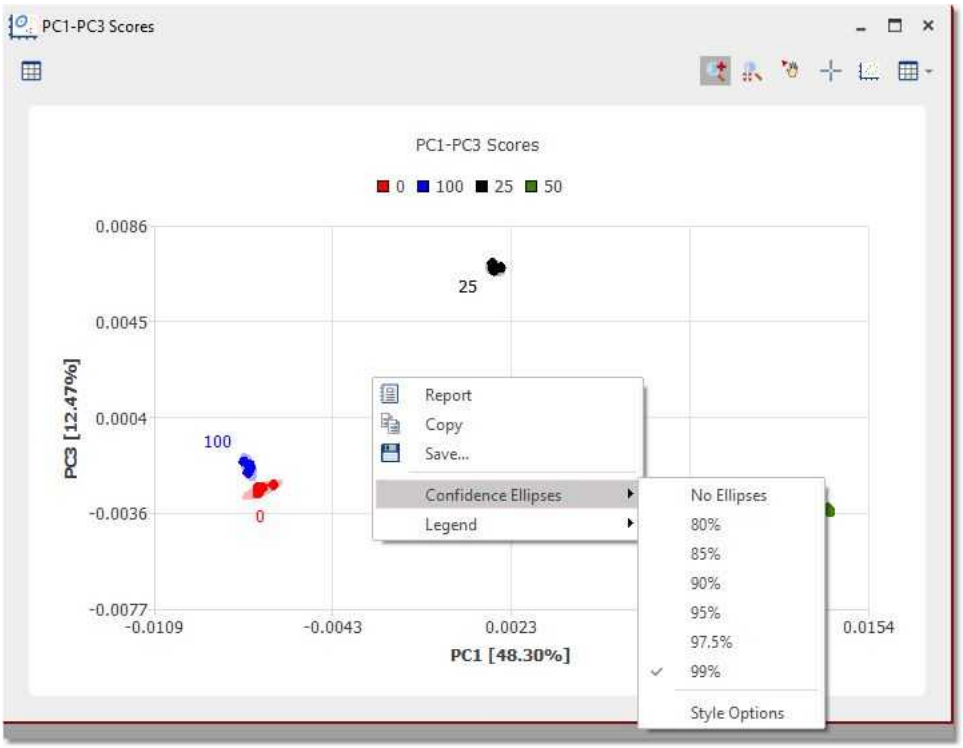
Clicking on the 'Show Table' button, will hide/display the applicable table with the relative weight for each component:



Selecting the 'Score Plot' tab from the "View/Scores" menu, will display a window like this:

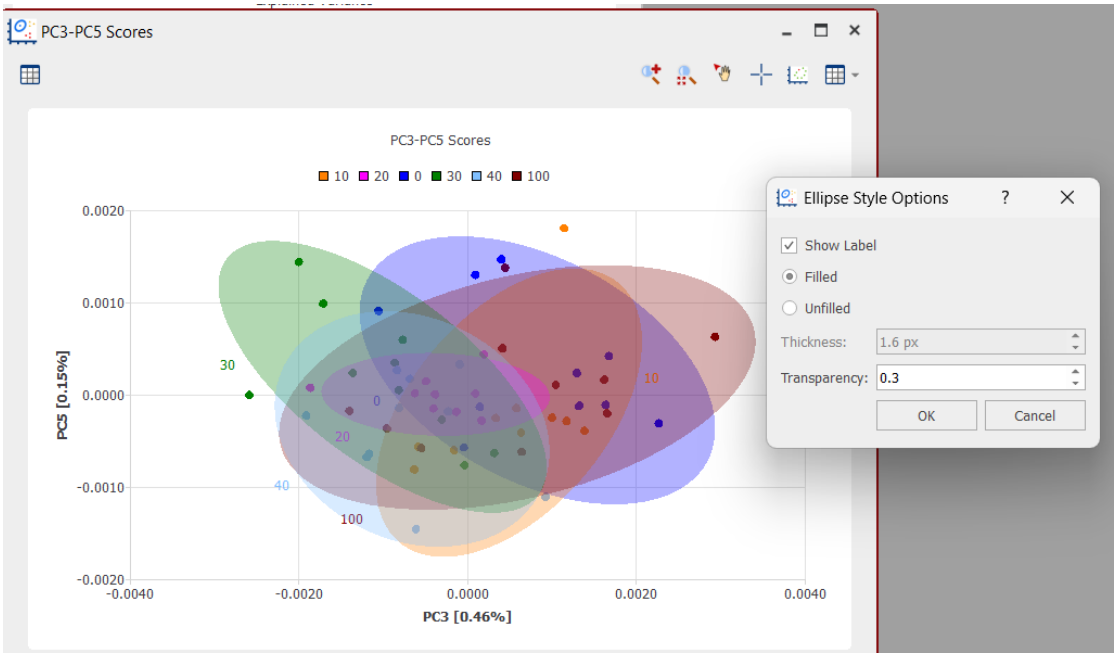


You can display the confidence ellipses by right clicking and selecting the desired confidence value:

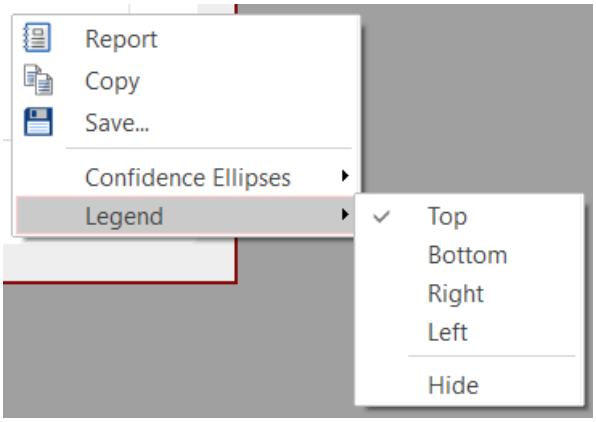


You can select some style options to show/hide the classes labels, to fill/unfill the ellipses and to choose the thickness and transparency:

© 2024 MESTRELAB RESEARCH



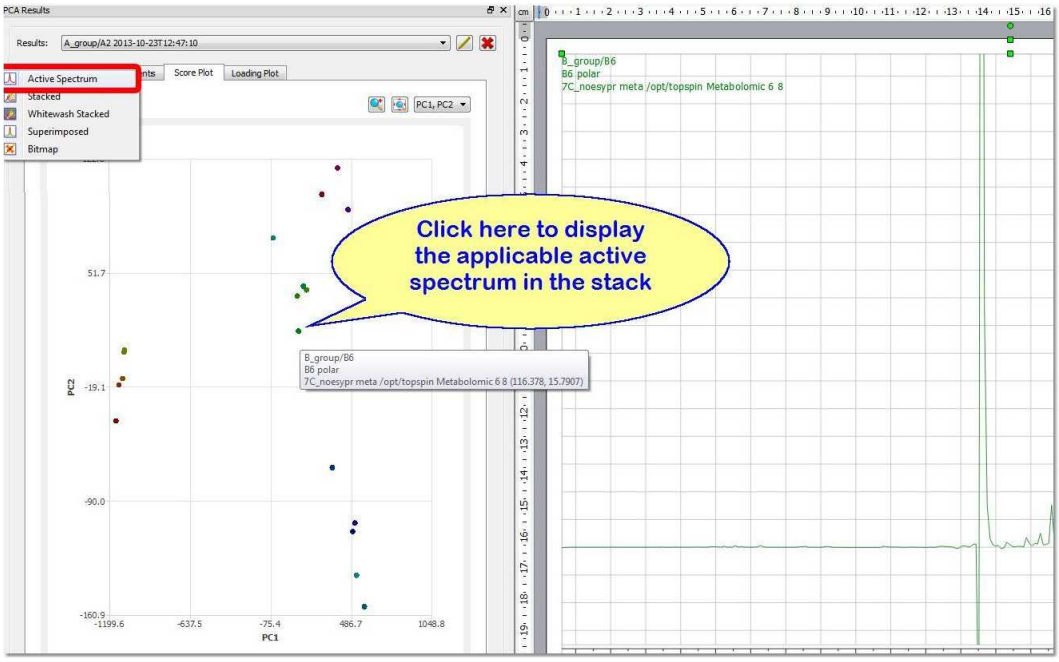
A legend can also be displayed and sorted from the context menu above:



For further information about how we are obtaining this value, please visit this link.

Each point of the 'Score Plot' is associated to a trace of the stack. If you are in the 'Active Spectrum' mode, you will get displayed the applicable spectrum when clicking on the point (of the Score Plot):

© 2024 MESTRELAB RESEARCH

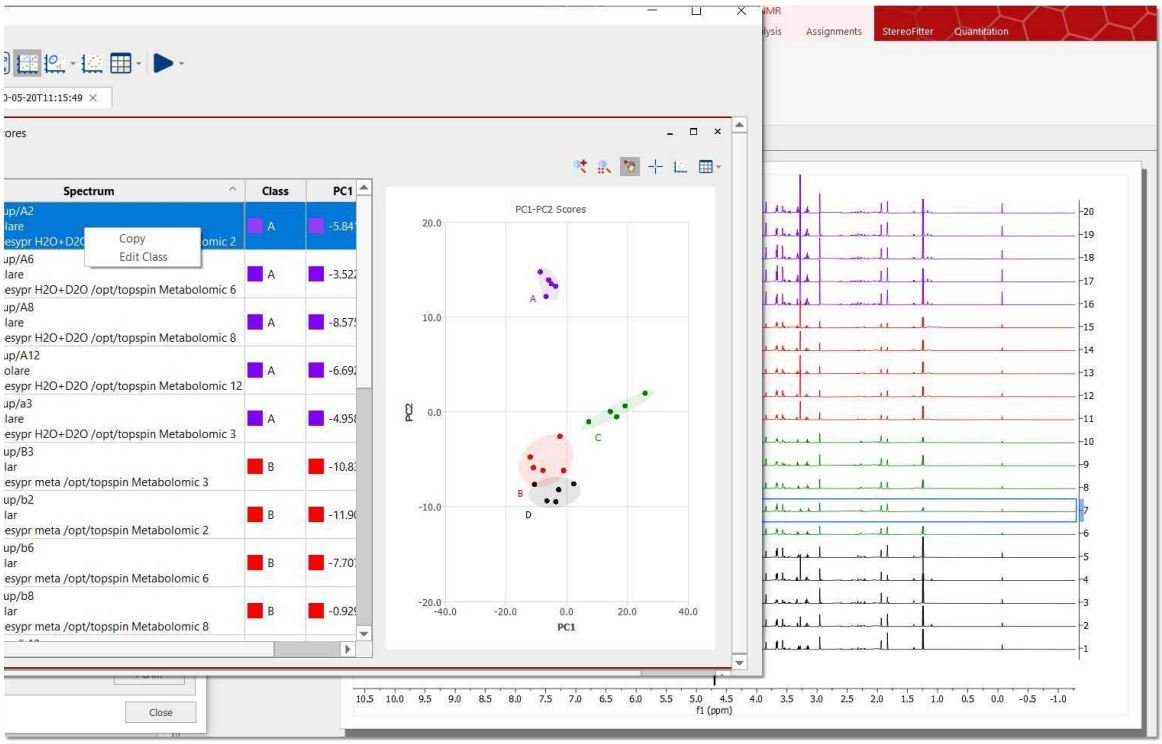


You can 'zoom in', 'reset zoom', use the crosshair or display the loadings plots by using the applicable buttons.

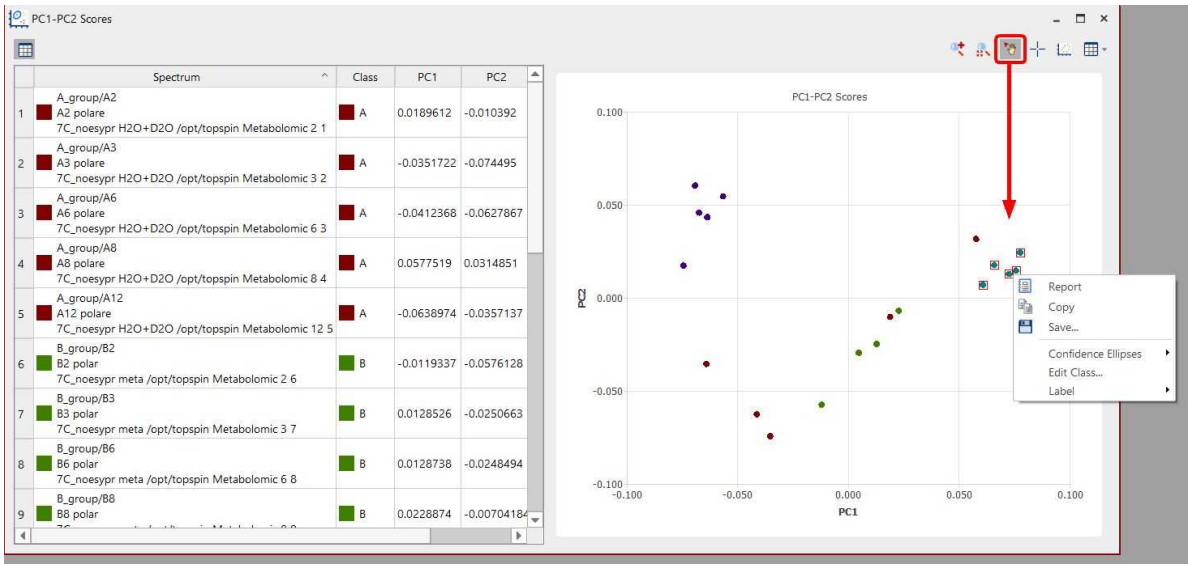


Clicking on the 'Show Table' button  will display the results panel. From here you can select several rows and change the class or the color by right clicking on them (you can also change the class from the 'Stacked Items Table'):

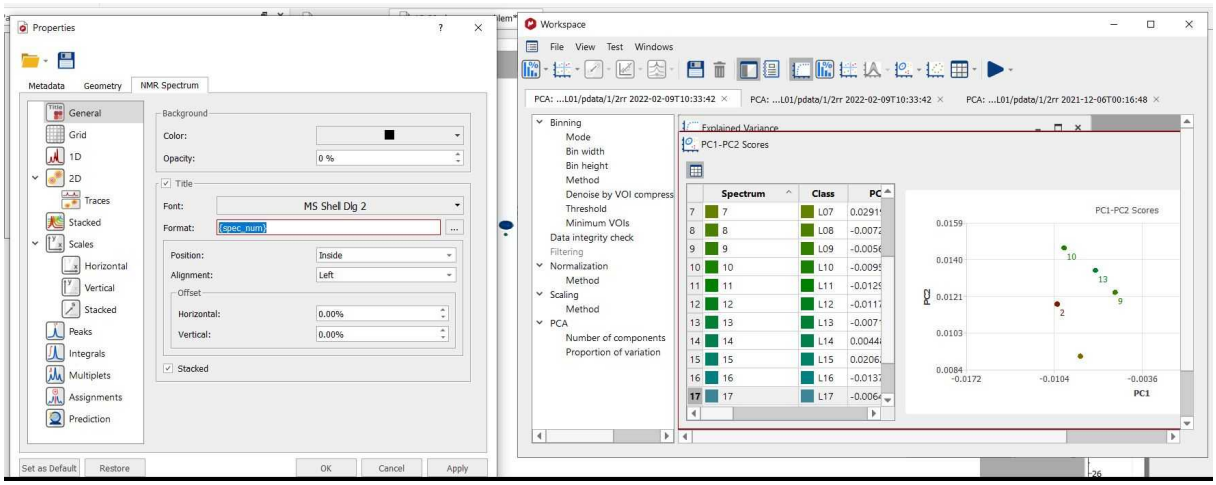
© 2024 MESTRELAB RESEARCH



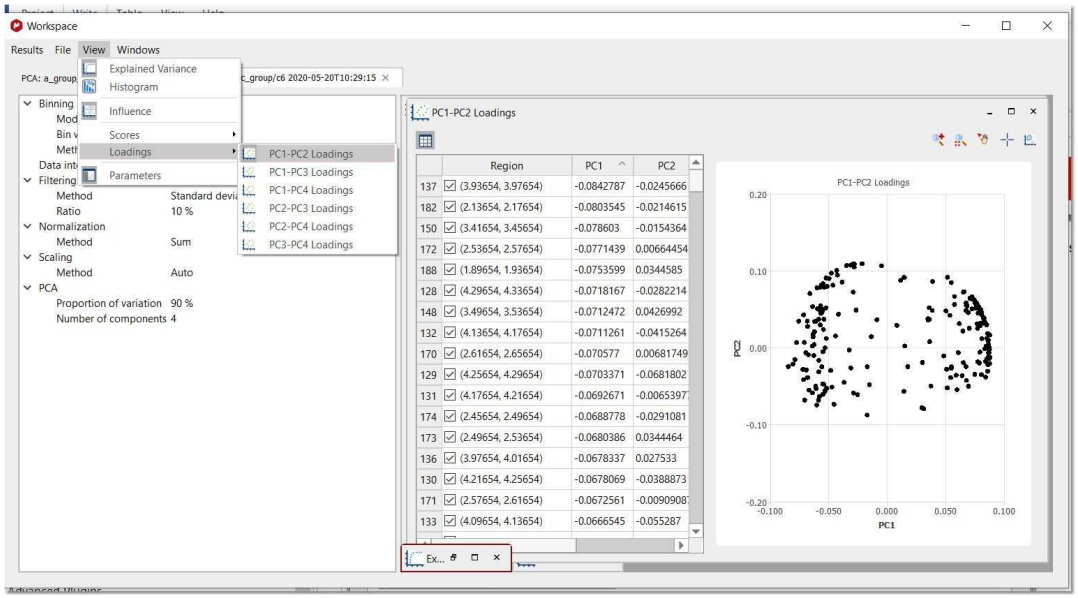
You can also click on the 'Select points' button, hold down the shift key to click on the points of interest and right click on the them to select the confidence elipses, edit the class or show the label:



It will be possible to show the Spectrum Number in that label, but for that, it would be necessary to configure the title of the spectra in the stack (from the Properties dialog box) so that Spec Number is displayed in the first line:

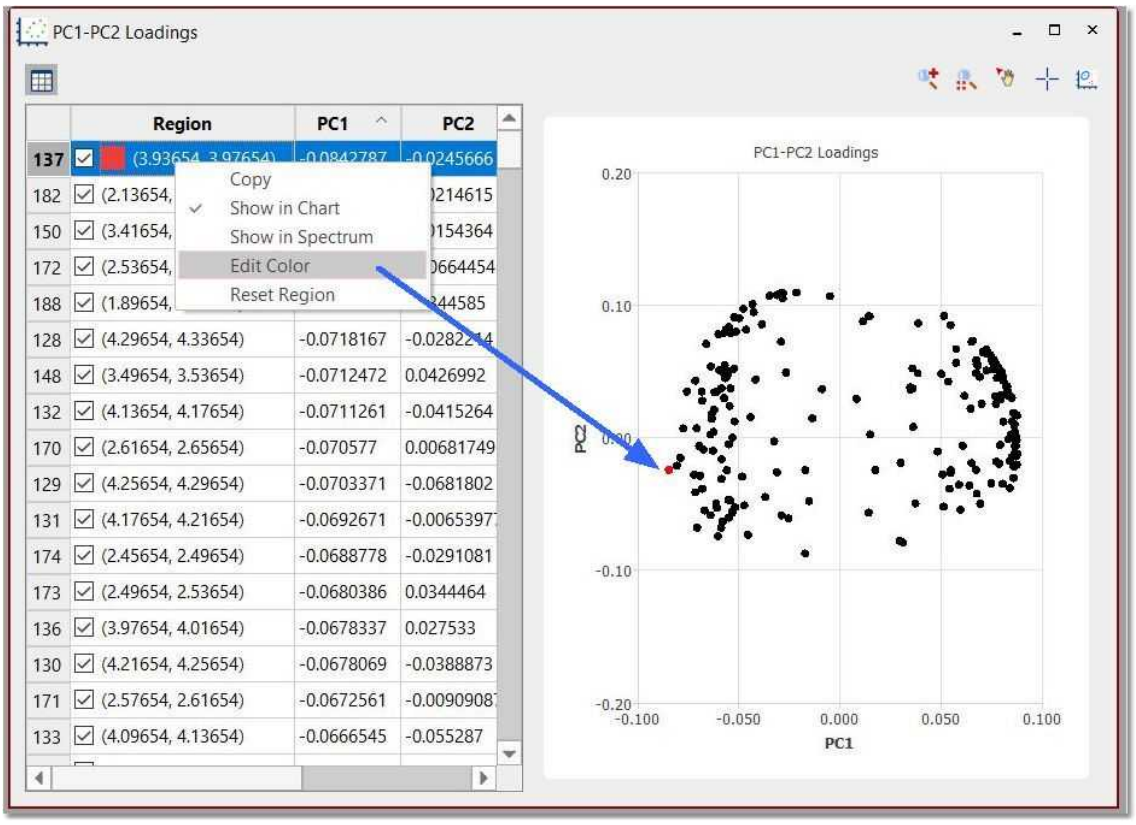


Select the applicable 'Loading Plot' from the View menu or by clicking on the applicable button of the Scores panel:

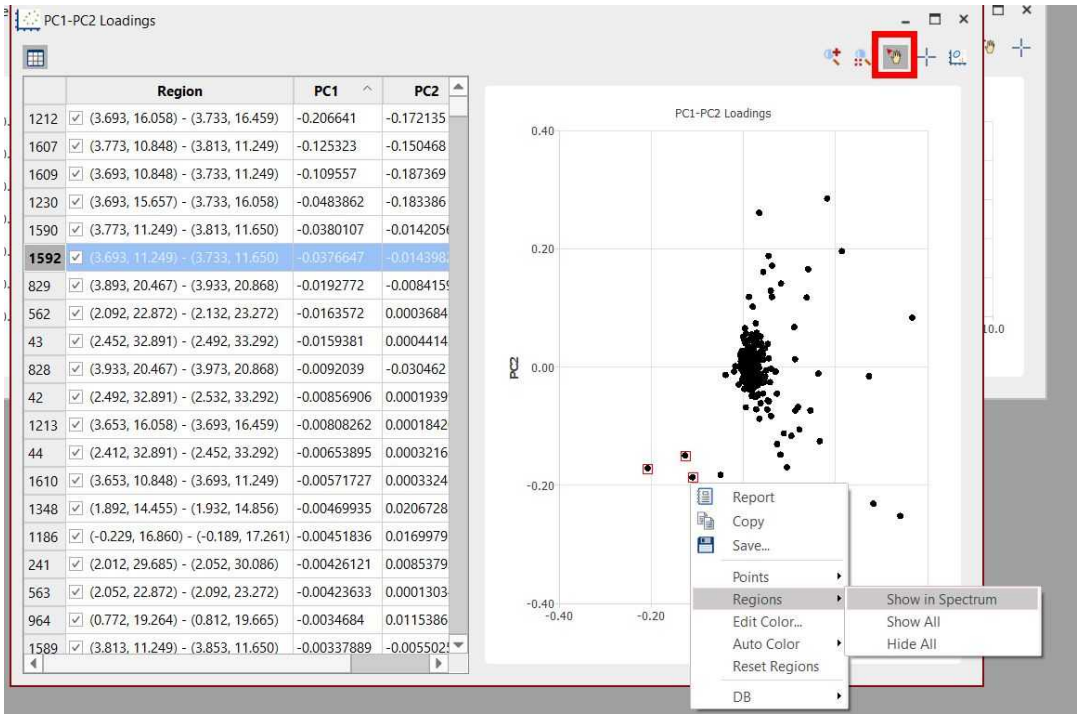


In this plot, each point will be associated to each region of the stacked spectra. You can change the visibility and colors of the bins by right clicking on the applicable row:

© 2024 MESTRELAB RESEARCH

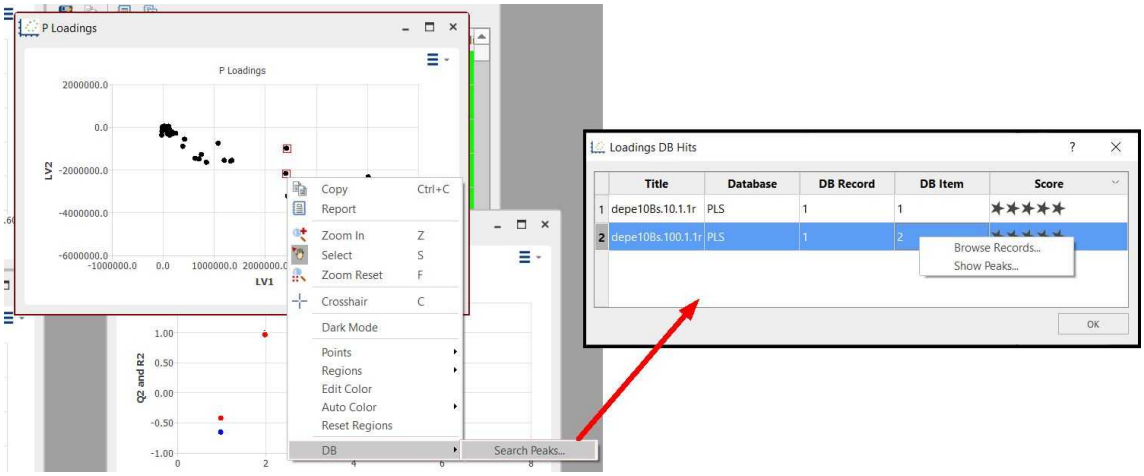


Using the feature to 'select points' in the loading plot and right clicking on them, will allow you to show the regions in spectrum or edit their color. Select 'Reset Regions' to get all of them in black.



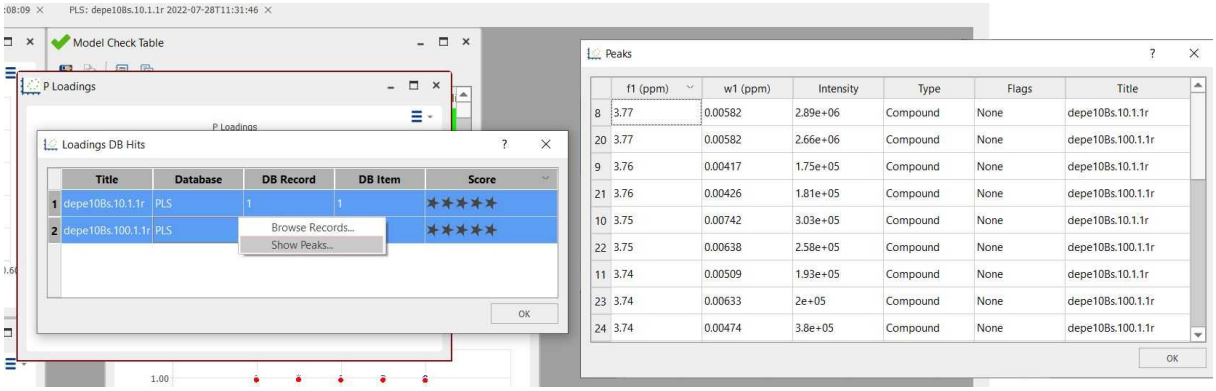
From the context menu, you can export the result as an image (png, svg, jpg or bmp) by clicking on the 'Save' option.

Click on the DB scroll menu to search for the Peaks in the Database:



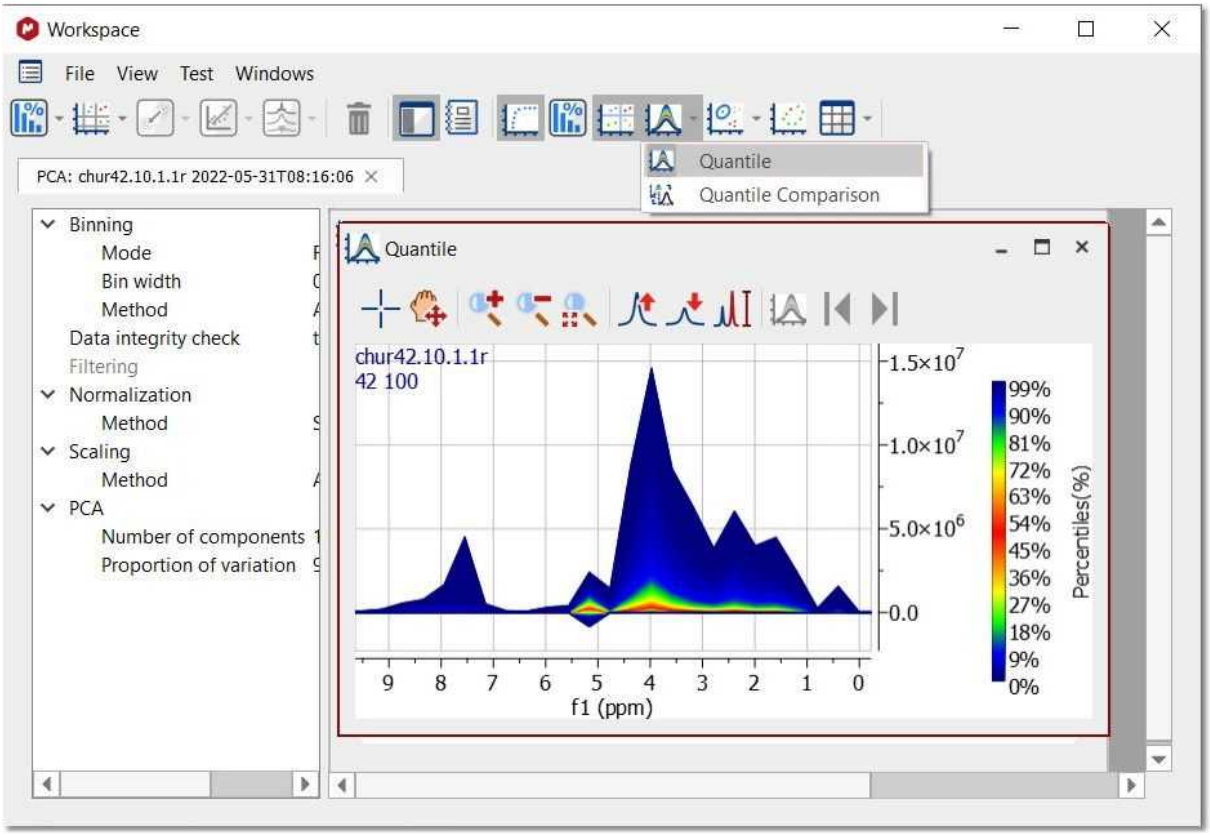
Double click on a row to display the record in the DB browser. Several records from several rows can be displayed by selecting the rows, right-clicking and selecting "Browse Records".

You can also display the list of found peaks by right-clicking and selecting 'Show Peaks':

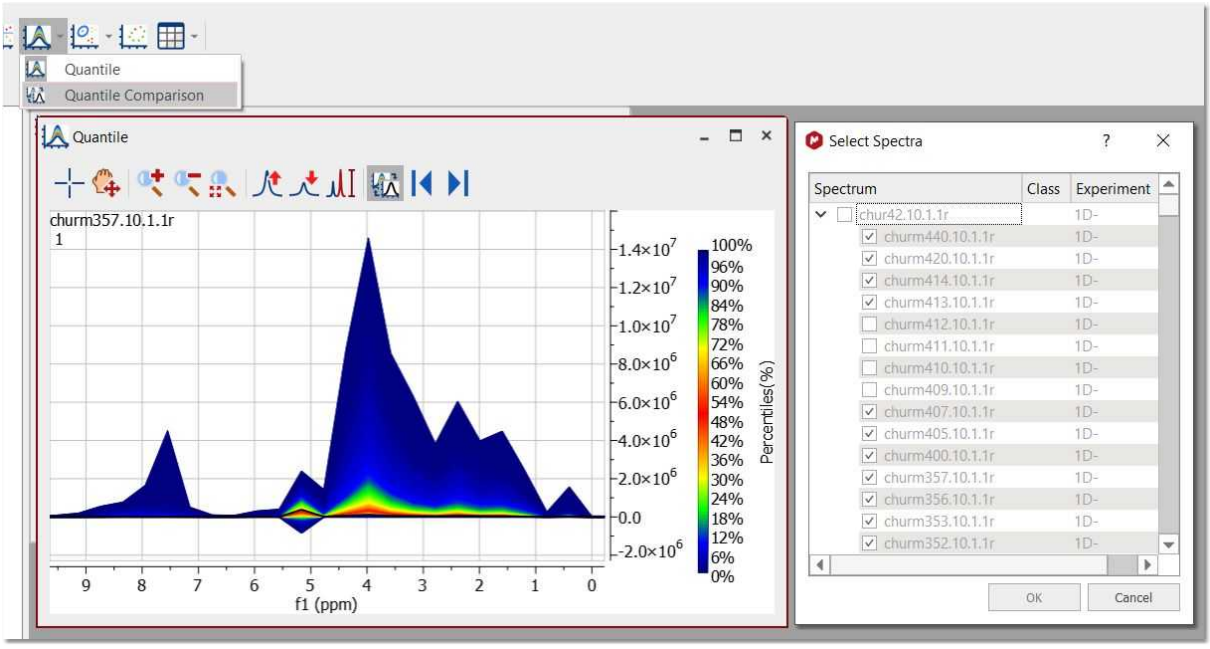


After having run the PCA, you can get the Quantile plot just by clicking on the applicable button:

© 2024 MESTRELAB RESEARCH



Quantile Comparison is available under Quantile scroll down menu. From here, you can select some/all the spectra in the stack to be overlaid one by one in the Quantile plot.



© 2024 MESTRELAB RESEARCH

If there are one or more spectra in the same document but not included in the stack, they can be also selected to be compared in the Quantile plot. Each overlaid spectrum shows in black color.

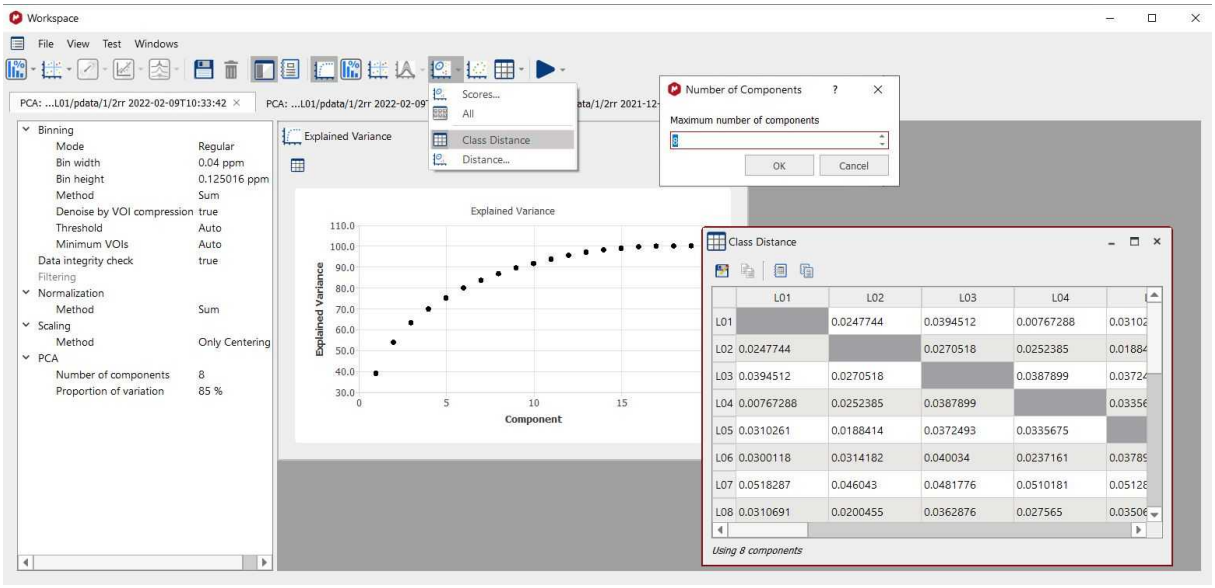
In the toolbar of the Quantile plot there are specific buttons for the comparison:

1. Button to toggle between Quantile and Quantile Comparison.
2. Previous/Next Compared Spectrum (shortcut: SHIFT+down and SHIFT+Up): when selecting more than one spectrum for the comparison, you can navigate through the spectra by clicking on these buttons.

Distance Calculations

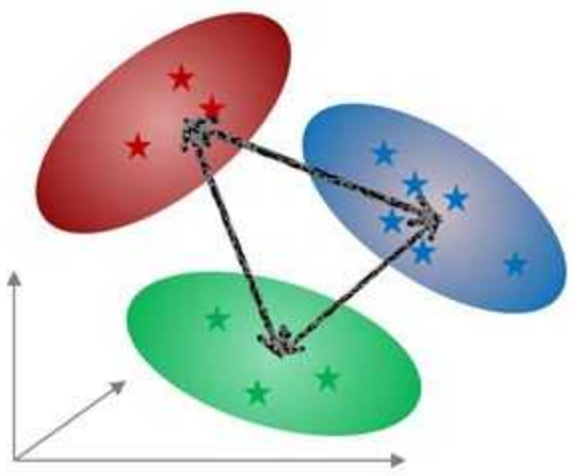
The Mahalanobis distance describes how many standard deviations a point is from the mean of a distribution. Distances are calculated between classes or between classes and an individual spectrum.

To compare classes, once you have defined several classes in one experiment, the less distance value will indicate that those classes will be 'more similar'. Once you have selected the feature, a new dialog will be displayed to select the number of components to be used (maximum number is the total number of components, 8 in this case):

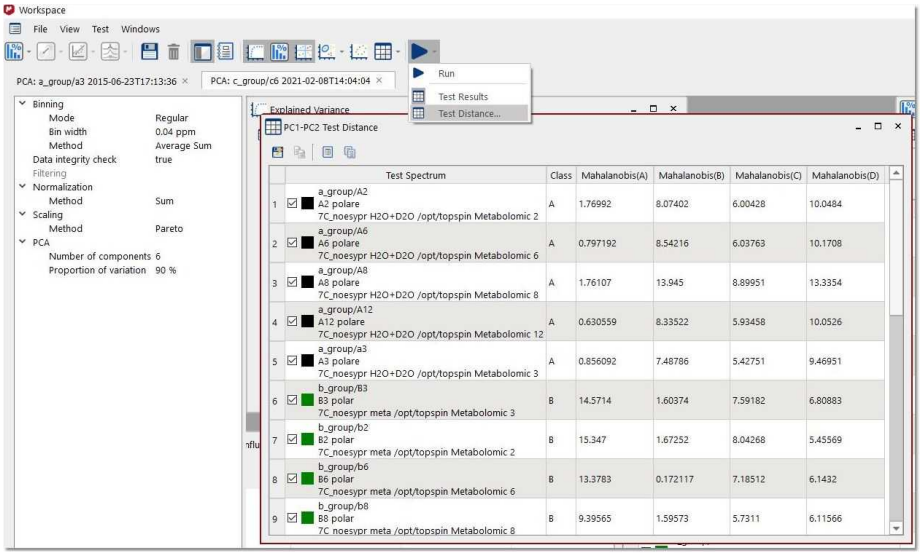


Global Class distances are calculated from multi-dimensional ellipsoids using either the selected number of principal components or two selected PCs.

© 2024 MESTRELAB RESEARCH

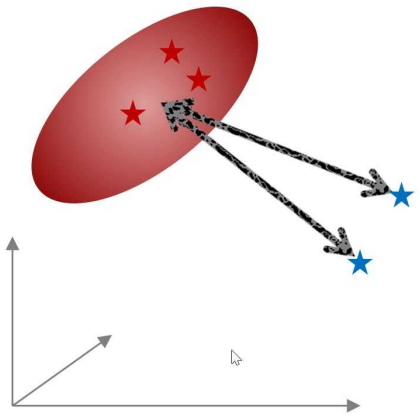


In the second case (to compare a test spectrum with different classes), once you have run the PCA and defined the classes, you could 'test' one (or several new spectra), the Mahalanobis distance against each of the classes (to get information about similar characteristics) .

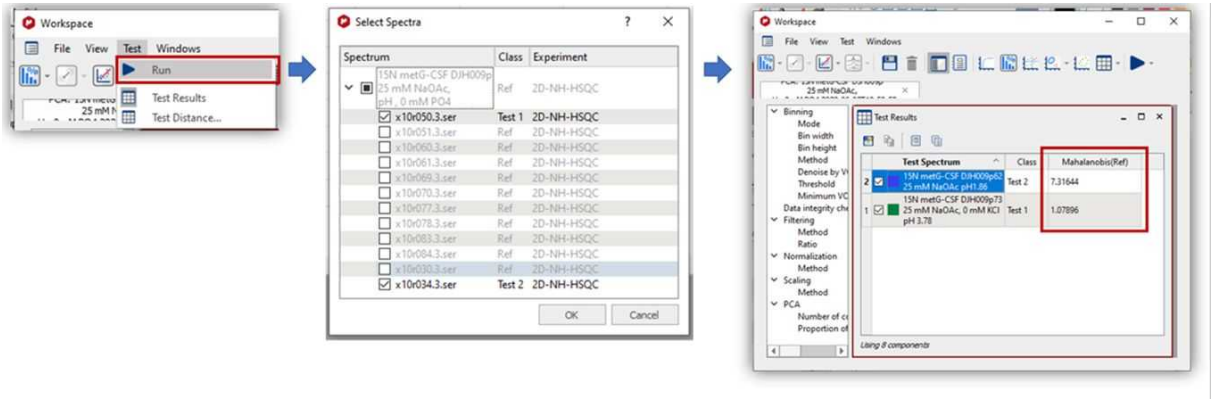


It is possible to measure the distance between classes and individual spectra. The spectra can be taken from any loaded document. It allows detecting to which class a spectrum belongs.

© 2024 MESTRELAB RESEARCH



This calculation is available via the *Run* command. Individual spectra can be selected.



For the cases where each spectrum is in its own class, the distances are not Mahalanobis but Euclidean distances.

Further information can be found in this paper http://dx.doi.org/10.1208/s12249-017-0911-1

See also this useful posts:
https://mestrelab.com/resources/pca-of-nmr-data-advantages-of-an-integrated-approach-2.html
http://nmr-analysis.blogspot.com.es/2014/07/pca-and-nmr-practical-aspects.html
http://mestrelab.com/blog/pca-of-nmr-data-advantages-of-an-integrated-approach/
And this video:
https://www.youtube.com/watch?v=kxVQKLY1KcU

© 2024 MESTRELAB RESEARCH

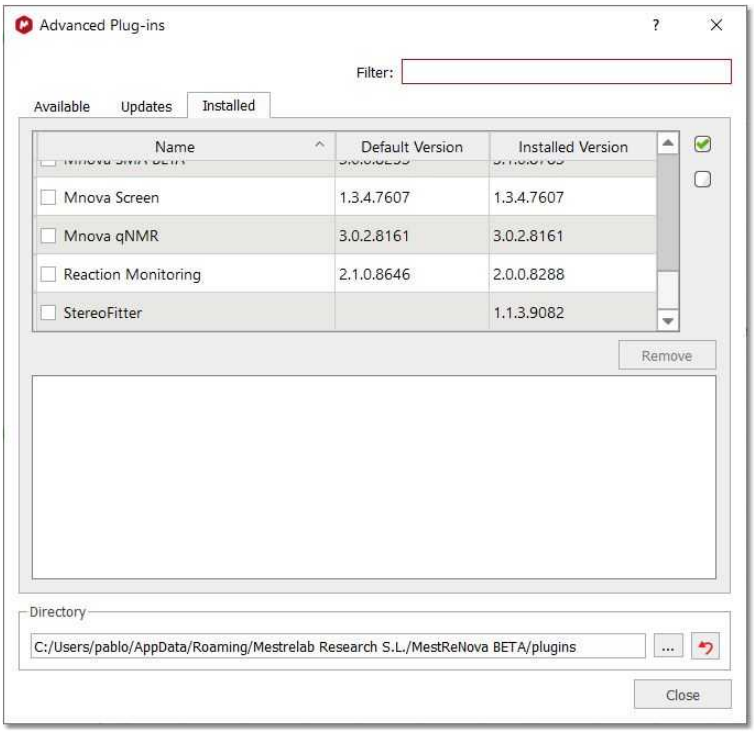
Part



19 Advanced Plugins

Most of the advanced plugins must be installed separately, by following the menu 'File/Advanced Plugins' and checking the applicable boxes from the 'Available tab'.

You can update those plugins from the 'Updates tab' and uninstall them from the 'Installed' tab:



You can find further information about it, here

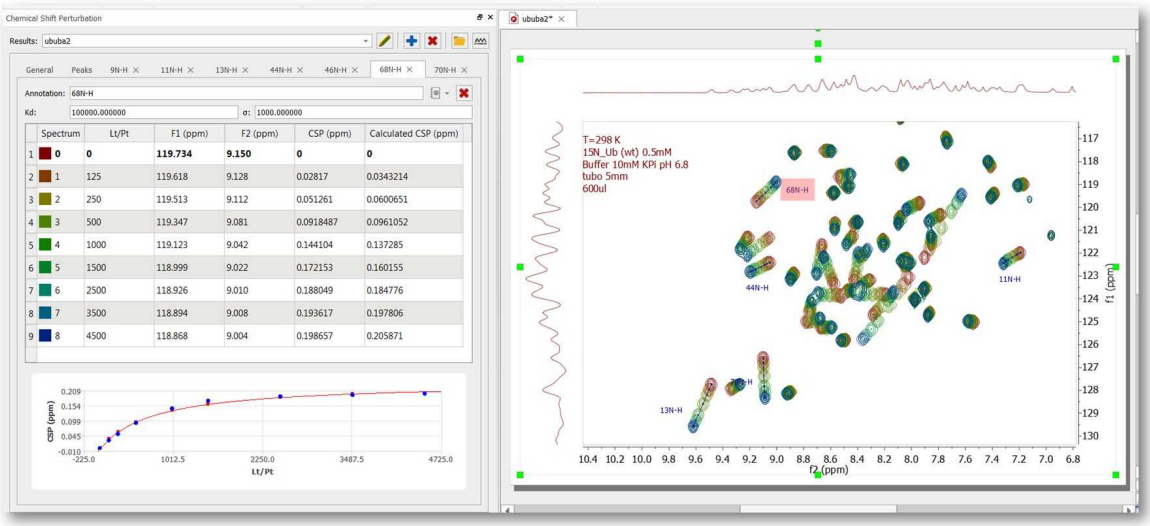
19.1 Binding

This advanced plugin needs a specific license (but it does not need to be installed apart).

It will be useful to analyse the chemical shift perturbation for fragment-based drug discovery.

It is a powerful tool that automatically processes 2D HSQC type of protein-ligand titration spectra, tracks the peak movement and computes the Kd's for multiple peaks.

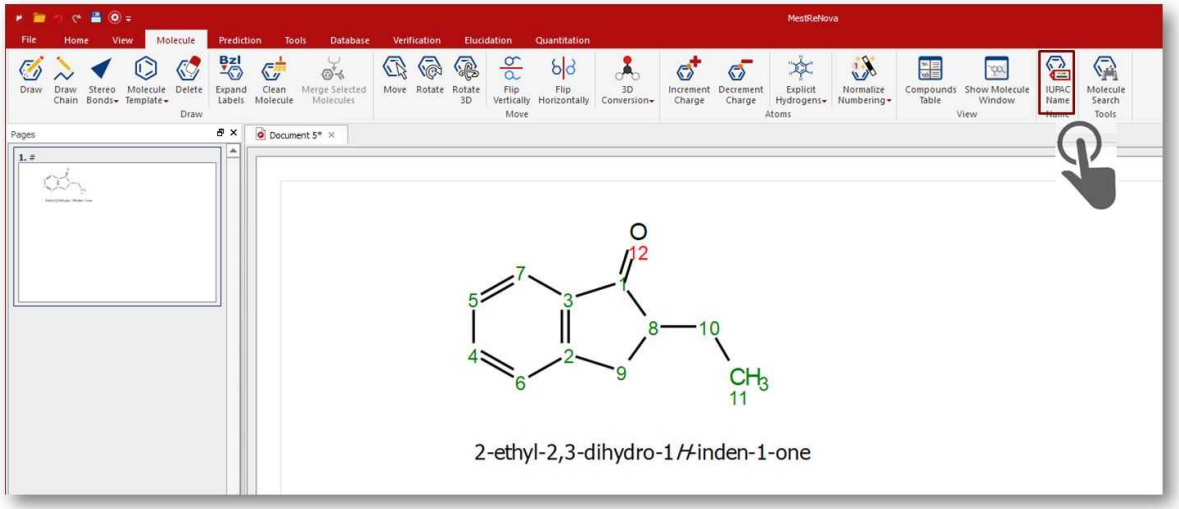
© 2024 MESTRELAB RESEARCH



More resources about this plugin can be found here. See also this paper.

19.2 IUPAC Name

This plugin needs a specific license (but it does not need to be installed apart). and will allow you to generate molecule IUPAC names from your molecular sketcher ribbon:

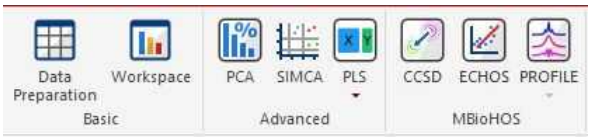


More information about this plugin can be found here

19.3 MBioHOS

This advanced plugin needs a specific license (on top of licenses for the NMR and Basic Chemometrics modules).

Mnova BioHOS is designed for the NMR analysis of biotherapeutics, like monoclonal antibodies. 2D or stacked NMR spectra of reference samples are compared with that of each test sample using a fingerprinting, or spectral similarity determination.

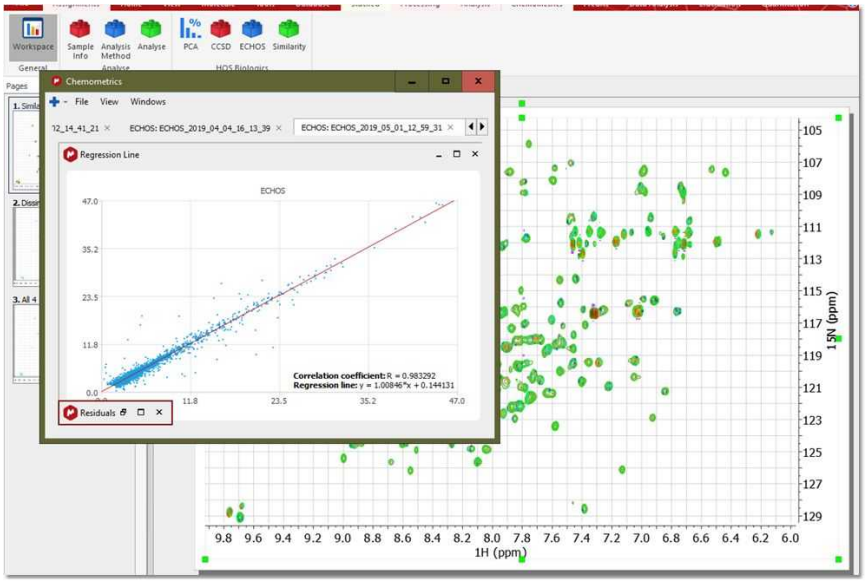


Includes four published and accepted methods to conduct analysis, each with its set of tailor-made visualizations allowing you to drill in results:

Combined Chemical Shift Difference, **CCSD** – number that represents the shifts in peak positions

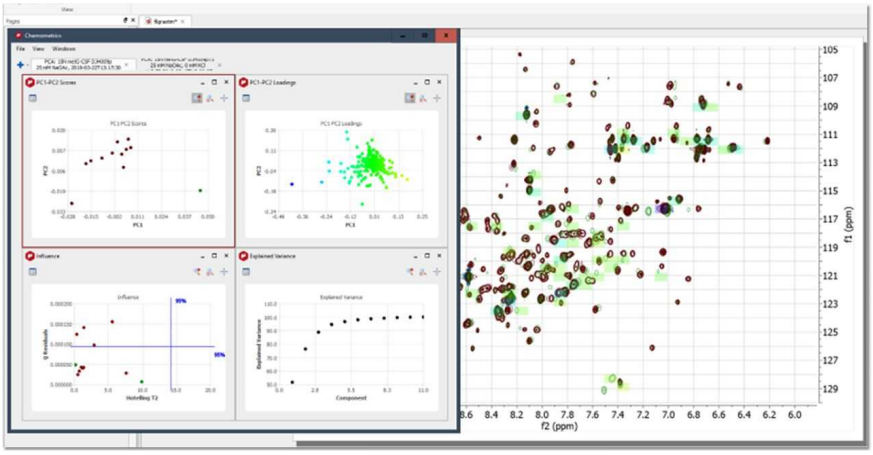


Easy Comparability of Higher Order Structure, **ECHOS** – simple representation of spectral differences



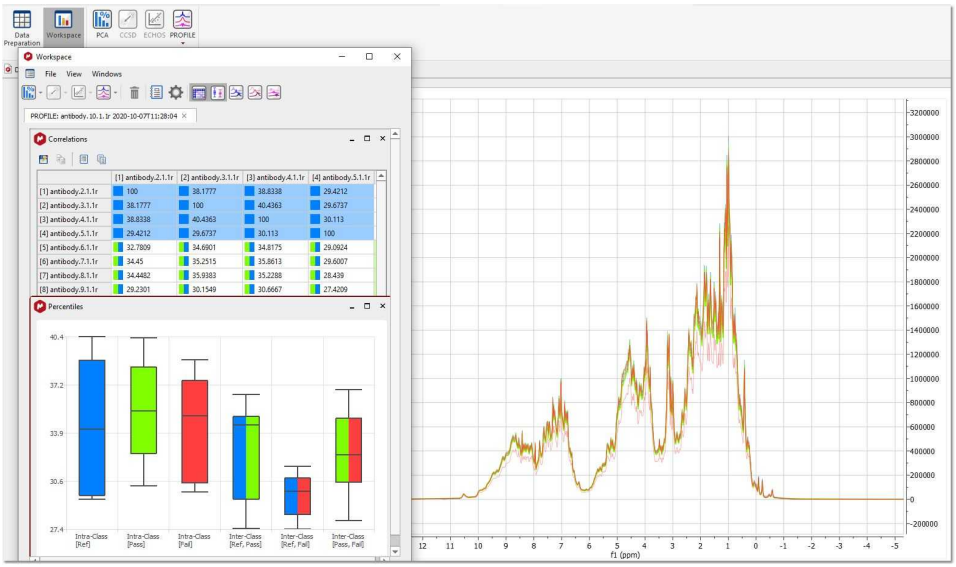
Principal Component Analysis, **PCA** – unsupervised chemometrics

© 2024 MESTRELAB RESEARCH



The **1D PROFILE** experiment consists in measuring the correlation between pairs of spectra by comparing their:

- **contours:** the broadened spectrum
- **fingerprints:** the result of subtracting the contour to the spectrum



The results of the PROFILE experiment are a table of **correlations** and a diagram of **percentiles**. These percentiles show the statistics of the intra-class correlations and inter-class correlations. To identify the class of each spectrum it is used the *Class* value, which can be edit in the *Stacked Items* table. Therefore, it is very important to classify the input spectra before running PROFILE.

The color code used in correlations table and percentiles diagram:

- only color: intra-class correlation
- two colors: inter-class correlation
- grey color: no-class defined

The resulting correlations and percentiles are shown after running the experiment. Correlations are normalized in a logaritmic scale, so the same pair of spectra should have the correlation value = 100, the maximum possible value. The higher the correlation, the more similarity between spectra. When the percentile of an inter-class is in similar ranges than its corresponding intra-classes, it means that the spectra of both classes are "similar".

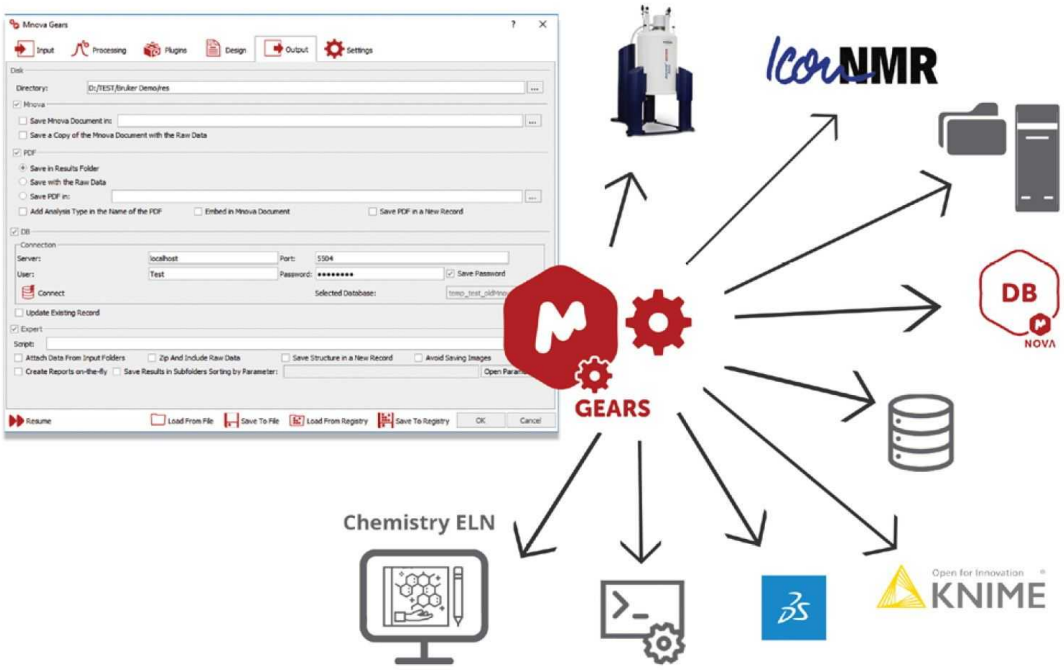
You will find further information and resources about this plugin, here and in this application note.

© 2024 MESTRELAB RESEARCH

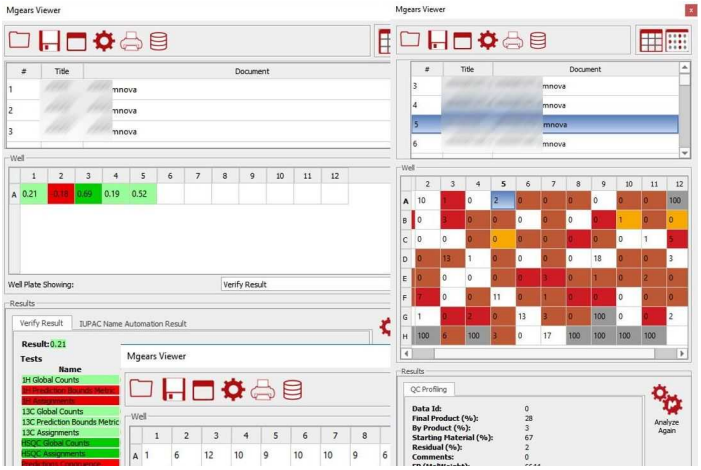
19.4 Mgears

This advanced plugin needs a specific license.

This module will allow you to build automation workflows for your analytical data, including NMR, MS, UV, IR and others.



Using Mnova features and advanced plugins as the starting bricks (such as Structure Verification, Purity, Concentration, QC Profiling, Batch Processing, Batch Prediction, IUPAC Name Automation, Reporting Screening, MPublish, MS Scan, DB Search, SQA, Peak and Multiplet report etc), you can replicate Standard Operating Procedures or those boring, repetitive tasks we humans hate so much!



See this chapters for qNMR and Verify automations.

More information about this Mgears can be found here, in this application note and in this whitepaper.

© 2024 MESTRELAB RESEARCH

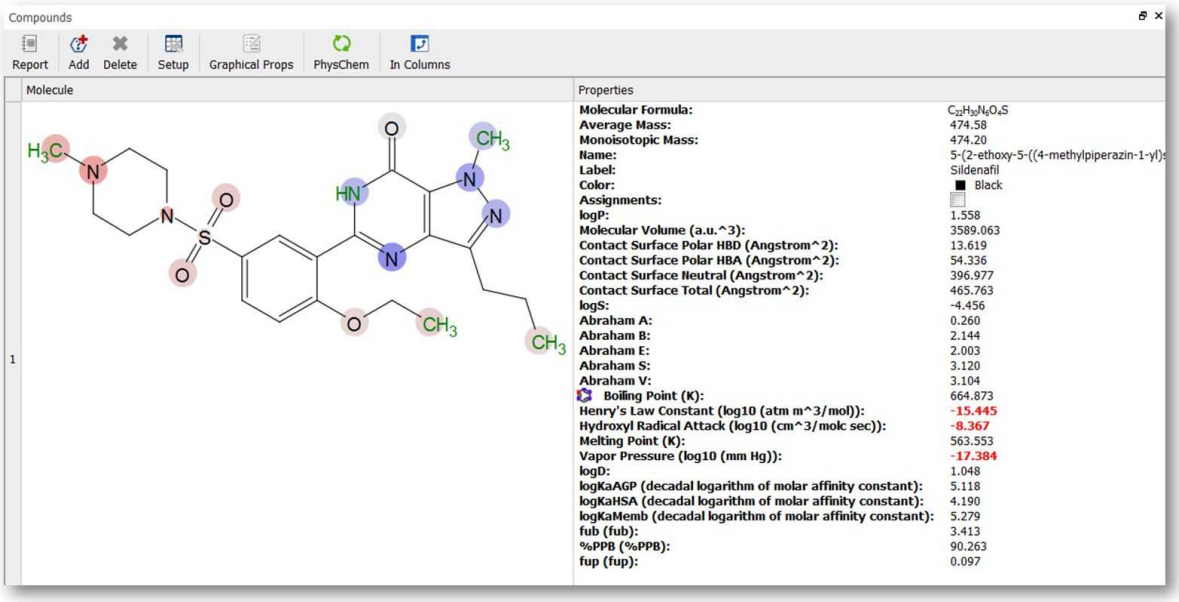
Further resources about the available bricks, can be found here:

See also this webinar.

19.5 PhysChem

This advanced plugin needs a specific license (but it does not need to be installed apart).

Validated and quantitative structure-property prediction integrated in Mnova under the Compounds Table:



More information about this plugin can be found here

19.6 qNMR

qNMR is the “gold standard” of compound quantification and has found many uses and applications. qNMR stands for “quantitative NMR”, and refers to the use of NMR to determine the concentration of one or more chemical species in solution.

The area of an NMR signal is directly proportional to its concentration, and this “response” will be the same for all molecules.

And this is also what sets qNMR apart from almost all other metrological techniques used to quantify compounds. Each suffers with problems such as compound-specific response factors (UV), chemical considerations (NCLD), or relative volatility (ELSD). The qNMR method only requires that (a) the sample dissolves completely in a (normally fully deuterated) solvent, and (b) it contains NMR-active nuclides – and that some care and attention is paid to data collection and processing.

Our new module provides a mechanism to quantify compounds that is:

- Accurate
- Versatile
- Automatic

© 2024 MESTRELAB RESEARCH

The basics of qNMR require simply the *Absolute Integral* per nuclide for one or more spectral regions, and a factor to *convert* this to concentration.

We have designed a competent, flexible, and sophisticated program for quantitation by NMR. It may be run fully automatically and is thereby incorporated into automated procedures and combined to streamline processes such as compound Verification and quantitation.

This new feature needs its own license and it is located under the Quantitation menu of Mnova.



You will need to install the qNMR plugin separately, by following the menu 'File/Advanced Plugins' and checking the applicable box.

Once you have installed the advanced plugin, you can move the folder to a shared location and create a defaults.ini file (in the Mnova installation folder) pointing to it. After doing that, all users accounts will have the advanced plugin installed.

Here you can see an example of a.defaults.ini file:

```
[%General]
AdvancedPluginsDir=C:\Your\Path\To\Plugins\Here
```

For further information about qNMR:
http://mestrelab.com/blog/qnmr-the-best-pulse-length-for-acquisition/
http://mestrelab.com/blog/edited-sum-integration-for-qnmr/
http://mestrelab.com/blog/determining-concentrations-when-using-nmr-to-model-chemical-reactions/
http://mestrelab.com/blog/nmr-quantification/
http://mestrelab.com/blog/automating-qnmr/
http://mestrelab.com/blog/qnmr-purity-recipe-book-i/
http://mestrelab.com/blog/qnmr-purity-recipe-book-ii/
http://mestrelab.com/blog/qnmr-purity-recipe-book-iii/
http://mestrelab.com/resources/getting-picky-with-q-nmr/
http://mestrelab.com/blog/new-qnmr-experiments/
and download article: http://pubs.acs.org/doi/abs/10.1021/ac400411q

For Mixtures Analysis:
https://mestrelab.com/main-product/sma-mixtures-analysis

19.6.1 Concentration Workflow

Generally, the workflow will be:

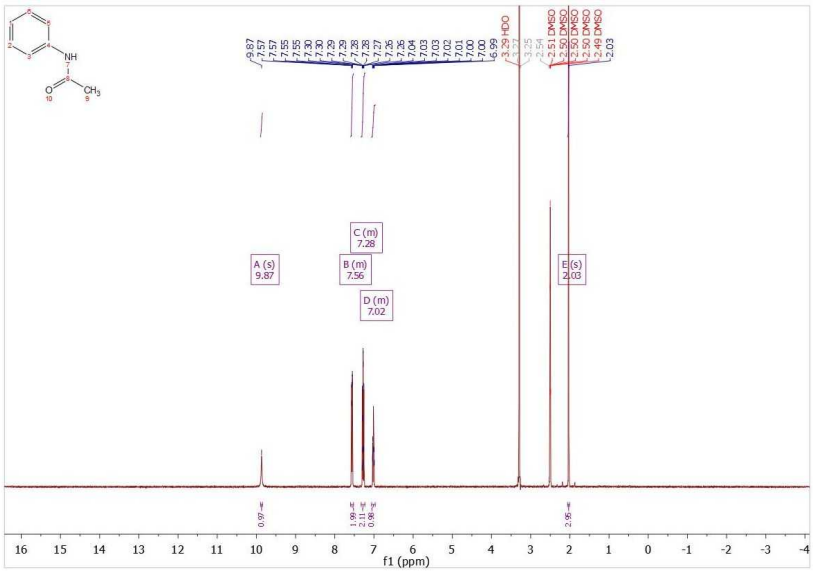
- Determine multiplets
- Run the script and select the qNMR variables
- The software will choose the multiplets to include
- Report

So first of all, we will load a 1H NMR spectrum and apply the necessary processing. (Depending on the level of accuracy required, you must pay attention to the number of data points used for the FT

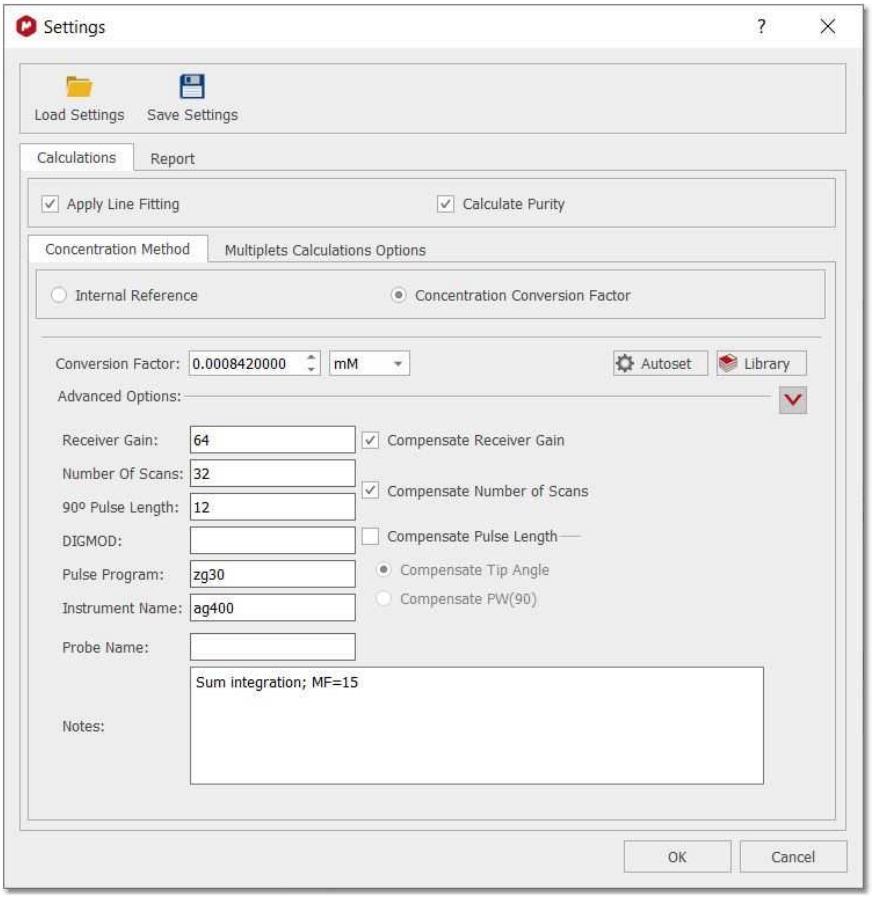
© 2024 MESTRELAB RESEARCH

(typically 64K) and to apply a phase correction. Sometimes a baseline correction could be also needed. If necessary (e.g., solvent saturation was used), blind regions should be used to mask these spectral regions).

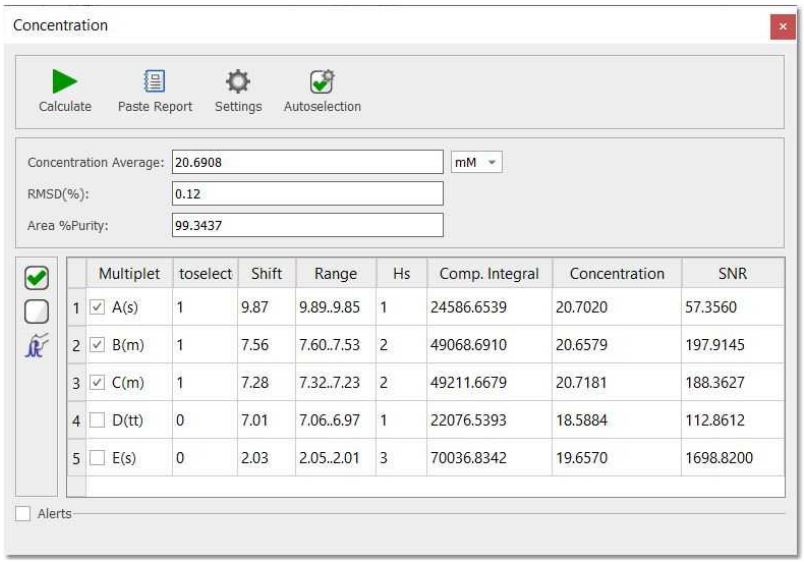
In the example shown below (which you can download from here), we have just loaded the dataset and applied an 'automatic multiplet analysis':



The qNMR functionality is selected from the “Quantitation” menu item. The software stores all settings in the registry, so you may not have to change things. But you will need to provide some information the first time you use this. This will be described in more detail, below. For this tutorial, we have clicked on the ‘Settings’ button of the qNMR panel and then on the ‘Load Parameters’ button to load the ‘1_acetanilide.data’ file (which you have downloaded together with the dataset in the previous step):



After having clicked on the OK button, the qNMR panel will be displayed with the information about the multiplets and compensated integrals found for their bounds. Having the correct number of nuclides is important and the software may do this better if the molecular structure is available and plotted.



	Multiplet	toselect	Shift	Range	Hs	Comp. Integral	Concentration	SNR
1	A(s)	1	9.87	9.89..9.85	1	24586.6539	20.7020	57.3560
2	B(m)	1	7.56	7.60..7.53	2	49068.6910	20.6579	197.9145
3	C(m)	1	7.28	7.32..7.23	2	49211.6679	20.7181	188.3627
4	D(tt)	0	7.01	7.06..6.97	1	22076.5393	18.5884	112.8612
5	E(s)	0	2.03	2.05..2.01	3	70036.8342	19.6570	1698.8200

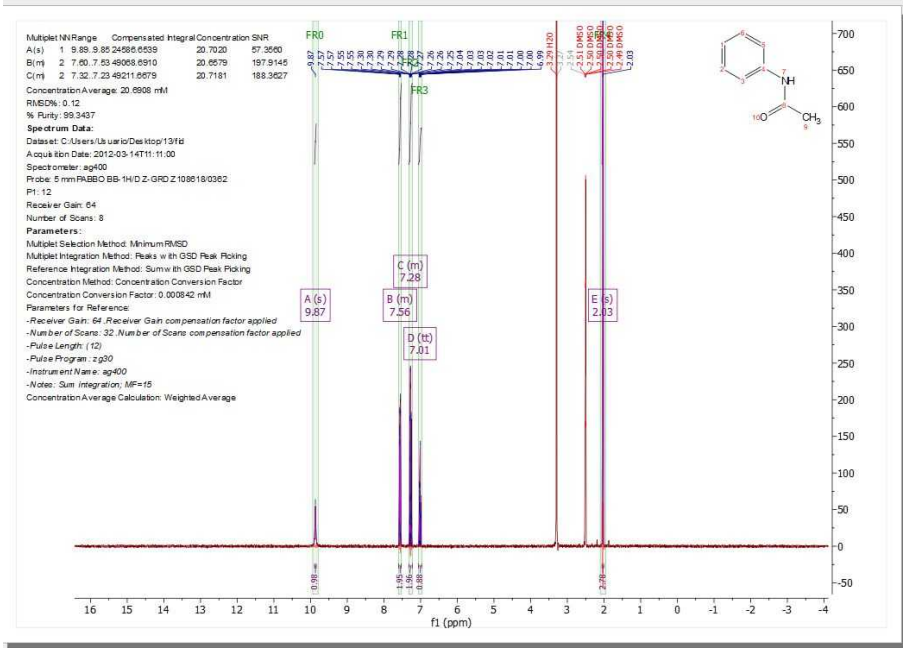
© 2024 MESTRELAB RESEARCH

Click on the Calculate button to run the concentration analysis. At this stage the qNMR process will be done automatically and the multiplets sorted in the table based on their perceived ranking as candidates for qNMR. The selected multiplets can be changed using the check boxes next to each multiplet, and the effect on the derived concentration will be updated. The concentration averaged calculated for this example is 21.5 mM (the units can be changed by using the applicable combobox)

You will find different button in the qNMR panel above:

1. 

Report qNMR results on the spectrum page, export the report into a txt file or copy the report to the clipboard (depending on the report options that you have selected in the settings)



2. 

Show the “Settings” dialogues

3. 

Selects best multiplets based on rules (see qNMR Settings)

4. 
Select all/no multiplets. (An alternative to 3)

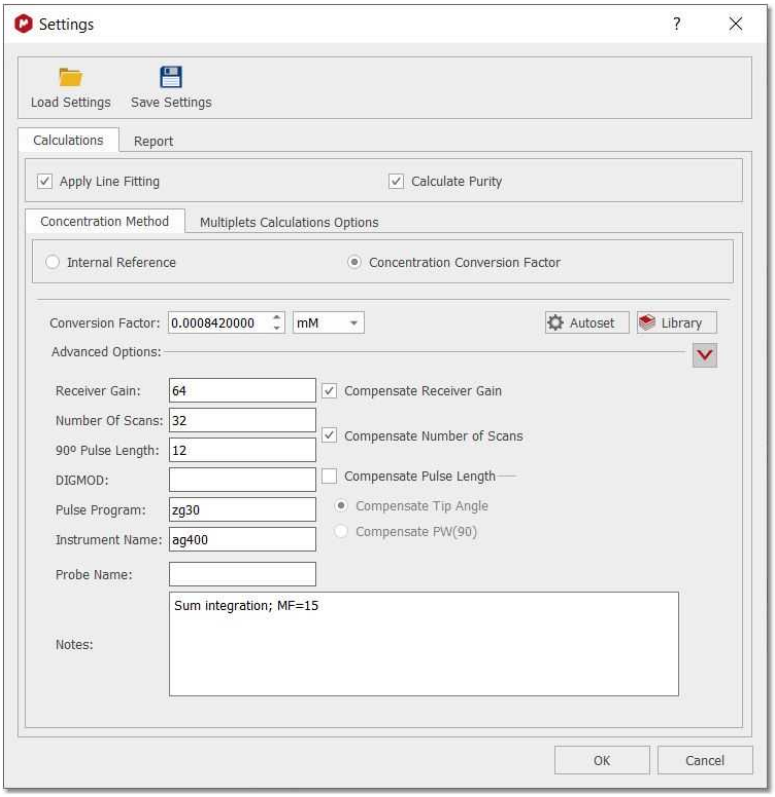
5. 
Normalize the number of protons

The user can check the applicable boxes to manually select/deselect the multiplets that will be used to calculate the concentration information. The “Hs” for a multiplet can be manually changed by clicking on the number.

© 2024 MESTRELAB RESEARCH

19.6.1.1 Settings

Selecting 'Settings' on the qNMR panel, will display the dialog box below, containing different tabs (Concentration, Options and Report):



Note first the “Save” and “Load” buttons. These will allow you to set up the correct settings for parameters and distribute amongst other users to ensure they do the analysis correctly

Please bear in mind that qNMR will use the integration calculation method that you have selected under the 'Multiplet Analysis' options.

Calculations
Apply Line Fitting. To apply the fitting over all the multiplets.
Calculate area purity. If checked, this will show with the results summary. It is calculated using the sum of areas for compound and impurities (from GSD), and is something of a rough value:
% Purity = (-A(C) / (A(C) + A(I)) * 100.0
A(C) is the sum of the GSD areas for peaks labelled as compound
A(I) is the sum of the GSD areas for peaks labelled as impurity

Concentration method

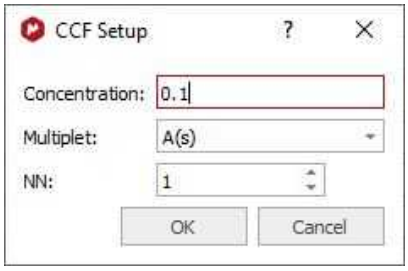
Fundamentally, there are two ways to determine concentration: using a signal as an “internal reference”, or a specified “Concentration Conversion Factor” (CCF). The internal reference may be a real signal area (e.g., residual solvent, TMS) or an artificial signal (e.g. ERETIC or QUANTAS). If a residual solvent is used then the “Reference peak is a solvent” box should be checked, and then only peaks of type “Solvent” in the chemical shift range will be used for area calculation. This can be useful to exclude errors that would result if impurity, analyte or other peaks were used, and this is a nice feature that follows from AutoClassify.

© 2024 MESTRELAB RESEARCH

Concentration Conversion Factor

Currently, we only support CCFs that have been determined using data processed within Mnova – there can be significant errors if Bruker 1r files are used. The CCF is determined for a particular experimental setup by processing the spectrum of a compound of known and specified concentration, and adjusting the "Conversion Factor" until the correct concentration is reported.

To calculate the CCF value, just load the spectrum with the multiplet analysis done and click on the 'autoset' button of the 'concentration settings' panel to input the concentration reference, the NN for this multiplet and the applicable multiplet in the spectrum:



The CCF will be calculated using:

CCF = [Reference]*(NN/AI)

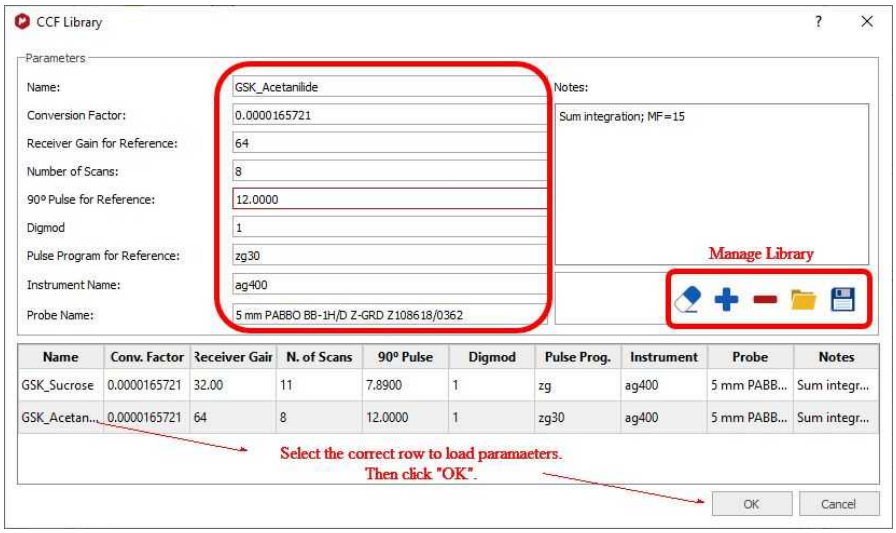
For further information about how to calculate CCFs with Mnova qNMR please take a look at this **document**.

Library of CCFs

It is important to account for the fact that a CCF value will have a fundamental, strong dependence on the spectrometer hardware: the particular NMR spectrometer and probe, principally. This must be determined each time empirically, as described above. After that is done, simple mathematical compensations can be made for differences in the spectrum acquisition parameters: receiver gain, number of scans, or 90° pulse length. If necessary, a different value can also be determined and saved for different solvents.

To facilitate this, the user can save these values to a file – and distribute them, of course.

© 2024 MESTRELAB RESEARCH

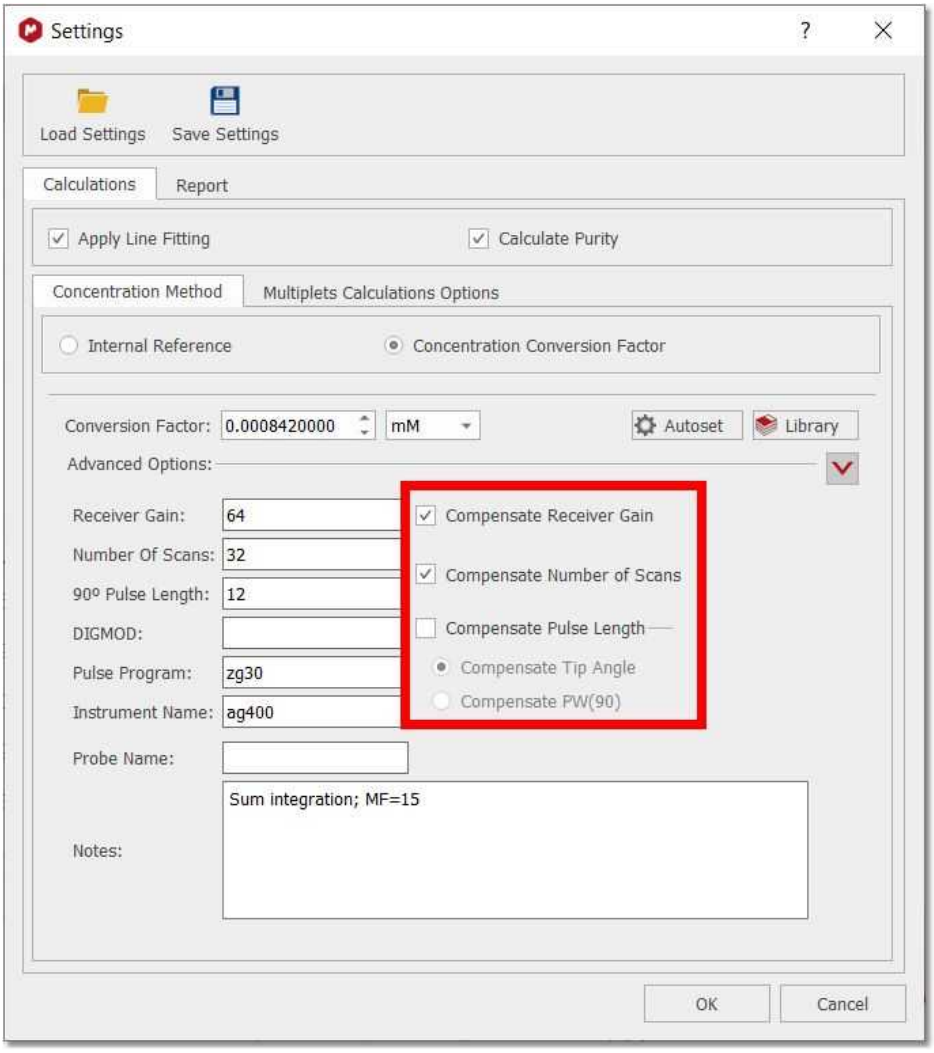


To add a new library condition the user will populate the parameter fields correctly, click the blue “+” button to add to the library, and save the updated data file.

To select a different CFF, highlight the row and click “OK”. The software will check that the parameters match if specified, and this information will be included in all reporting.

It is intended that the sample and references are acquired under same conditions, but you can compensate the receiver gain, number of scans and pulse lenght by checking the applicable radio buttons:

© 2024 MESTRELAB RESEARCH



Here you can find how the compensations are applied and calculated:

Compesated Integral = Absolute integral * Factor.

- Factor to compensate Receiver Gain:
  Bruker format:
  $Factor = RG_{reference} / RG_{experiment}$
  Others:
  $Factor = [10^{RG_{reference} - RG_{experiment}}]^{1/20}$

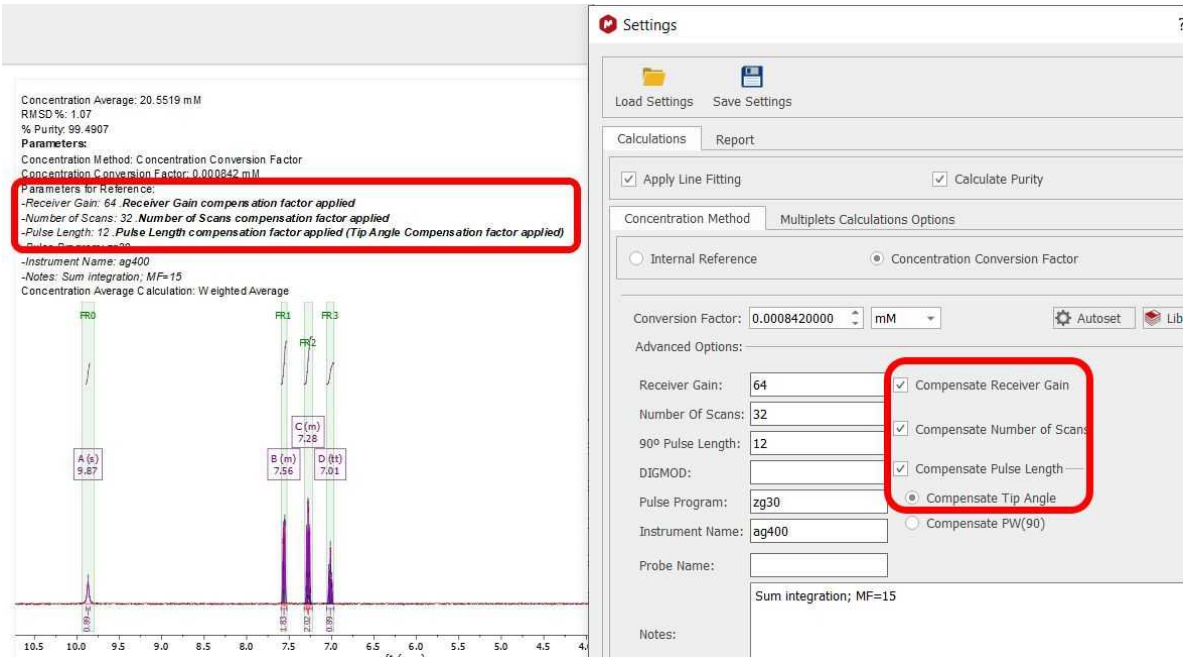
- Factor to compensate Number of Scans:
  $Factor = NS_{reference} / NS_{experiment}$

- Factor to compensate Tip Angle:
  $Factor = PW_{reference} / PW_{experiment}$

- Factor to compensate PW:
  $Factor = PW_{experiment} / PW_{reference}$

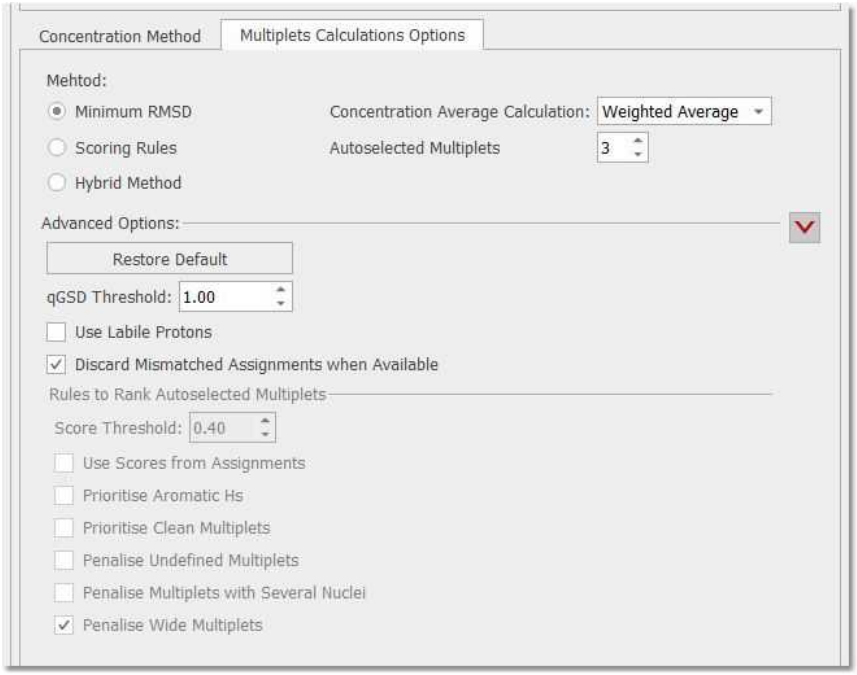
© 2024 MESTRELAB RESEARCH

The concentration report will include information about the compensation factors applied in the analysis.



Multiplets Calculation Options

This is where the settings for the multiplet selection criteria are grouped:



These methods will all try to select the number of multiplets in, whilst respecting the score threshold.

© 2024 MESTRELAB RESEARCH

Minimum RMSD. This option will ignore the multiplet scores and simply chose the multiplets that give the smallest RMSD in the average concentration. It may be used for very pure compound spectra. The maximum number to be used is the user-selectable “Autoselected Multiplets”.
Scoring rules. This applies the rules selected in section 3, below, and then uses the top multiplets to determine the average concentration (and RMSD). The maximum number to be used is the user-selectable “Autoselected Multiplets”.
Hybrid method. This method first applies the scoring rules and then chooses amongst the highest scoring multiplets. Using the multiplets whose final score exceeds the score threshold, the multiplets that give the smallest RMSD are identified and used for the final average concentration calculation (and RMSD).
Concentration average calculation. Difference in how the average is calculated. Simple average will calculate the mean average of the concentrations. Weighted Average will calculate the concentration with the sum of the integrals, divided by the sum of the nuclei and multiplied by the scaling factor.
Autoselected multiplets. The target (max.) number of multiplets that will be used for the final concentration determination. The score threshold, labiles use, etc. will be respected.

Advanced Options
qGSD Threshold. Multiplets with qGSD residuals over this value Threshold will be ignored.
Use labile protons. The default is to ignore labile protons (if categorised), but if you check this box then the restriction is lifted. The thinking is that signals from labile protons almost always under-integrate, and should not be included in qNMR calculations. This offers a partial workaround if the user really wants to use labiles. This rule is applied if “Use Labile Protons” is checked; multiplets considered to be or contain labile protons will be penalized by a factor of 0.25. If unchecked, labiles have a score of 0.0 and are therefore omitted from all concentration calculations.
Discard mismatched assignments when available. This test uses nuclide information from AutoAssignment and checks that it matched with NN from the multiplets. If they are not in disagreement, the multiplet will be discarded.

Rules to rank autoselected multiplets

a. Prioritise aromatic Hs. Non-aromatic multiplets will have their scores penalised. Especially useful when the aliphatic region is complicated by large residual/impurity peaks or when solvent saturation is used. If the user checks the option, the aromatic Factor will be 1 for an Aromatic multiplet, and a non-Aromatic H is multiplied by 0.5 (Aromatic range=5.5-12ppm).
b. Penalise multiplets with several nuclei. Prioritises multiplets having the fewest NN. This assumes that impurities can lead to a false NN. The score is multiplied by 1/NN: the larger NN, the lower the score.
c. Use scores from assignments. If Verify/AutoAssign was used, the individual multiplet scores will be taken into account. A multiplet with a higher assignment score Q(-1 to 1) will be scored higher. *Assignments Factor = 0.75 + Q/4*
d.
e. Penalise undefined multiplets. The thought is that a multiplet marked as “m” may be a clear solute multiplet but complicated by impurity peaks. In this case the “m” is penalised. The factor will be 0.6 for ‘m’ or 's' multiplet types, and 1 for all others.
f. Prioritise clean multiplets. The presence of impurity peaks will cause a 10X increase in their relevance when determining the score. Use when spectra are very clean.

Clean Multiplets factor= multipletPeaksSum/(multipletPeaksSum+noMultipletPeaksSum)

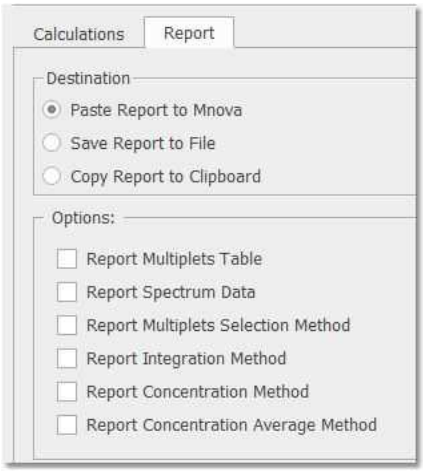
multipletPeaksSum is the total sum of the areas of all the peaks
noMultpletPeaksSum is the sum of the areas of the peaks not assigned to the multiplet
There is a further correction for peaks labeled as impurities: the area for this peak type will be increased x10.

© 2024 MESTRELAB RESEARCH

g. Penalise Wide multiplet. Penalises a multiplet having a width >60 Hz. This case can arise when the multiplet picks up impurity peaks. Consider using with dirtier spectra. The score is divided by 2 if the width of the multiplet is greater than 60Hz.
h. Score Threshold. Multiplets having a score below this number will not be used in the average concentration determination. A value of 0.3 is typical. (Scores are between 1 and 0.).

Reports

This is where you can select how to report your results; by pasting the report into the Mnova document, by exporting to an ASCII file or by copying to the clipboard.



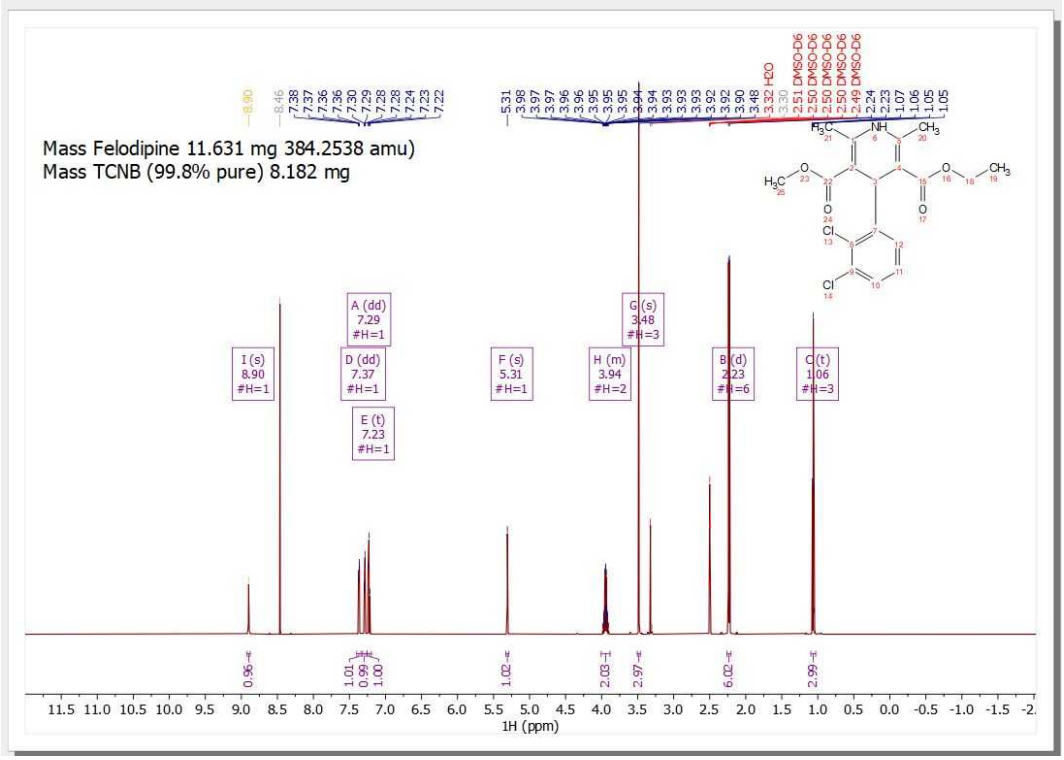
You will find also some options to choose which parameters you want to report.

19.6.2 Purity

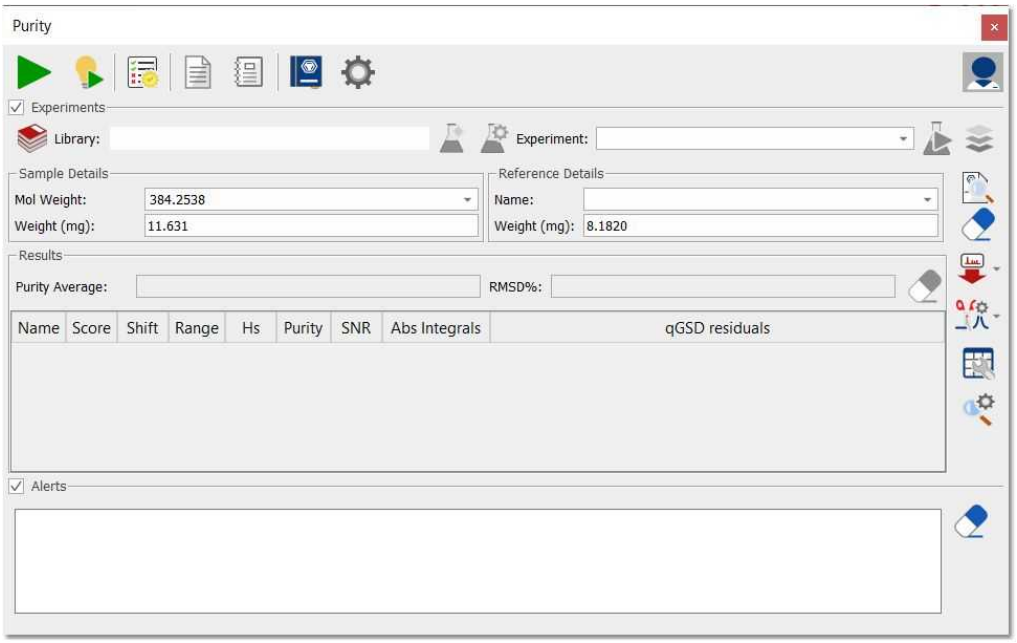
This new feature is an enhancement of the Purity calculator script by using the qNMR engine to automatically select the best multiplets in a spectrum and use them to calculate *concentration* using a number of industry standard approaches. Analysing sample replicates is facilitated.

The workflow would be the expected. We describe the process for a spectrum of Felodipine with TCNB (tetrachloronitrobenzene) as an internal purity reference. Open your dataset with the molecular structure and apply an automatic multiplet analysis (you can download the dataset and the libraries from this link):

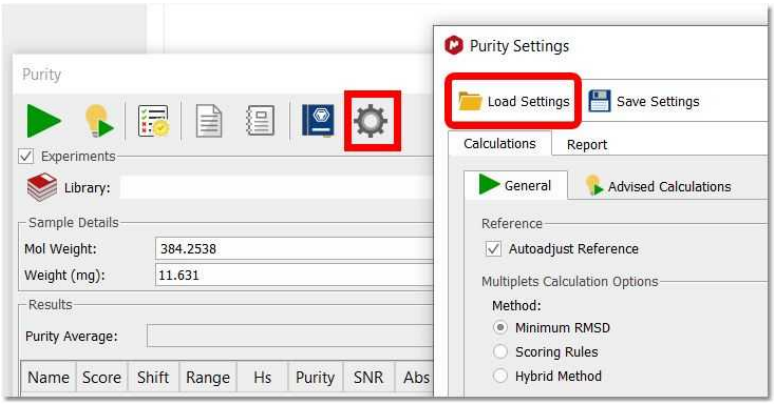
© 2024 MESTRELAB RESEARCH



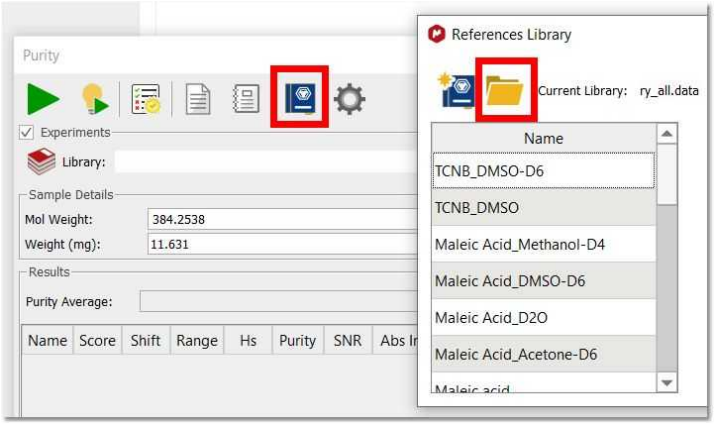
Next, follow the menu 'Quantitation/qNMR/Purity' to display this dialog box:



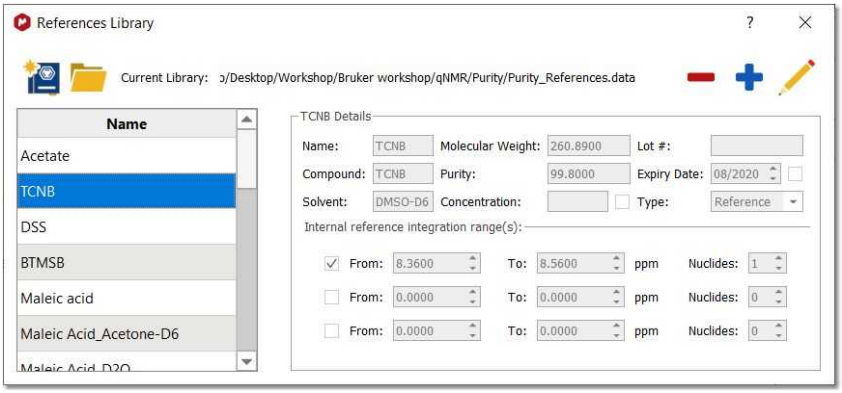
The next step will be to define the settings, by clicking on the applicable button. For this example, we can load the 'Purity_settings.data file (by clicking on the load button highlighted in red in the picture below)



We will also need to define our 'References Library' by clicking on the 'References' button of the 'Purity panel'. From here, you could create your own 'References Library' or click on the 'Load' button to import the 'Purity_References.data' file (From the zip file):



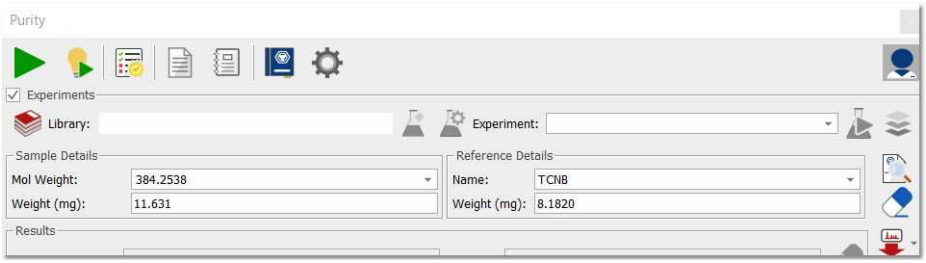
Once the library has loaded, we can click on the 'TCNB' row to use the applicable values. You could also type the reference compound details by hand (instead of loading the settings):



Note that if your purity standard is not in the list, you will have to manually add it. From the References library panel, you could enter the information of your reference compound (Molecular weight, purity, ppm range(s), etc.); next, click on the blue “+” button to add it to the library and finally click on the save button to the keep the changes.

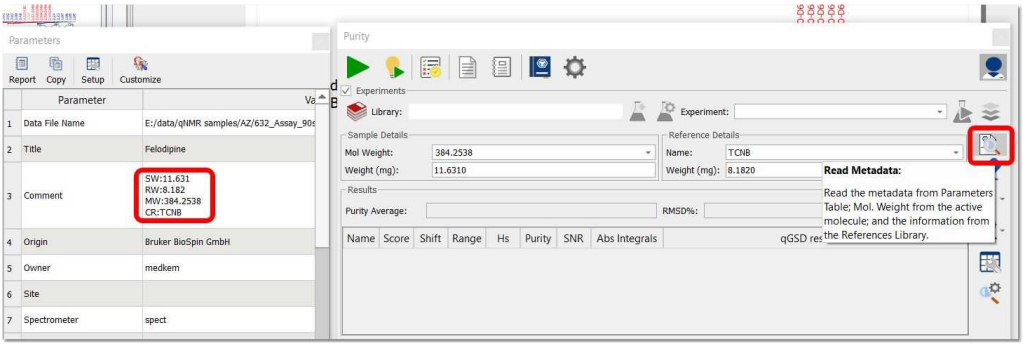
Click on the OK button to fill the 'Purity' dialog box with the information about the 'reference compound'

© 2024 MESTRELAB RESEARCH



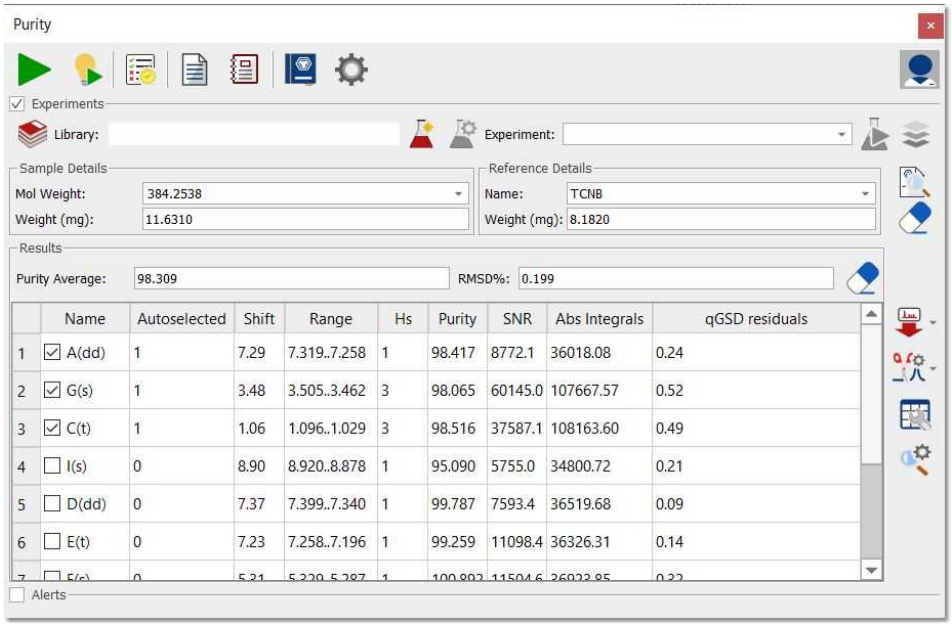
The next step would be to fill the reference weight (8.182 mg of TCNB for this example), the molecular weight of our compound (384.2538 g/mol) and the sample weight (11.631 mg of Felodipine).

Please bear in mind that if the spectrum was acquired including the sample and the reference details under the 'Comment' field of the 'Parameters Table' (by using the format displayed in the screenshot below: SW for Sample Weight; RW, for Reference Weight, MW: for Sample molecular Weight and CR: for compound reference (or RN or even Reference Name), or even SM: followed by a molecule in smiles format, or NS: for Number of scans), we could parse them just by clicking on the 'Read Parameters' button, so we will not need to manually type them on the Purity panel. If your document contains the molecular structure, the MW will be obtained from there (and not from the comment field).



Finally, click on the 'Calculate' button to get your purity result (98.3%) and paste the result in Mnova by clicking on the 'Paste Report' button:

© 2024 MESTRELAB RESEARCH



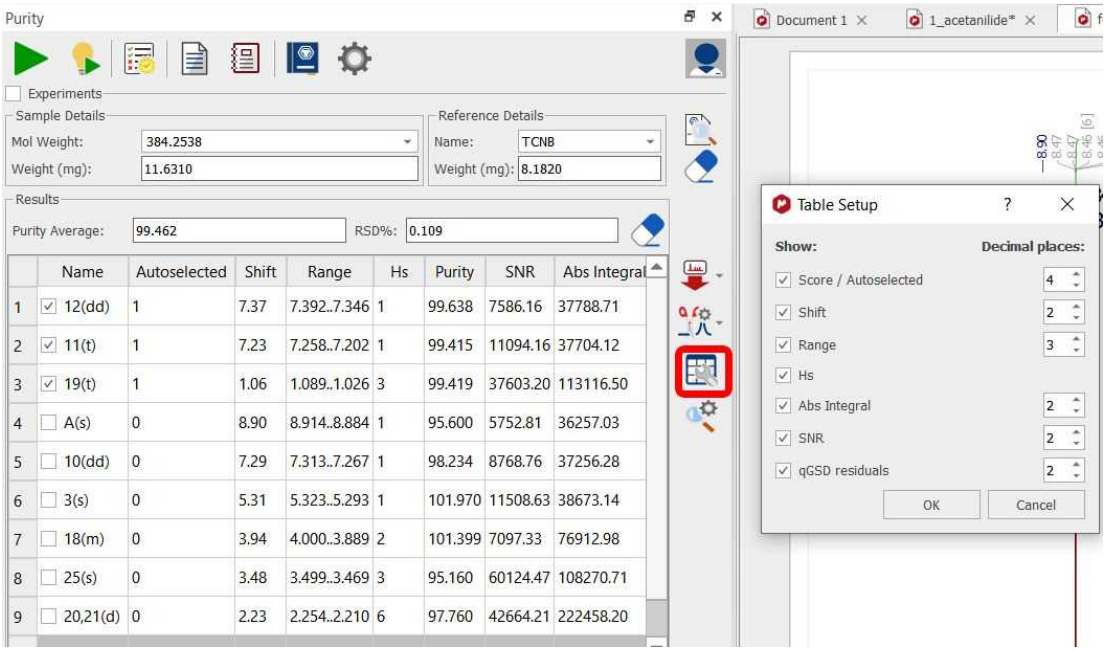
	Name	Autoselected	Shift	Range	Hs	Purity	SNR	Abs Integrals	qGSD residuals
1	A(dd)	1	7.29	7.319..7.258	1	98.417	8772.1	36018.08	0.24
2	G(s)	1	3.48	3.505..3.462	3	98.065	60145.0	107667.57	0.52
3	C(t)	1	1.06	1.096..1.029	3	98.516	37587.1	108163.60	0.49
4	I(s)	0	8.90	8.920..8.878	1	95.090	5755.0	34800.72	0.21
5	D(dd)	0	7.37	7.399..7.340	1	99.787	7593.4	36519.68	0.09
6	E(t)	0	7.23	7.258..7.196	1	99.259	11098.4	36326.31	0.14

Clicking on the Results Manager button will display a drop down menu which will allow you to 'Load Results' (in the Purity Results panel) or to 'Open the Results Manager'



Clicking on the Table Setup button will allow you define the number of decimals places:

© 2024 MESTRELAB RESEARCH

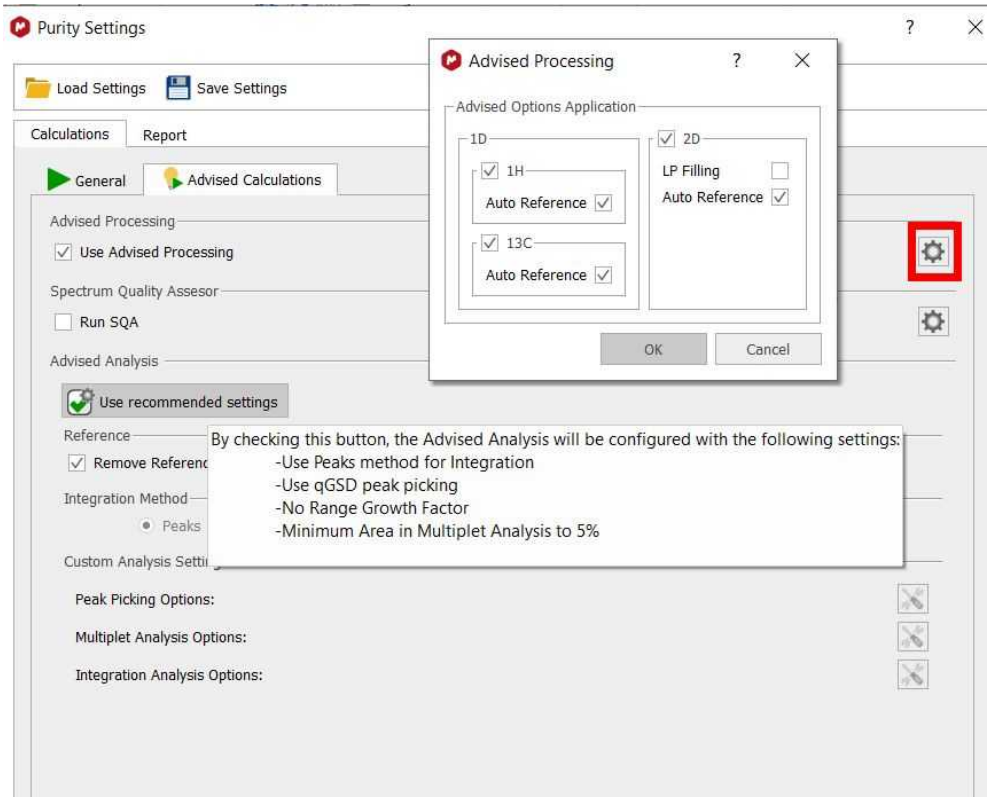


Advised Calculations

You could also use the **'Advised Calculation'** button which will apply an Automatic Multiplet Analysis (Peaks as integral calculation method) with Reference Compound Integration (Peaks as integral calculation method). The **'Advised Calculations'** feature will also read the parameters (SW, RW, SW, CR) from the metadata (if they are included in the 'Comments' field of the 'Parameters table'). It will also run an Auto Assignment if a molecule is present; if not, an automatic multiplet analysis will be performed. Finally, it will display the 'Purity results'.

From the settings, you will be allowed you to define the 'Advised Processing' options (Auto Reference and LP) and the integration method (Sum, Peaks or Edited Sum), Peak Picking and Multiplet analysis settings:

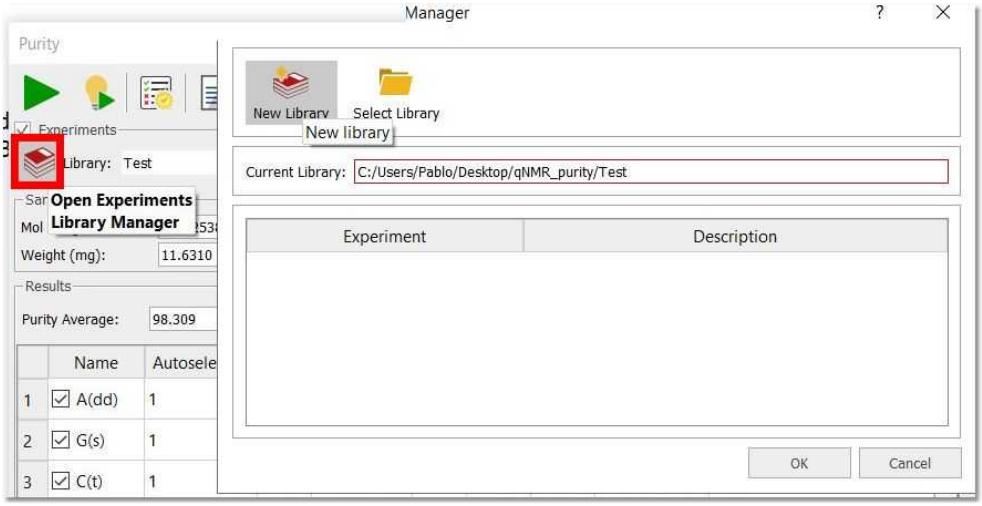
© 2024 MESTRELAB RESEARCH



Replicates

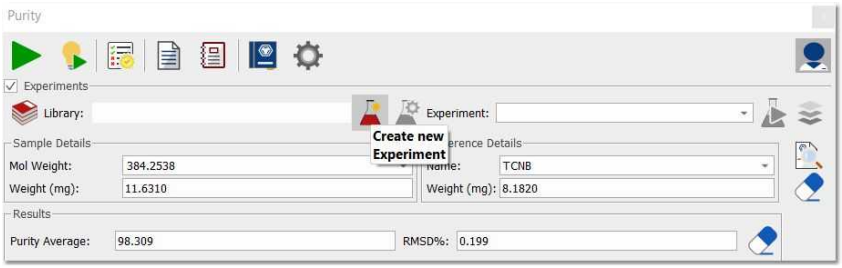
It is quite often the case that an analysis is performed with replicates: duplicate, triplicate, etc. samples. That is, 2 or 3 samples of the same compound and reference standard are made, and their spectra recorded. In this case the analysis of the replicates is facilitated allowing the user to apply the same analysis on all samples, and determine an average purity for the replicates.

After performing the analysis on the first sample, you could need to create a library of experiments. To do it, just check the 'Experiments box' and click on the 'Open Experiments Library Manager' button and select 'New Library' (which will be saved at the desired folder):

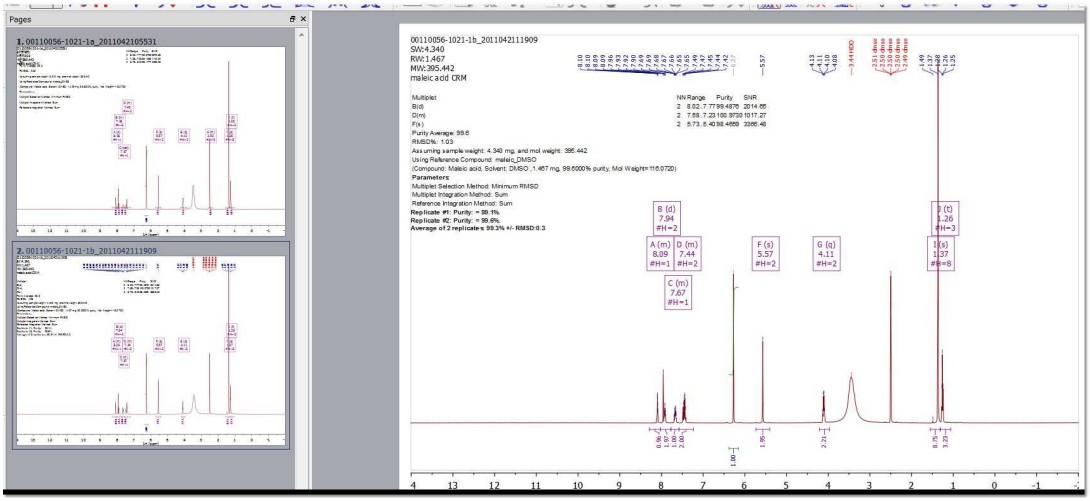


© 2024 MESTRELAB RESEARCH

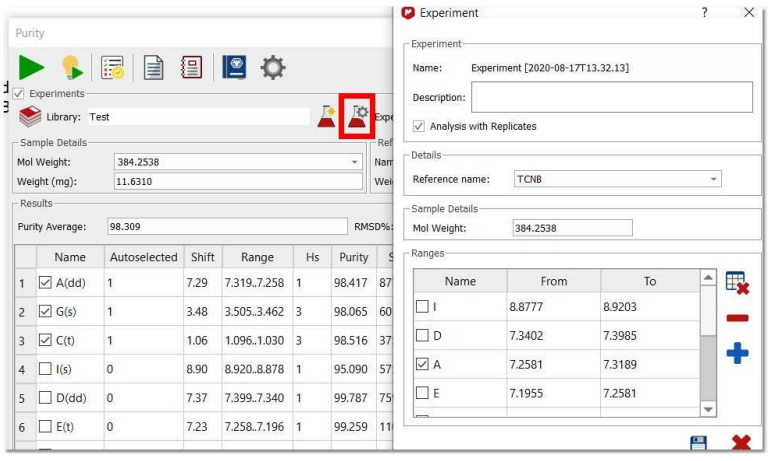
Next click on the 'Create New Experiment' button':



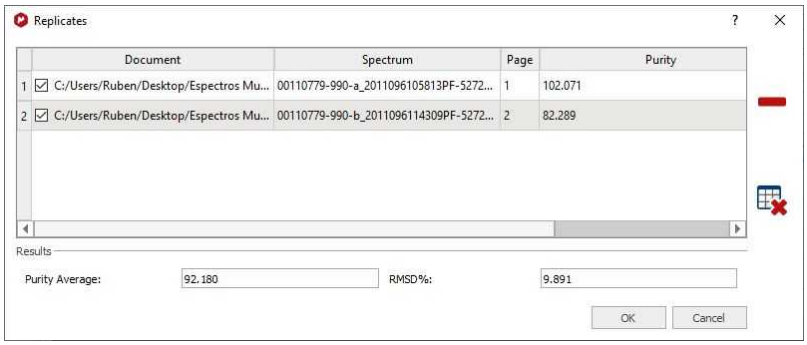
That will save the selected multiplets, ref. compound, compound MWt - things that stay the same from sample to sample in the replicates. When the 2nd, 3rd, etc. sample is active, press "Apply experiment" button to replicate all the qNMR Purity parameters except the weights of sample and reference. In the last replicated sample you will get the averaged purity value.



Clicking on the 'Edit Current Experiment' button, will allow you to change the current experiment:



Clicking on the 'Open Replicates Manager' button will allow you to see (select/deselect/delete) the results obtained:



Here you can find the formulas used for the replicates statistics calculations and for the global results calculations:

Formulas used in Replicates Statistics Calculations

$$\text{Purity}_\text{s} = \frac{1}{M_s}\sum_{m=1}^{M_s} p_{ms} \qquad \text{Purity}_\text{m} = \frac{1}{S_m}\sum_{s=1}^{S_m} p_{ms}$$

$$\text{SD}_\text{s} = \sqrt{\frac{1}{M_s}\cdot\sum_{m=1}^{M_s}(p_{ms}-\text{Purity}_\text{s})^2} \qquad \text{SD}_\text{m} = \sqrt{\frac{1}{S_m}\cdot\sum_{s=1}^{S_m}(p_{ms}-\text{Purity}_\text{m})^2}$$

$$\text{RSD}_\text{s} = \frac{\text{SD}_\text{s}\cdot 100}{\text{Purity}_\text{s}} \qquad \text{RSD}_\text{m} = \frac{\text{SD}_\text{m}\cdot 100}{\text{Purity}_\text{m}}$$

$p_{ms} =$ purity for multiplet m and sample s $\qquad$ $S_m =$ number of total samples for multiplet m

$M_s =$ number of total multiplets for sample s

Formulas used in Global Result Calculations

In the Global Result table it is shown, in each row:

- *SampleID*
- *Multiplets Average Method*: chosen by the user in Settings → Calculations → General → Multiplets Calculations Options → Multiplets Average Calculation.
- *Purity*: mean of the multiplet purities for this sample, calculated according to *Multiplets Average Method*
- *RSD (%)*: Relative Standard Deviation = $\frac{\text{SD}\cdot 100}{\text{Purity}}$

In the last row, the Global Mean; SD and RSD are shown:

$$\text{GlobalMean} = \frac{1}{Number of Replicas}\sum_{1}^{Number of Replicas}\text{Purity}$$

$$\text{SD} = \sqrt{\frac{1}{Number of Replicas}\cdot\sum_{1}^{Number of Replicas}(\text{Purity}-\text{GlobalMean})^2}$$

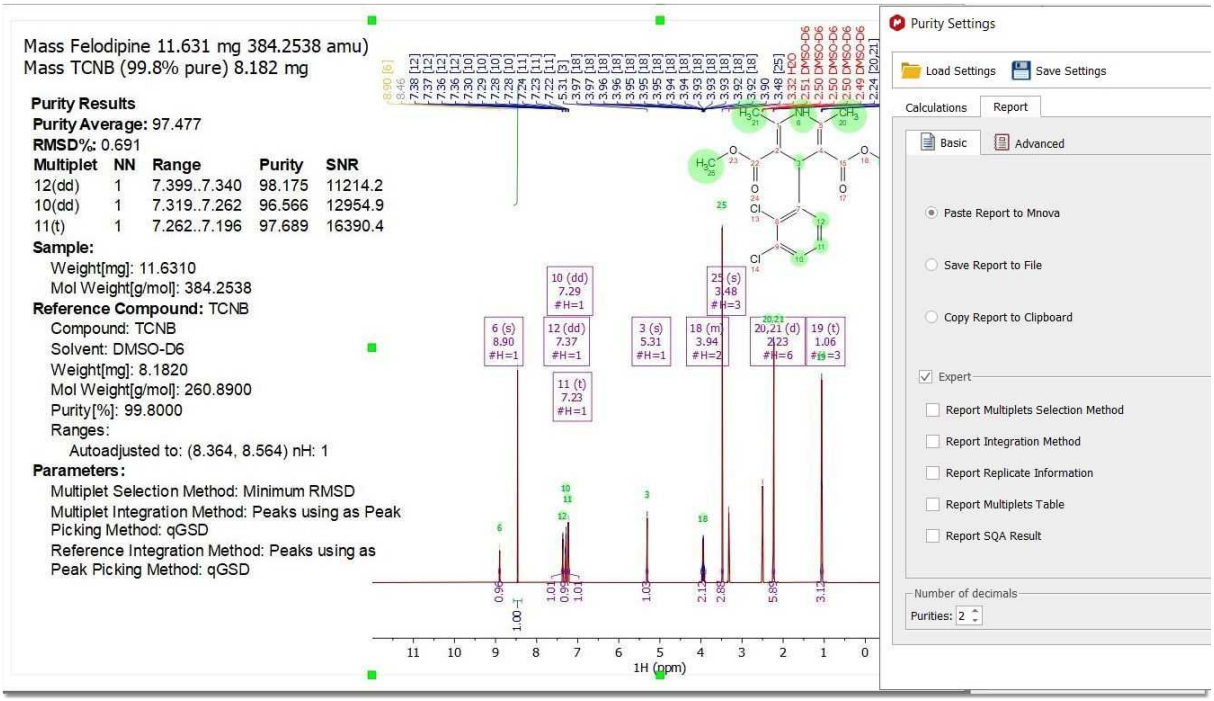
$$\text{RSD} = \frac{\text{SD}\cdot 100}{\text{GlobalMean}}$$

Reports

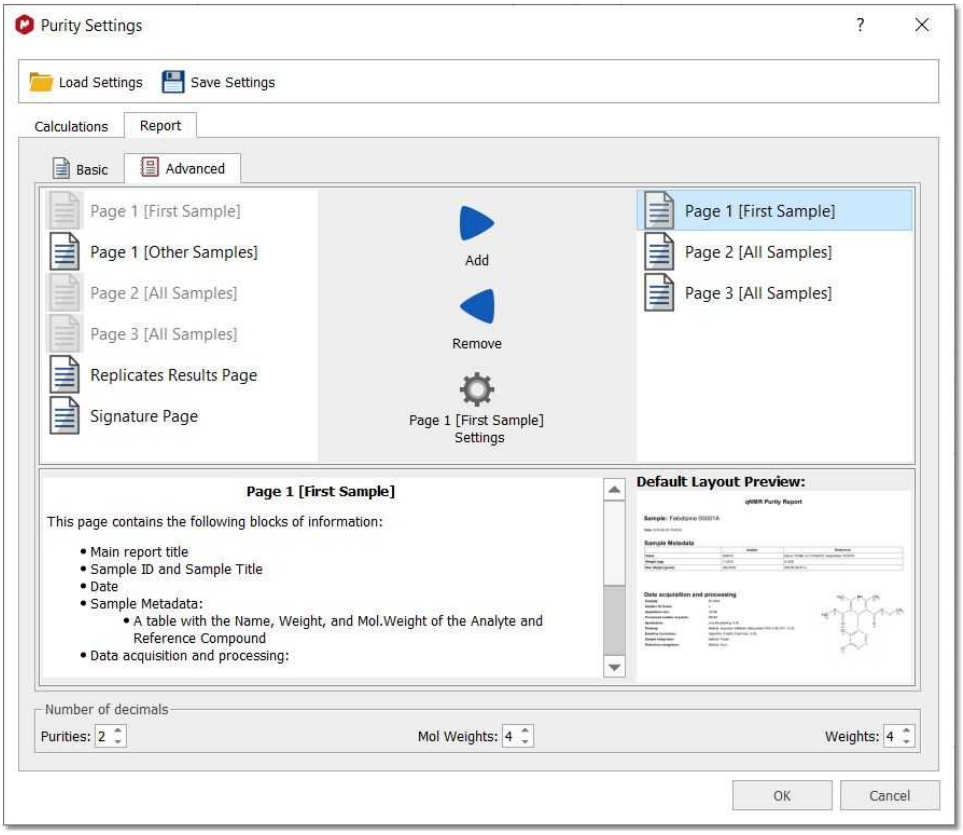
© 2024 MESTRELAB RESEARCH

You can easily report your purity results into your Mnova document or export them to a file. Clicking on the 'Settings' button will allow you to customize the 'Basic' and the 'Advanced Reports':

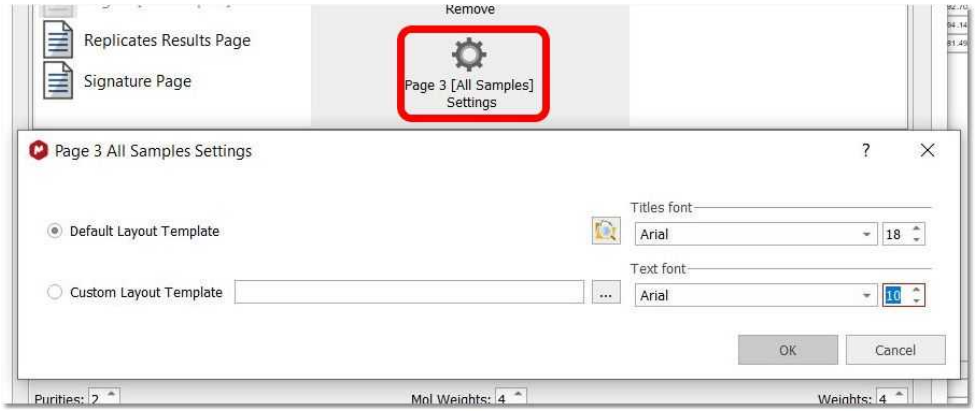
Here you can find an example of a basic report:



You can also create templates for more advanced results:

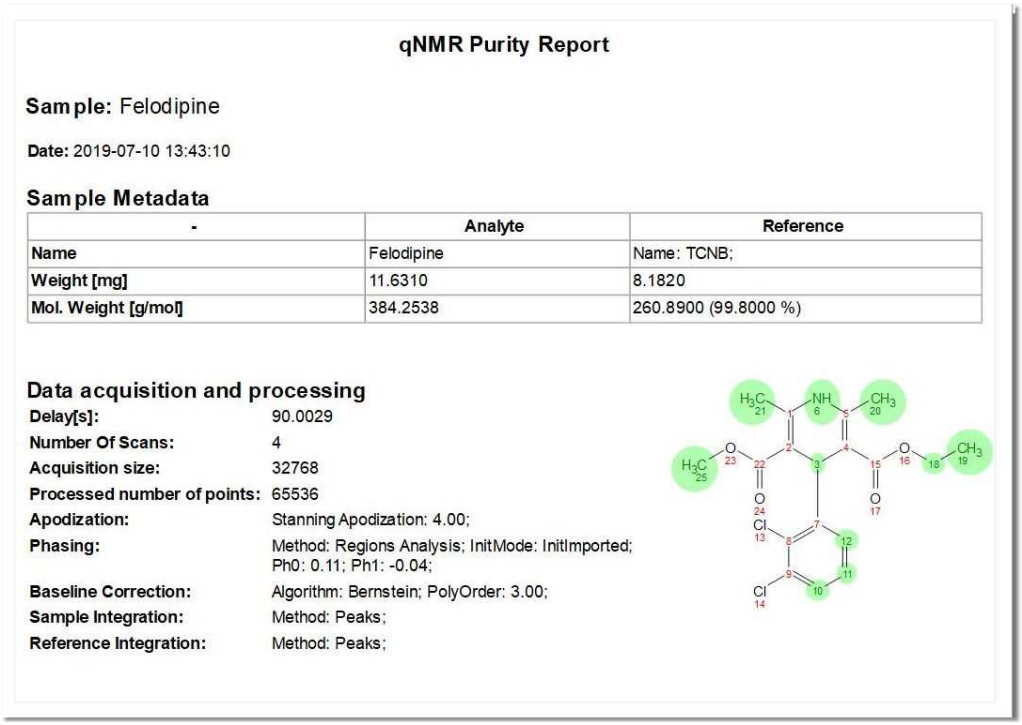


Clicking on the settings button will allow you to customize the fonts or to use your own layout template:



Here you can find an example of an advanced report:

© 2024 MESTRELAB RESEARCH



qNMR Purity Report

Sample: Felodipine

Date: 2019-07-10 13:43:10

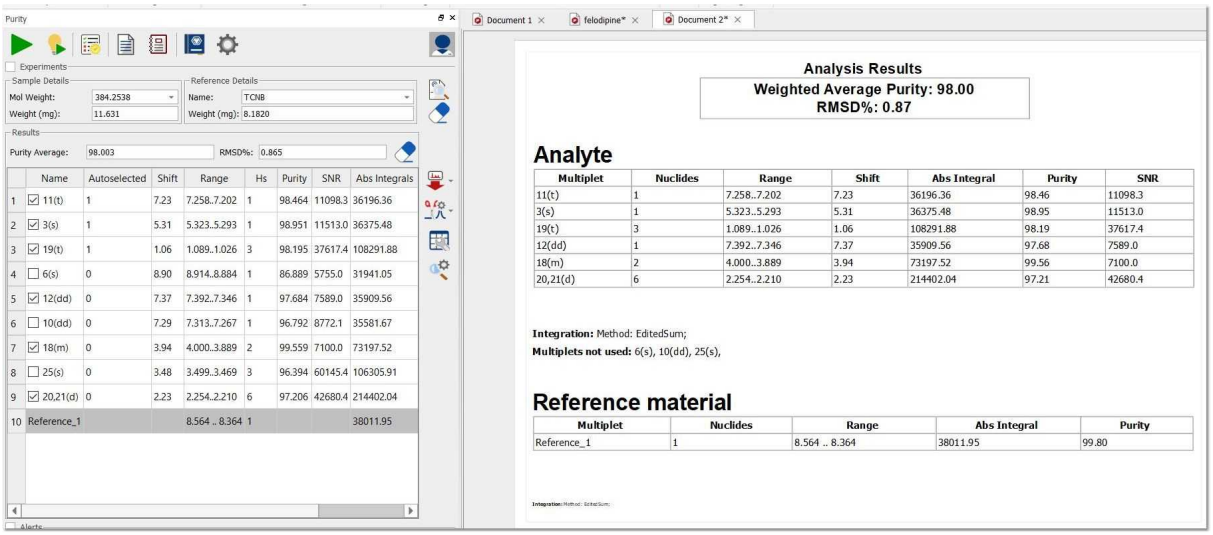
Sample Metadata

-	Analyte	Reference
Name	Felodipine	Name: TCNB;
Weight [mg]	11.6310	8.1820
Mol. Weight [g/mol]	384.2538	260.8900 (99.8000 %)

Data acquisition and processing

Delay[s]:	90.0029
Number Of Scans:	4
Acquisition size:	32768
Processed number of points:	65536
Apodization:	Stanning Apodization: 4.00;
Phasing:	Method: Regions Analysis; InitMode: InitImported; Ph0: 0.11; Ph1: -0.04;
Baseline Correction:	Algorithm: Bernstein; PolyOrder: 3.00;
Sample Integration:	Method: Peaks;
Reference Integration:	Method: Peaks;

Please note that the advanced reports will be automatically updated if you change the analysis of your spectrum (for example the selection of your multiplets):



Analysis Results

Weighted Average Purity: 98.00

RMSD%: 0.87

Analyte

Multiplet	Nuclides	Range	Shift	Abs Integral	Purity	SNR
11(t)	1	7.258..7.202	7.23	36196.36	98.46	11098.3
3(s)	1	5.323..5.293	5.31	36375.48	98.95	11513.0
19(t)	3	1.089..1.026	1.06	108291.88	98.19	37617.4
12(dd)	1	7.392..7.346	7.37	35909.56	97.68	7589.0
18(m)	2	4.000..3.889	3.94	73197.52	99.56	7100.0
20,21(d)	6	2.254..2.210	2.23	214402.04	97.21	42680.4

Integration: Method: EditedSum;

Multiplets not used: 6(s), 10(dd), 25(s),

Reference material

Multiplet	Nuclides	Range	Abs Integral	Purity
Reference_1	1	8.564 .. 8.364	38011.95	99.80

Visit also our blog:

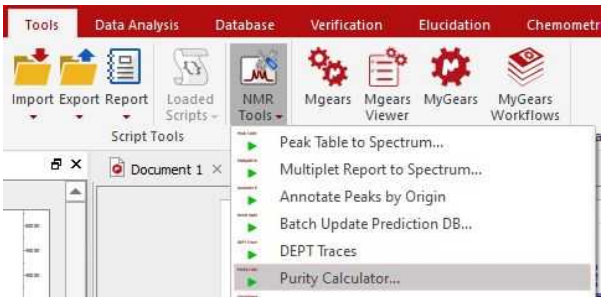
http://mestrelab.com/blog/what-is-qnmr/
http://mestrelab.com/blog/qnmr-the-best-pulse-length-for-acquisition/
http://mestrelab.com/blog/edited-sum-integration-for-qnmr/
http://mestrelab.com/blog/determining-concentrations-when-using-nmr-to-model-chemical-reactions/
http://mestrelab.com/blog/nmr-quantification/
http://mestrelab.com/blog/automating-qnmr/
http://mestrelab.com/blog/qnmr-purity-recipe-book-i/
http://mestrelab.com/blog/qnmr-purity-recipe-book-ii/
http://mestrelab.com/blog/qnmr-purity-recipe-book-iii/

© 2024 MESTRELAB RESEARCH

http://mestrelab.com/resources/getting-picky-with-q-nmr/
http://mestrelab.com/blog/new-qnmr-experiments/

19.6.3 Purity Calculator script

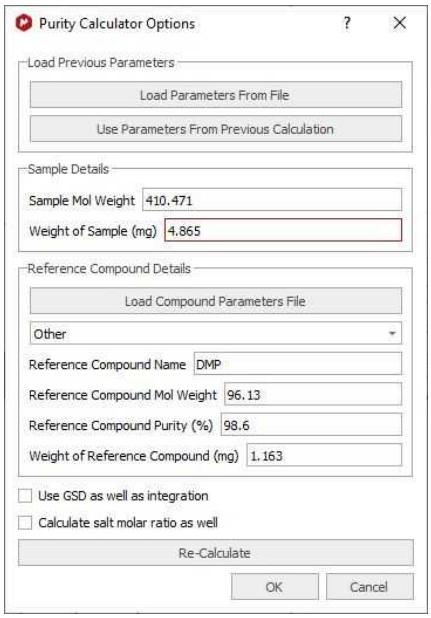
This new script can be run without a license of the qNMR plugin. You will only need a license of the NMR module. This script is located under the 'Tools/NMR Tools' menu:



The script method is still heavily used in some Development labs as a final check of compound purity. The spectra used must be of high quality with good shimming, acquired with sufficient time between pulses for full relaxation (typically 20-40 s in DMSO-d6). For processing the spectrum should be well very well phased (manually), and baseline corrected.

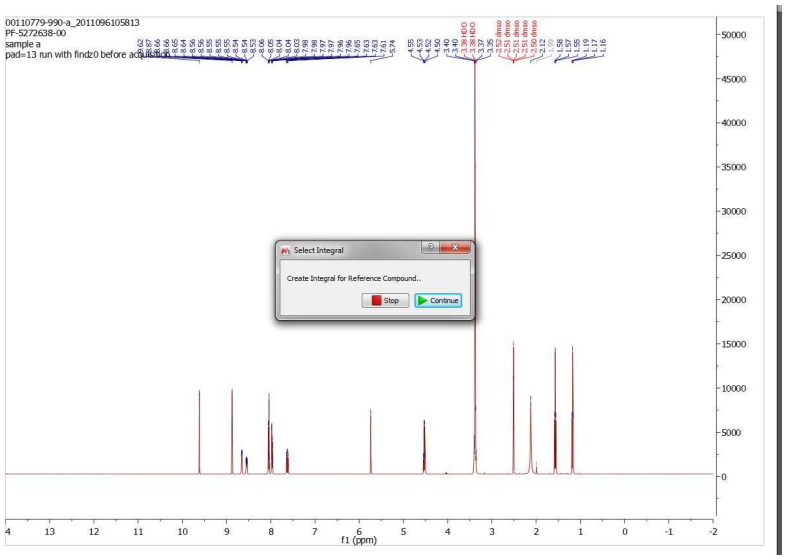
The example shown here is typical and doesn't present problems. Practical problems that may arise could be a large broad OH signal (which can sometimes be sharpened by adding a little TFA), or careful baseline correction. Other examples also require that you determine the salt ratio – but we don't deal with that here.

Just load the sample spectrum (which you can download from **here**) and follow the menu 'Tools/NMR Tools/Purity Calculator' in order to fill in the sample details and choose the reference compound with the applicable molecular mass of the internal reference and its purity (you can load, modify and add to the reference compound details by loading the csv file that you downloaded in the previous step):

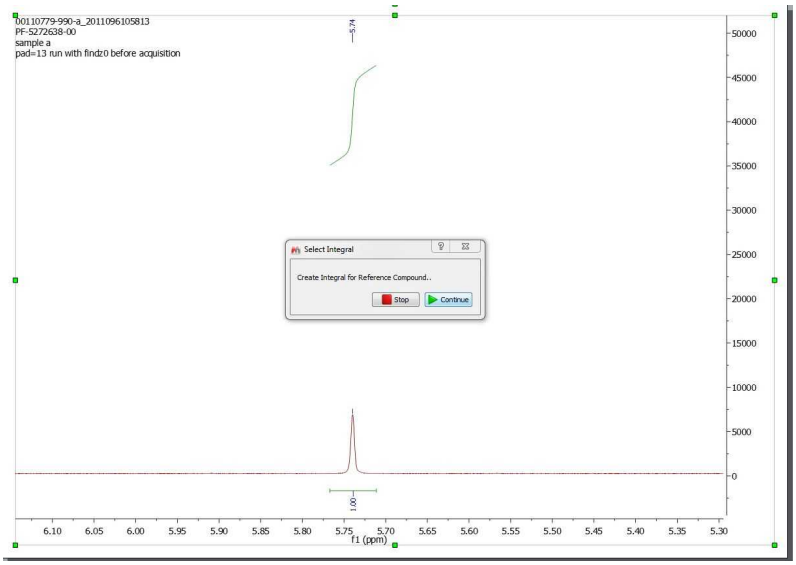


Clicking on OK, you will be prompted to integrate the signal that will be used as reference compound.

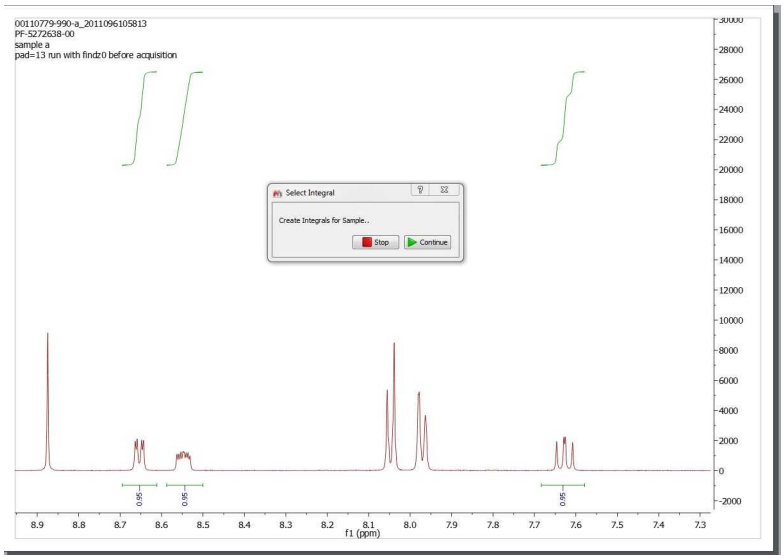
© 2024 MESTRELAB RESEARCH



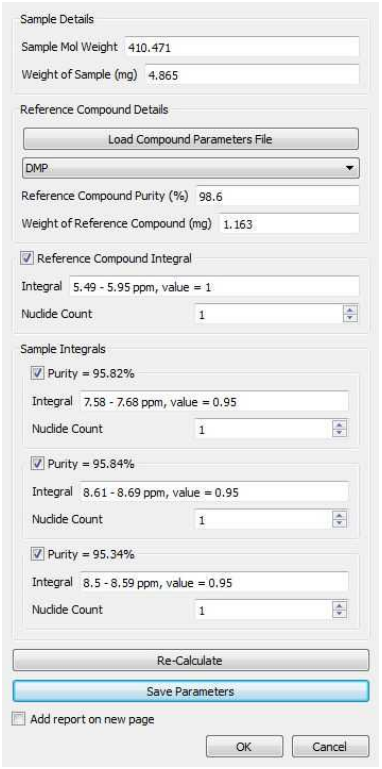
In that case, we will manually integrate the signal at 5.74ppm and click on the 'Continue' button:



Now, we will integrate 3 multiplets for the analyte (choosing the aromatics close by). Once these have been integrated, click “Continue” again.

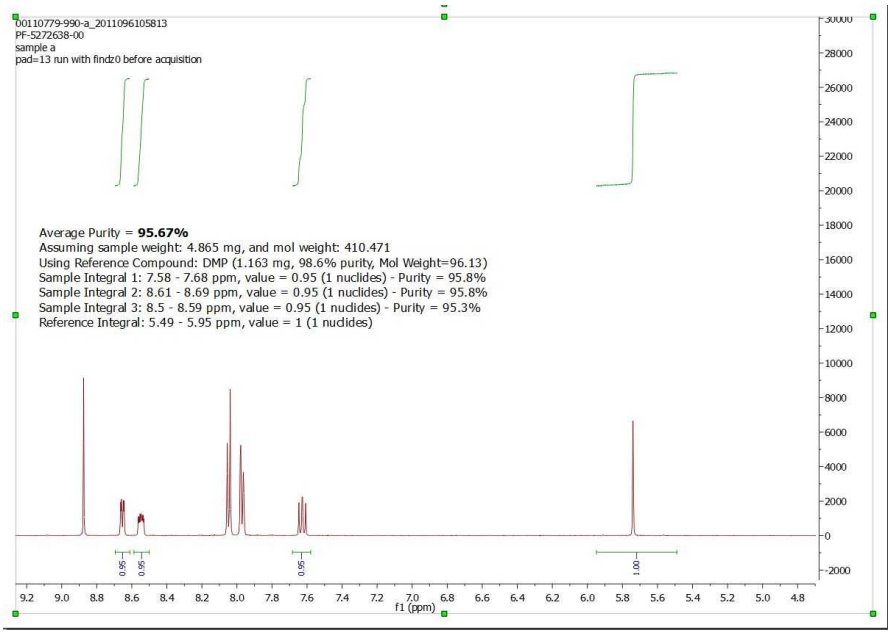


Clicking on the 'Continue button will display the dialog box below:



From that dialog box, if it is necessary, you will be able to adjust masses, see the purity result for each multiplet (they should be similar), see the number of nuclides values and you will be able to change the analyte peaks to be included in the calculation. Once all is OK, click on OK and the report will be added to the spectrum page (if the applicable box was checked) :

© 2024 MESTRELAB RESEARCH



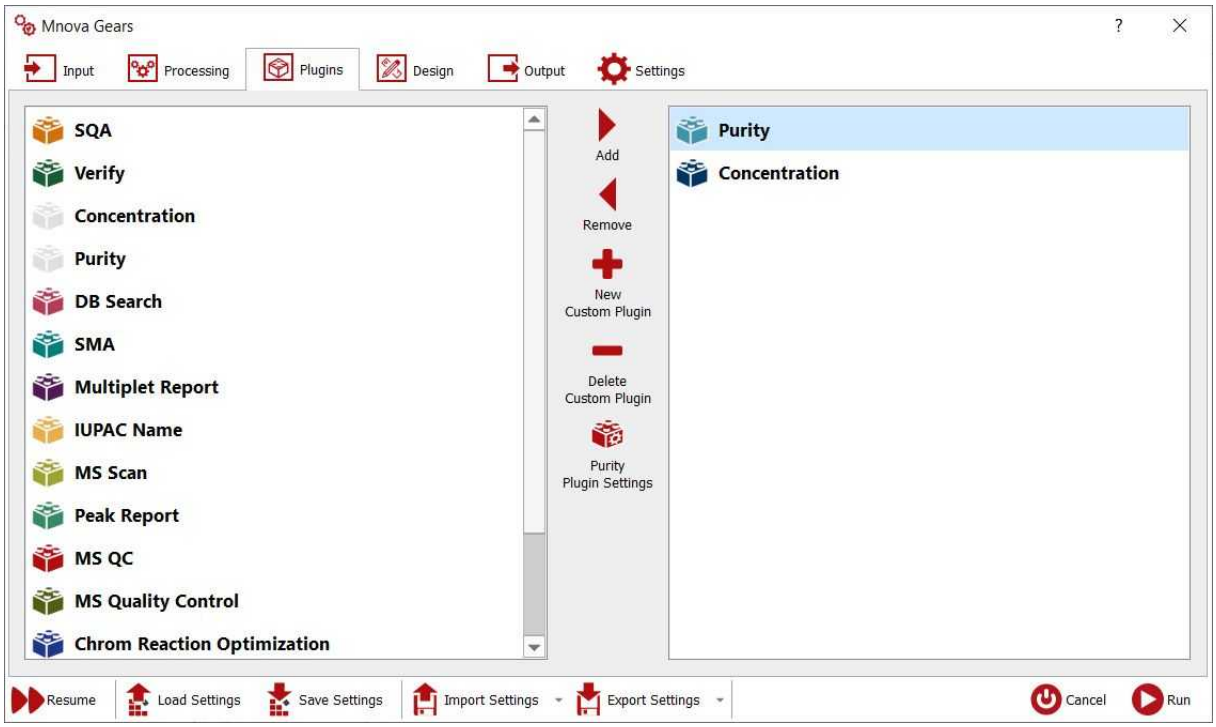
After that, if you plan to work with a similar dataset, you can load the spectrum and re-run the 'Purity Calculator' script, selecting "Use parameters from previous calculation". The only values you would need to change will be the masses.

For further information, please visit our blog:
http://mestrelab.com/blog/article/purity-calculation/

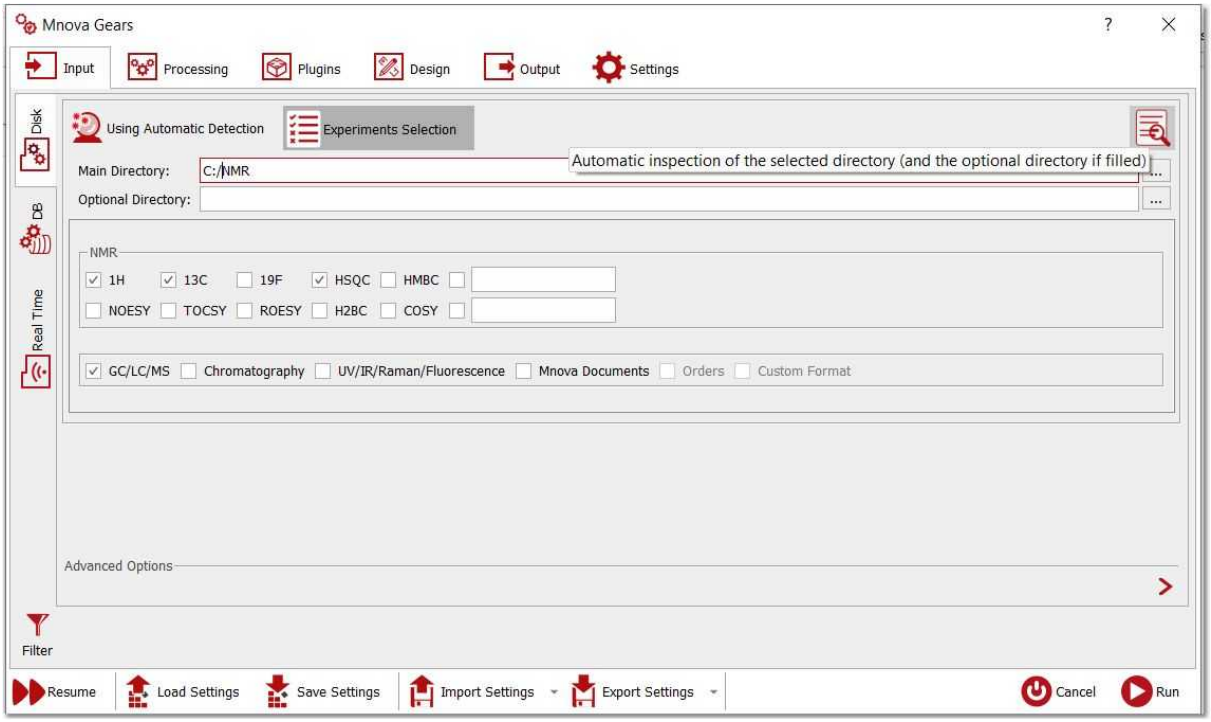
19.6.4 Mgears qNMR

You will need to install Mgears and the Batch qNMR plugin separately (with the applicable licenses), by following the menu 'File/Advanced Plugins' and clicking on the 'blue plus' button or just by dragging&dropping the XML file into Mnova.

Having the applicable licenses, you can run qNMR analysis in batch mode by following the menu 'Automation/Mgears' and making sure that you have selected the 'Concentration' and/or the Purity bricks (under the 'Plugins' tab):



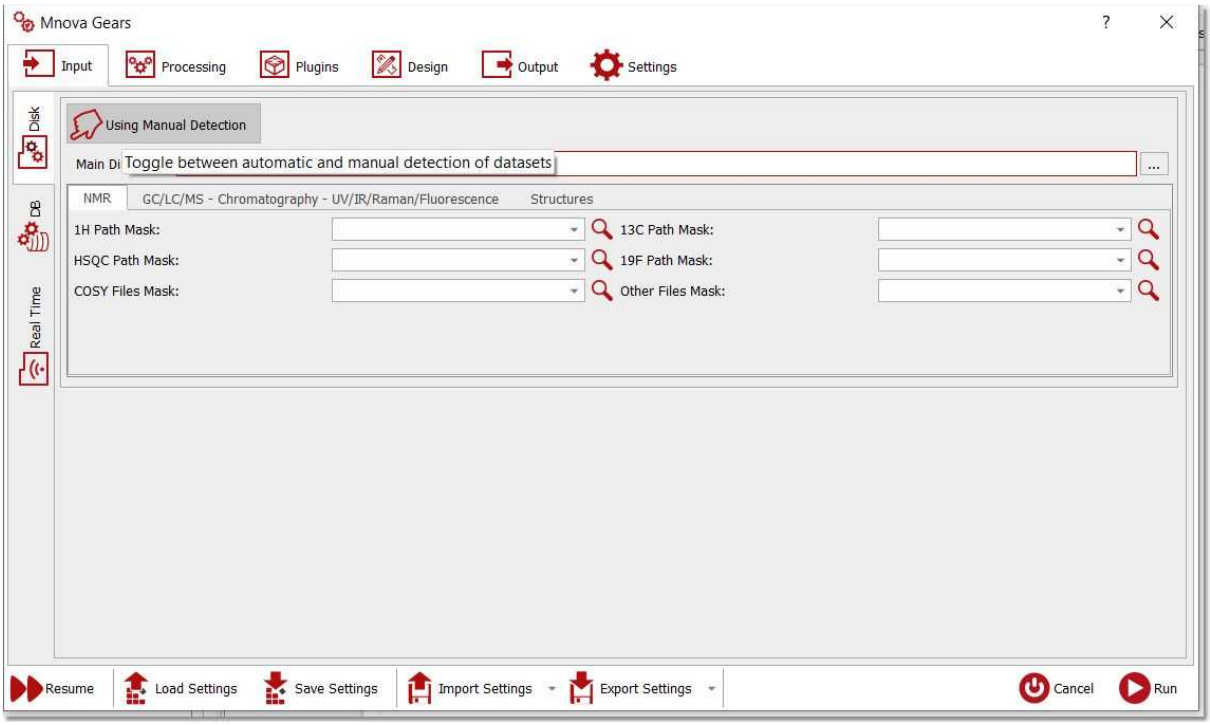
From Input/Disk tab, you will be able to select the file locations in your hard disk. Clicking on the 'Automatic Inspection' button will check the datasets included in the Base Directory:



.

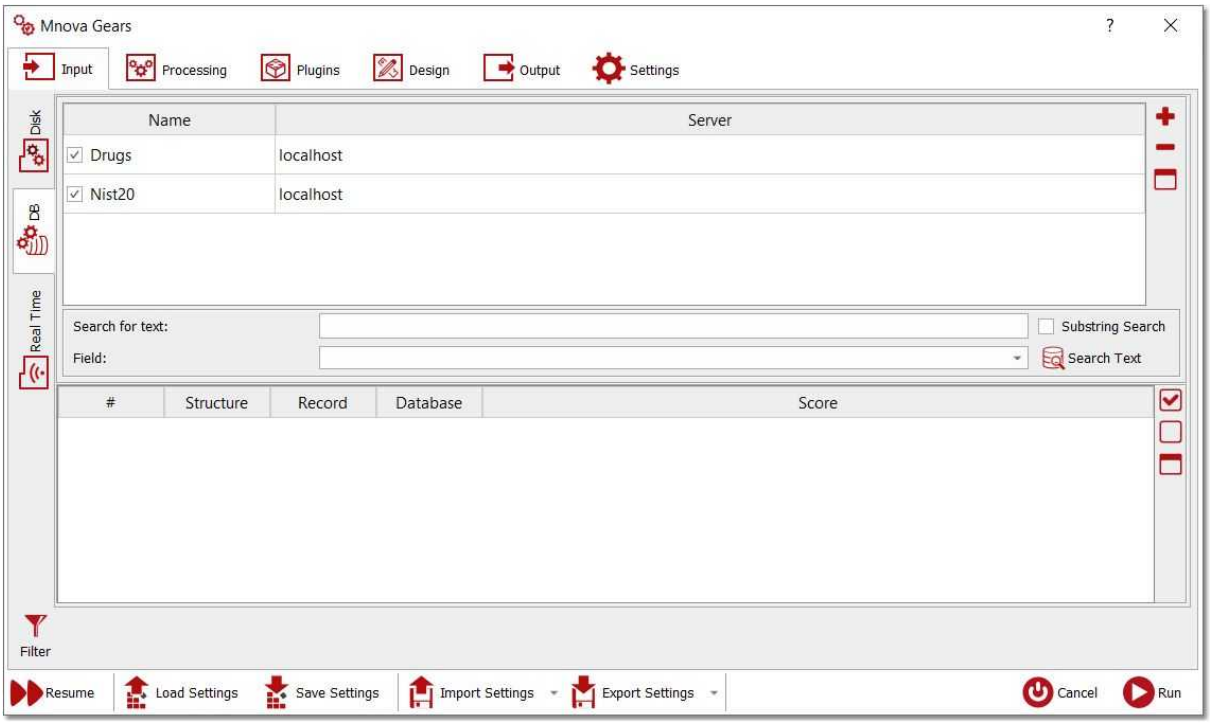
If you click on the 'Toggle' button, you could select the masks for the spectra and molecules (instead of an automatic detection):

© 2024 MESTRELAB RESEARCH

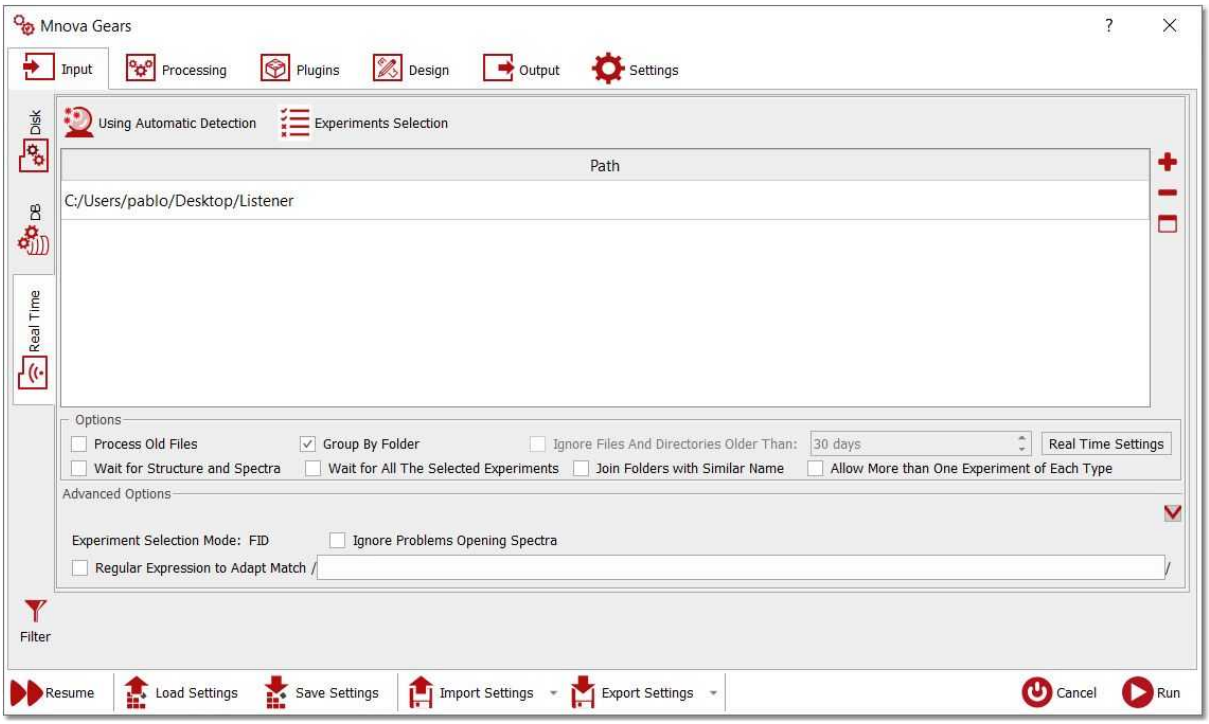


Mask: From here you will need to type a 'Path Mask' for your datasets describing how to open each of the spectra. All Operating System standard conventions apply (*, ?, !, etc.).

Selecting the Database tab will allow you to choose the input from existing Mnova databases:



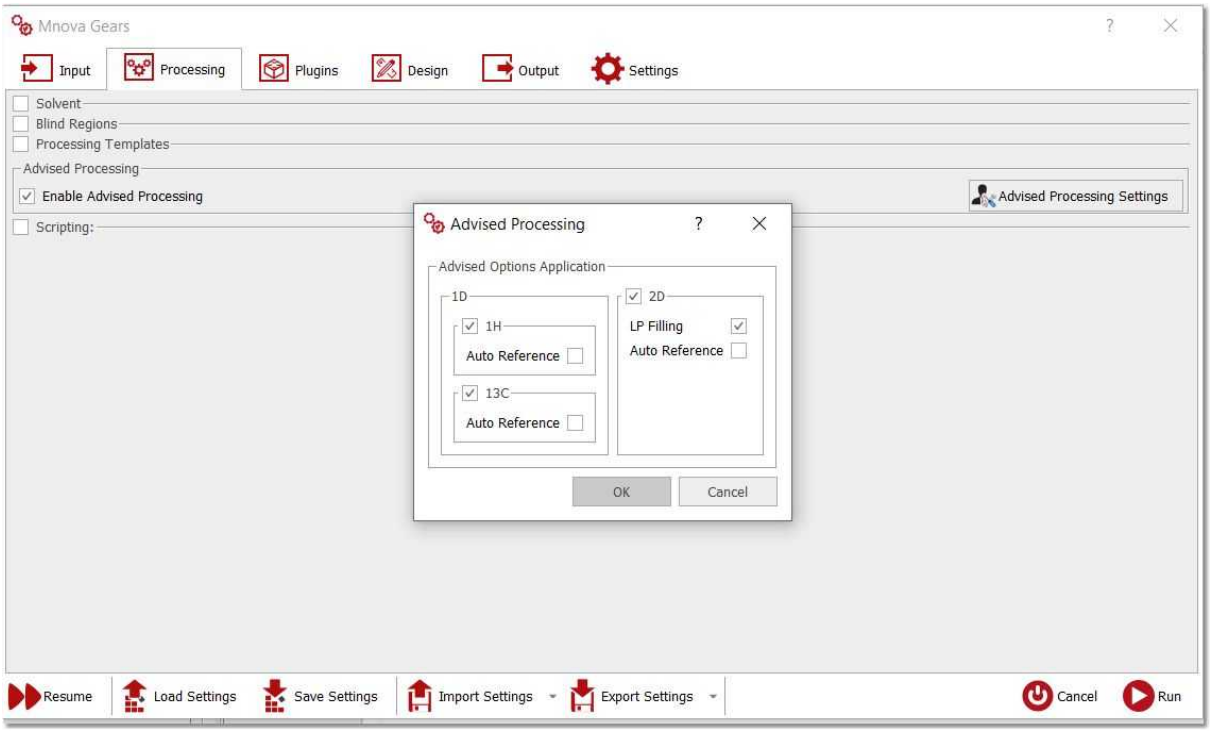
You can also run Batch qNMR as a **listener**. Mnova can ´listen´ to a series of folders or drives and can grab data as is acquired and saved, and run the qNMR analysis automatically, saving the result in the selected folder. The settings for the qNMR listener can be selected under the 'Real Time' tab:



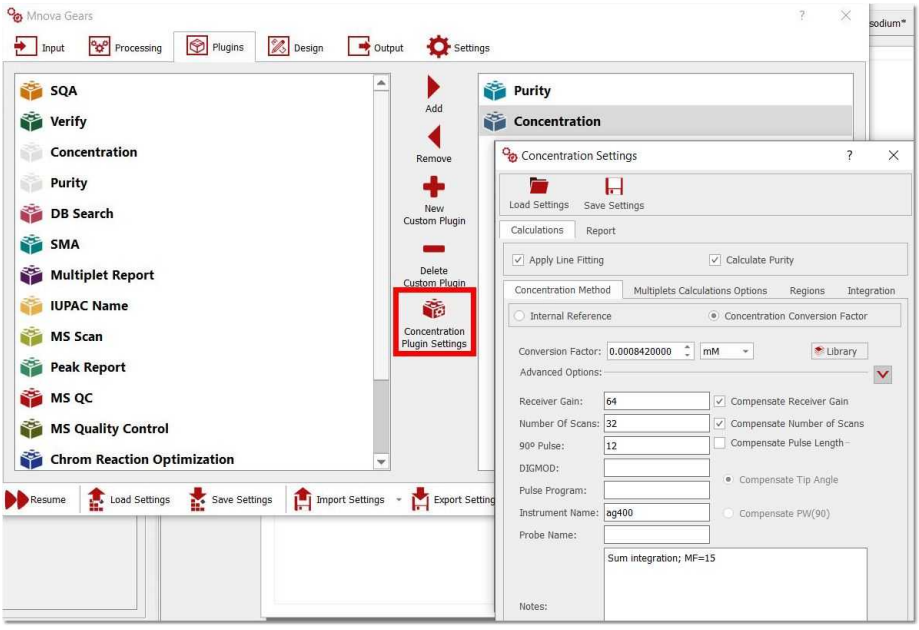
In this case, you will need to select the path of the dataset folder. If the folder contains a .mol file, the applicable molecule will be also loaded.

From the 'Processing' tab, you could replace the default solvent (this allows you to handle situations in which the solvent may be wrong in the parameters), add blind regions, use processing templates or enable your Advised processing settings:

© 2024 MESTRELAB RESEARCH

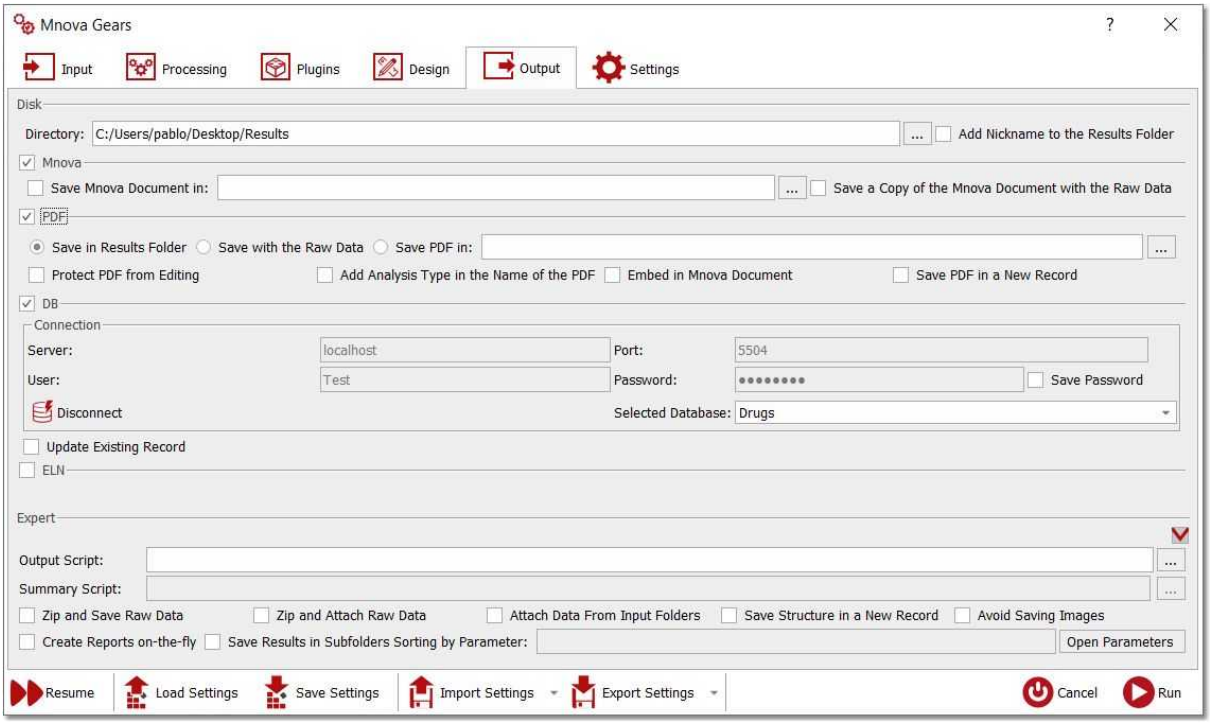


Under the Plugins tab, you could select your Concentration or Purity settings:

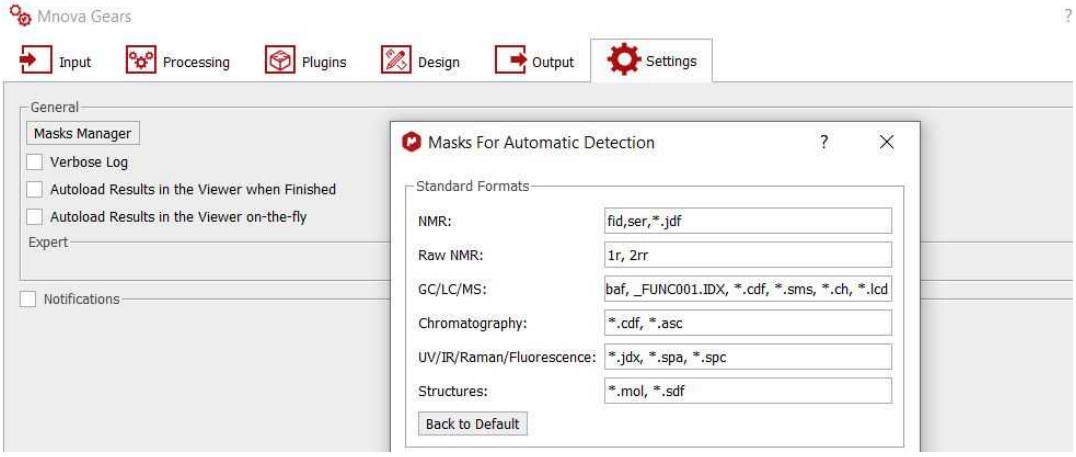


You will find several tabs for your 'Calculations', 'Report', 'Concentration method', 'Multiplets Calculations options', 'Regions' and 'Integrations' settings (as you have in the qNMR single mode).

The Design tab will allow you to use a Layout template, whilst the Output tab will be used to select the folder containing your results (with the option to save them as Mnova documents, PDF, or to an existing Database):

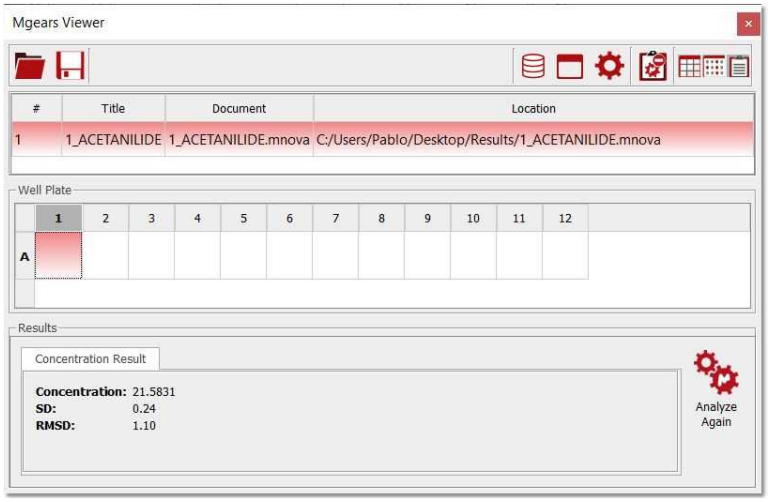


The settings tab will be useful to select the masks for the automatic detections of your input (datasets and molecules):



Once the analysis has done the Mgears Viewer can be used to check the results. You would need to navigate to the “results” directory and select the 'data' subfolder”:

© 2024 MESTRELAB RESEARCH



Clicking on each row, will load the dataset in Mnova to allow you to make changes if necessary. The changes made in the Mnova document will be automatically updated in the Viewer.

From the viewer, you can load/save/clear or copy the report to the clipboard (by default).

See also this application note.

19.7 Reaction Monitoring (R-M)

Many experiments require obtaining a series of FIDs, related to each other through the variation of one or more parameters.

For example, in a kinetics or reaction monitoring (R-M) experiment, a series of spectra are recorded at predetermined time intervals to follow the progress of the reaction.

There are two ways to acquire these kind of experiments:

- Acquiring all spectra at different times and storing those individual spectra
- Instead of acquiring separate data sets, it is very common to keep all acquired spectra into a single NMR experiment, the so-called **Arrayed mode**

In general, arrayed mode is the most commonly used, although sometimes, acquiring the spectra as individual spectra can offer more flexibility. Of course, **Mnova supports both modes.**

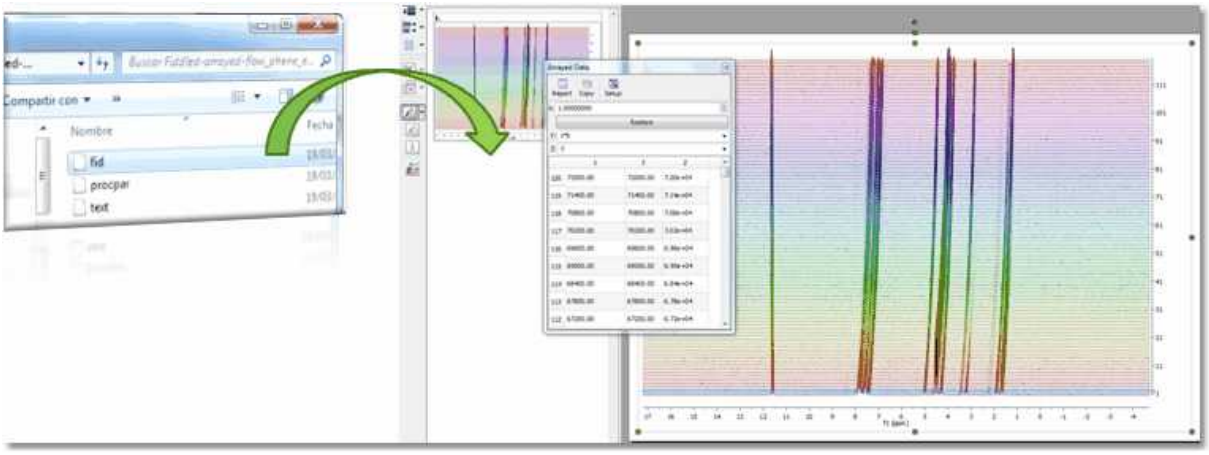
See this video for further information and also this post

19.7.1 Importing Datasets

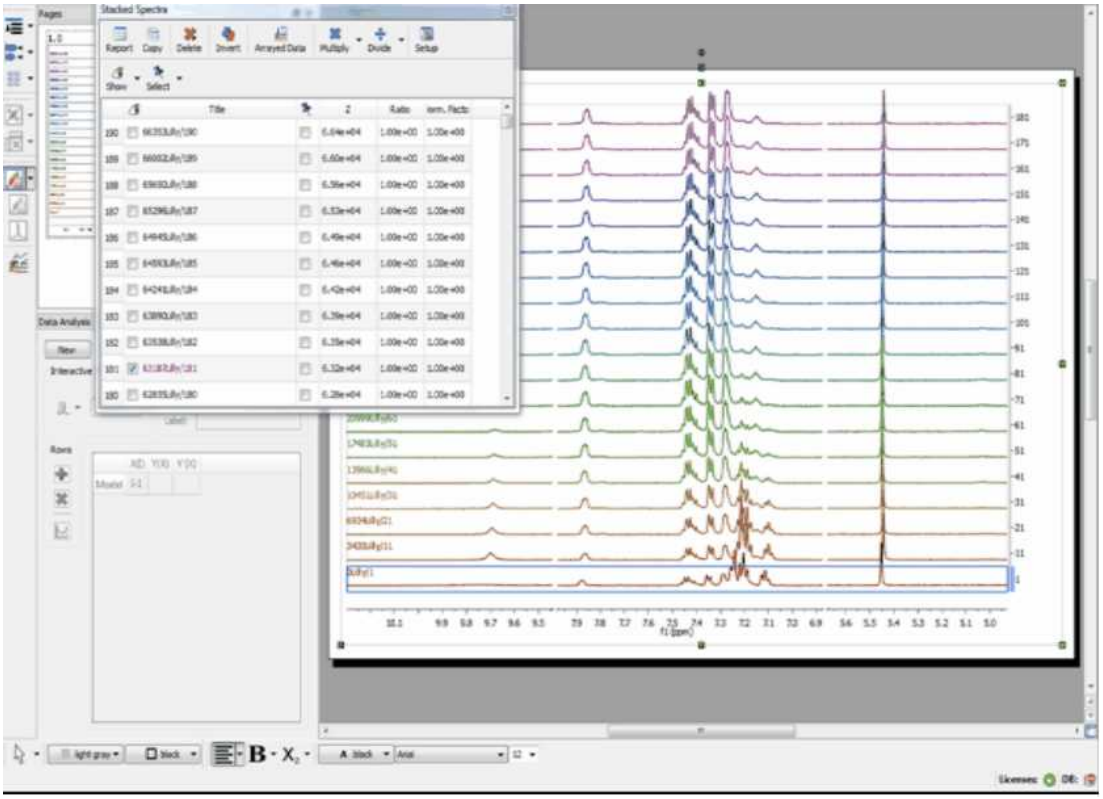
Importing of R-M data sets acquired in arrayed mode

If your dataset has been acquired in arrayed mode, it will be enough to drag&drop the FID (Varian) or SER file (Bruker) to Mnova.

Mnova supports, in addition to Reaction Monitoring, Relaxation (T1/T2) and PFG (DOSY) experiments (in other words, it will parse the arrayed parameter automatically).



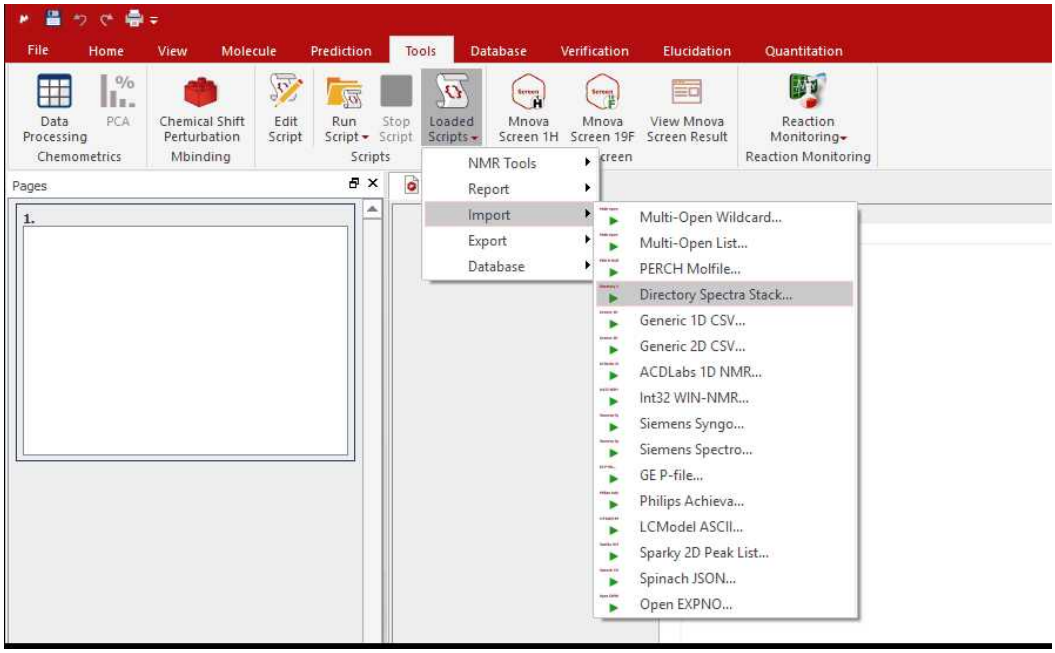
In either case, Mnova will automatically read the values of the arrayed parameter, e.g. time stamp and populate the corresponding arrayed data table



Importing of R-M data sets acquired on individual basis

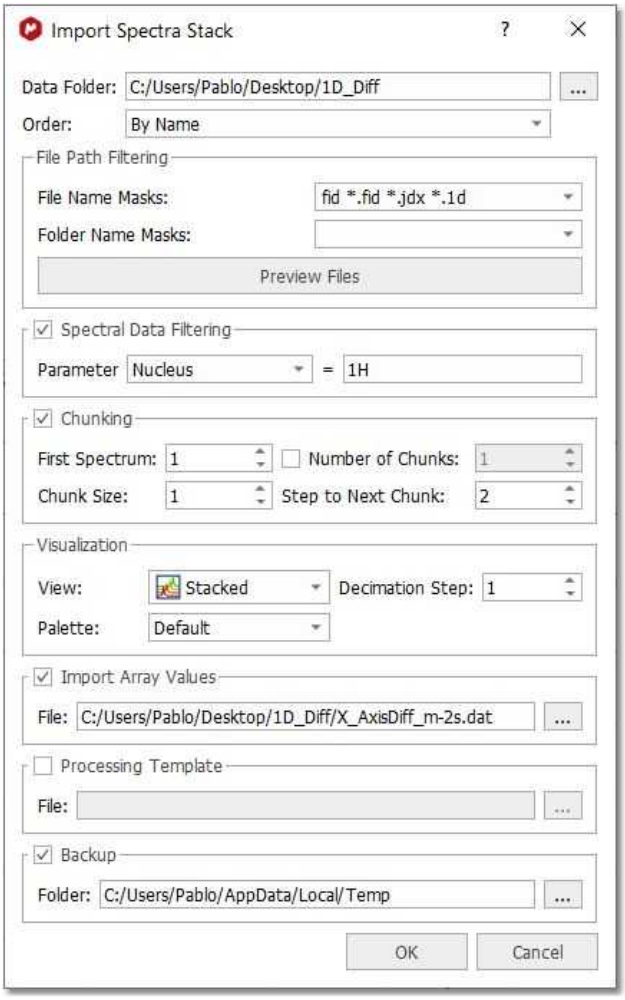
Mnova includes a powerful script which makes it possible to import all spectra contained in a folder and automatically stack them for forthcoming R-M analysis. This script is under the Scripts menu and it is called 'Directory Spectra Stack'. It will allow the user to select the directory where the spectra are contained. Then Mnova will open and process all those spectra (FIDs) automatically in bunches of 20 spectra and finally will stack all of them.

© 2024 MESTRELAB RESEARCH



Note: This script has also the option to decimate the spectra while loading (i.e. the program can skip every 10 spectra in the data set) and to apply a processing template (or to load the 1r processed data):

© 2024 MESTRELAB RESEARCH



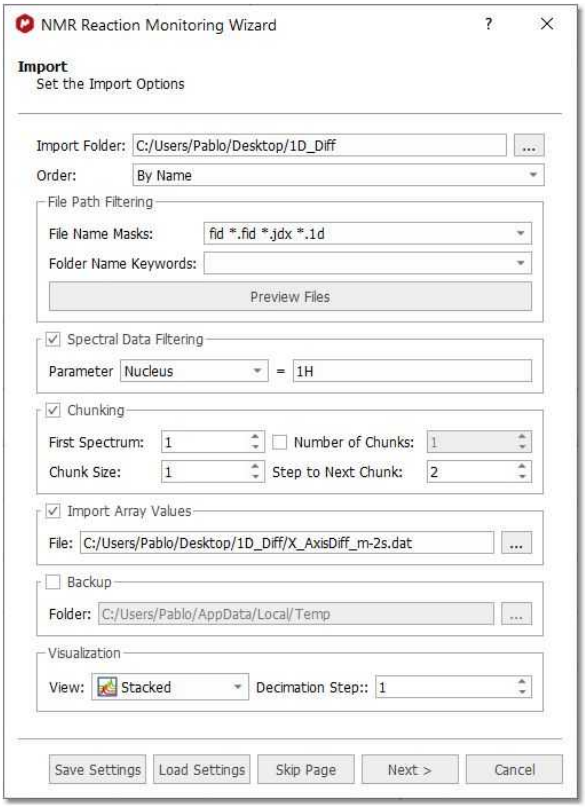
Reaction Monitoring Wizard :

This wizard is a simple way to quickly get Reaction Monitoring data into Mnova and analyse it. It will allow you the save and recall procedures in the future.

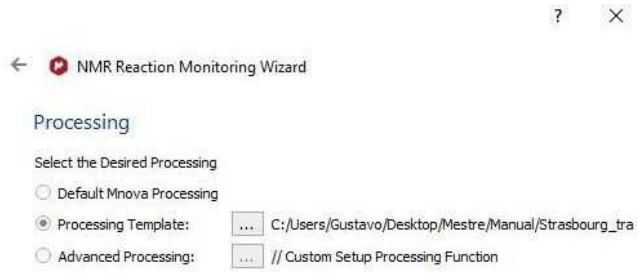


In the screenshot below you can see how to select the directory of your dataset, to sort the spectra by name or date, to display them in stack or superimposed mode and to use decimation.

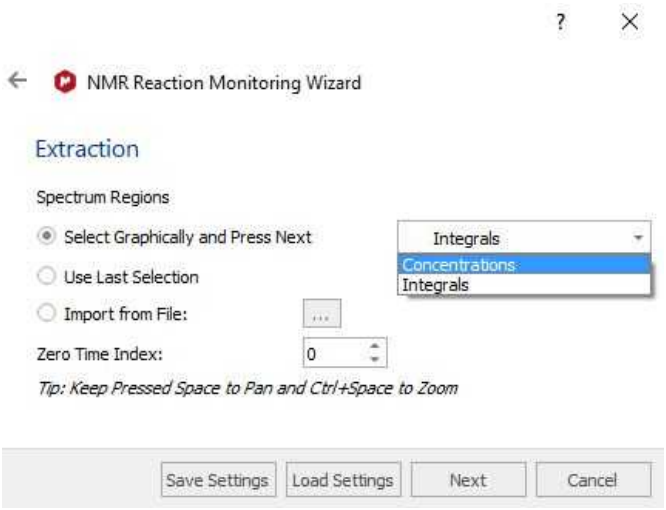
© 2024 MESTRELAB RESEARCH



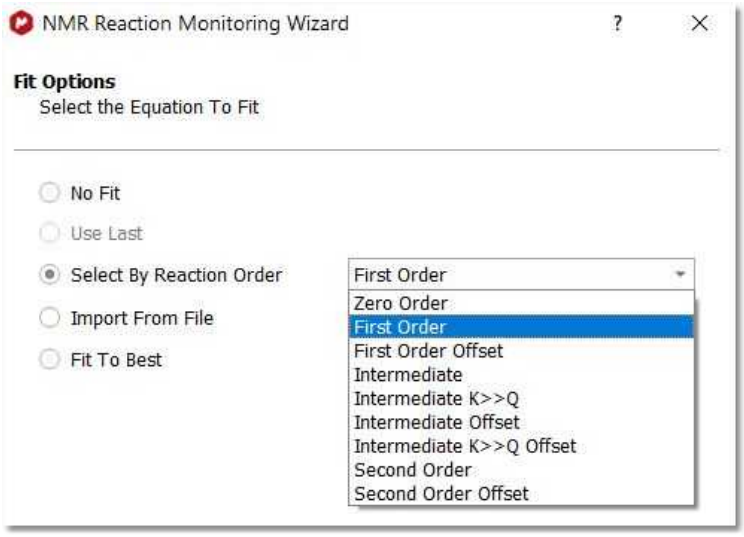
You can also 'Save or Load Settings' from this dialog box. Clicking on the 'Next' button will display the dialog below:



This new window will allow you to apply 'processing templates' to your datasets. Clicking on the 'Next' button will display the 'Extraction' dialog:

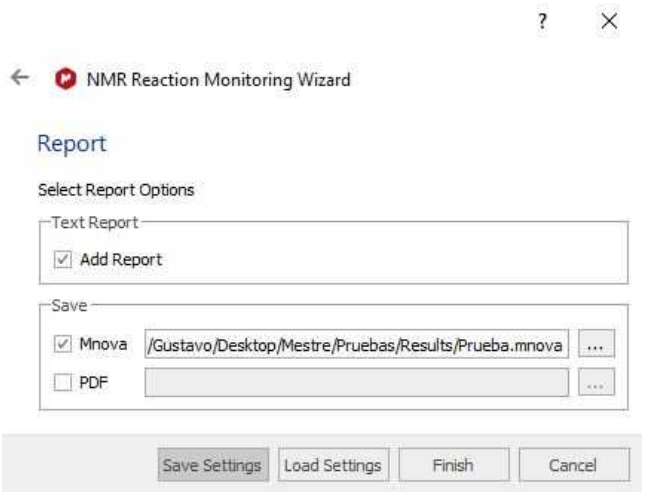


From the dialog above, you will be able to select graphically the regions (with concentrations or integrals), import a XML file with the regions of interest or use the latest selection. From here you can also select the Zero Time Index. Clicking on the 'Next' button will display the 'Fit Options' dialog:



Finally, click on the Next button to display the Report dialog box which will allow you to save the document in Mnova or PDF format:

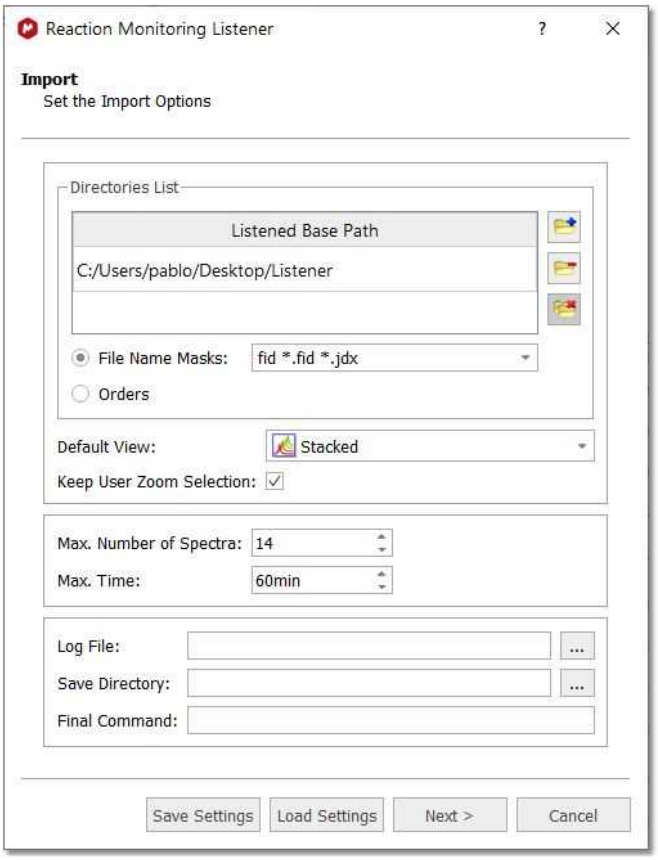
© 2024 MESTRELAB RESEARCH



See also this video

Reaction Monitoring Listener:

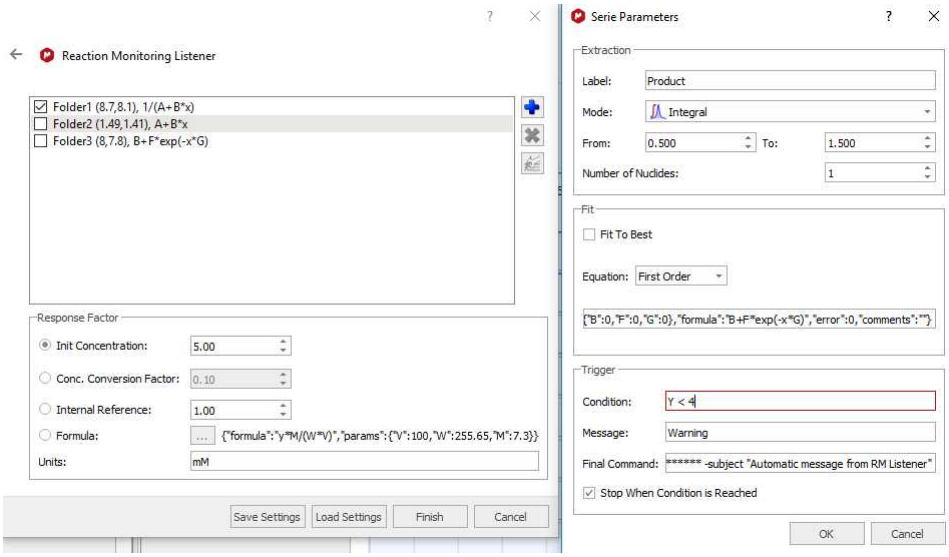
The listener will automatically import, stack and save any dataset that arrive to the selected folder according to the selected file masks:



You can define a default view (stack or superimpose), keep the zoom selection (by checking the applicable box) and select a maximum number of spectra or time.

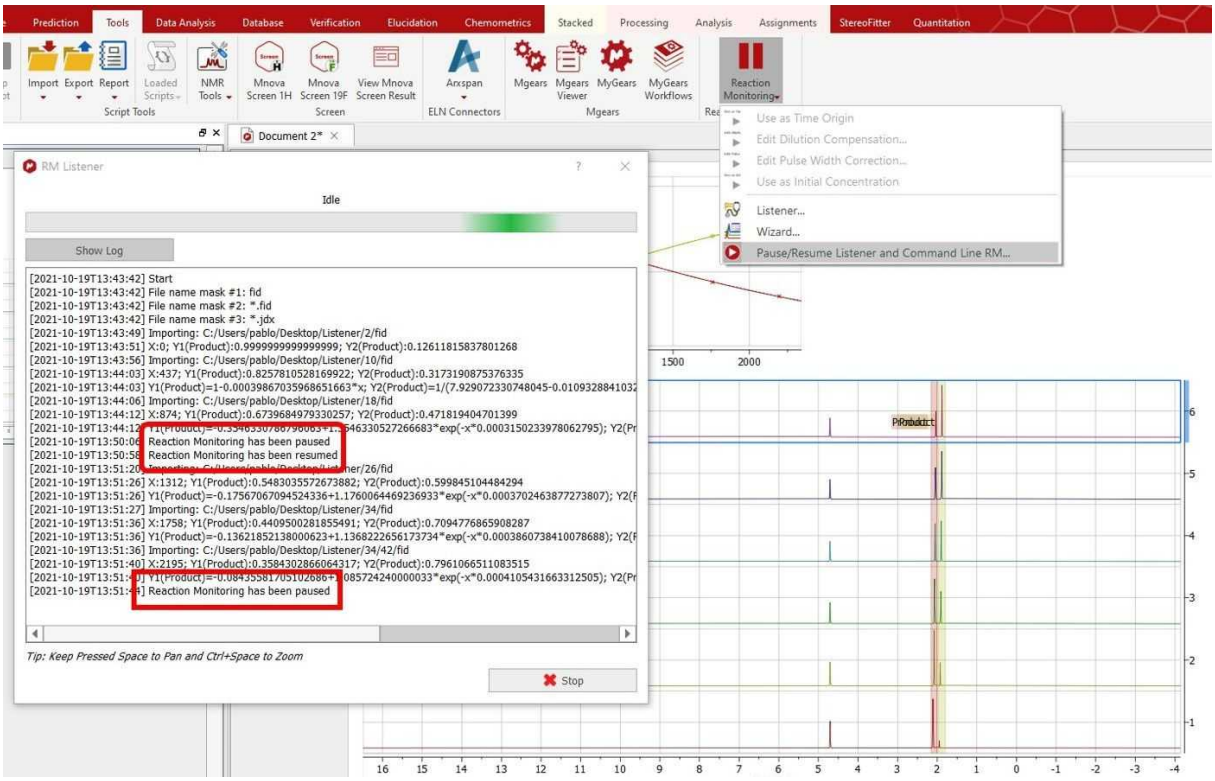
You can also use a final command to get whatever you want. For example, to receive an email when the listener stops completely, because all the conditions has reached. You can define a final command when an specific condition has reached (but not necessary all of them).

In the example below, we have used the application 'blat exe' to send an email when a condition in a serie has reached. The initial concentration was 5 mM. After the condition is reached (when concentration is lower than 4), the listener stops, shows a message of "Warning" and an email is sent. (the final command used was:
c:\temp\blat.exe -body "Condition reached" -to support@mestrelab.com -u guest@mestrec.com -pw ****** -subject "Automatic message from RM Listener")



There is a button in the ribbon which will allow you to pause/resume the listener (and the command line tools), very useful if you are interacting with the stack whilst the listener is running:

© 2024 MESTRELAB RESEARCH



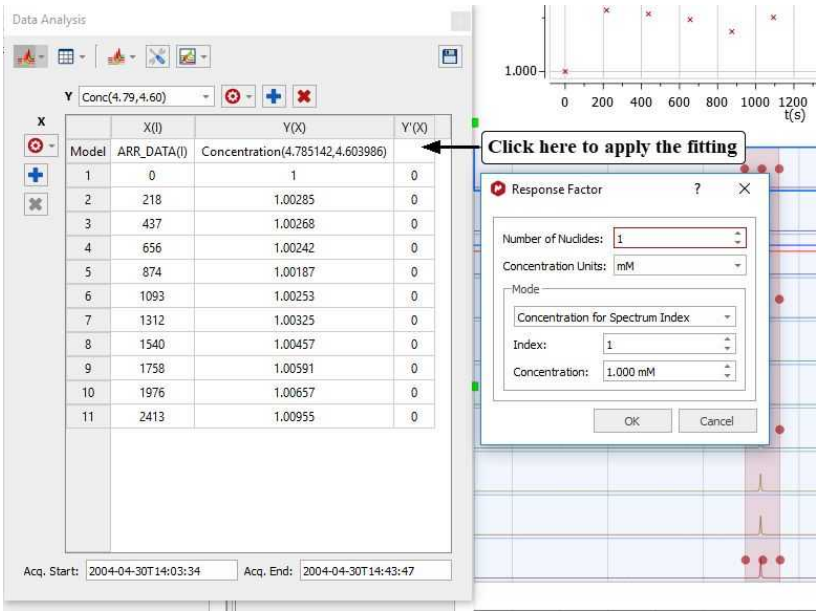
19.7.2 Selecting Regions

Once you have imported your datasets, you can select the 'Reaction Monitoring' regions of interest by following the menu 'Analysis/Data Analysis/Create Concentration Graph' to select the desired regions:

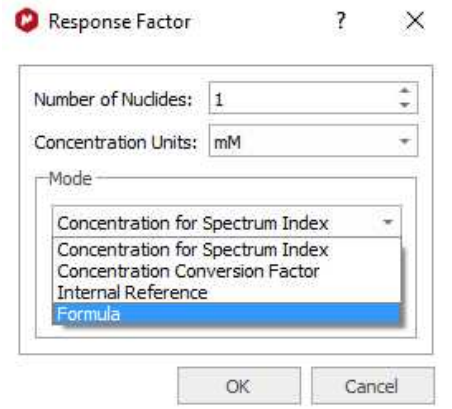


Once you have selected the first region in your stacked plot, you will be asked to type the number of nuclides and concentration value. Once you have typed the values, the Data Analysis panel will be filled.

© 2024 MESTRELAB RESEARCH

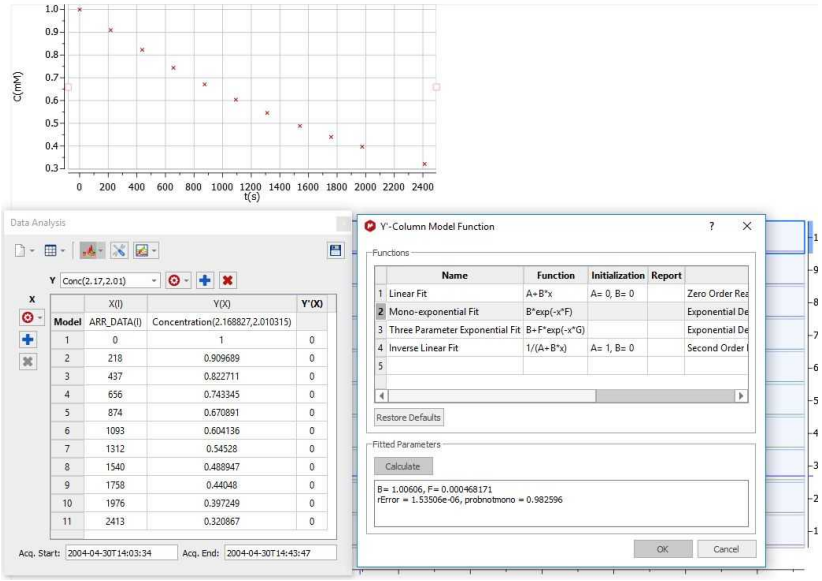


You can select different modes: Concentration for spectrum Index (by area of peak of the selected spectrum in the array), Concentration Conversion factor (CCF), Internal Reference (to include a reference compound of constant, known concentration) or to use a Formula:



Next click on the '3 dots' button of the Y'(x) column to select the desired fitting (Mono-Exponential in the picture below). Next, click on the 'Calculate' button to generate the parameters of the fitting and finally click on the OK button to draw the fitting curve.

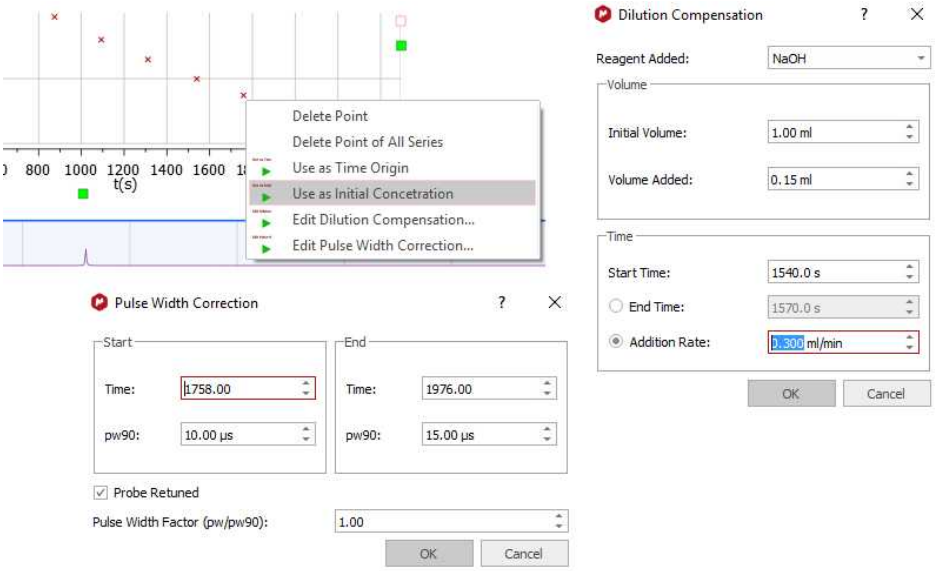
© 2024 MESTRELAB RESEARCH



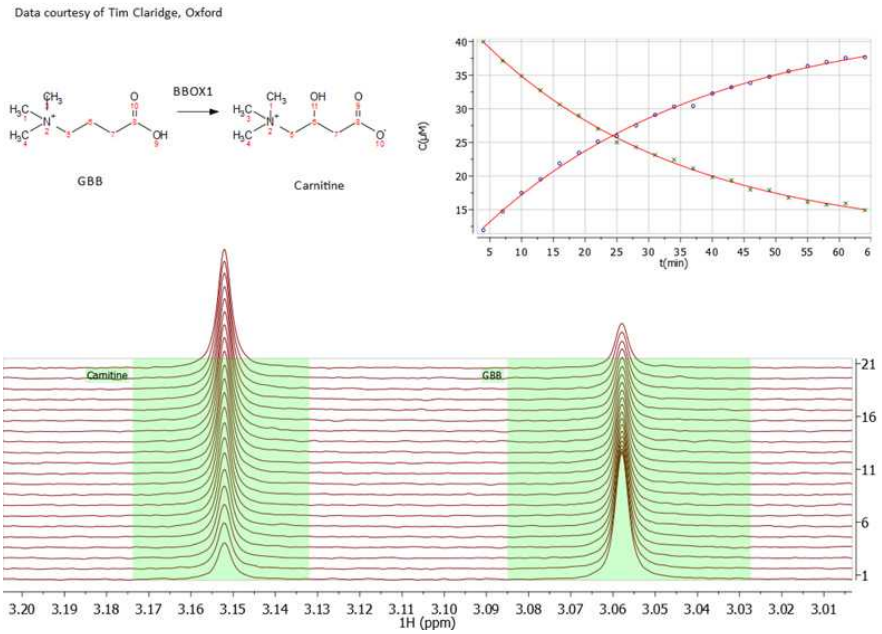
Follow the menu ' Analysis/Data Analysis/Edit/Fit to Best' and Mnova will try to guess the order of the reaction; fitting the data to standard equations and comparing the mean square error:



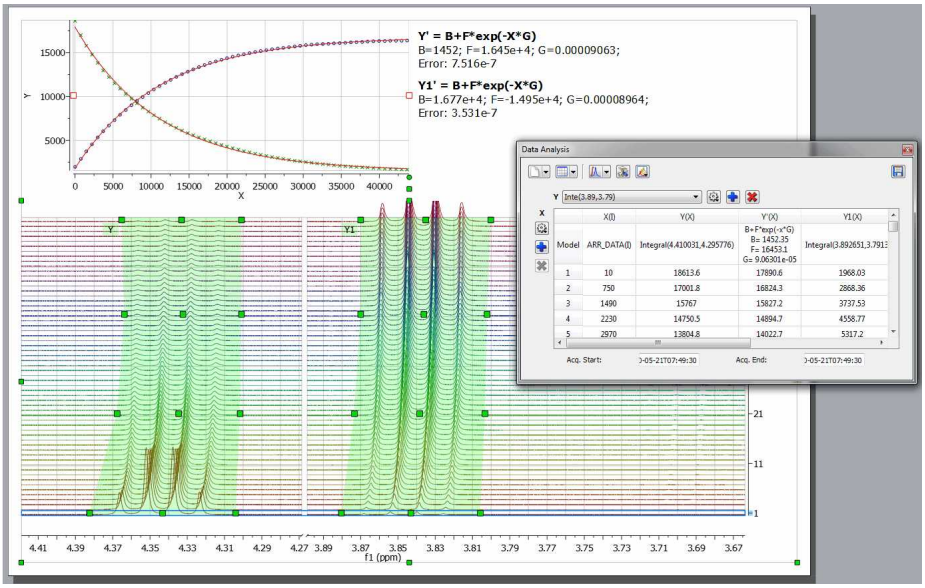
With the *Reaction Monitoring* plugin enabled, if you right click on any point of the Data Analysis Graph (or Table), you will be allowed to 'use that point as time origin or as 'Initial Concentration', or to set the Dilution Compensation Parameters' and 'Pulse Width Correction':



Here you can see an example following an enzymatic reaction:



and here an ester hydrolysis:



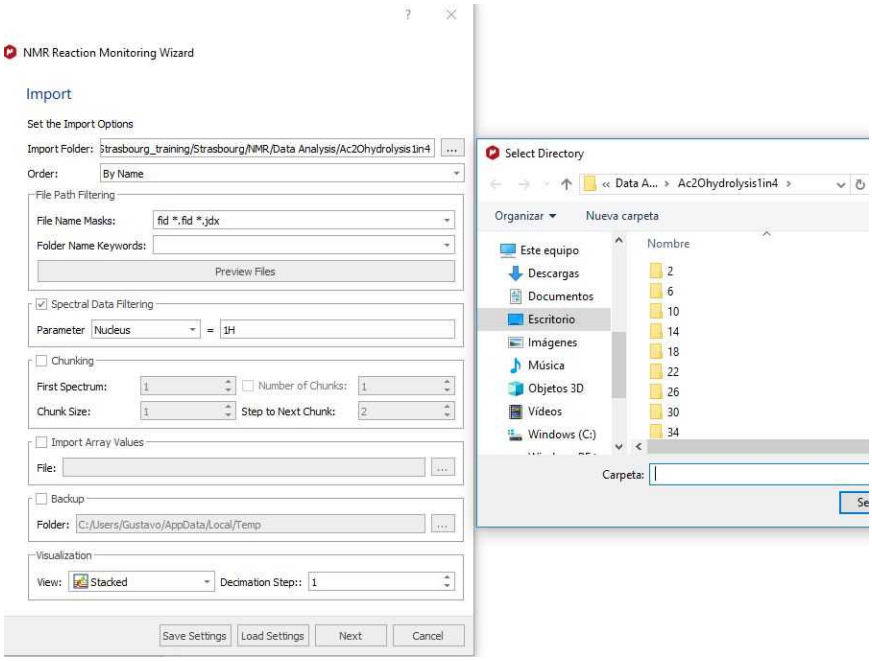
19.7.3 Practical Example

This tutorial will show you how to use the Reaction Monitoring plugin for your spectra.

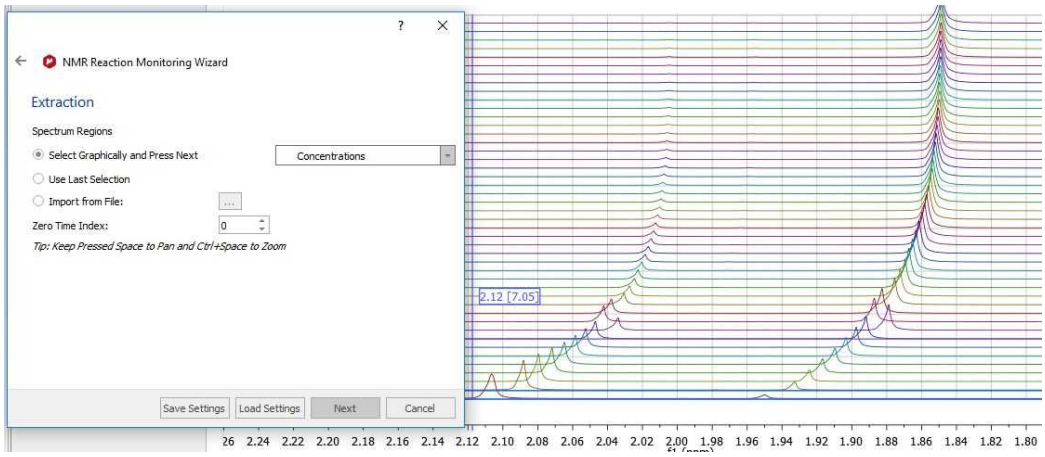
You will need to import your dataset into Mnova just by dragging and dropping the stacked spectra (if it has been acquired in arrayed mode) o by using the Reaction Monitoring Wizard (Advanced/Reaction Monitoring/Wizard) if the datasets have been acquired on individual basis (you can also use the Scripts/Import/Directory spectra Stack).

© 2024 MESTRELAB RESEARCH

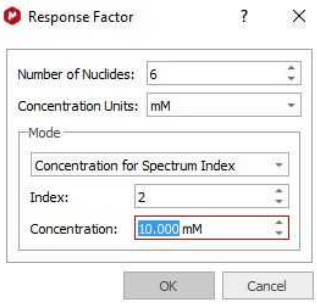
In this tutorial we will use the RM Wizard. You can download the dataset from this link.



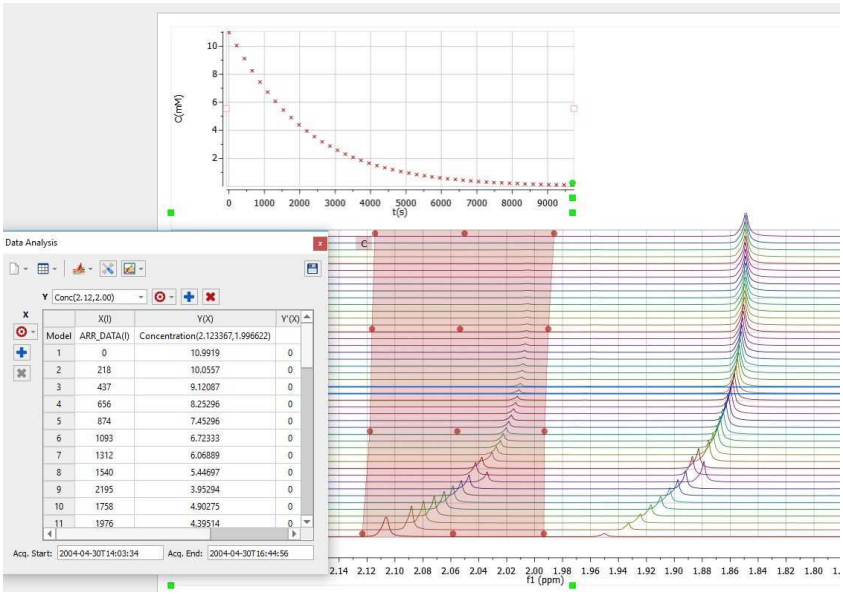
Once Mnova has loaded all the individual spectra, it will show you the stacked plot. Zoom in (by using 'Ctrl+Space' (or Space+Cmd) to activate the zoom in mode temporarily) the spectral region of interest and select graphically the signals that you want to analyze



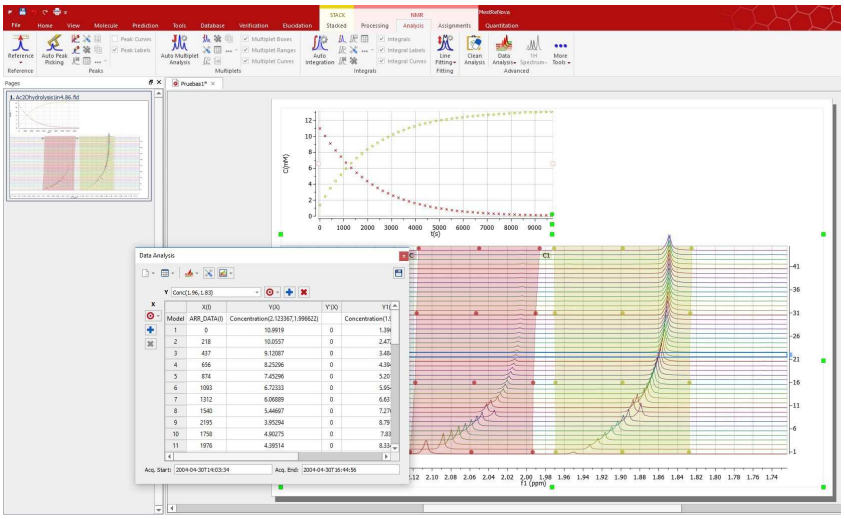
Once we have selected the first region (signal at around 2.10 ppm), a dialog box will be displayed to enter the 'response factor' values:



Clicking on OK, the graphic will appear on your spectral page and the 'Data Analysis' panel will be displayed:

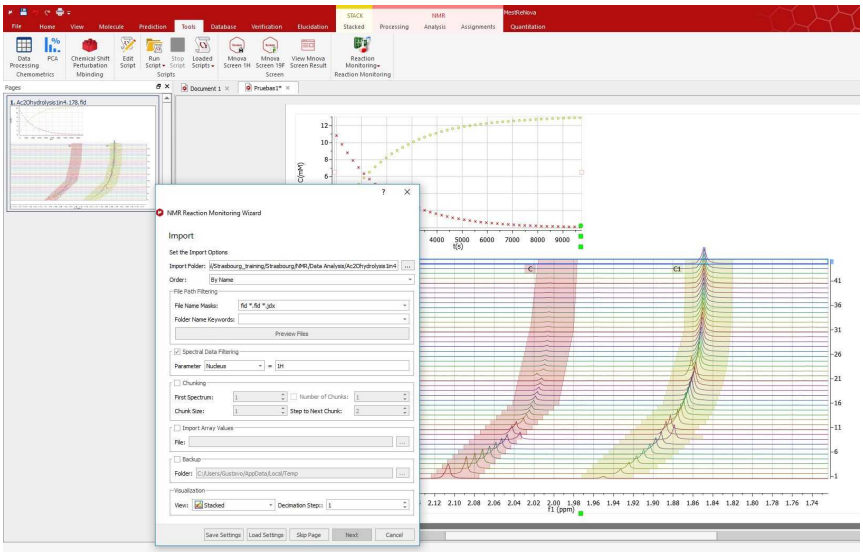


The next step will be to select the second region of interest (around 1.90 ppm) and the number of nuclides (6):

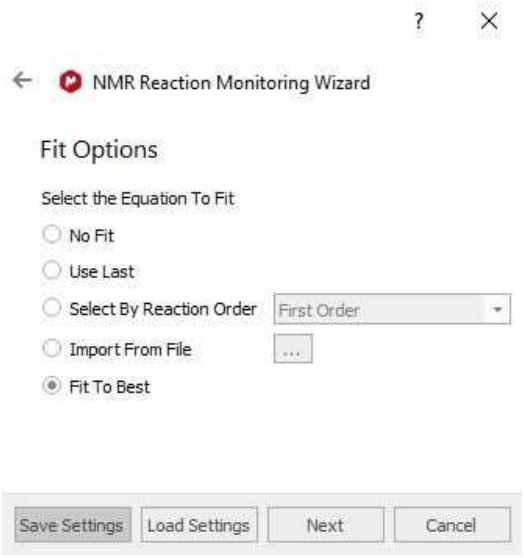


© 2024 MESTRELAB RESEARCH

We can use the green square handles to adjust the regions of interest with the deviation of the signals in the stacked (you could also use the 'Align Spectra' feature)

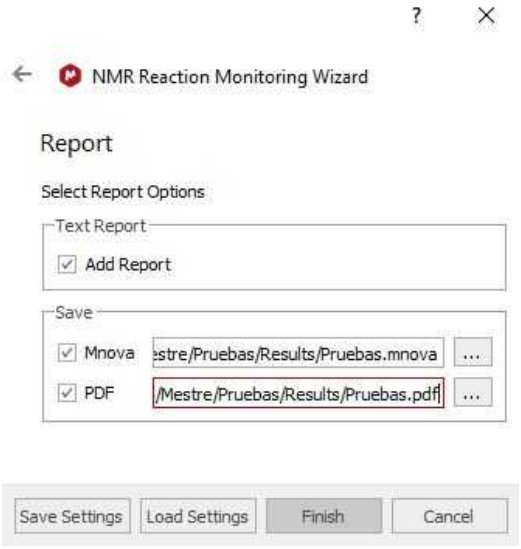


Clicking on the 'Next' button of the RM Wizard will allow us to select the equation for the fitting (or to ask to Mnova to search for the Best Fitting):

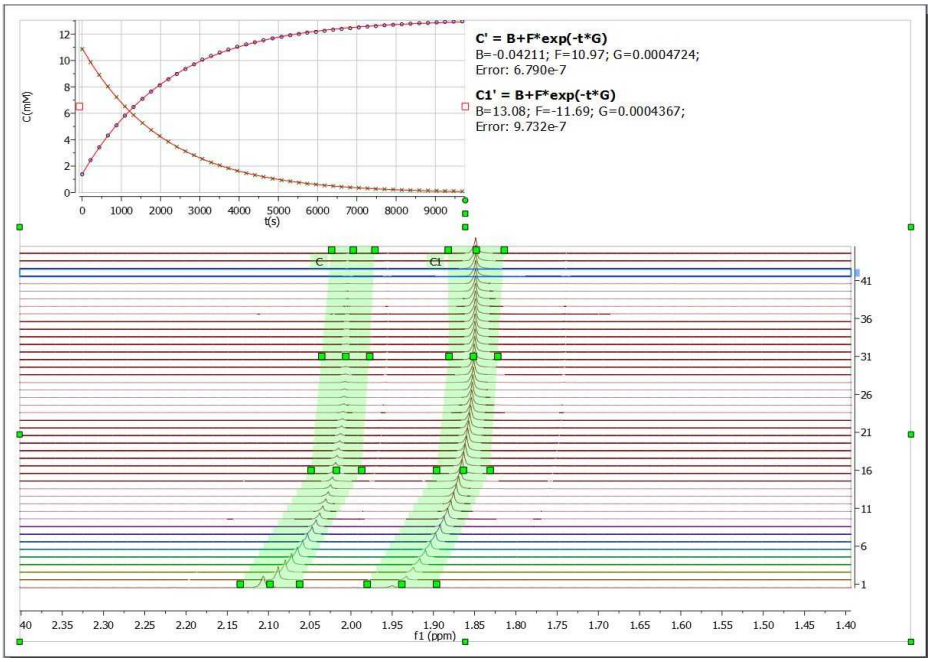


Clicking on the 'Next' button again, we will be allowed to paste the report into the Mnova document and to save the result in Mnova and PDF documents:

© 2024 MESTRELAB RESEARCH



Clicking on the 'Finish' button, will do the job. You can 'save the settings' to be used in the future with similar datasets.

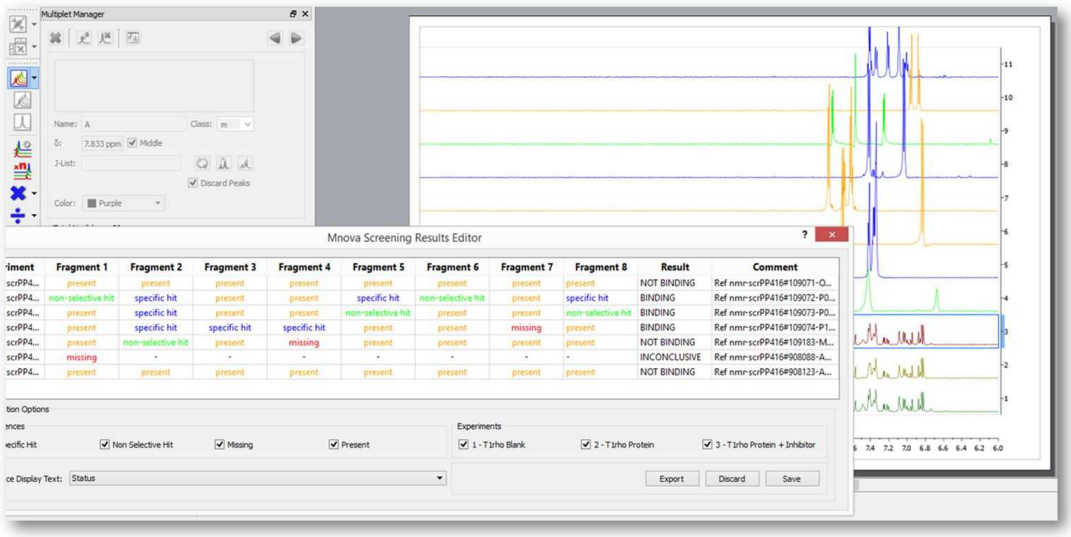


19.8 Screen

This advanced plugin needs a specific license.

The objective of this module is to allow the simple and efficient analysis of Ligand Binding Screening experiments (WaterLOGSY, STD, T1rho, CPMG) –^{1}H, ^{19}F

© 2024 MESTRELAB RESEARCH

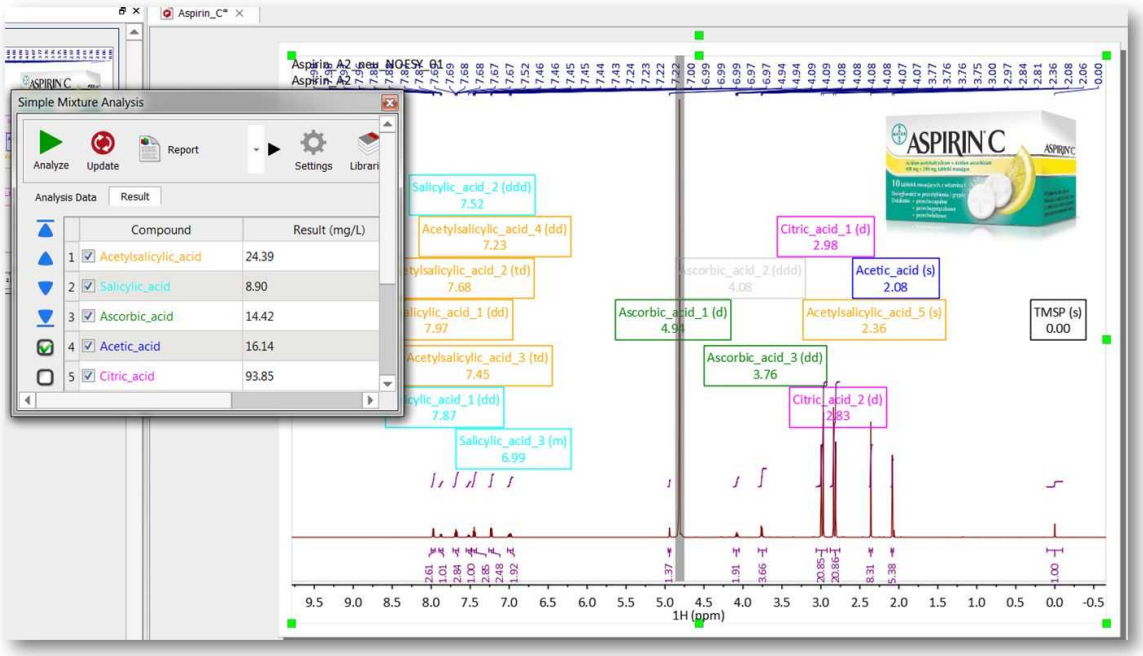


More resources can be found here

19.9 SMA

This advanced plugin needs a specific license (on top of a license for the NMR module).

A versatile, functional program for the quantitation of mixture components by NMR based on chemical shift range integrals.

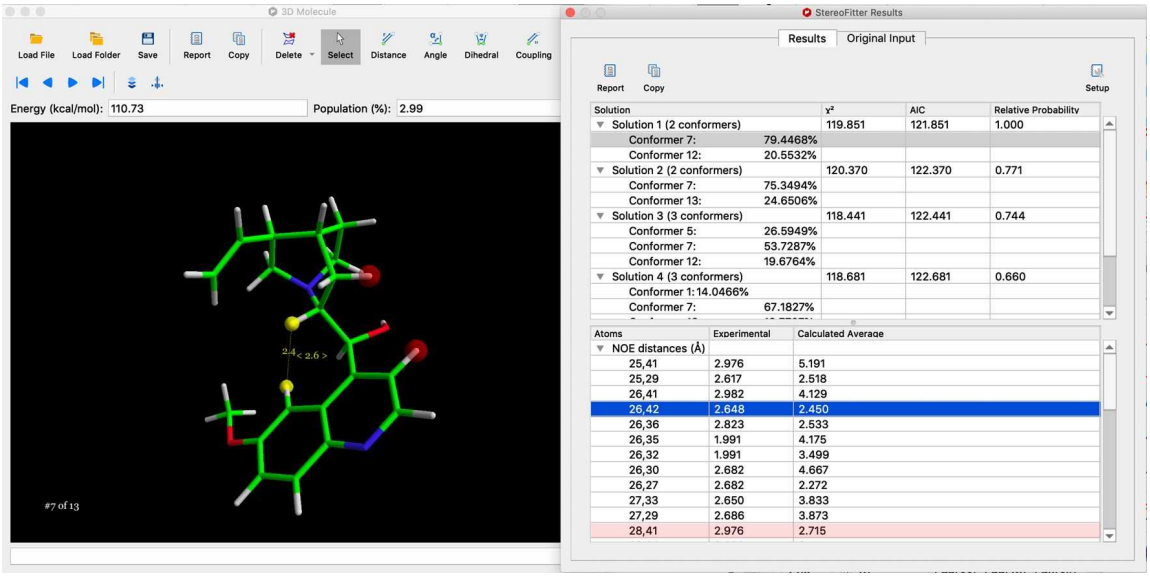


More resources can be found here

19.10 Stereofitter

This advanced plugin needs a specific license and an independent installer. You can download it from here. To install it, just run Mnova and load the zip file containing the xml installer.

StereoFitter computes the probability of 3D structural configurations and/or conformations, based on various forms of experimental data input. Currently, StereoFitter can accept four distinct types of input in order to calculate the best 3D structure candidate(s): NOEs, RDCs, Js, and/or chemical shifts.



Information can be supplied from one or more of the techniques in parallel, and it is this potential for combining information from different techniques in one calculation that makes StereoFitter uniquely powerful. Theoretical measurements of configurations/conformers are compared to experimental ones, each given a score, and presented in order of best fit.

StereoFitter starts with a set of possible 3D configurations and/or conformations of a molecule. These can be imported from external programs, or generated within StereoFitter using Balloon or GMMX. All possible stereochemical isomers can also be computed within StereoFitter from a 2D structure if the stereochemistry of any chiral centers are not known. Various formats of external structure file formats are compatible: SDF; XYZ; MAE. 3D structures can be viewed, manipulated, and their properties measured within StereoFitter using an interactive tool.

Chemical shifts can be computed within StereoFitter using results from DFT calculations. The calculations are done on an external server, due to the intense computational requirements required to make calculation times more acceptable.

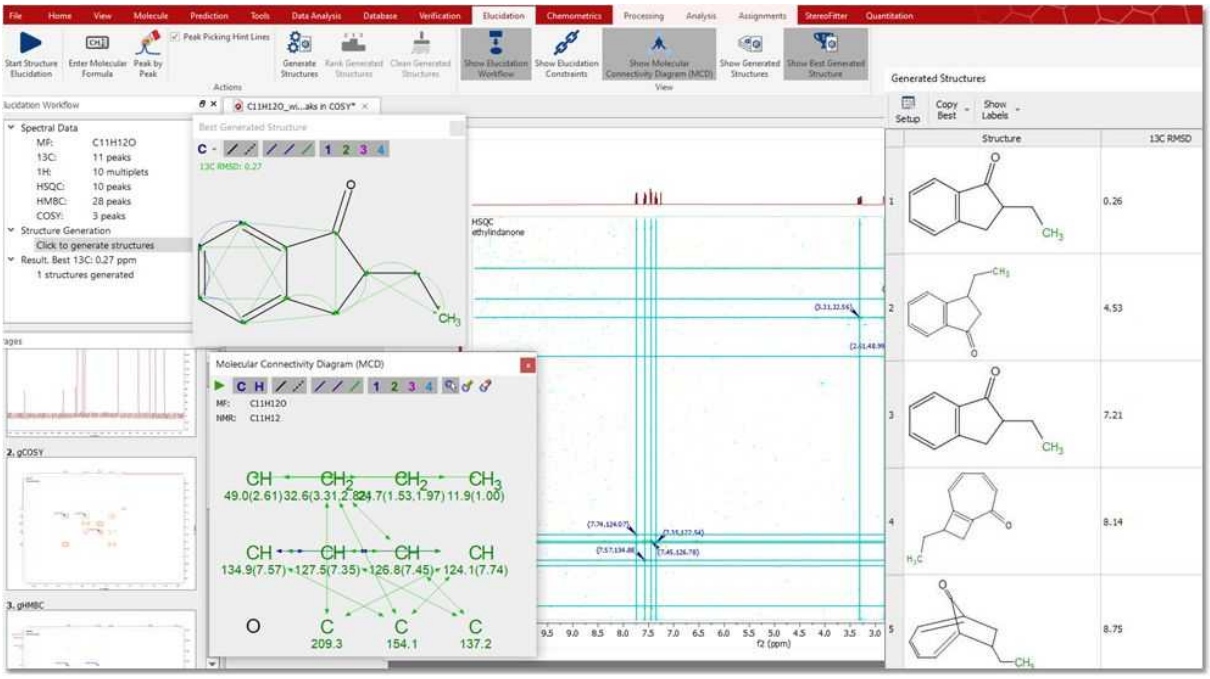
For further information and a complete manual of this plugin, please check this link and this one.

19.11 Structure Elucidation

This advanced plugin needs a specific license.

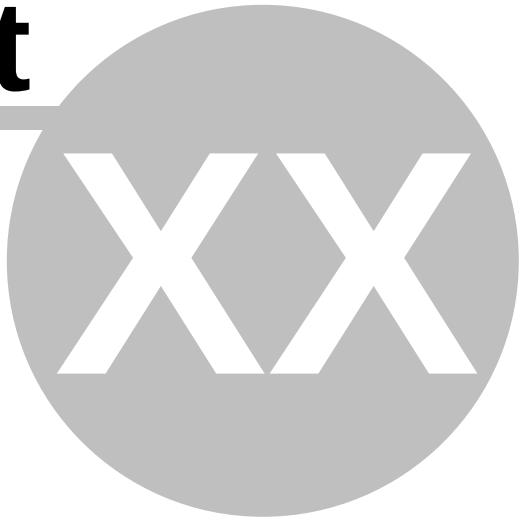
Speeding up the elucidation workflow and making them available to non-expert NMR users. It combines latest advances in 1D and 2D NMR peak picking strategies (GSD deconvolution, filtering, smoothing), new approaches to constraint generation and handling and the latest methods for structure generation based on the constraints.

© 2024 MESTRELAB RESEARCH



Further information can be found here

Part



20 Tutorials

The goal of the present chapter is to give you a fast introduction to the basic analysis tools which are commonly used to solve structural problems. Not everything is explained in detail; the rest of the manual provides a more comprehensive description of all topics covered here.

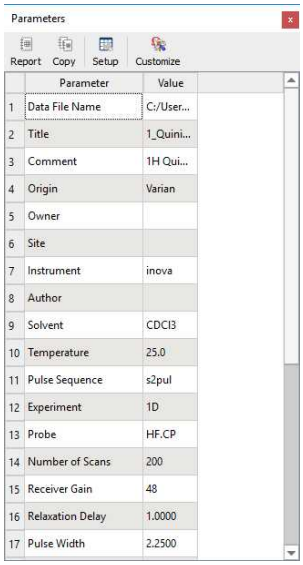
20.1 Importing Spectrum Parameters

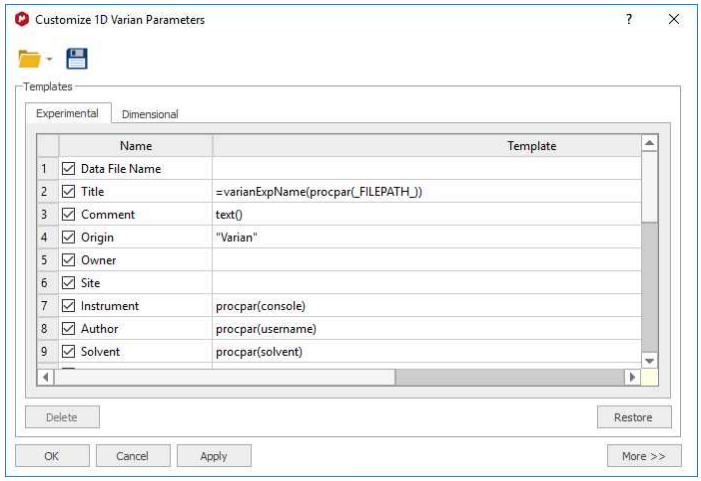
(Not available in Mnova Lite)

The 'Importing Spectrum Parameters' feature will allow Mnova users to include any variable into the Parameters Table from the raw data file. In addition, the users will be able to create 'Parameters Templates' in order to obtain the same parameters in all of their processed spectra. Before this feature was implemented, only standard spectrum parameters could be included in the corresponding table.

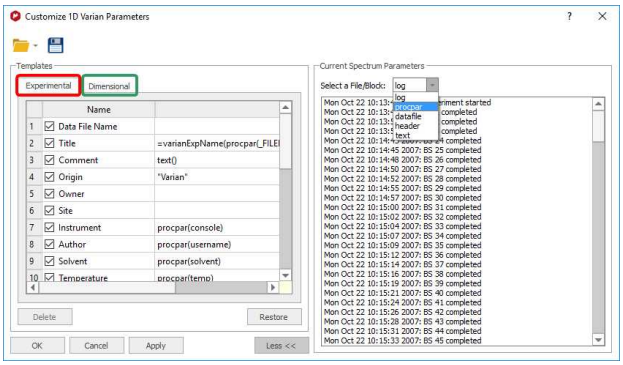
The user will easily be able to add, create, remove or modify any spectrum parameter or even to create templates in order to obtain the same parameters in any processed spectra.

To customize the parameters table just follow the menu: View/Parameters and click on the ‘Customize’ icon to display the 'Customize Parameters’ dialog box.



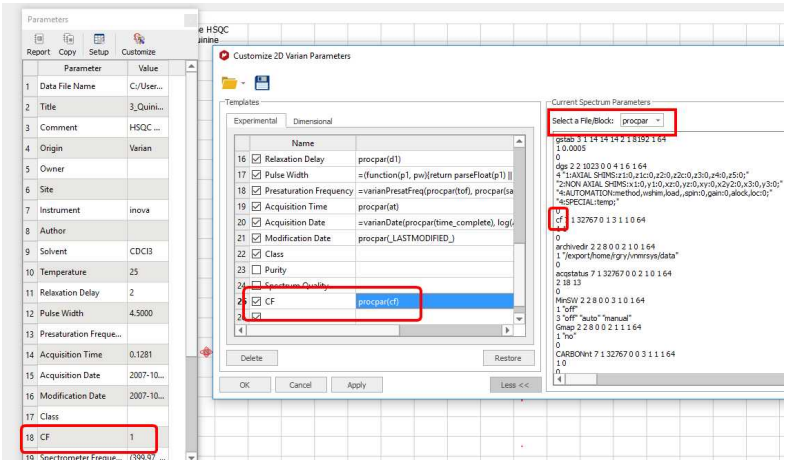


Clicking on the 'More>>' button will display the 'Current Spectrum Parameters' window, as you can see in the picture below. The user will be able to obtain any parameter from any raw data file (procs, acqus, title, etc...) by selecting the desired file on the 'File/Block' box (see the picture below):



In the 'Customize Parameters' dialog box, the user will see an ‘Experimental Parameters Template’ (red square in the picture above) which will be common for 1D and 2D (with information about: Title, Origin, Owner, Solvent, Temperature, etc.) and the ‘Dimensional Parameters Template' (green square in the picture above) with information about the 'Spectrometer Frequency', "Acquired Size', 'Spectral Width', etc.). Both sections have blank rows in their last position to allow for addition of further parameters.

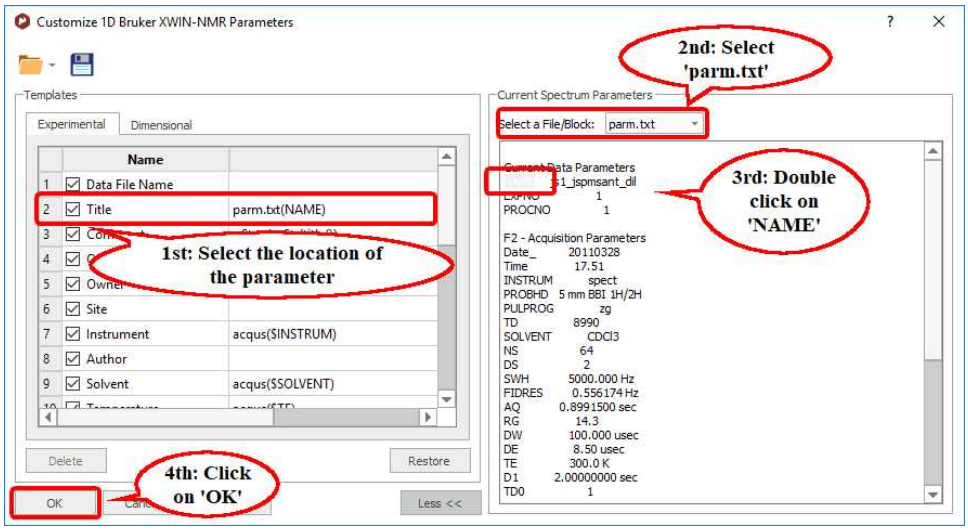
You can easily add any existing parameter from the raw data. Start by selecting the location of the parameter in the Experimental or Dimensional Parameters Template by clicking on the relevant cell of the 'Template' column on the table (cell number 25 in the screenshot below). Once this has been selected, double click on the desired parameter (from the 'Current Spectrum Parameters' box; procpar in the example below). This will add the chosen parameter (CF in the screenshot below) to the template.



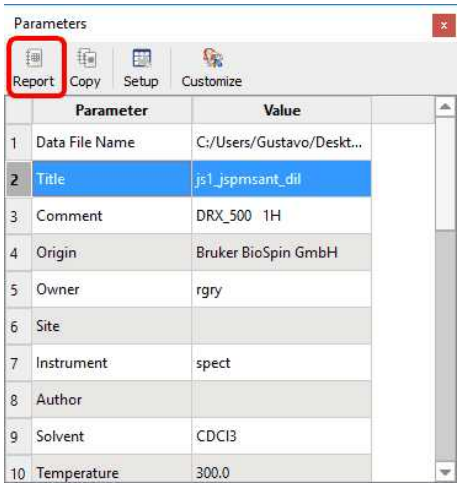
Let´s see now **how to add the title from the ‘parm.txt’ file** (Bruker):

1st: Select the location of the parameter in the Experimental Parameter Template.
2nd: Select ‘parm.txt’ in the ‘Current Spectrum Parameters’ combo box.
3rd: Double click on the desired parameter (NAME, in this case).
4th: Click on ‘OK’ to add the parameter to the template.

© 2024 MESTRELAB RESEARCH



Then, you will obtain the title on the parameters table:



Finally you will be able to add the Parameters Table to the spectrum by just clicking on the 'Report' button (highlighted in red in the picture above).

You can use the first line of a title which contains several lines. To do it, just type this function under the parameter Title template: =(function(aTitle){return aTitle.split("\n")[0];})(title())

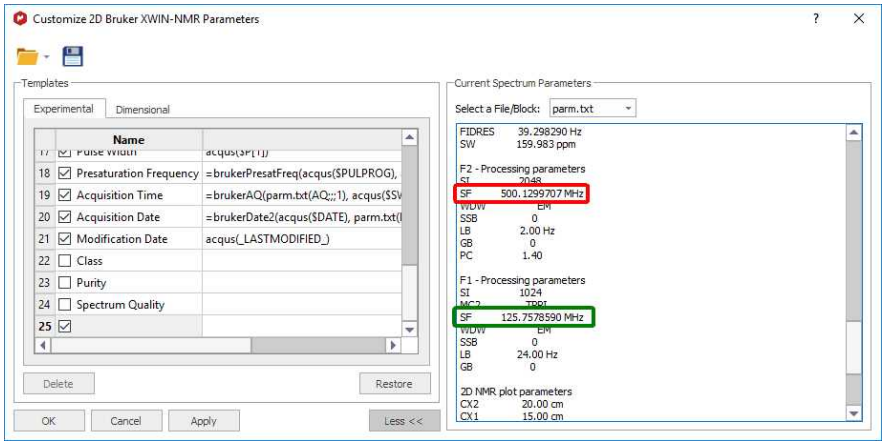
This is the way to add the 'easy' parameters to the 'Parameters Table'. However, there are other parameters which need a different procedure to be added from the 'parm.txt' file to the table. For example, some parameters with the same name i.g. for 2D can be written two times (for example the Spectral Frequency for f1 and for f2). Also some parameters have units. The syntax of the *parm.txt* file parsing allows handling both situations (allowing either to get a number, or a number with the units, or even only the units).

Please bear in mind that the syntax of the 'parm.txt' parsing is:

parm.txt(PARAMNAME[;OCCURENCE;FIRST_WORD;NUMBER_OF_WORDS])

© 2024 MESTRELAB RESEARCH

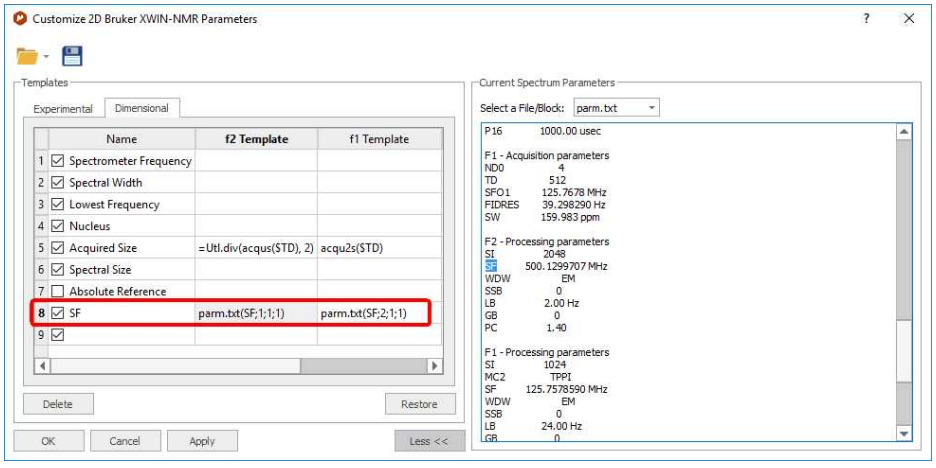
Let´s see how it works with a real example where we want to include the Spectral Frequency value for f1 and f2. As you can see in the picture below there are two values for the parameter 'SF' in the *parm.txt* file (one for f2 and another for f1):



We can not follow the same procedure that we followed a few pages before to insert the title on the parameters table, because now, we have two different parameters (one for f2 and another for f1) with the same name (SF).

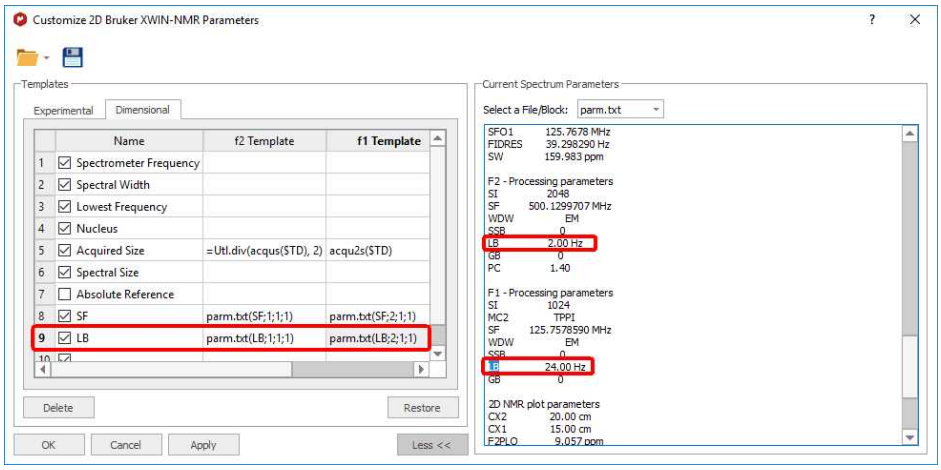
For this reason we need to use the syntax of the *parm.txt* parsing, typing in the 'f2 Template' cell: **parm.txt(SF;1;1;1)** where **SF** is the name of the parameter, the first number ('1') indicates the order of appearance (The f2 parameter appears before in the parm.txt file than the f1). The second and the third number indicate the first word and the number of words of the string, respectively. In this case, the parameter has two words: the value (500.1299707) and the unit (MHz), but we are not interested in getting the units, so type (SF;1;1;1). If we were interested in also include the units, we must type: (SF;1;1;2).

In the 'f1 Template' cell; we would type: **parm.txt(SF;2;1;1)**, because the SF parameter for f1 appears in the second position (after the SF value for f2):

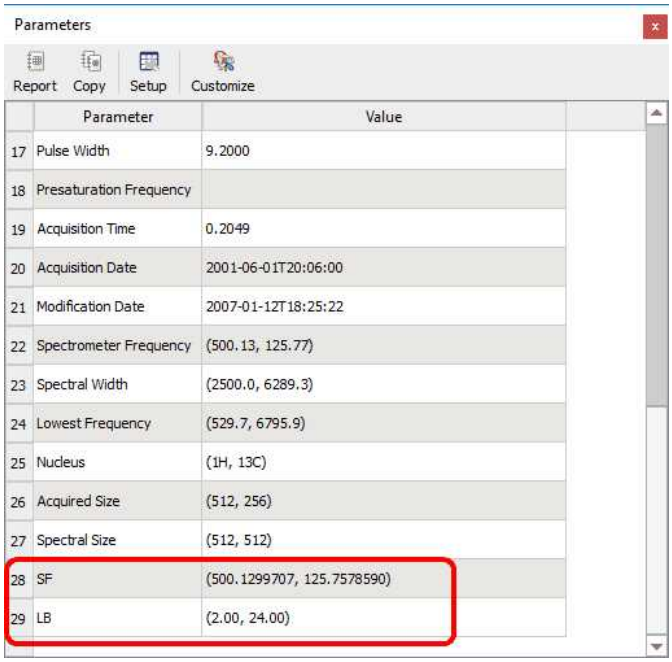


Similar procedure will be followed, for example, to include the LB parameter for f2 and f1, as you can see in the picture below:

© 2024 MESTRELAB RESEARCH



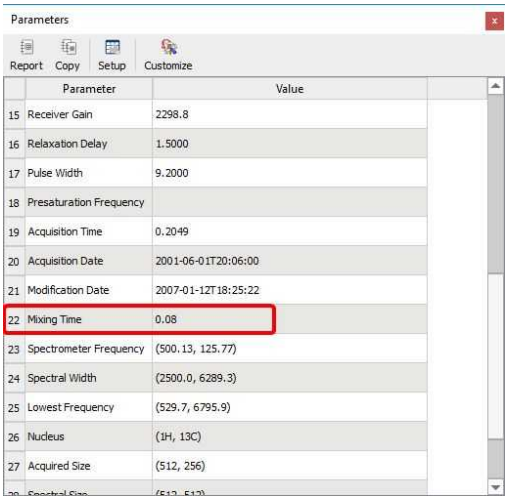
And here it is the result:



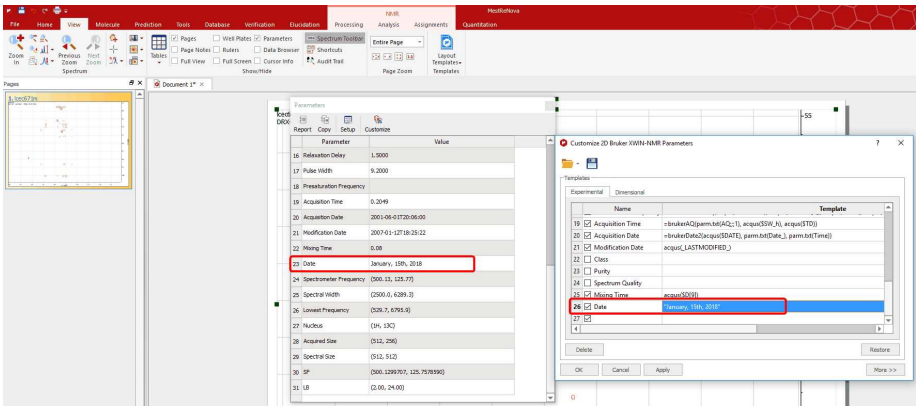
Imagine, that you have a parameter which appears in the middle of another parameters. For example the 'Mixing Time' parameter of a NOESY spectrum (from Bruker); which appears in the acqus file with this format:

```
##$D= (0..63)
3e-06 0.9643645 0 0 0 0 0 0 0 0.06 0
0.03  2e-05  3e-06  0  0  0.0002  0  0
0.00025
1e-05 1e-05 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0
```

You want to display in this case, the D9, so you will need to type **acqus($D[9])** in the Parameters template list as it is shown in the picture below:



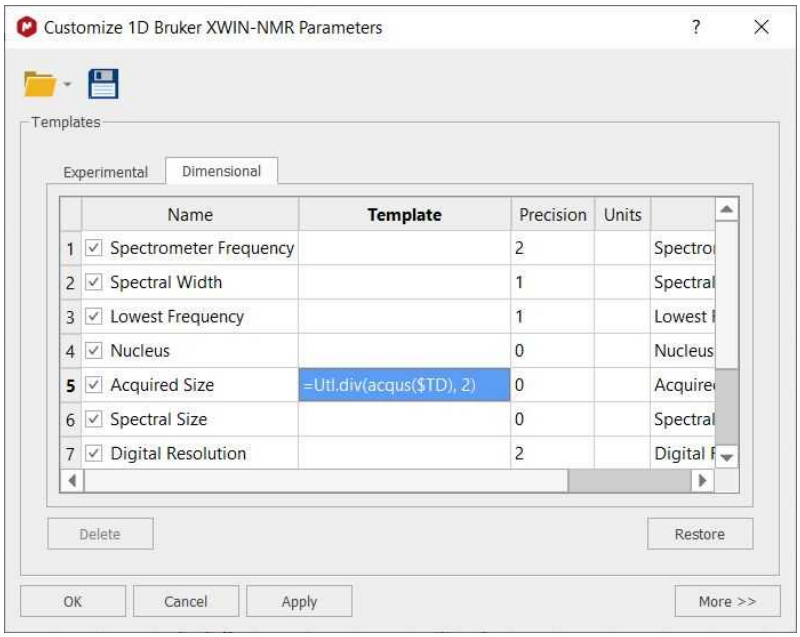
The user will also be able to add his/her own parameters by double clicking on the table and typing the corresponding parameter name (between "quotation marks") and value. In the picture below, we have added manually the text "January, 15th, 2018" in the field: 'Original Name' and 'Date' as the 'Visible Name'



The user will also be able to introduce formulas (available in the script) by typing an *equal* symbol followed by the desired formula (numbers or parameters), for example: =sum(a, b), or =div(acqus($TD), 2). Please note that formulas are case sensitive and should be lower case.

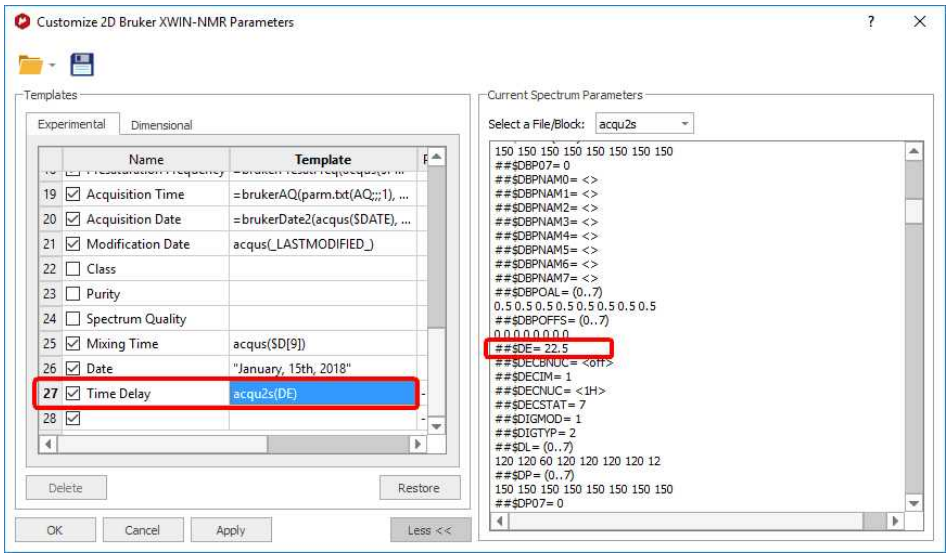
For example, by default, the Acquired Size value is obtained by dividing the TD value by 2 (as TD is the total number of real and imaginary points), so we are using this template for the calculation *=Utl.div(acqus($TD), 2)*

© 2024 MESTRELAB RESEARCH



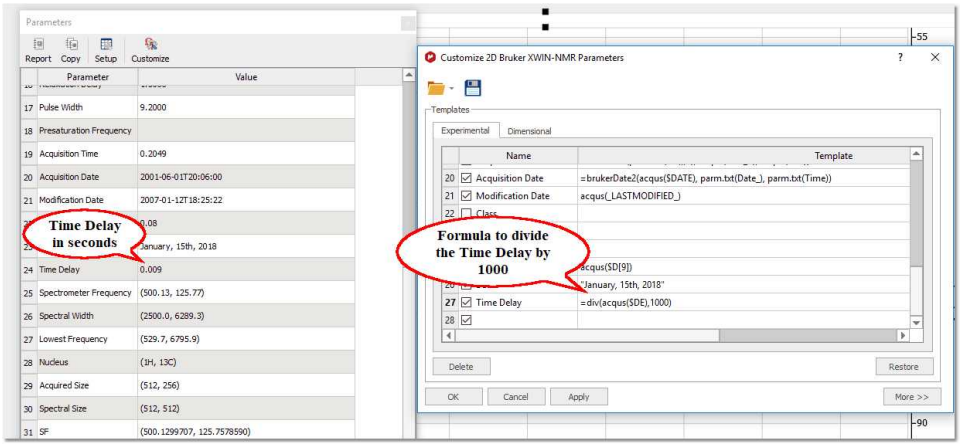
but if for some reason, you want to display just the TD value as Acquired size (instead of dividing the TD value by 2), you can leave the template as this: *acqus($TD)*

The following example shows the procedure to show the 'Time Delay' in seconds. Firstly, we need to double click on the desired parameter in the 'Current Spectrum Parameters' list to add it to the 'Parameters Template':



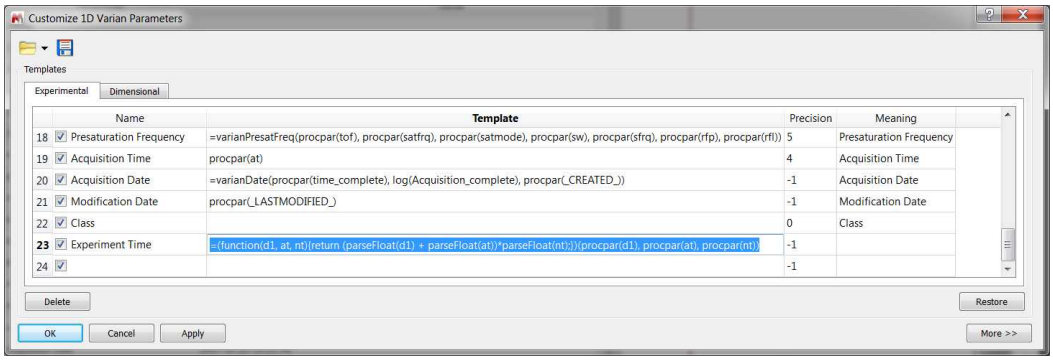
By default the Time Delay is showed in milliseconds, so we need to divide the value by 1000 to obtain it in seconds. So, the user will need to type '=div(acqus($DE),1000)' in the 'Parameters Template' to get the division. In this way the user will obtain the 'Time Delay' value in seconds in the Parameters Table, as you can see in the picture below:

© 2024 MESTRELAB RESEARCH



To get the **'Experiment Time'** in a Varian dataset; obtained by adding the '*d1'* plus the '*at'* and multiplying the result by the *'nt'*, we could use this function:

=(function(d1, at, nt){return (parseFloat(d1) + parseFloat(at))*parseFloat(nt);})(procpar(d1), procpar(at), procpar(nt))



The parameters will be hidden in the table display if the user removes the corresponding tick mark on the 'Visible' Column. The user can also delete any parameters from the table by highlighting each of them and clicking on the 'Delete' button.

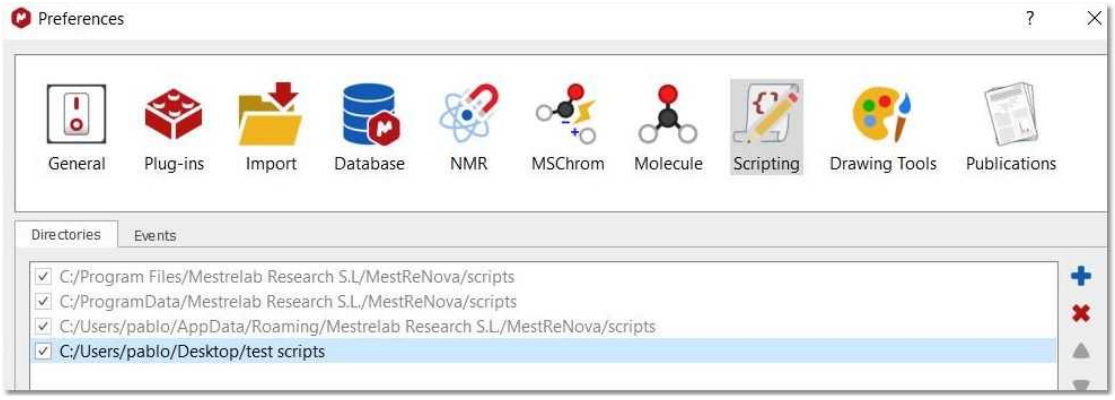
The 'Restore' button is used to recover the default template. The user will be able to save his/her own template by clicking on the 'Save Template' icon . To load a template, just click on the 'Open Template' button . Clicking on 'OK' will save the template in the registry and Mnova will remember it for futures uses. The 'Apply' button is used to view the effect of any changes directly on the parameters table without needing to close the 'Customize Parameters' dialog box.

You can also use the scripting engine to parse any parameter from any other file of the raw dataset. For example, the script below will be useful to parse the ICON-NMR user from the audita.txt file:

```
function iconNMRUser(aFilePath) {
    "use strict";
    var newPath = aFilePath.replace(/\\/g, "/"),
        f, str, audita = "", re=/^\s*ICON-NMR User ID:\s+(.+)$/gm, result;
    f = new File(newPath);
    f.open(File.ReadOnly);
    str = new TextStream(f);
    audita = str.readAll();
    f.close();
    result = re.exec(audita);
    if (result && result.length>1) {
        return result[1];
    }
    return "";
}
```

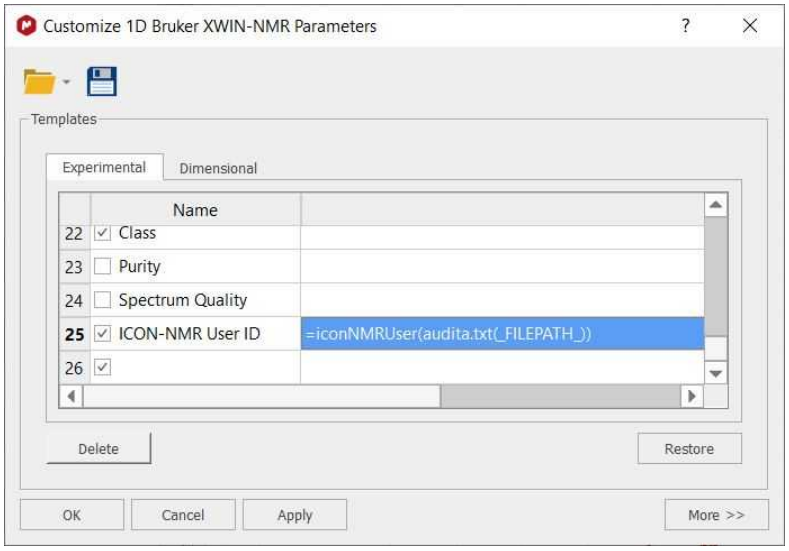

© 2024 MESTRELAB RESEARCH

To use it, copy the script into one of your folders in your hard disk. Next run Mnova, follow the menu 'File/Preferences/Scripting' and add that folder to the scripting directories:

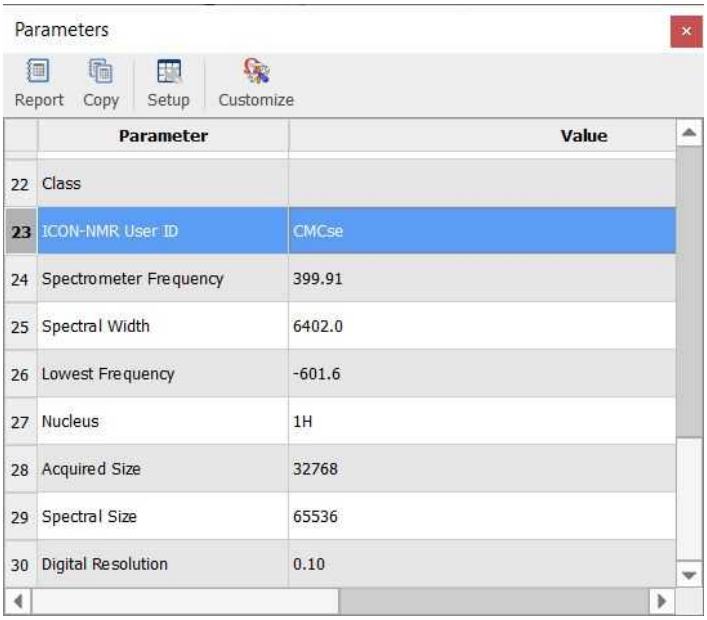


Next, restart Mnova, load your NMR dataset and click on the 'Customize' button of your Parameters Table to add the new Parameter with this template:

=iconNMRUser(audita.txt(_FILEPATH_))



Click on the OK button and you will find the new parameter added to your table:



See also:

Scripts

20.2 Basics on Arrayed-NMR Data Analysis

In this tutorial we shall cover some basic concepts on the analysis of a very important class of NMR experiments, the so-called Arrayed NMR spectra. The concept is very simple: an arrayed experiment is basically a set of individual spectra acquired sequentially and related to each other through the variation of one or more parameters and finally grouped together to constitute a composite experiment. These experiments are also known as 'pseudo-2D'. For example, in the case of Bruker spectra they have the same file name as 2D spectra, that is ser files (ser = serial spectra) . In the case of Varian, the file name is fid (Varian uses the same name for 1D, 2D, 3D, … and arrayed spectra). However, unlike with actual 2D spectra, arrayed spectra are only transformed along the F2 –horizontal or direct- dimension (assuming 1D arrayed spectra only).

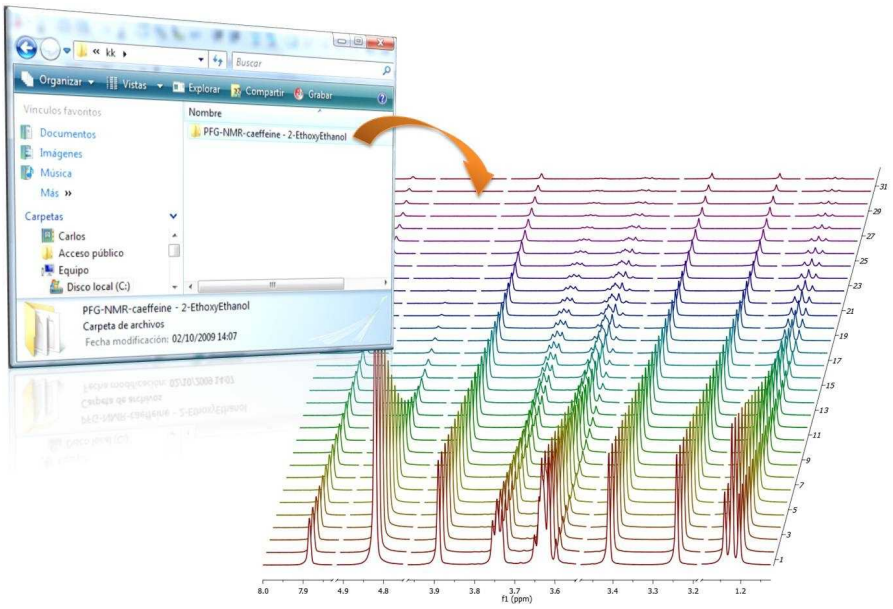
The modus operandi is better explained with an example: let's suppose it is necessary to acquire a pulse field gradient (PFG) experiment. Instead of acquiring independent spectra, it is more convenient to create an array with increasing PFG amplitudes. All resulting spectra are now treated as a single experiment. This grouping greatly facilitates processing as, in general, all subspectra require the same processing operations (apart from some occasional minor adjustments of one or several spectra).

Well known examples of NMR arrayed experiments are, among others:

- Relaxation (T1, T2)
- PFG experiments (DOSY)
- Kinetics and reaction monitoring by NMR

The figure below illustrates the results obtained when a Bruker arrayed folder is dragged and dropped into Mnova:

© 2024 MESTRELAB RESEARCH



What has happened here are basically 2 things:

1. First, Mnova detects that the dropped folder contains an arrayed experiment

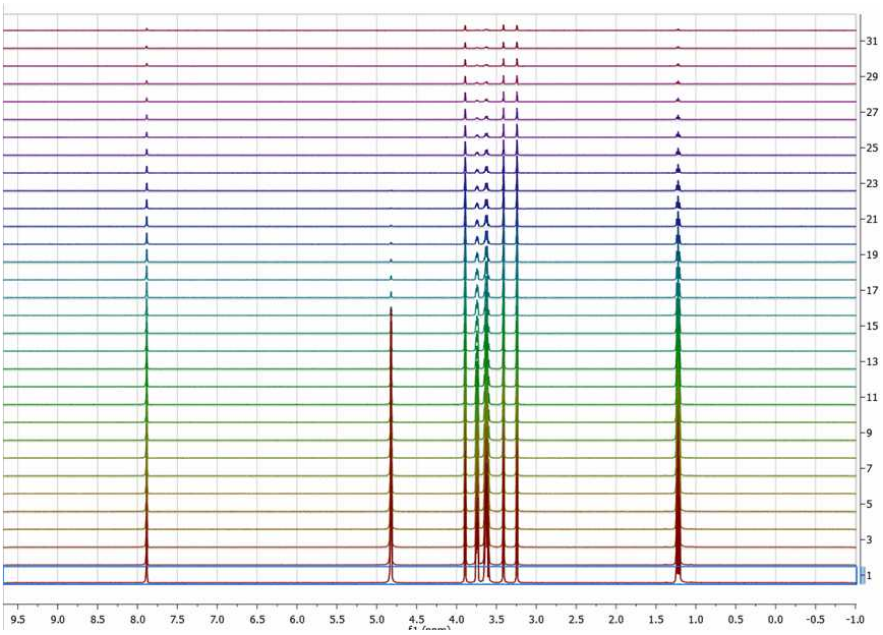
2. With that knowledge in hand, Mnova proceeds to process all the individual spectra, one after the other and of course, along the only valid dimension (F2). So for every spectrum, Mnova applies appropriate weighting, zero filling, FT, phase correction, etc and stacks all the spectra as shown in the picture above

As a result, all individual spectra grouped within one composite item (i.e. arrayed item) have been processed in the same way. However, it's very common that some subspectra might require independent tuning. For example, many PFG NMR experiments present gradient dependent phase shift so that it becomes necessary to adjust the phase of some individual spectra separately. This is very easy to accomplish with Mnova as you will see below.

Practical hints

When an arrayed experiment has been detected, all subspectra are grouped together and plotted in the stacked display mode in Mnova (see figure below).

© 2024 MESTRELAB RESEARCH



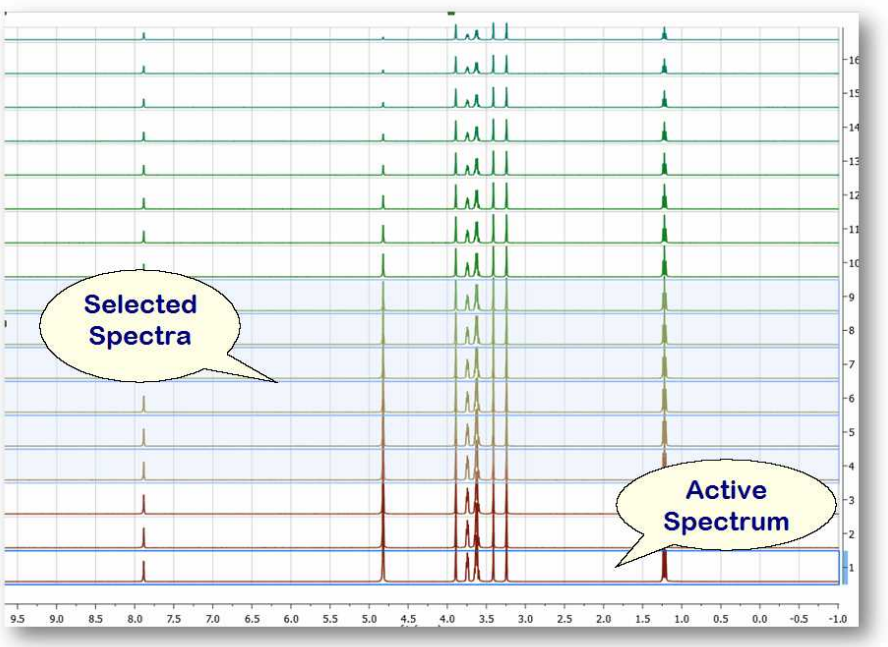
Several points are worth mentioning:
(A). Take a look at the blue box in the figure above: it shows the so-called 'active spectrum'. What does this mean?

The concept of active spectrum is easier to illustrate with the following example: as we said before, in general all the spectra in an arrayed item are processed exactly with the same processing operations. For example, same level of zero filling, same apodization function, same FT type, same phase correction, etc. However, it i's possible that some particular spectra require a slightly different processing, independently from the others. In order to do that, it is necessary to select the applicable spectra. See below the difference between selected and active spectrum:

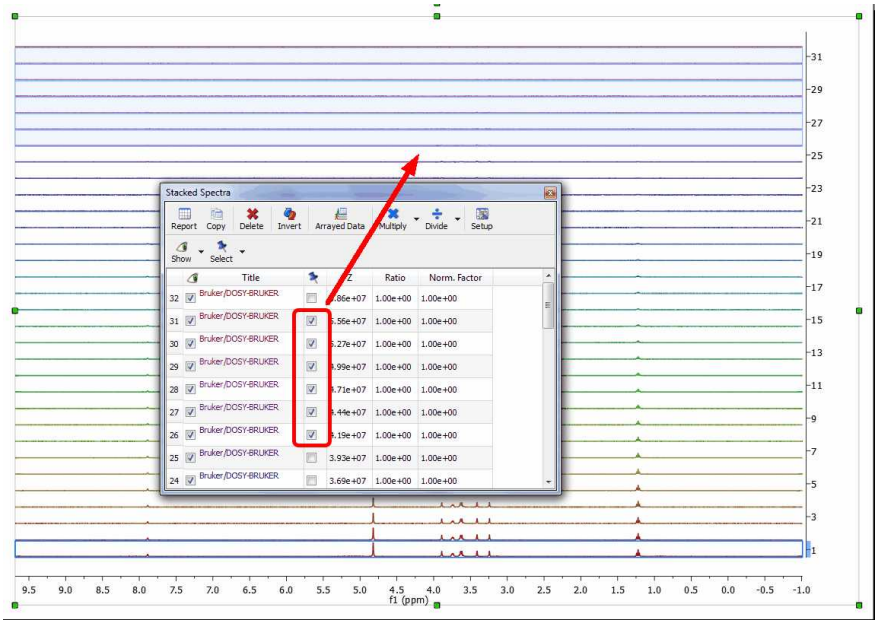
Selected spectra: when the spectra are selected, they are highlighted with a light blue background. Any processing operation will take place only for those selected spectra

Active Spectrum: active spectrum is marked with a blue box and it indicates the spectrum which will be displayed in the *active spectrum* display mode. It's also the reference spectrum used in some processing operations (e.g. alignment)

© 2024 MESTRELAB RESEARCH

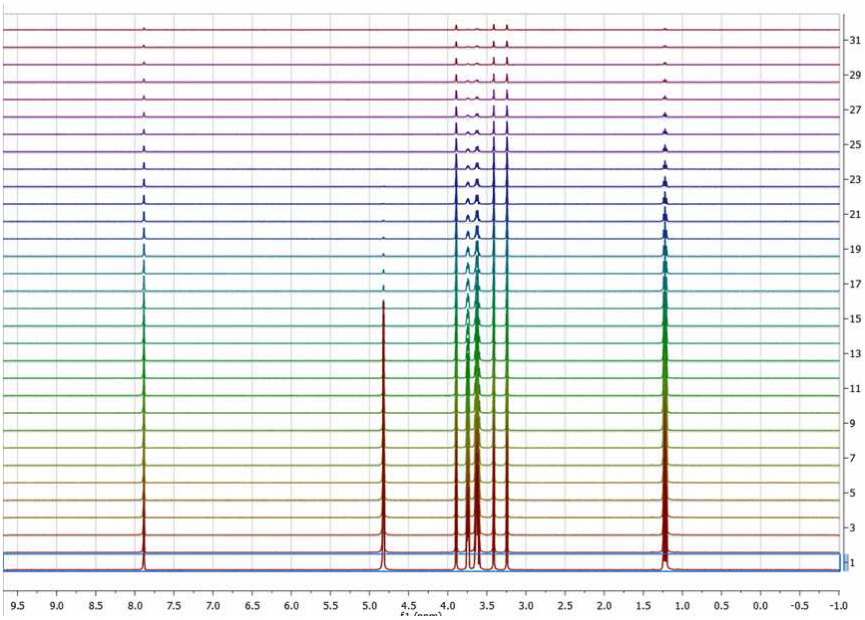


You can also **select** several slides by using the check boxes on the right of the 'Stacked Items Table'. When the spectra are selected, they are highlighted with a light blue background. Any processing operation will take place only for those selected spectra.

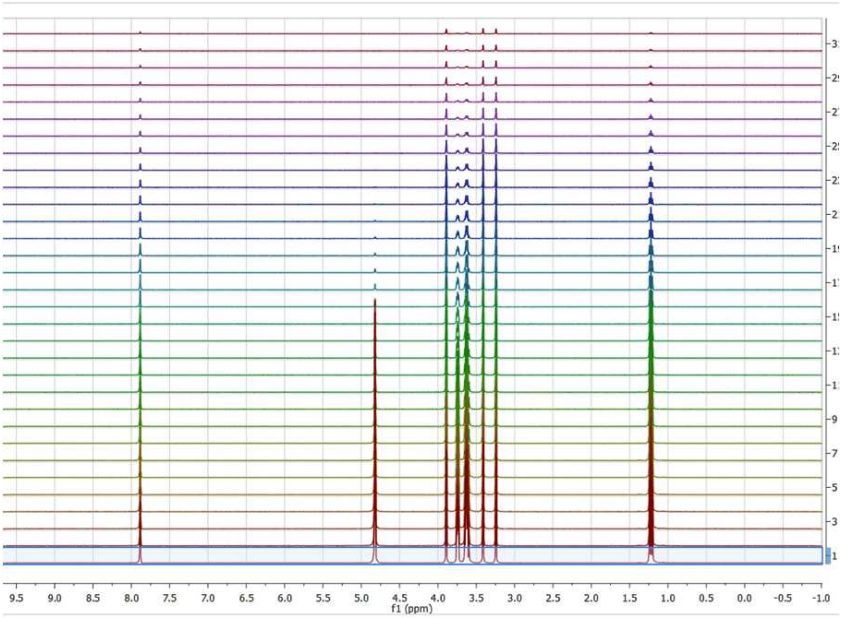


Example #1:
If there are no spectra selected and the active spectrum is not either selected (i.e. no spectra have a blue background) then ***any processing operations will be applied to ALL spectra (***Active spectrum is not selected)

© 2024 MESTRELAB RESEARCH

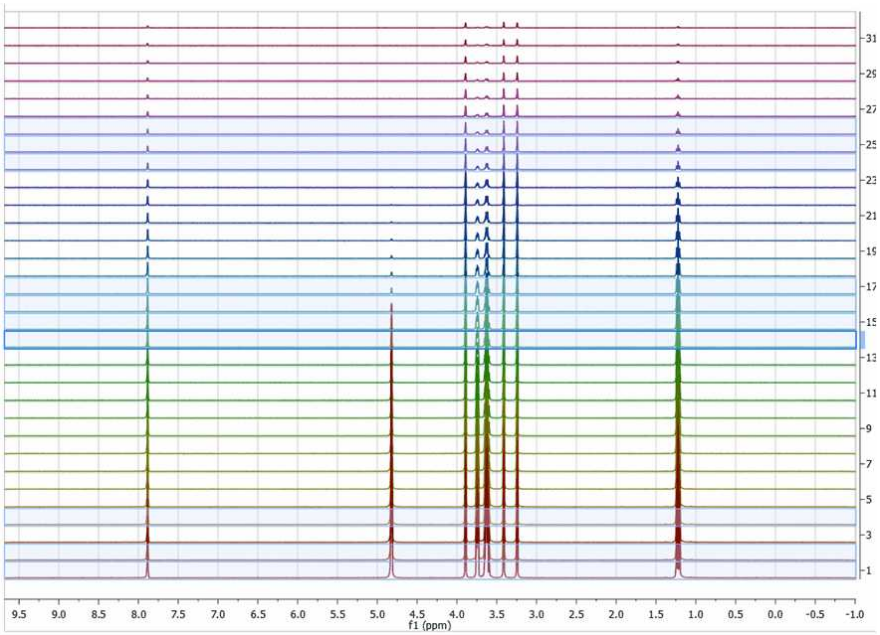


Example #2:
If only the active spectrum is selected, ***any processing operations will be applied ONLY to the active spectrum.*** (Remember, to select a spectrum: left click + Alt)



Example #3:
In general, ***any processing operation will be applied only to the selected spectra*** (and remember that if no spectra are selected, All spectra will be processed):

© 2024 MESTRELAB RESEARCH



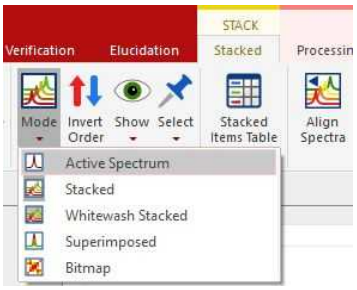
So, how can we change the active spectrum? There are 3 different ways:

1. Just click on the spectrum you want to be active.

2. Use SHIFT + Mouse Wheel to navigate throughout all the spectra in the stack, one after the other.

3. Use SHIFT + Up/Down arrow keys. This is analogous to point 2)

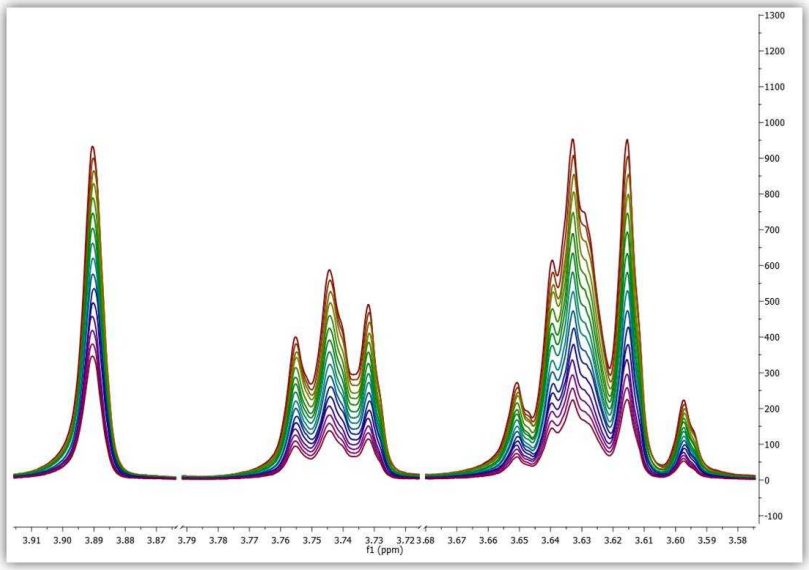
(B). If the number of subspectra (traces) is large (e.g. > 10), working in the stack mode might not be very practical. Quite often, working only with the active spectrum on the screen will be a much better option. This mode can be activated as shown in the image below.



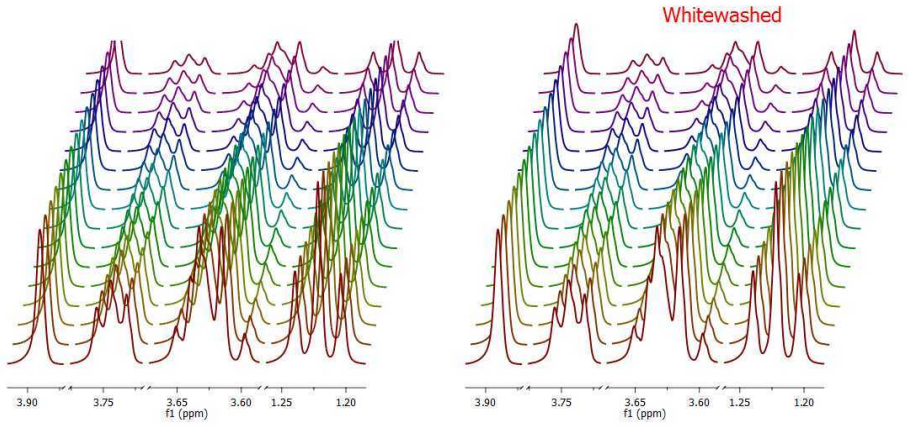
While working on this mode, you will see on the screen just the active spectrum. Should you want to move to another spectrum without resorting to the stack mode, just use methods 2) and 3) described above (Shift+Mouse Wheel or Shift + Up/Down keys).

(C) Another useful display mode consists of superimposing all subspectra (see figure below). This method is very useful, for example, when you want to check whether some peaks shift their position (for instance, due to differences in pH, temperature, etc., as is common in biofluids spectra).

© 2024 MESTRELAB RESEARCH



Finally, there is an additional display mode, the so called whitewashed stacked plot. The whitewashing effect means that the spectra at the front of the display hide the spectra behind them from view, as depicted in the figure below.



This plotting mode can be useful to create nice reports, but it's important to emphasize that drawing time will be significantly higher than with the other plotting modes, so it is not recommended when processing the spectra in real time (e.g. interactive phase correction).

Extracting and calculating useful NMR related molecular information

After the basic introductory lines on arrayed NMR experiments, it's now time to get some action and see how to extract relevant information from these experiments and calculate useful NMR related parameters such as diffusion, relaxation times, kinetics constants, etc.

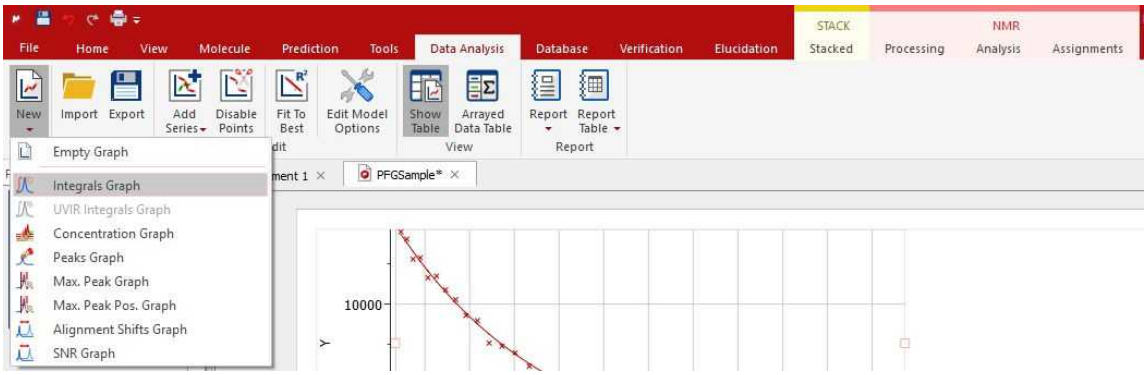
© 2024 MESTRELAB RESEARCH

Actually, in this tutorial we will cover the first case, that is, the analysis of PFG experiments to calculate diffusion coefficients.

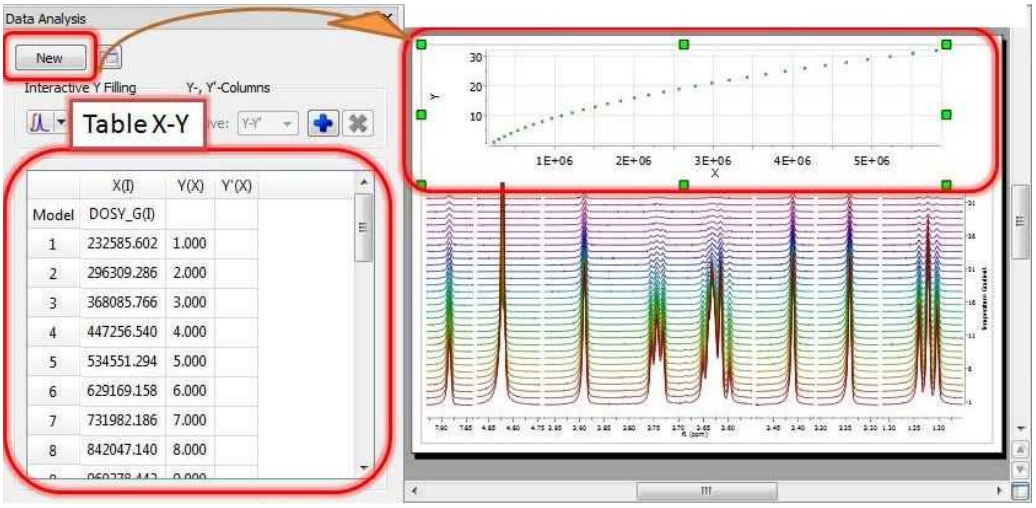
This is how a PFG (Pulsed Field Gradient) experiment can be analyzed with **Mnova** with the Data Analysis module (for your information, Mnova includes a DOSY-like processing algorithm based on a Bayesian Algorithm. For further infomration about it, please take a look at this tutorial and this post.

You can download the full PFG data set used in this tutorial from here.

Once the arrayed spectra has been loaded into Mnova, open Data Analysis. This will be the central control panel (see figure below) for anything related to the analysis of arrayed experiments.



Its operation is very simple. The first thing you have to do is click on the New button. As a result, Mnova will populate the X-Y Table with some initial values (as described below) and create a new item, the so-called Data Analysis Plot. This new item will display the values from the X-Y Table which in general are the values extracted from the arrayed spectra, both experimental (Y) and fitted (Y').



The X-Y Table

This table is composed by one X column, X(I), one or several Y-columns (Y, Y1, Y2, etc) to hold the experimental values extracted from the arrayed spectra and one or several Y'-columns which hold the fitted values of their Y counterpart columns.

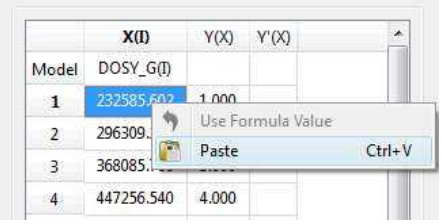
X-Column

When the table is initialized, in the case of PFG experiments the X-column is populated with the Z values from the Diffusion table, that is, the gradient strengths scaled by taking into account the constants from the selected Tanner-Stejskal model. In the case of a relaxation experiment, the X column will contain the time delays. Of course, it is possible to change the contents of the X-column by following any of these methods:

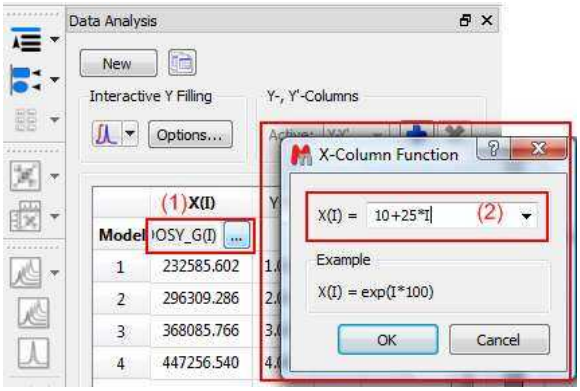
- Manual editing of the individual cells



- Copy & paste from a text file. For example, you can put the values for the X-column into a text (ASCII) file and then paste its contents into the table. To do that, just right click on the first cell you want the paste action to start from.



- Enter a formula into the Model cell. Double click on the X(I) model cell (1) and then enter the appropriate equation to populate the X column (2). For example, if you simply enter I, the X column will be filled in with numbers 1, 2,3, etc. If you enter a formula like 10+25*I, the X column will be filled with numbers 35,60, 85, etc.



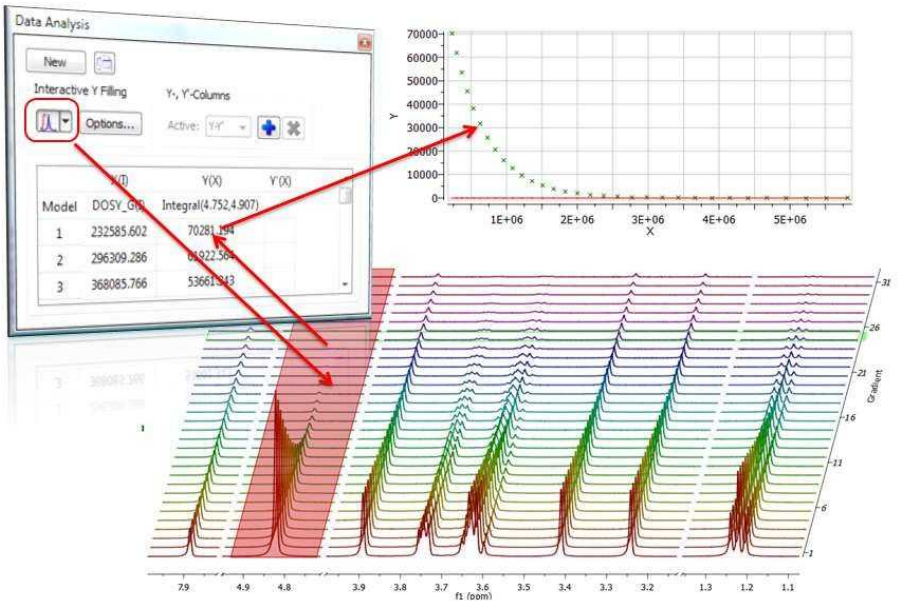
Y-Column

In all cases, when the table is initialized, the Y-column is automatically filled in with values 1,2,3, etc. The purpose of this column is to hold the experimental values from the arrayed experiment. For example, in the case of a PFG experiment, it may contain how the intensity (or integral) of a given peak (or set of peaks) evolves as the applied pulse field gradient changes. Likewise, in the case of T1/T2 experiments, this column will show the relaxation profile of a given resonance (or set of resonances). So the question is: **how to populate the Y-column with actual information from the spectra?**

© 2024 MESTRELAB RESEARCH

This is again very easy. There is a graphical way (mouse driven) and a manual one. Let's start with the graphical method:

- **Graphical Selection:** Click on the 'Interactive Y Filling' button (see red-highlighted button in the image below). After doing this, the cursor will change into an integral shaped cursor expecting you to select the region from where you want the integrals to be extracted across all the subspectra in the arrayed item. After the selection is done (see figure below), all the integrals will be placed in the Y column and those values will be displayed in the X-Y plot as green crosses (note: the shape, color, etc of these crosses can be customized from the X-Y plot properties).



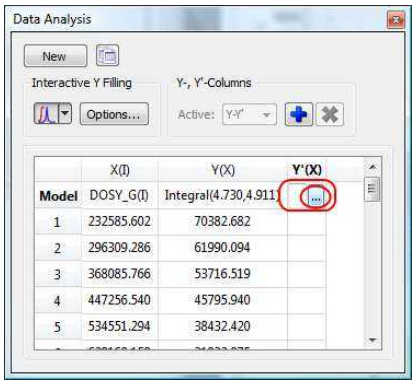
- **Manual selection:** if you take a closer look at the Data Analysis table in the figure above, you can appreciate that once the integral region has been selected, the program shows the following text: Integral(4.752, 4.907). This means that we have selected an integral covering that range. This value can be edited manually so that you can specify the limits by simply editing that cell.

Y'-Column

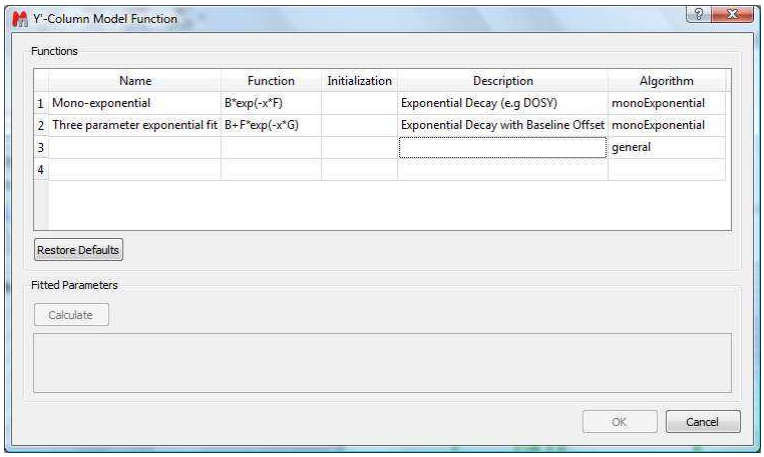
The Y' column is reserved for the fitted values assuming a particular theoretical model (e.g. a exponential decay). In this particular case, as we are dealing with PFG experiments, we will be interested in the calculation of the Diffusion coefficients and thus, our fitting model could be a mono-exponential decay (multi-exponential decays can also be handled with this module, but I will not address this problem in this post). The process is very simple:

First click on the Y'(X) cell and then on the small button with 3 points as indicated in the figure below:

© 2024 MESTRELAB RESEARCH

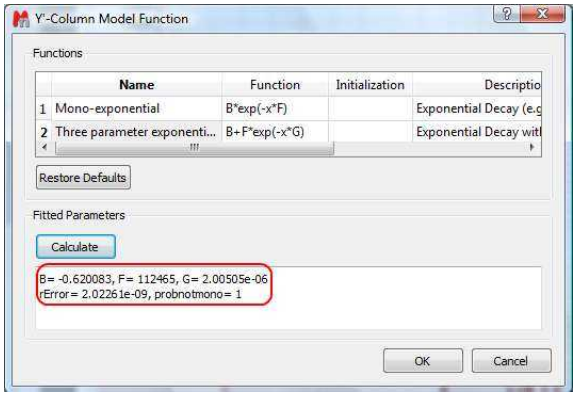


This will launch a dialog box with powerful fitting capabilities.



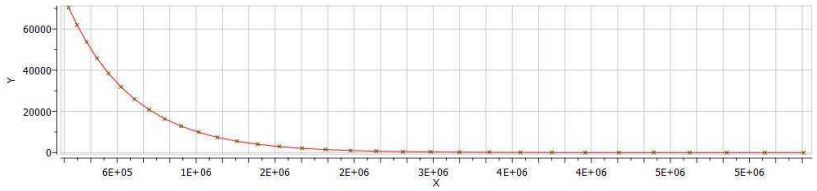
This dialog provides two predefined functions useful for fitting mono-exponential data (such as PFG and Relaxation NMR experiments) using either a 2- or a 3-parameter fit. Furthermore, this dialog offers the possibility to enter user customized functions.

For the time being, suffice to say that if your problem regards mono-exponential functions, just select any of the 2 predefined functions in the dialog box and click on the Calculate button. Mnova will immediately compute the optimal values, returning these optimal values as well as the fitting error and the probability that the acquired series follows the chosen monoexponential model.

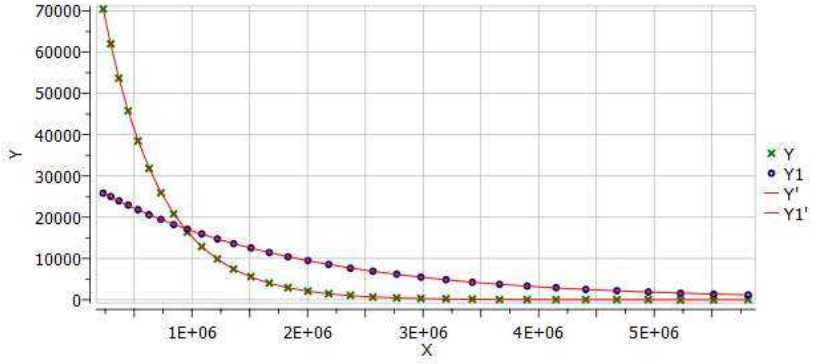


© 2024 MESTRELAB RESEARCH

Finally, after closing the dialog, it will populate the Y' column and the X-Y will be updated with the fitted curve (Red line in the figure below).



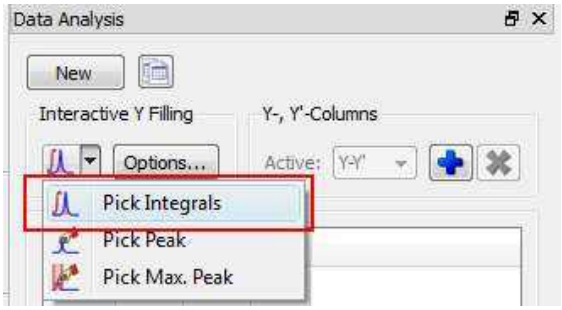
One nice feature of the Data Analysis module is its ability to handle multiple series. For example, it's possible to analyze the decay of several resonances within the same experiment. In order to do that, just click on the (+) button to add a new series and repeat the same process to select the desired resonance range and fit the values. For example, in the figure below I'm showing two curves with different decay rates.



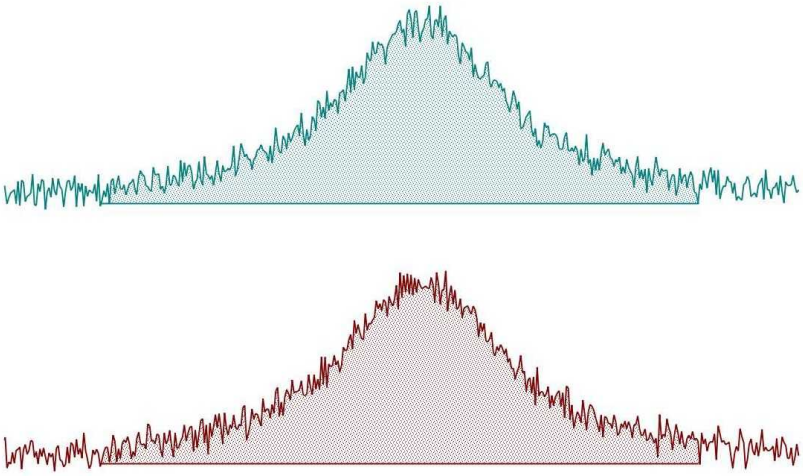
Of the different existing methods for the extraction of peak intensities from arrayed NMR spectra, Mnova provides the following ones:

(1) Peak area integration

This is the default method in Mnova Data Analysis module (see figure below)



This method consists of a standard numeric integration over the whole peak. Basically, the program is summing up all the points within the selected area of interest) as illustrated in the figure below:



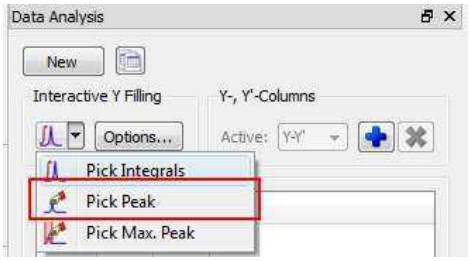
This figure has been created as follow: two identical Lorentzian lines (green & red) were simulated and then noise was added. The noise level is the same in both spectra but obviously, the actual numbers are different (more technically, noise in both spectra was calculated using a different seed in the random number generator).

This peak area method for data extraction is quite robust to noise (provided that the noise level is more or less constant across the different spectra in the arrayed experiment) and more importantly, insensitive to chemical shift fluctuations from trace to trace in the experiment, a situation which is more frequent than generally realized. For these reasons, and for its simplicity of use, this is method of choice for well-resolved peaks.

If the peaks of interest exhibit some degree of overlap, this method is not very reliable and some of the next methods will be more convenient

(2) Peak Height Measurement

This is the second method for data extraction (see figure below) and it finds the peak height at a given chemical shift across all the spectra in the arrayed experiment.



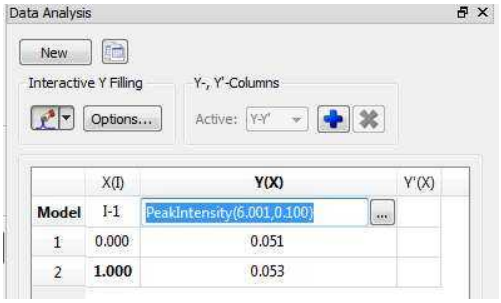
By default, the program will find the peak intensity at the position indicated by the user (using a vertical cursor) and then it will perform a parabolic interpolation in order to refine the value. In addition, the user can specify an interval in such a way that the program will find the maximum peak within that region. This can be done in 2 different ways:

© 2024 MESTRELAB RESEARCH

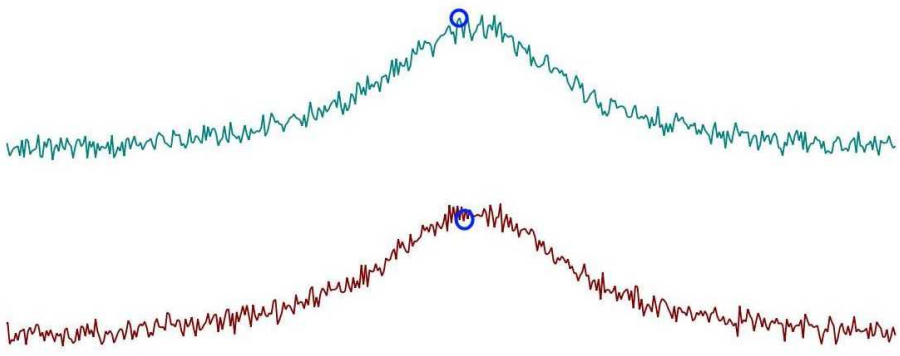
- i) If you click in the Options button, you can define whether you want to use Parabolic and the interval in which the maximum should be found (in ppm



- (ii) Alternatively, once a peak has been selected, you can change the interval by direct editing of the peak selection model. In the figure below, you can see how the peak selection model is PeakIntensity. The first number (6.001 in the figure) corresponds to the central chemical shift whereas the second number (0.100 in the figure) represents the interval for the peak maximum search.



Parabolic interpolation is useful because it minimizes the problems caused by the random noise. For example, let's assume that Parabolic interpolation is not used so that peak heights extraction will be done always at the same fixed chemical shift position (see figure below). When this method is used the values are seen to be quite different in the two cases: here the precision of the measurement will depend strongly on the noise.



Parabolic interpolation and/or measurement of the intensity as the maximum height within a fixed box around the peak will help to minimize the effects of movements on the chemical shift position of the

peaks due to, for example, temperature instability, pH changes, etc.

For convenience, Mnova includes the so-called Pick Max. Peak method which is totally equivalent to the previous one but it allows the graphical selection of the left and right boundaries in which the maximum peak will be searched for.

In a nutshell, peak height measurement can be used in those cases in which peak overlap might represent a problem. However, it should be noted that if for some reasons the line widths of the peaks under analysis change from trace to trace, peak heights will not represent a reliable measurement and peak integrals should be used instead.

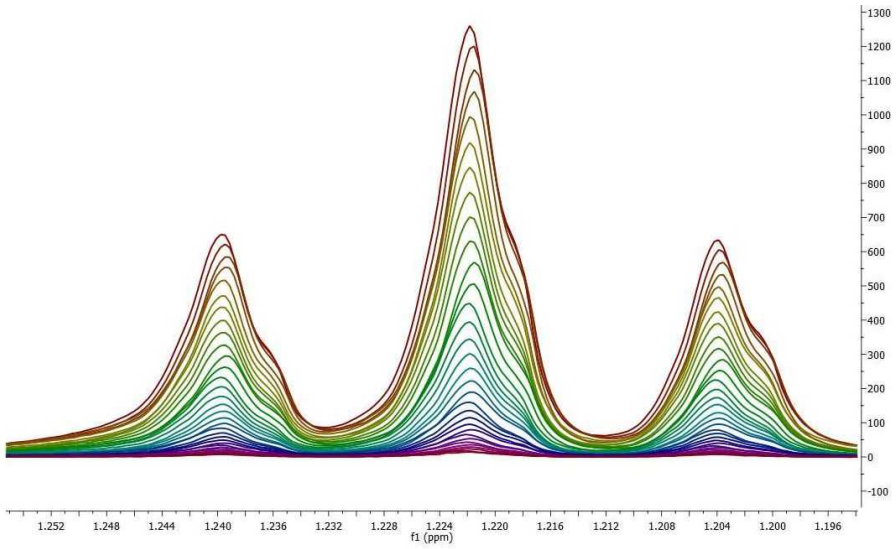
In general, we would recommend peak integrals as the most general-purpose method for quantitation of peak intensities in arrayed experiments.

Binning and NMR Data Analysis

It is well known that many NMR arrayed experiments suffer from unwanted chemical shift variations due to fluctuations in experimental conditions such as sample temperature, pH, ionic strength, etc. This phenomenon is very common in NMR spectra of e.g. biofluids (metabonomics/metabolomics) but also exists in many other experiments such us Relaxation, Kinetics and PFG NMR spectra (diffusion).

This problem negatively affects the reliability of quantitation using, for instance, peak heights, and for this reason integration is, in general, a more robust procedure as these spectral variations are mitigated by averaging data points over the integral segment. We will try to show you one simple trick which helps to understand, in a pictorial way, why integration is useful to remove the major part of chemical shift scattering.

First, consider the following experiment depicted in the figure below. It shows a triplet and as you can see, some minor peaks shifts are present from spectrum to spectrum:

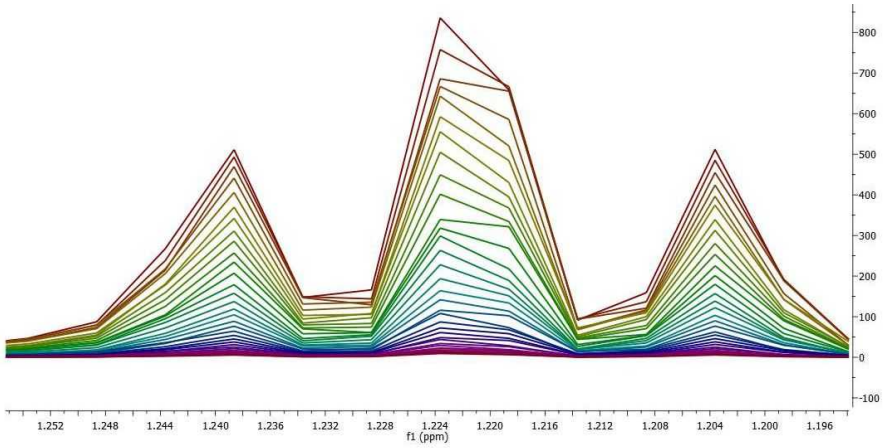


If peak heights are determined at a fixed position, this might introduce appreciable errors in the posteriori quantitative analysis (e.g. exponential fitting). As described before, this could be circumvented in some extent by using parabolic interpolation or peak searching of the maximum in a predefined box.

Nevertheless, integration is a very simple solution as can be appreciated in the figure below. Instead of using the Peak Integrals tool in the Data Analysis module, we will show now a complementary

© 2024 MESTRELAB RESEARCH

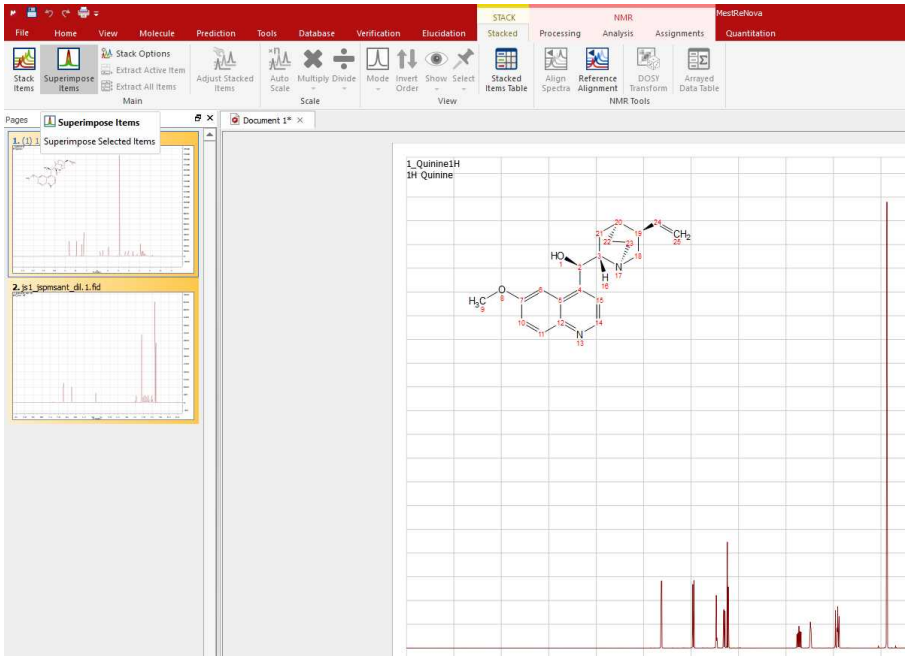
procedure. Basically, what we have applied to all spectra is the well-known binning operation which consists of dividing each spectrum in equally sized (e.g. 0.01 ppm in this case) bins, so that integral (area) of each bin represents a new point in the binned spectrum.



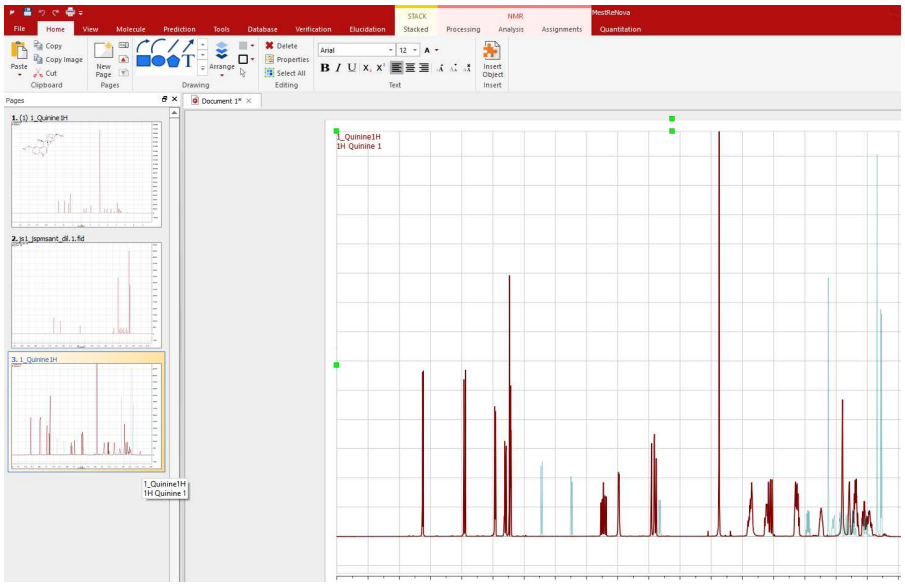
As seen in the figure above, binning clearly removes the effect of chemical shift changes but of course, at the cost of a significant reduction in data resolution.

20.3 Superimposed Spectra

It is very easy to **Superimpose Spectra** with Mnova. Just select the desired spectra on the page navigator (by holding down 'CTRL/Cmd key' while clicking on each spectrum) and then issue the command 'Stack/Superimpose spectra'.

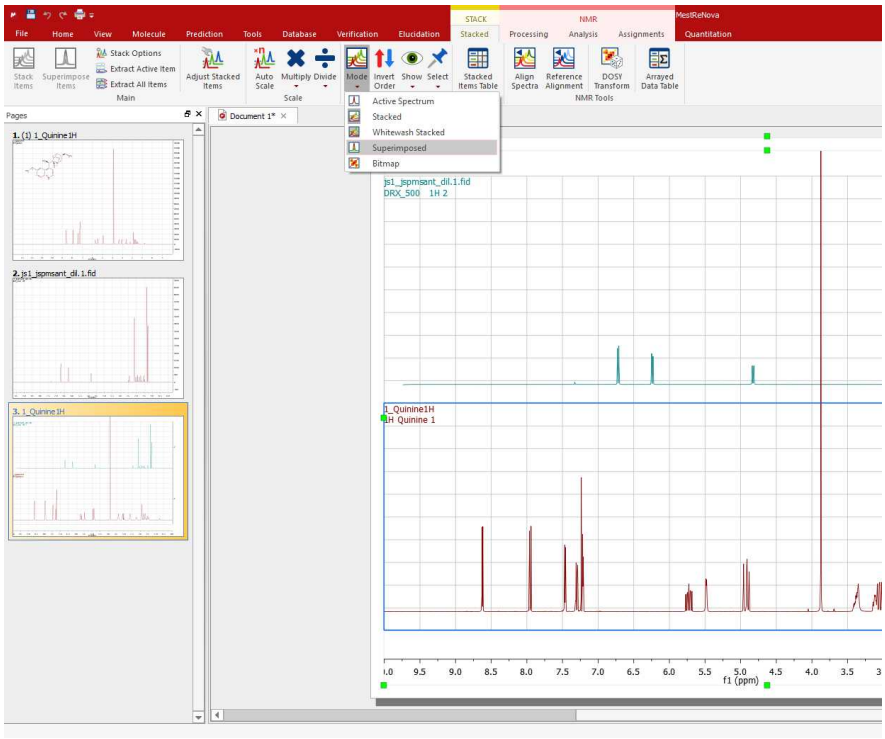


Finally, you will obtain the overlaid spectrum (one spectrum on top of the others):



It is possible to apply any analysis feature (peak picking, integration or multiplet analysis) to the superimposed spectra and then take a look at the result over each trace by selecting 'Active Spectrum' in the 'Stacked' scroll down menu. To navigate through the traces, press and hold down **SHIFT** and use the up or down arrow key (or use the mouse scroll wheel)

You will also be able to obtain the superimposed spectrum from a stacked one by selecting **'Superimposed'** on the stacking mode (vertical-left toolbar) or by following the menu 'NMR/Stack/Stacked/Mode/Superimposed':



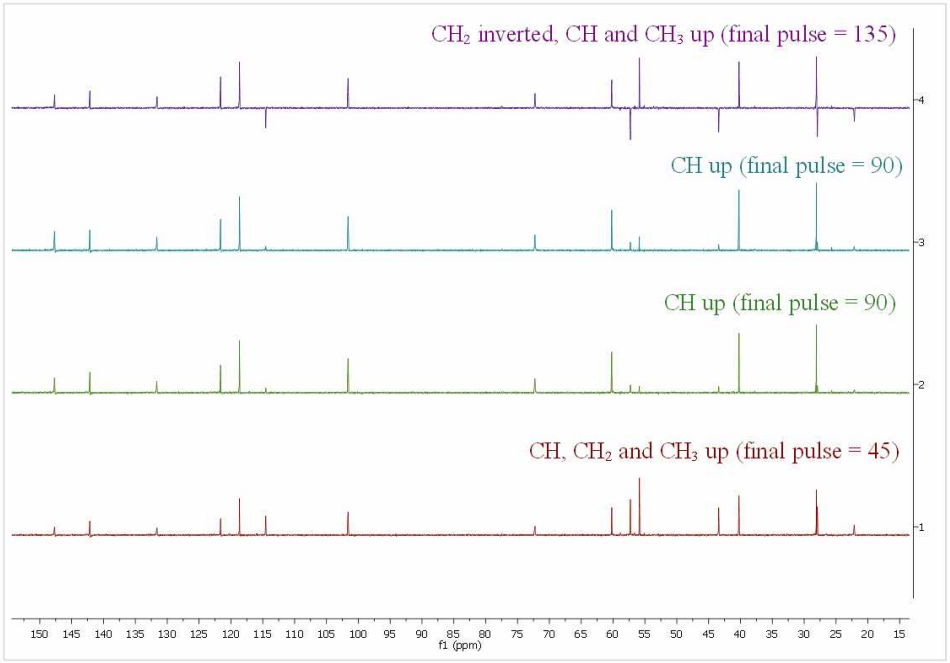
© 2024 MESTRELAB RESEARCH

If you have several spectra on the same page (to obtain it, just paste the desired spectra to one page, by using Ctrl/Cmd+C, to copy, and Ctrl/Cmd+V, to paste), you can also superimpose them by following the menu 'Edit/Alignment/Align Left (or by using other alternatives in the vertical-left toolbar) after having selected all of them (by Ctrl/Cmd+A, or Edit/Select All').

20.4 How to process Varian DEPT spectra?

This tutorial will show you how to process a Varian DEPT spectrum. As you well know, the DEPT pulse program in Varian generates a stacked plot with four slices, acquired with phase of the last pulse equal to 45, 90, 90, 135 degrees.

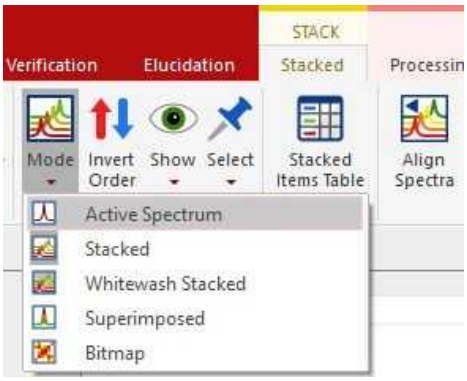
When you load this kind of spectra into Mnova, you will obtain something like this (if in the '**File/Preferences/NMR/Import'** you have the VnmrJ DEPT processing **unchecked**):



As you can see, you will have in the first slice (red slice with final pulse: 45 degrees) all the protonated signals, in the second and third slices you will get only the CH signals up and in the forth slice (purple slice with final pulse of 135 degrees) a spectrum with the CH and CH_3 up and the CH_2 down

Then, please make sure that the phase of all the slices is right. If not, you will need to apply a phase correction. We recommend you to select 'Active spectrum' from the stacked ribbon toolbar:

© 2024 MESTRELAB RESEARCH

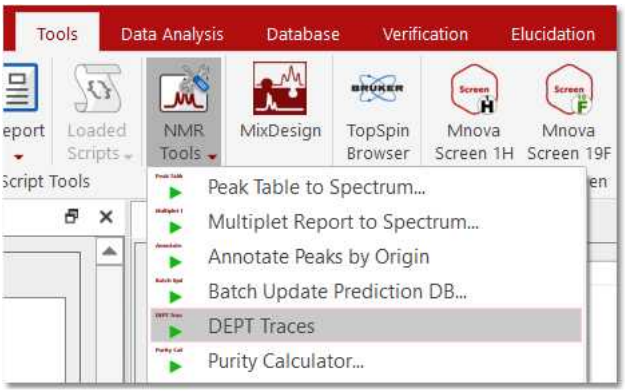


While working on this mode, you will see on the screen just the active spectrum (the spectrum which is highlighted in green in the stacked plot). Should you want to move to another spectrum without resorting to the stack mode just press Shift+Mouse Wheel or Shift + Up/Down keys.

IMPORTANT

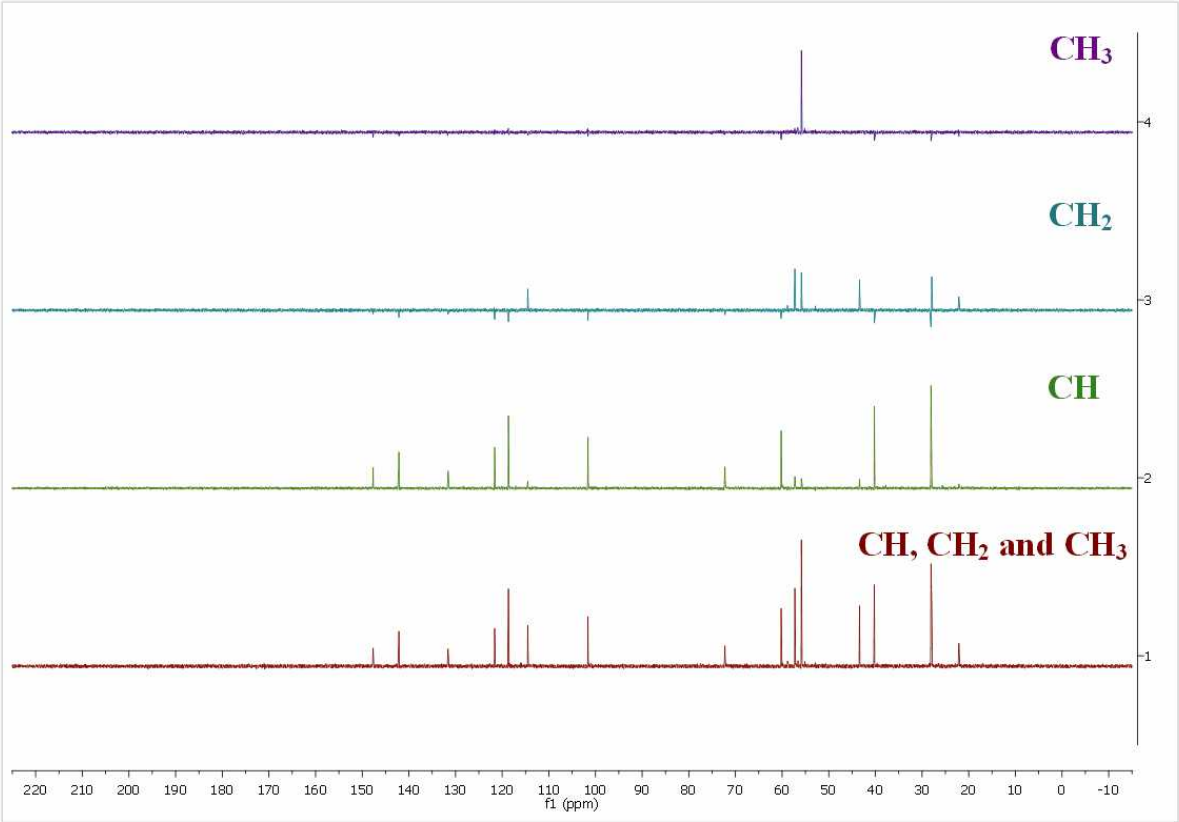
Remember that even if only the active spectrum is visible, unless the "Apply Processing to All spectra in Stack" option is off, all spectra in the stack will also be processed.

Finally, follow the menu 'Tools/NMR Tools/DEPT Traces':



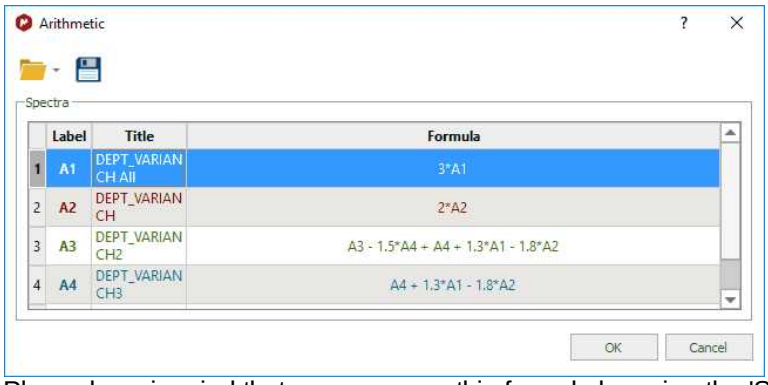
After having applied the script, you will obtain all the protonated carbons in the first slice (red), the CH in the second slice (green), the CH_2 in the third one (blue) and the CH_3 in the forth slice (purple), as you can see in the picture below:

© 2024 MESTRELAB RESEARCH



Please bear in mind that the relative intensities of the signals are not exactly the same in all the slices, so it is possible that you get small residues in some of the slices.

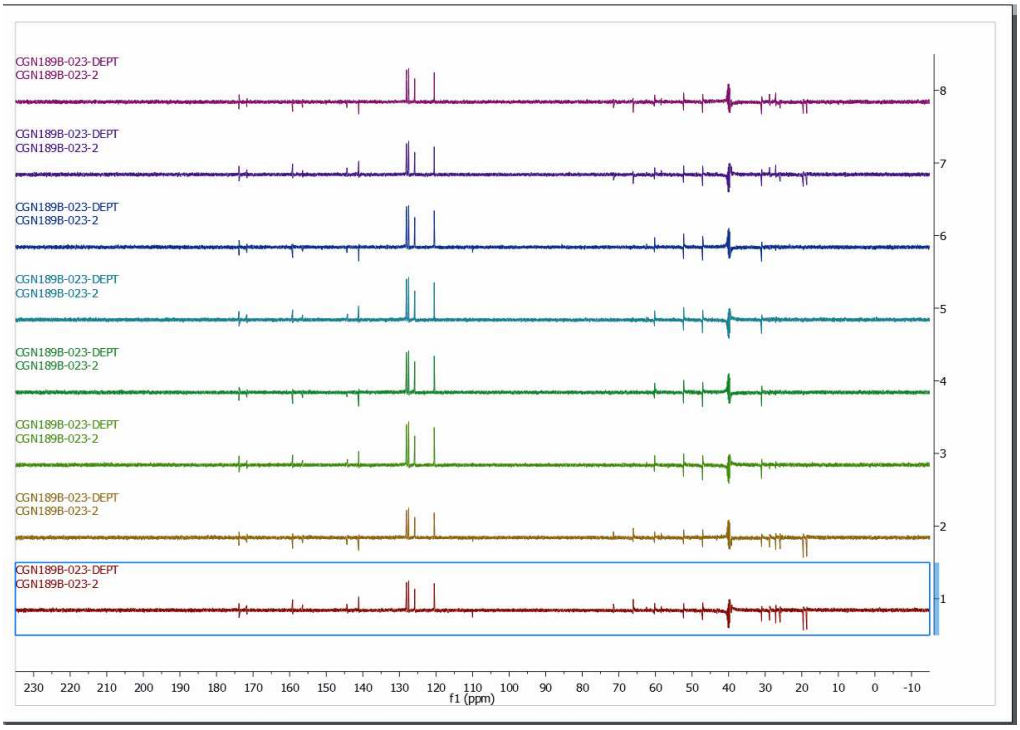
You could also use the Arithmetic feature to process your Varian DEPT spectra, by following the menu 'Analysis/More Tools/Arithmetic' and enter the following formula:



Please bear in mind that you can save this formula by using the 'Save' button .

If your dataset shows **eight spectra** means that it contains two arrayed variables "mult" and "qphase".

© 2024 MESTRELAB RESEARCH



The arrayed variable mult '0.5 1 1 1.5' refers to the type of DEPT spectrum, in this case DEPTQ 45º, DEPTQ 90º, DEPTQ 90º and DEPTQ 135º, respectively
The arrayed variable qphase '1 -1' refers to the sign of the quaternary carbons + and -, respectively.

This is the meaning of the eight spectra:

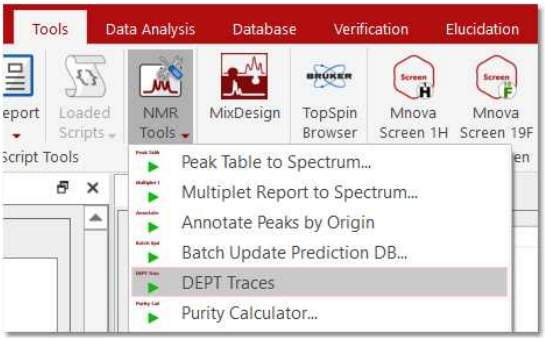
spectrum1: +CH + CH2 + CH3 + Cq (deptq45 qphase 1)
spectrum 2: +CH + CH2 + CH3 -Cq (deptq45 qphase -1)
spectrum 3: +CH 0 0 +Cq (deptq90 qphase 1)
spectrum 4: +CH 0 0 -Cq (deptq90 qphase -1)
spectrum 5: +CH 0 0 +Cq (deptq90 qphase 1)
spectrum 6 : +CH 0 0 -Cq (deptq90 qphase -1)
spectrum 7 : +CH -CH2 +CH3 + Cq (deptq135 qphase 1)
spectrum 8 : +CH -CH2 +CH3 - Cq (deptq135 qphase -1)

By doing aritmetics with the 8 spectra you can obtain the edited spectra.
For instance the following combinations:

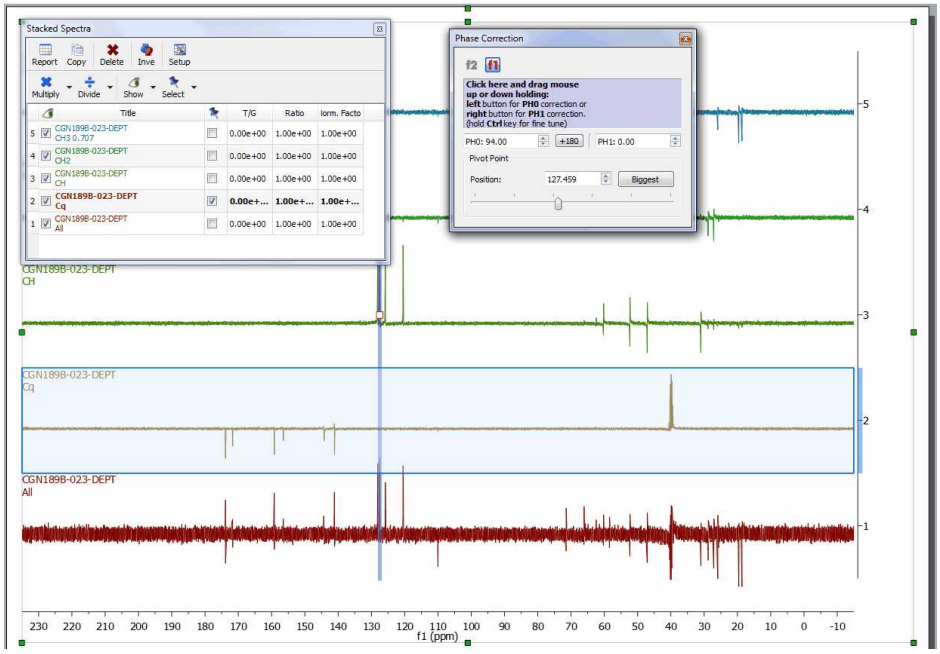
Only Cq: 1 -2 +3 -4 +5 -6 +7 .7
Only protonated carbons: 1 + 2
Only CH = 3 +4 +5 +6
Only CH2 = 1 + 2 -7 - 8
All carbons = 1

You will be able to process those datasets by following the menu 'Tools/NMR Tools/DEPT traces'.

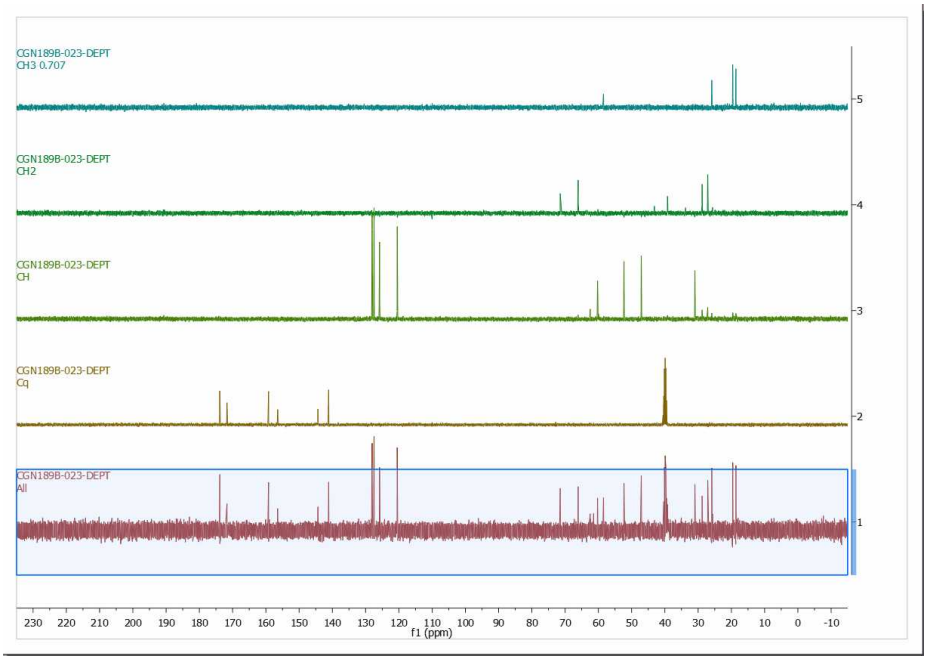
© 2024 MESTRELAB RESEARCH



After having run the script, a phase correction may be needed. If you need to apply a different phase correction for each trace, just hold down the Alt key and click on the desired trace (or open the Stacked Items Table) to finally correct the phase:



After having corrected the Phase to all the traces, this will be the result (from up to down: CH3, CH2, CH, quaternary and All carbons):



20.5 Mnova for metabonomics

Mnova allows you to easily process large datasets with the best quality in order to obtain the most precise results. Once datasets have grown to 20-200 spectra, **manual processing is no longer an option**.

Mnova can automate the processing of **large datasets of biofluids** to extract an ASCII file ready for the statistical measurement.

Mnova includes powerful specific **Phase and Baseline Correction algorithms for** 1D and 2D **biofluid spectra** in order to get the correct quantification

Getting Started

Mnova can be used on large datasets of biofluid spectra in two main ways, depending on the size of the dataset.

Graphical (<100 spectra): this mode allows all processing commands to be executed on a group of spectra simultaneously making possible the interaction with all spectra at the same time.

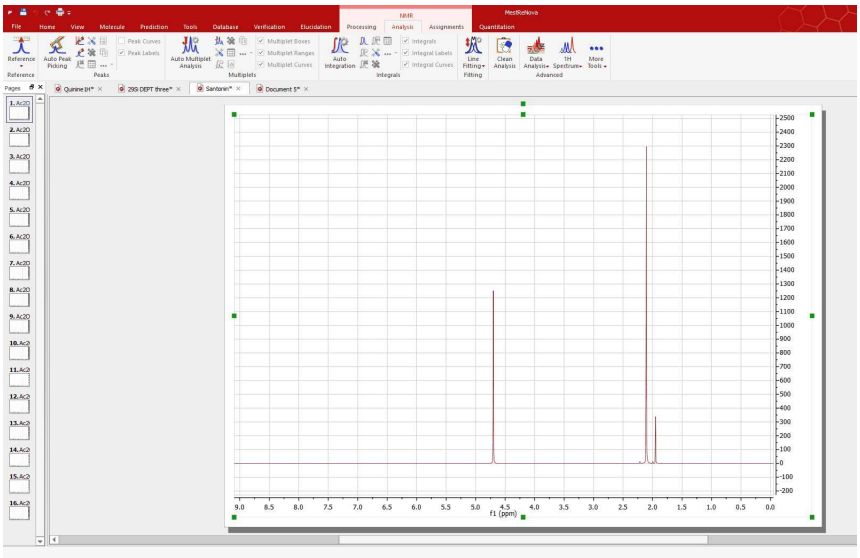
Scripting (>100 spectra): the user will only need to open a single spectrum of the dataset and optimize the processing in order to apply it to the remaining spectra by using the scripting capability implemented in Mnova.

Processing Basics

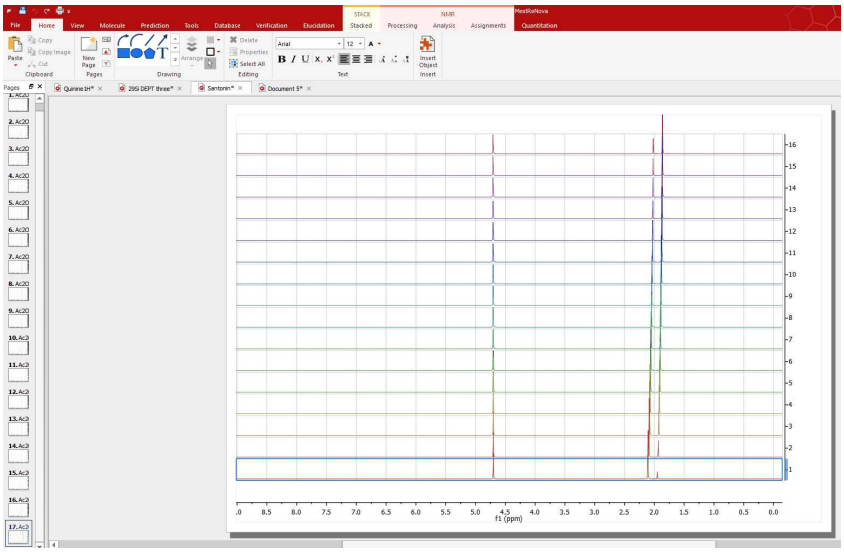
Let´s take a look at the '**graphical method**'.

1. When the dataset is small, we can drag the spectra folders to Mnova in order to get all the spectra fully processed (you can also use the multiOpen script). In this case I have dragged 16 spectra folders to the Mnova icon. After about 5 seconds, I got all the spectra fully processed as you can see in the picture below

© 2024 MESTRELAB RESEARCH

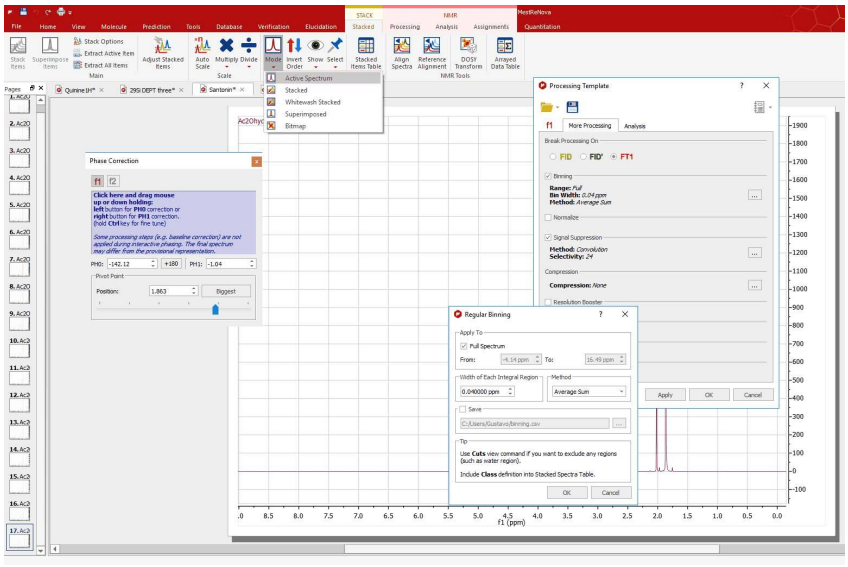


2. Select all the spectra in the page navigator in order to create the stacked spectra. We can do it by pressing ‘Ctrl/Cmd+A’ or by following the ‘Stack/Stack Spectra’ menu.



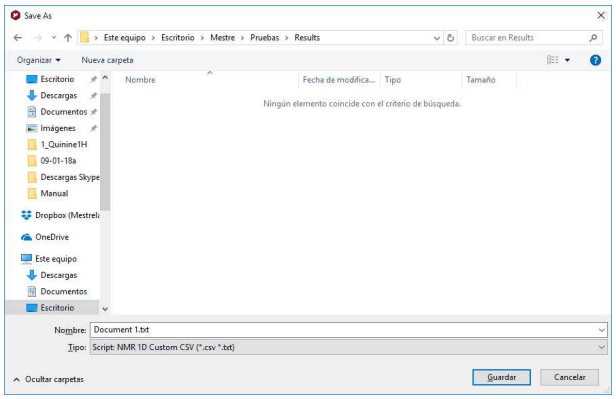
3. Select one of them (by selecting ‘Active Spectrum’ in the Stack scroll down menu of the left-vertical button) and apply the necessary processing and analysis (phase and baseline corrections, binning, signal suppression…). The operations to the first spectrum will be applied simultaneously over the whole dataset.

© 2024 MESTRELAB RESEARCH



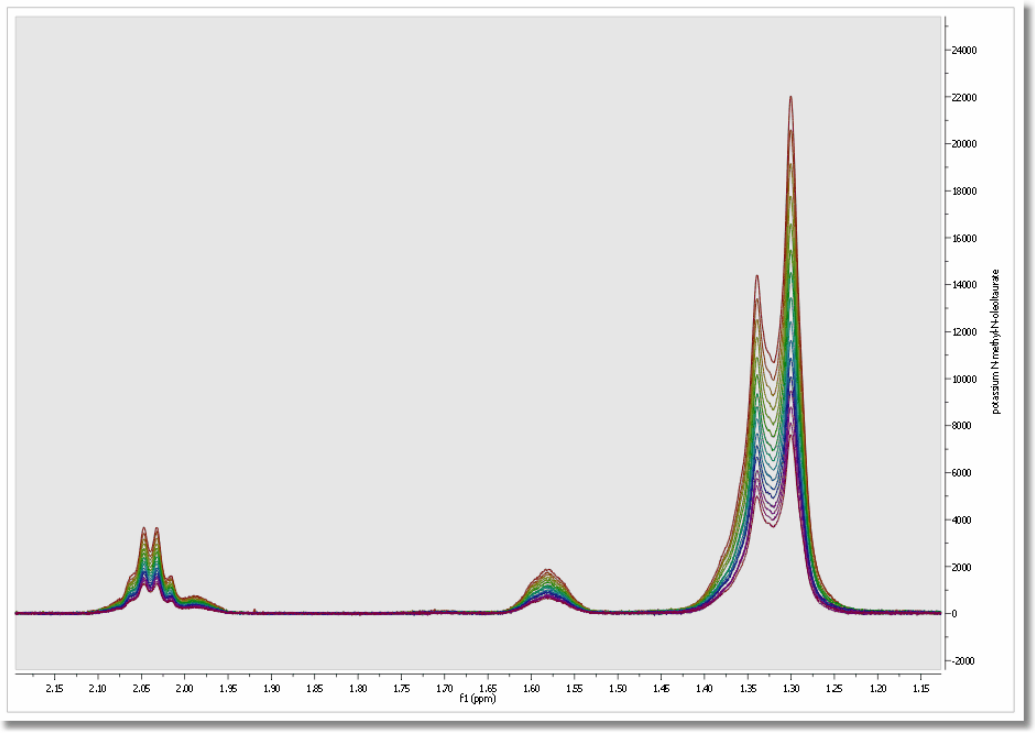
Mnova incorporates several specific features for biofluid samples; such as **Automatic Phase and Baseline corrections algorithms**, **Solvent Suppression** (to remove, for example, the dispersive signal of the water), **Auto-tuning referencing**, **Binning** (or bucketing), **Normalize**, **Global Spectral Deconvolution** (work in progress).

4. Once the processing and analysis have been made, we can export to an ASCII file for the PCA or PLS, just by following the menu 'File/Save As/NMR CSV File (*.csv *.txt)'; making sure that you type the extension *.txt in the filename field:



Highlight the stacked spectra and follow the menu 'Stack/Superimpose Spectra' to overlay them (useful for example to check that all spectra are perfectly aligned of referenced):

© 2024 MESTRELAB RESEARCH



Scripting Method

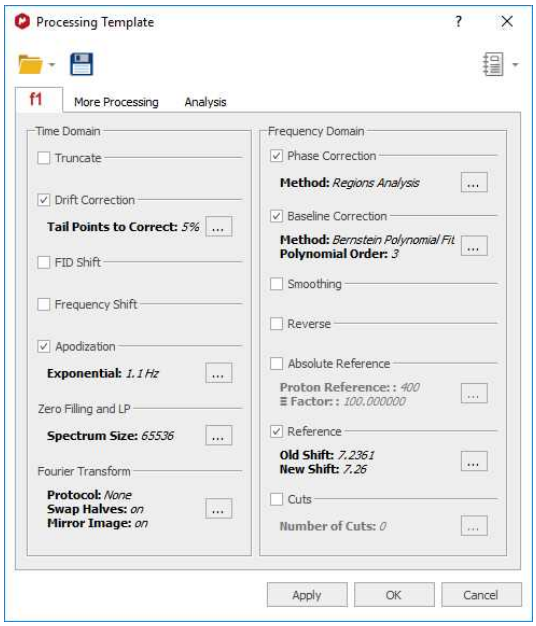
Mnova includes **Scripting capabilities** to automate the previous process with the ability to create Processing Templates which can be used with our Script engine to perform batch processing.

Mnova includes a powerful and flexible feature which allows the user to easily automate the full processing of both 1D & 2D NMR data sets. This is ideal for the batch processing of spectra of the same type, and can also incorporate analysis operations.

For example, let's say you have acquired 20 proton spectra which you want to process using the same processing operations. The procedure is very simple:

1. Load one spectrum and process it as desired.
2. Go to ***Processing/Processing Template***. The following dialog appears:

© 2024 MESTRELAB RESEARCH

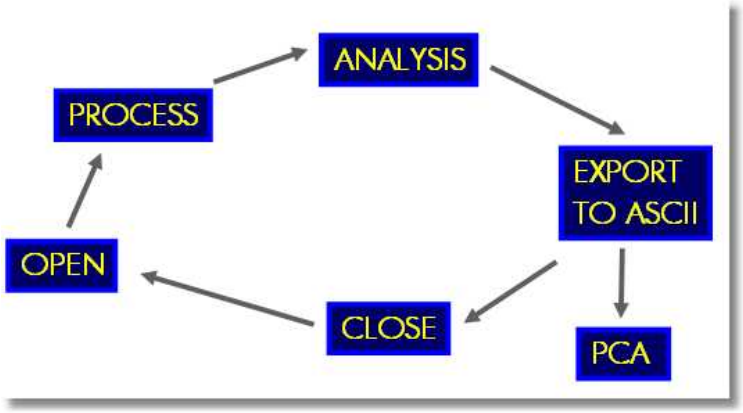


This dialog includes all the processing and analysis features implemented in **Mnova**. You can choose the required options by selecting the check boxes and, if necessary, specify options by clicking the button next to the corresponding command.

3. Once you are happy with the result, **save** the script file to a Mnova Processing file (*.mnp) by clicking on the save button in the dialog box.

Once you have saved this template, you will be able to run a script to process all the spectra in the same way.

If the number of datasets is too large, Mnova is able to open, process (apodization, FT, Zero Filling, LP, phase and baseline Correction…), analyze (binning, etc.), save to an ASCII file and close the spectrum, in order to open the next one and continue with the processing (to avoid an excessive use of memory). If the number is not large, Mnova can keep all the spectra fully processed on screen.



You can use the below script to automate the above process and save all the result into the same ASCII file. You will be able to download it here

Copy this script to the scripts folder of Mnova (that you have selected by following the menu "File/Preferences/Scripts/Directories'), restart the software and the script will appear under the 'Scripts' menu.

© 2024 MESTRELAB RESEARCH



Please bear in mind that you can introduce this script into the toolbar by using the 'Toolbar Customization' feature (View/Customize User Interface/Customize Toolbars').

To read more about Mnova scripting, please click here

```
//<GUI menuname="import_process_saveOneAscii_loop"shortcut="Ctrl+I"tooltip="Auto Process and
Save as ASCII"/>
/*****************************************************************************
Copyright (C) 2008 Mestrelab Research S.L. All rights reserved.
This file is part of the MNova scripting toolkit. Authorized users of MNova Software may use
this file freely, but this file is provided AS IS with NO WARRANTY OF ANY KIND, INCLUDING THE
WARRANTY OF DESIGN, MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE.
*****************************************************************************/
// This example function demonstrates how to apply some processing to a set of spectral files
and to save the result in a single ASCII file.
function import_process_saveOneAscii_loop()
{
	const procName = "c:/procTemplates/proc.mnp"; // Processing file. It must be created in
the Processing Template dialog of MestReNova.
	const fileMask = "fid"; // This mask specifies files to be processed.
	const dirMask = "*"; // This mask specifies directories to be scanned for spectra.

	// Select a directory with spectral data
	const specDirKey = "import_process_saveOneAscii_loop/SpectraDir";
	var dirName = FileDialog.getExistingDirectory(settings.value(specDirKey, Dir.home()),
"Select Spectra Directory");
	if (!dirName)
		return;
	var dir = new Dir(dirName);
	dir.cdUp();

	// Select a file to save the output
	const outDirKey = "import_process_saveOneAscii_loop/OutputDir";
	var saveDir = settings.value(outDirKey, Dir.home()); // Get last used directory name
	var fileName = FileDialog.getSaveFileName("ASCII Files (*.txt)", "Select Output File",
saveDir);
	if (!fileName)
		return;

	// Open the selected file and create a text stream mapped to it
	var fout = new File(fileName);
	settings.setValue(outDirKey, fout.absDirPath); // Save the directory name to be used
another time the script is called
	var sout = new TextStream(fout);
	sout.precision = 10;

	// Temporary file name
	const lastOutName = Dir.temp() + "/import_process_saveOneAscii_loop.lastOut.tmp";
	if (File.exists(lastOutName))
		File.remove(lastOutName);
```

```
	// The below function getMaskFiles is located in the files.qs script.
	var files = getMaskFiles(dirName, dir.absPath, fileMask, dirMask, true);
	for(var fi = 0, length = files.length; fi < length; fi++)
	{
		var dw = new DocumentWindow(Application.mainWindow.newWindow()); // Create new
document window
		if (serialization.open(files[fi]))
		{
			nmr.process(procName); // Apply processing template
			var spec = new NMRSpectrum(nmr.activeSpectrum());

			fout.open(File.WriteOnly);

			var flastOut = new File(lastOutName);
			var slastOut = new TextStream(flastOut);
			if (!fi)
			{	// Put fist column of the file in ppm. The scale is taken from
the first spectrum.
				flastOut.open(File.WriteOnly);
				slastOut.precision = 10;

				var ppm = spec.hz()/spec.frequency();
				var dPpm = spec.scaleWidth()/spec.count()/spec.frequency();
				for(var si = spec.count()-1; si >= 0; si--)
				{
					slastOut.write(ppm, "\n");
					ppm += dPpm;
				}
				flastOut.close();
			}
			flastOut.open(File.ReadOnly);

			for(var si = spec.count()-1; si >= 0; si--)
				sout.write(slastOut.readLine(), "\t\t", spec.real(si), "\n");

			fout.close();
			flastOut.close();
			flastOut.remove();
			if (fi < length-1)
				File.copy(fileName, lastOutName);
		}
		dw.close(); // Close document window of processed spectrum
	}
}
```

20.6 Show Traces in 2D-NMR

The user can show the traces of the 2D-NMR spectrum, just by clicking on the **'Show Traces'** icon  (or by using the contextual menu by right clicking over the spectral window). On the same icon, you will find a scroll bar menu to setup the vertical and horizontal traces of a bidimensional spectrum. The user can increase or decrease the intensity of the traces simply by scrolling the mouse wheel, after hovering the mouse over the desired trace (or by using Ctrl/Cmd+Shift+Up/Down for the horizontal trace and Ctrl/Cmd+Shift+Left/Right for the vertical trace).

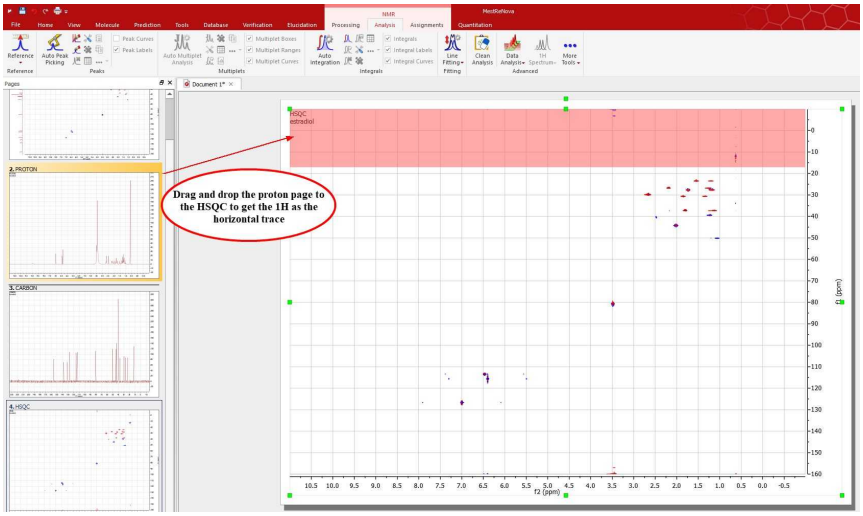
To move the trace, just hover the mouse over the top-right areas of the slices (the arrow cursor will change to a vertical panning cursor) and then click & drag your mouse in the desired direction. You will

© 2024 MESTRELAB RESEARCH

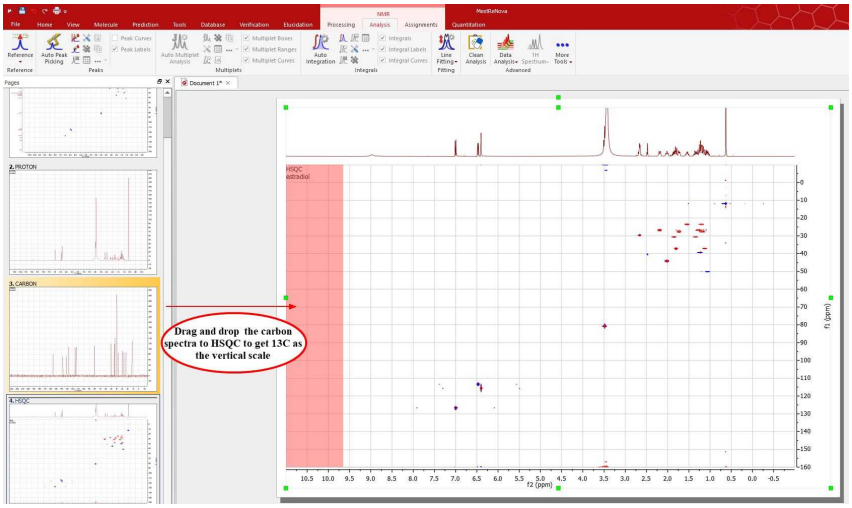
obtain the same result by holding the 'Ctrl' (Cmd in Mac) key while scrolling the mouse wheel over the trace.

Mnova automatically detects the 1D spectra and use them as external traces of a 2D plot if the applicable option is checked under the 'File/Preferences/NMR/Import' menu.(All the spectra must be opened in the same document). Any phase, baseline correction or reference applied to the 1D spectra will automatically update the applicable trace in the 2D plot.

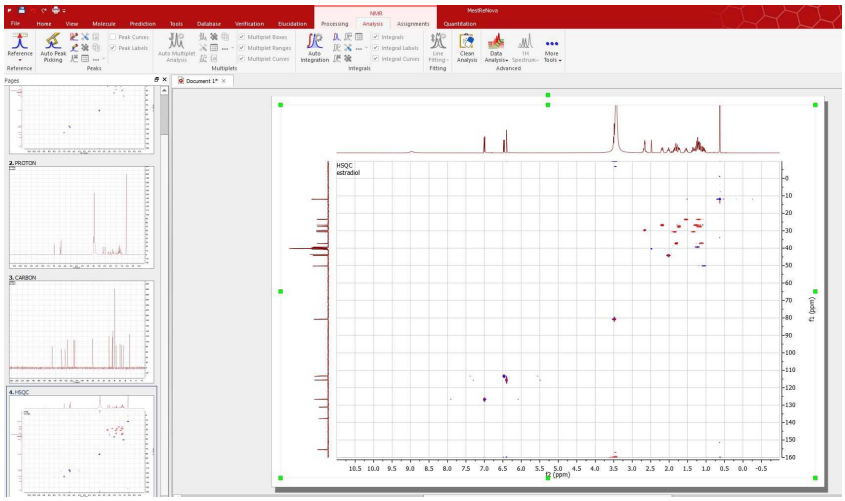
If the 'Auto Attach Traces' option is not checked, you can drag and drop any currently opened 1D-NMR spectrum to a 2D NMR dataset in order to get them as traces. Here you can see how the page number 2, containing a 1H dataset is dropped to the HSQC to get the horizontal trace:



The same can be done with the page 3 (containing a 13C spectrum) to get the vertical trace:

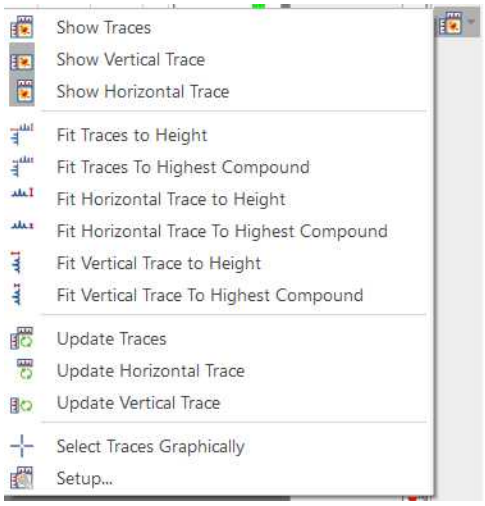


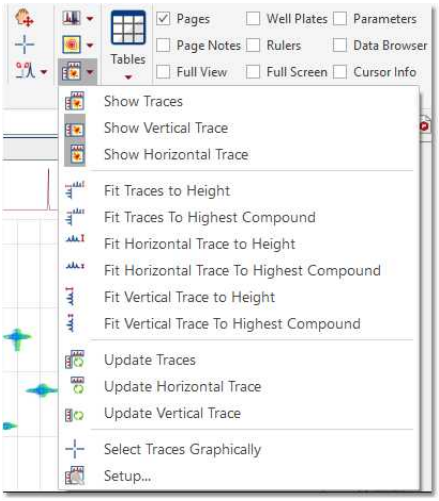
Here you can see the result:



There is also a script under the menu 'Tools/NMR Tools/Auto Attach Traces' which will automatically setup the 1D spectra as external traces of a 2D.

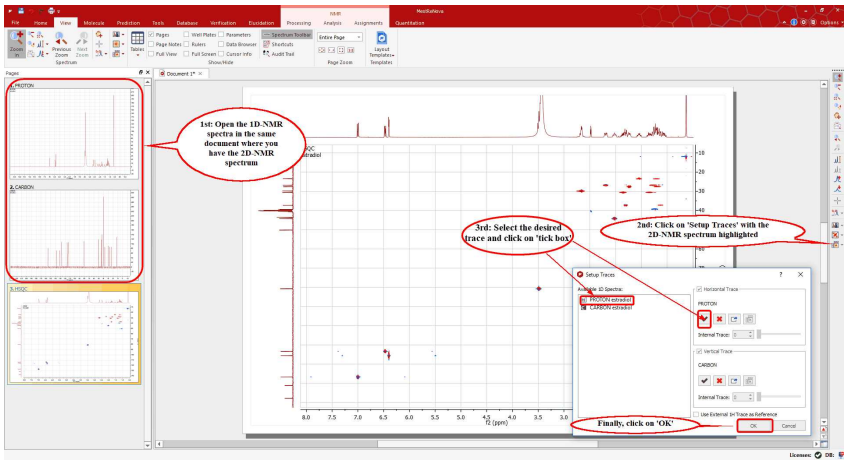
You can also choose any currently opened 1D-NMR spectrum as a trace by clicking on 'Setup' on the **'Show Traces'** scroll bar menu (or by using the traces contextual menu by right clicking over one of the traces).





and next selecting the desired 1D-NMR spectrum for the horizontal and then for the vertical trace.

© 2024 MESTRELAB RESEARCH



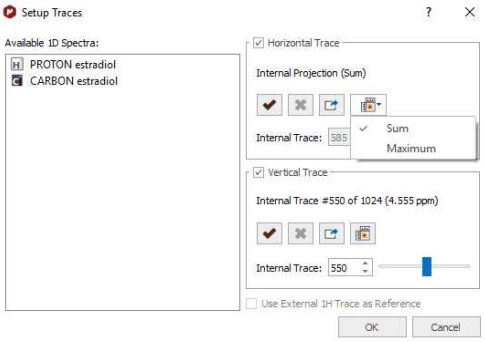
ATTENTION

Any phase, baseline correction or reference applied to the 1D spectra will automatically update the applicable trace in the 2D plot.

Use the 'update traces' tool if you have applied any other processing feature on your 1D spectra, to update the changes in the traces.

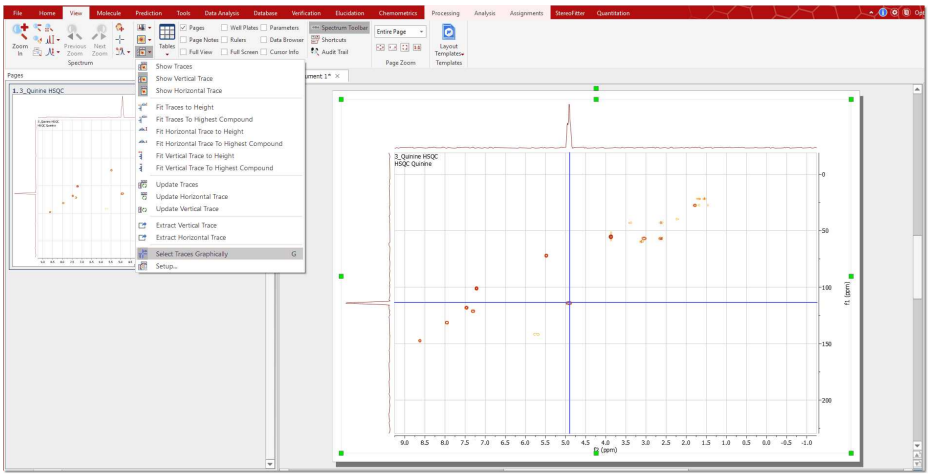
The user will be able to remove the external trace by clicking on the **'Delete'** icon .

You can show the **'Internal Projections'** in 2D spectra as either a calculation of the sum of all traces across a given dimension (Sum) or by computing the maximum of every trace (Maximum), just by clicking on the 'Projections icon' . Click again on this icon to show the **'Internal Traces'**.



The user can also select the traces directly from the 2D-NMR spectrum by using the **'Select Traces Graphically'** tool (shortcut: G) in the 'Show Traces' scroll bar menu. By pressing the key several times the tool toggles between both dimensions, only horizontal and only vertical (as it works for Zoom and Cut tools). Clicking and dragging will extract the sum of the traces of the selected region.

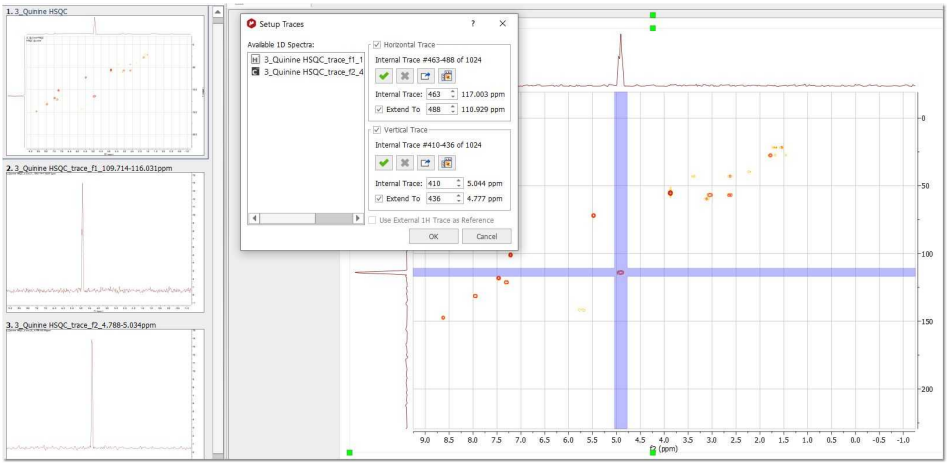
© 2024 MESTRELAB RESEARCH



You can also extract a trace without adding it previously as a horizontal/vertical trace in the 2D spectrum using the Select Traces cursor and keeping 'Shift' key pressed. It allows you to select just one trace (horizontal or vertical) or 2 traces (one at each dimension) just with one click. The selected traces are extracted and added to new pages in the document.

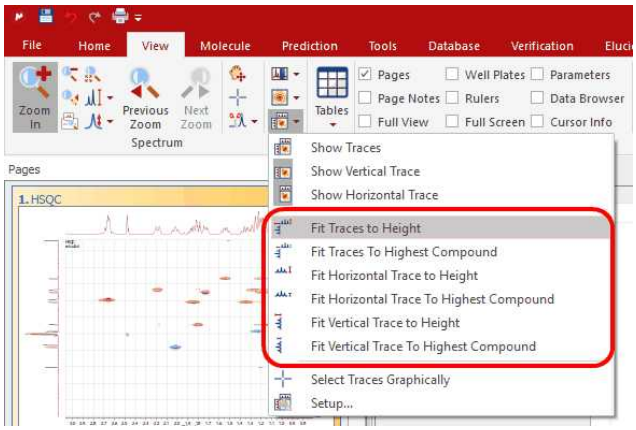
Each extracted spectrum contains the title of the original 2D spectrum and additionally the dimension and position of the extracted slice.

You can also extract the sum of several slices of the closest points around a signal by clicking&dragging, instead of just clicking a position, while keeping 'Shift' pressed:

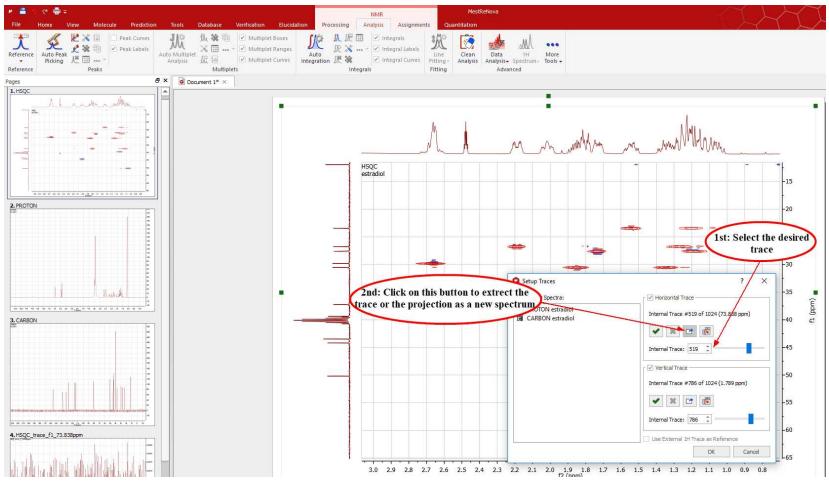


The user will be able to **'Fit Traces to Height'** (the vertical, the horizontal or both), just by following the menu 'View/2D Traces' or by using the **'Show Traces'** scroll bar menu on the left vertical toolbar:

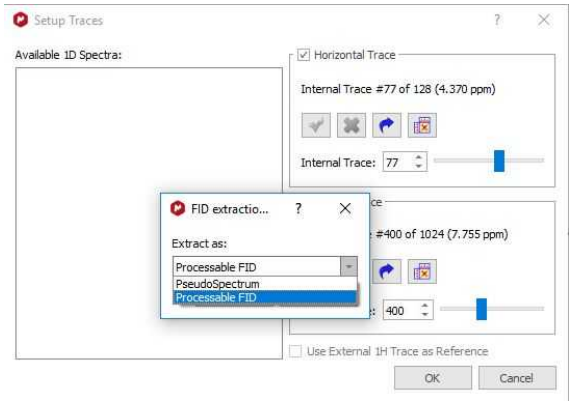
© 2024 MESTRELAB RESEARCH



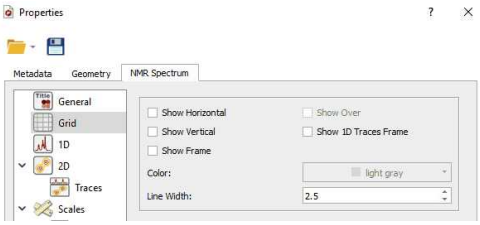
The user can also extract a current trace (or projection) as a new spectrum by clicking on the button  in the 'Setup traces' dialog box. In the figure below, you can see how to extract the vertical trace (# 517).



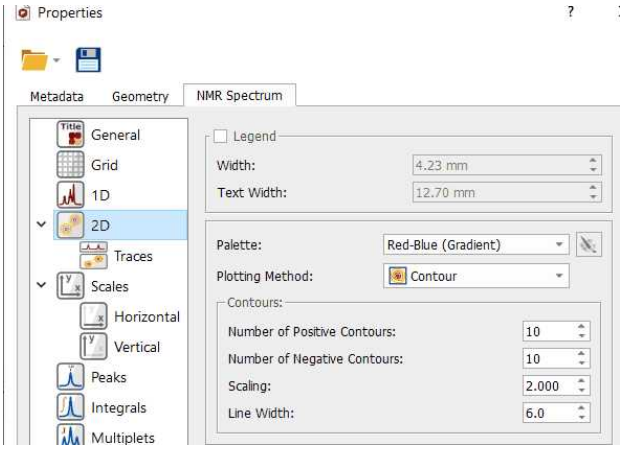
You can extract the vertical trace in a 2D spectrum and apply a FT on it. To get it:
- load a 2D spectrum and select "Spectrum after 1st FT" (from the 'Processing/Break Processing on' menu).
- select the slice to extract with "select traces graphically" and then open "setup" dialog for traces.
- if select "Extract current Vertical Trace as New Item" a new dialog is open. You can get the trace as "PseudoSpectrum" (not posibility FT) or "Processable FT" (posibility to do FT):



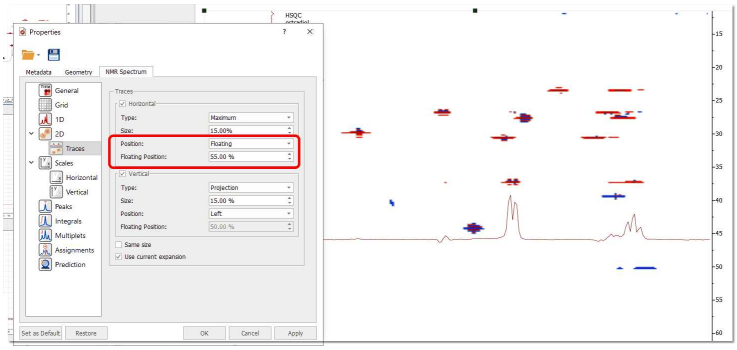
The user will be able to add or remove the 1D traces frame from the 'Grid Properties' dialog box (displayed by double clicking on the 2D-NMR spectral window):



You can select the size of the traces (or to show them by default) by selecting the applicable option on the 'Properties' dialog box:



You can move the traces across the 2D spectrum to freely to compare peaks, as floating traces:



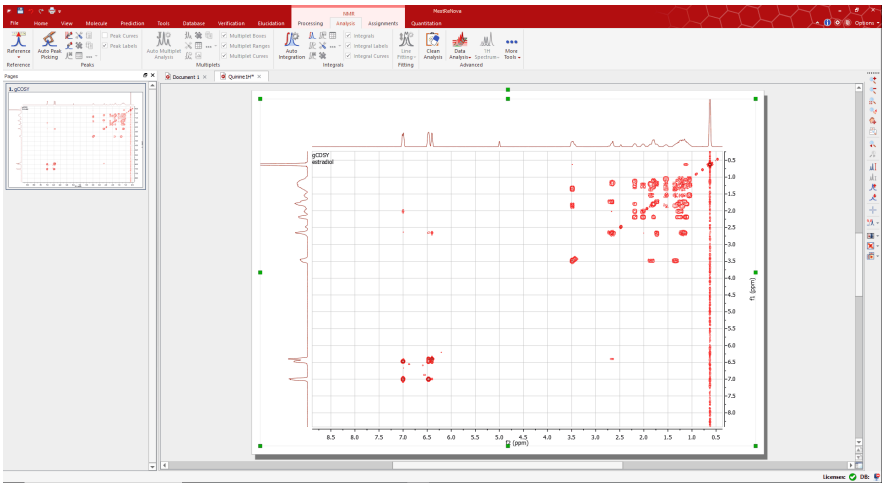
Make sure that the "Use current expansion" property is checked and you could see the Sum and Maximum traces of only those peaks displayed in the zoom region.

Automatic reference of 2D-NMR spectra with the 1D external trace.

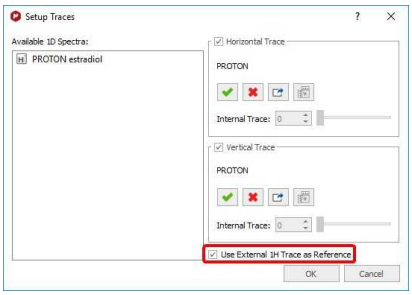
You can reference the 2D-NMR spectra directly with the 1D external trace. Currently this feature only works which those bidimensional experiments which internal traces are equivalent with the external ones. For example: COSY, NOESY, etc...

Let´s see how this feature work with a unreferenced COSY spectrum:

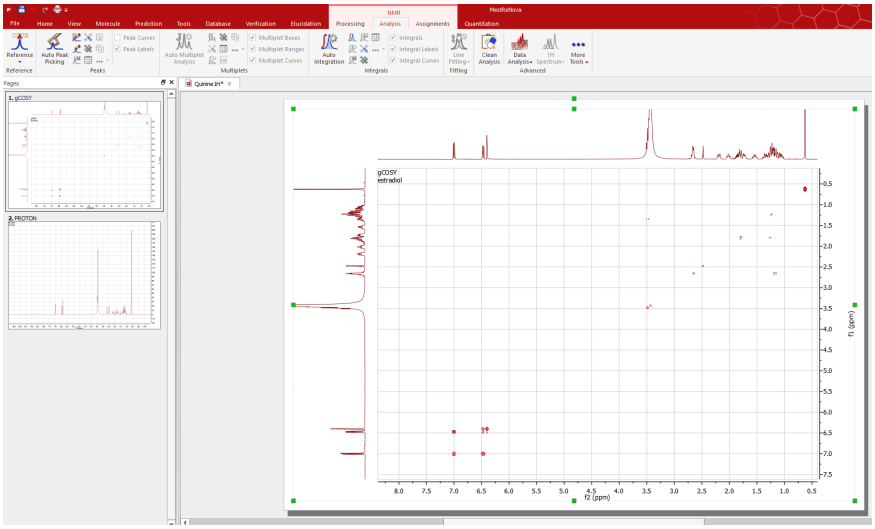
© 2024 MESTRELAB RESEARCH



As you can see, this spectrum is not referenced. To apply an automatic referencing, just right click on one of the traces and select 'Setup' to display the 'Setup Traces' dialog box. Once there, select the desired external trace and check the applicable box (highlighted in red in the picture below):



Automatically, you will obtain your COSY spectrum referenced, as you can see in the screenshot below:



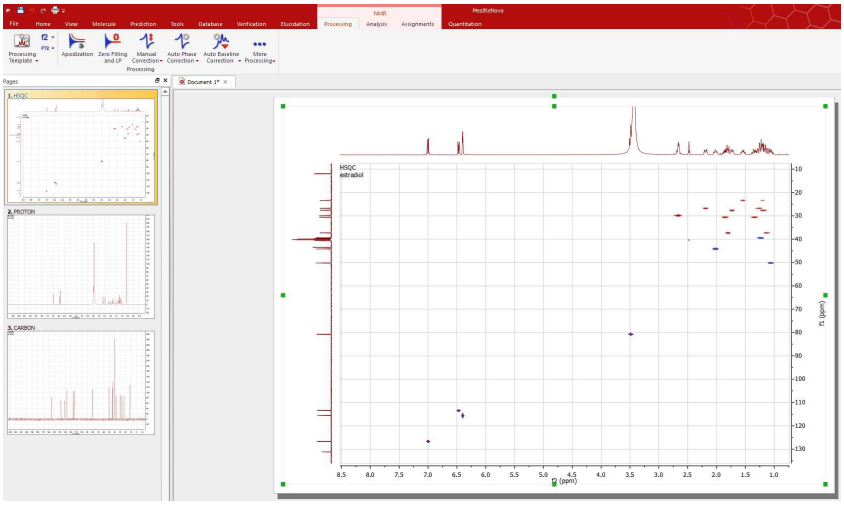
See also:

Miniclip of this functionality at this link

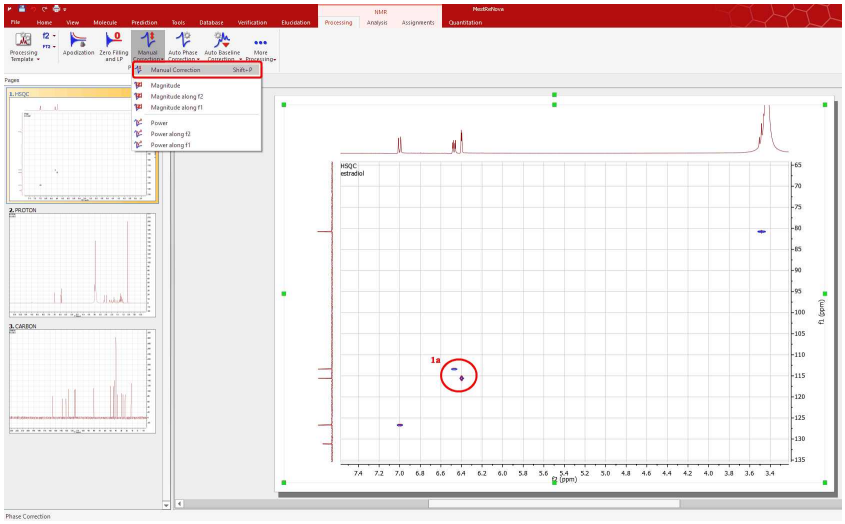
20.7 Manual Phase Correction in 2D-NMR

In this tutorial, we will explain how to phase the spectrum in real time, directly on the 2D spectrum.

We will show how to correct the phase of a HSQC experiment (see figure below).

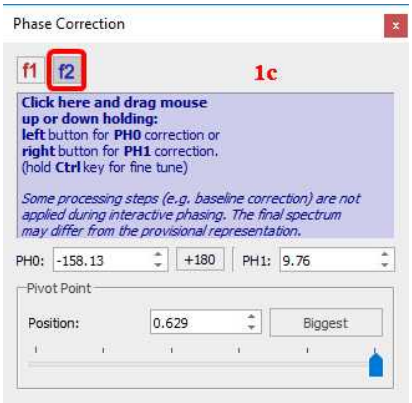


Once you have opened the spectrum; *'zoom in'* the area of interest:



1. Then, take a look at the signal, or the group of signals (**1a**), which appear in a corner of the spectrum (for example, the signals highlighted with a red circle in the capture above); select 'manual correction' (**1b**) from the drop down menu on the 'Phase Correction' toolbar button and adjust the zero order (PH0) phase parameter along f2 (by dragging the mouse up or down over the blue rectangle in the 'Phase Correction dialog box' whilst holding the left mouse button (**1c**)).

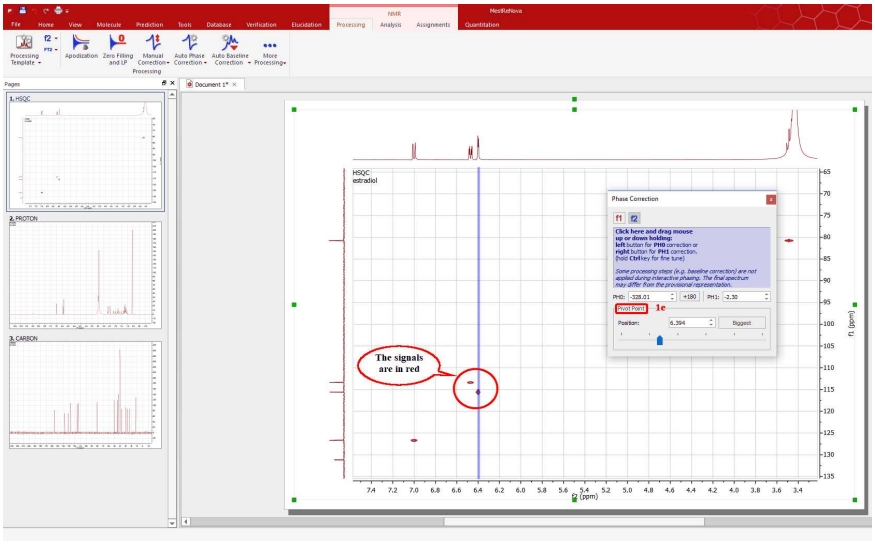
© 2024 MESTRELAB RESEARCH





Please note that the Phase Correction dialog is **modeless**, so you will be able to use any other tool (i.e. zooming) whilst you are applying the correction.

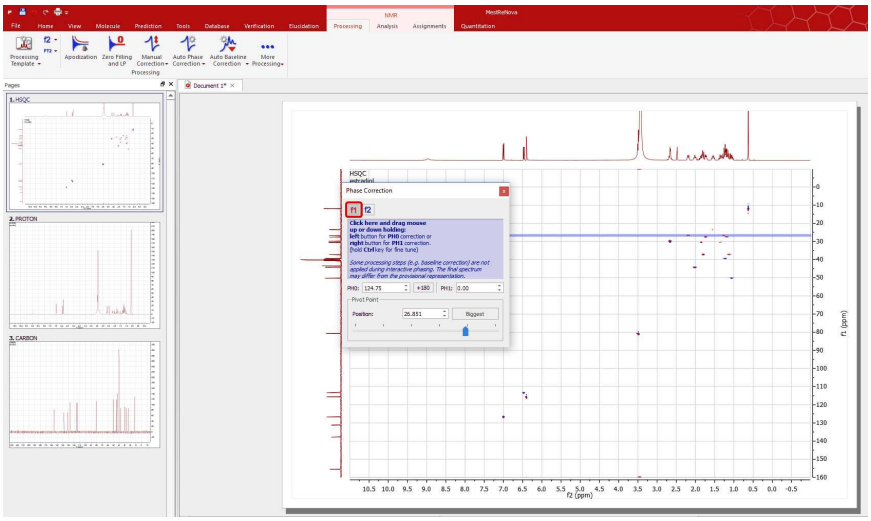
The goal (**1d**) will be to make all the **signals of the area of interest** (highlighted with the green circle in the picture above) positive (note that in this example, positive signals are red whereas negative are blue). Remember that we are adjusting the phase along f2, so do not worry if the phase of the peaks along the indirect (vertical) dimension (f1) is not correct.



Next, if some peaks on the right side of the area of the spectrum shown on screen require phase correction (remember, still along f2), you can place the pivot point (**1e**) over the signal we have selected as reference (in the green circle) and carry out the first order phase correction PH1 (by dragging the mouse up or down over the blue rectangle in the 'Phase Correction dialog box' whilst holding the right mouse button). In this particular case, the spectrum does not require first order correction.

2. Finally, if necessary (as in this case), you can proceed in the same manner to correct the phase along **f1.** Just click on the f1 button on the dialog box and then proceed as explained along f2, by starting with a zero order phase correction and following with a first order phase correction (remember: along **f1**):

© 2024 MESTRELAB RESEARCH



Bear in mind that this procedure is exactly the same as with 1D-NMR spectra, but it is necessary to correct the phase along 2 dimensions.

20.8 Pasting Spectra into Reports

When the user copies a spectrum, he is copying a graphic. A graphic is any kind of image displayed by a computer. There are two kinds of graphics: vector graphics and raster graphics. A vector graphic (metafile) is a graphic drawn as lines, polygons and text while a raster graphic is drawn as an array of colored dots (bitmap).

A ***metafile*** is a list of commands that can be played back to draw a graphic. Typically, a metafile is made up of commands to draw objects such as lines, polygons and text and commands to control the style of these objects. So, internally, a metafile is an array of variable-length structures called metafile records. The first records in the metafile specify general information such as the resolution of the device on which the picture was created, the dimensions of the picture, and so on. The remaining records, which constitute the bulk of any metafile, correspond to the graphics device interface (GDI) functions required to draw the picture. These records are stored in the metafile after a special metafile device context is created. This metafile device context is then used for all drawing operations required to create the picture. When the system processes a GDI function associated with a metafile, it converts the function into the appropriate data and stores this data in a record appended to the metafile.

NOTE: Some people equate metafiles with vector graphics. In most cases this is correct but, strictly speaking, a metafile can contain any mix of vector and raster graphics. For example, a metafile could contain just one command to display a bitmap. This is the approach used in Mnova, which by default pastes the spectrum as a metafile which contains a bitmap in order to reduce the file size (without too great a loss in resolution). Mnova also allows the user to use (only in Windows) the graphical format known as ***Enhanced Windows Metafiles*** in order to keep the maximum graphical resolution possible (at the expense of an increase in the file size). This will usually be the preferred way to copy NMR spectra.

Enhanced metafiles provide true device independence. You can think of the picture stored in an enhanced metafile as a “snapshot” of the video display taken at a particular moment. This “snapshot” maintains its dimensions no matter where it appears—on a printer, a plotter, the desktop, or in the client area of any application.

You can use enhanced metafiles to store a picture created by using the GDI functions (including new path and transformation functions). Because the enhanced metafile format is standardized, pictures which are stored in this format can be copied from one application to another; and, because the

© 2024 MESTRELAB RESEARCH

pictures are truly device independent, they are guaranteed to maintain their shape and proportions on any output device. In addition, the user is able to work with the copied spectrum in the document just by double clicking on it (Mnova will automatically open due to the Object Linking and Embedding technique; OLE).

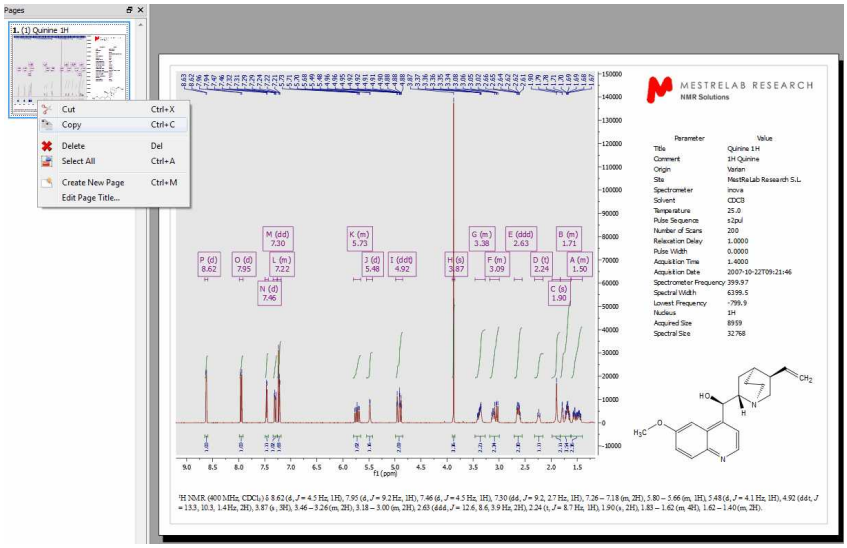
A ***bitmap*** is another name for a raster graphic. In Windows, these are typically saved in a BMP file. However, other common bitmap formats include PC Paintbrush (PCX), Tagged-Image File Format (TIFF), Graphics Interchange Format (GIF) and JPEG.

In general, vector graphics take longer to display but can be scaled to any size with no degradation, whilst raster graphics are faster to display but image quality suffers when they are scaled up or down.

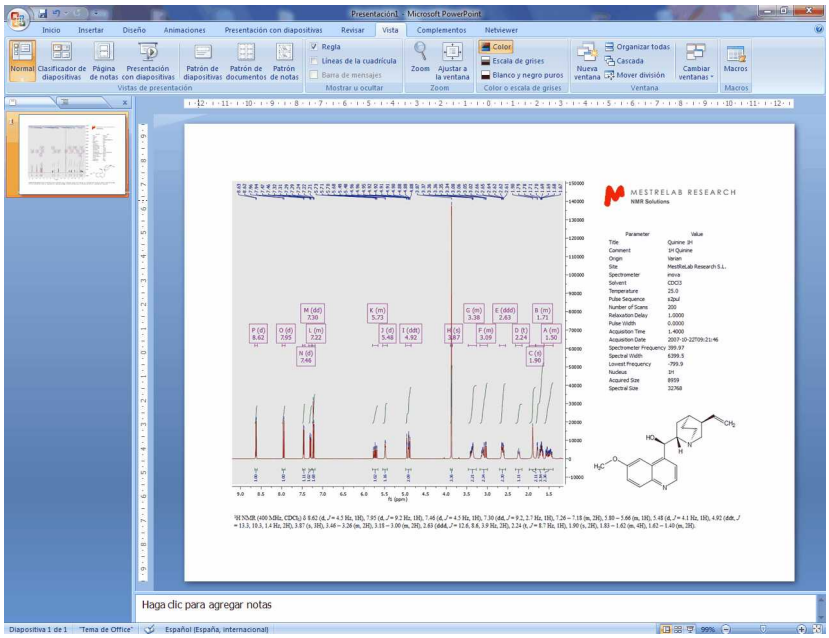
See also this post:
http://mestrelab.com/blog/making-publication-quality-images/

How to paste a spectrum in a Report?

Mnova provides the ability to copy&paste spectra into other applications (e.g. MS Office apps) via the clipboard. Just be sure that you have highlighted the desired objects (Edit/Select All, if you want to select all the objects) and then follow the menu 'Edit/Copy' or alternatively use the shortcut 'Ctrl+C' (or 'Cmd+C' in Mac). You can also select the page on the page navigator and do Ctrl/Cmd+C (or right click->Copy) to copy to the clipboard all the content of the page.

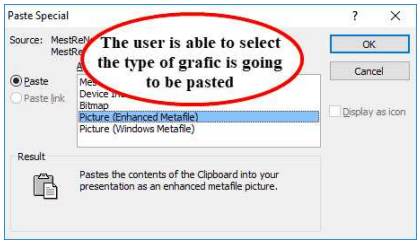


Next, open the document (PowerPoint in this case) where you want to paste the spectrum and follow the menu Edit/Paste or alternatively use the shortcut, Ctrl+V (or Cmd+V in Mac).

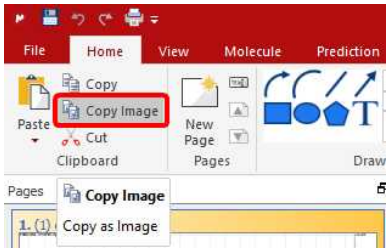


Mnova will copy to the clipboard, by default, the object as a metafile. This object will have OLE properties in Windows, so the user will be able to work with the copied spectrum in the document just by double clicking on it (Mnova will automatically open due to the Object Linking and Embedding technique; OLE). Please bear in mind that the size of this 'image' will be bigger than a regular image, because you are storing the FID to allow Mnova to reprocess the NMR spectrum in the future if it is needed.

However, the user can change this default by following the menu 'Paste/Paste Special' (or Ctrl/Cmd+Alt+V) before pasting (in the corresponding document; PowerPoint in this case). It will open the 'Paste Special' dialog box where the user will be able to select between Mnova Document (by default), Enhanced Windows Metafile, Bitmap, etc.

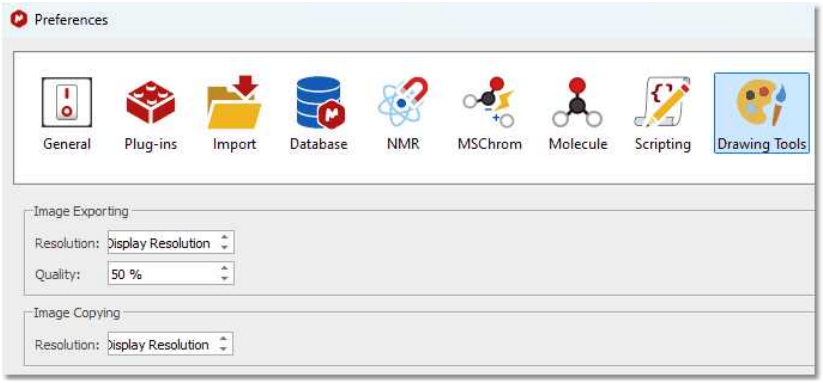


The user can also copy the spectra just as an image (very useful for large datasets) by following the menu Home/Clipboard/Copy as an image'. **This will reduce the file size of the document but the image will not contain OLE properties.**

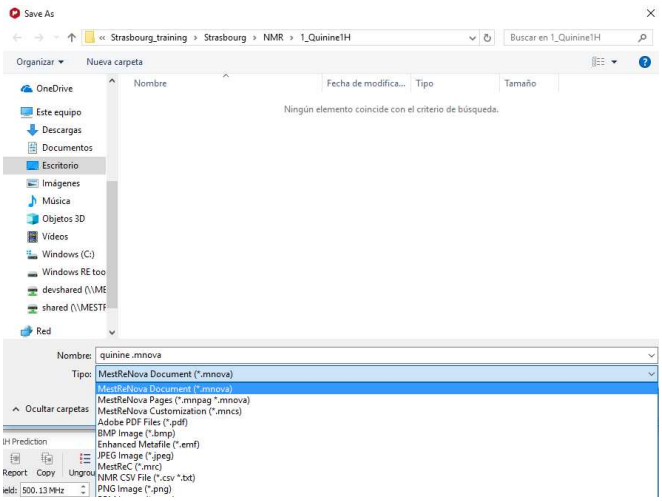


From the '**Drawing Tools Preferences**', you will be able to select the exporting 'Resolution' and 'Quality' of the graphics and also the resolution of the image copying:

© 2024 MESTRELAB RESEARCH



The user can also save the spectrum by following the menu 'File/Save as' and then 'paste it' in the corresponding document by selecting 'Insert/Picture'. Mnova allows the user to save the data in Mnova (*.Mnova) or MestRe-C (*.mrc) formats as well as in ASCII text (*.txt), adobe PDF or several kinds of image files, such as bitmap (bmp), enhanced metafile (emf), JPEG, PNG, SVG, TIFF, XBM, XPM, etc...



'ASCII Text File' (*.txt): It is essentially raw text just like the words you are reading now. Each byte in the file contains one character which conforms to the standard ASCII code. This kind of text file becomes a common denominator between applications which do not import each other's formats. If both applications can import and export ASCII files, you can transfer your files between them. Almost all word processors import and export ASCII files as well as many database and spreadsheet programs.

'Adobe Text File' (*.pdf): Portable Document Format (PDF), is an open file format used for representing two-dimensional documents in a device independent and resolution independent fixed-layout document format. Each PDF file encapsulates a complete description of a 2D document which includes the text, fonts, images, and 2D vector graphics which compose the document.

'JPEG Image File' (*.jpg): JPEG stands for Joint Photographic Experts Group; it is a lossy compression algorithm which has been conceived to reduce the file size of natural, photograph-like true-colour images as much as possible without affecting the quality of the image as perceived by the human sensory engine.

'PNG Image File' (*.png): PNG (Portable Network Graphics) is another bitmapped image format which employs lossless data compression.

© 2024 MESTRELAB RESEARCH

XPM Image Files (*.xpm): XPM stores image data in the form of ASCII text formatted as a Standard C character string array. This type of format allows XPM files to be edited easily with any text editor, to have comments inserted at any point within the file, to be included as data in C and C++ programs, and to be easily transmitted via electronic mail.

20.9 How to predict a spectrum with NMRPredict Desktop?

Carrying out of predictions in Mnova with the **NMRPredict Desktop** plugin is very easy.

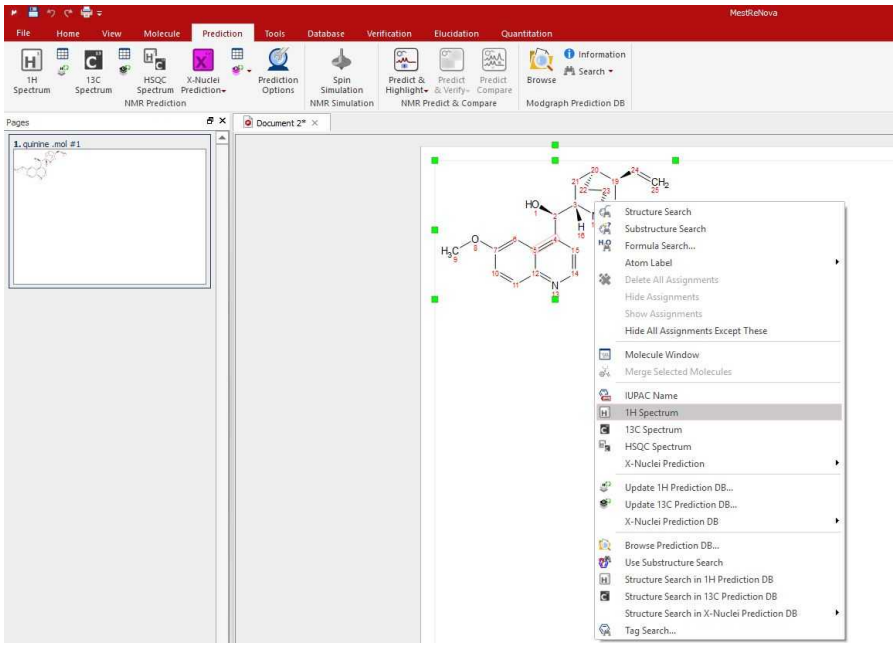
1. Just, import a molecular structure. Currently, molecular structures can be imported as a **.mol** file by simply using the Open command exactly in the same way as this would be applied to a spectrum. Bear in mind, that you are actually able to copy&paste **.cdx** structures from **ChemDraw** and **.mol** from **IsisDraw** but not drag&drop them. Support to 'drag & drop' operations are currently limited to **ChemSketch.**



ATTENTION

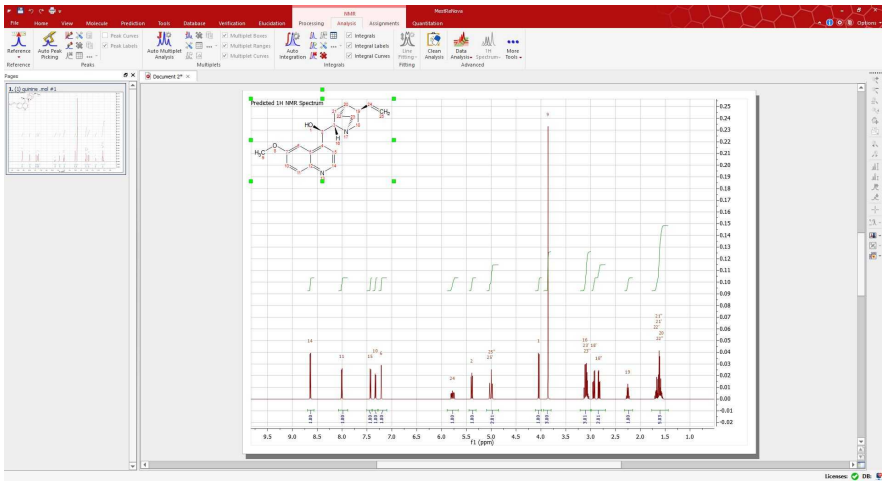
Bear in mind that, in terms of stereochemistry, drawing a structure as a chair or as a boat means nothing to the .mol file and the computer. In those cases the user must draw all the corresponding bonds to show the stereochemistry of the molecule, i.e., you must explicitly draw up and down bonds using wedges.

2. Right click on the molecule structure and select ‘Predict 1H Spectrum’ or ‘Predict 13C Spectrum’.



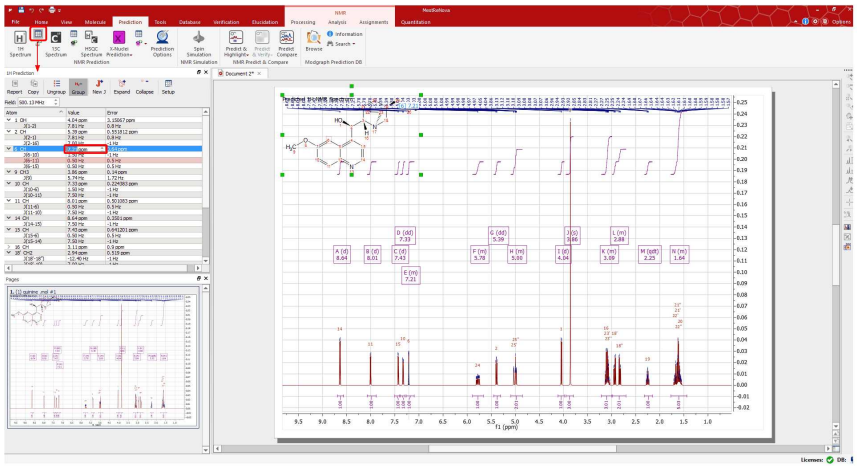
3. After that, you will obtain the desired predicted spectrum which can be analyzed as a real one (e.g. it can be integrated, peak picking, etc).

© 2024 MESTRELAB RESEARCH



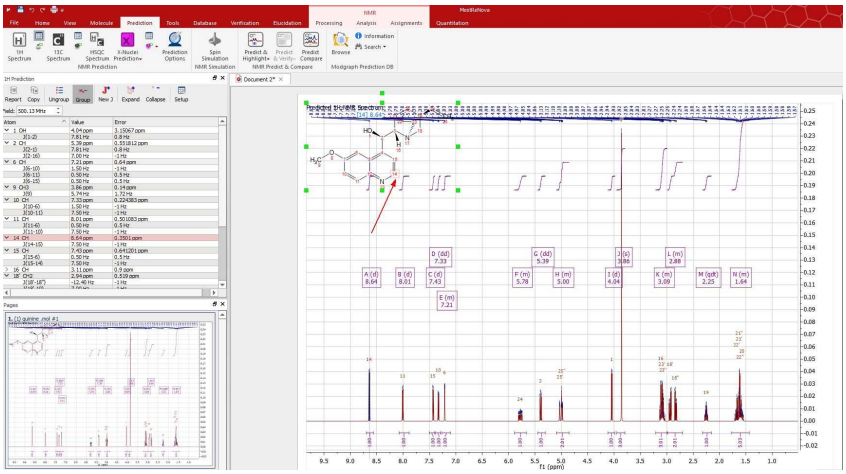
You can also modify the chemical shift and the coupling constant values from the table of the predicted spectrum. Just, highlighted the spectrum, follow the menu 'Prediction/1HPrediction Table' and then double click on the value that you want to edit. The changes made on the table will take effect automatically in the simulated spectrum.

Please bear in mind that the peak labels and the multiplet boxes will show the corresponding atom numbers of the molecule.

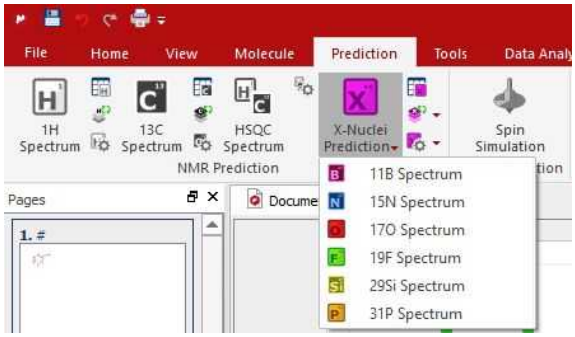


Finally, if you want to know the "predicted assignment", just hover the mouse over the desired atom, (so that it becomes highlighted in a red box) and the corresponding signal in the spectrum will automatically highlight in blue (increasing slightly its intensity); or vice versa (hovering the mouse over the peak will highlight the corresponding atom on the molecular structure).

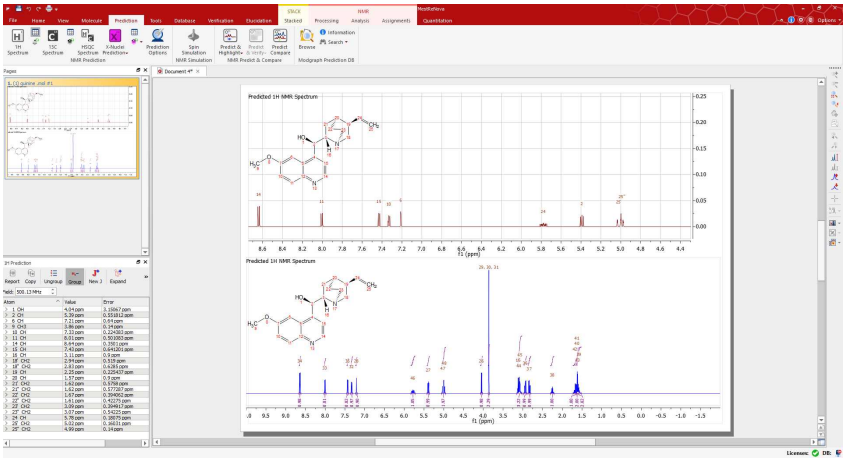
© 2024 MESTRELAB RESEARCH



A similar procedure will be followed to run X-Nuclei predictions: ^{31}P, ^{19}F, ^{15}N, ^{17}O, ^{29}Si. In this case, just follow the menu 'Predict' and select the desired option:



Of course you will be able to change the appearance of your simulated spectra as you prefer. Here is a sample:



© 2024 MESTRELAB RESEARCH

Thank you!

Thank you for reading this manual, and for purchasing this release version of Mnova. We will be very keen to read your feedback on the application, to hear about any bugs you may find and to also listen to any additional ideas or suggestions you may have.

Please remember that you can send all those, and any queries about the software, or requests for help, to:

support@mestrelab.com

Keep checking our web site (www.mestrelab.com) for additional information on our range of software packages, and for news on our company.