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Mnova Training - Basics

Version 12.0.3

Oct. 23, 2018

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Main Topics

- Installation and Activation of Mnova NMR
- Opening and processing 1D ^{1}H NMR
- Multiplet analysis for 1D ^{1}H NMR
- Opening and processing 1D ^{13}C NMR
- Peak picking for 1D ^{13}C NMR
- Opening and analyzing LC-MS
- Reporting and publishing results
- Saving the results

Note: This tutorial covers the NMR, NMRPredict and MS plugins of Mnova

Specifics for <xxxxxx> University (To be completed by instructor)

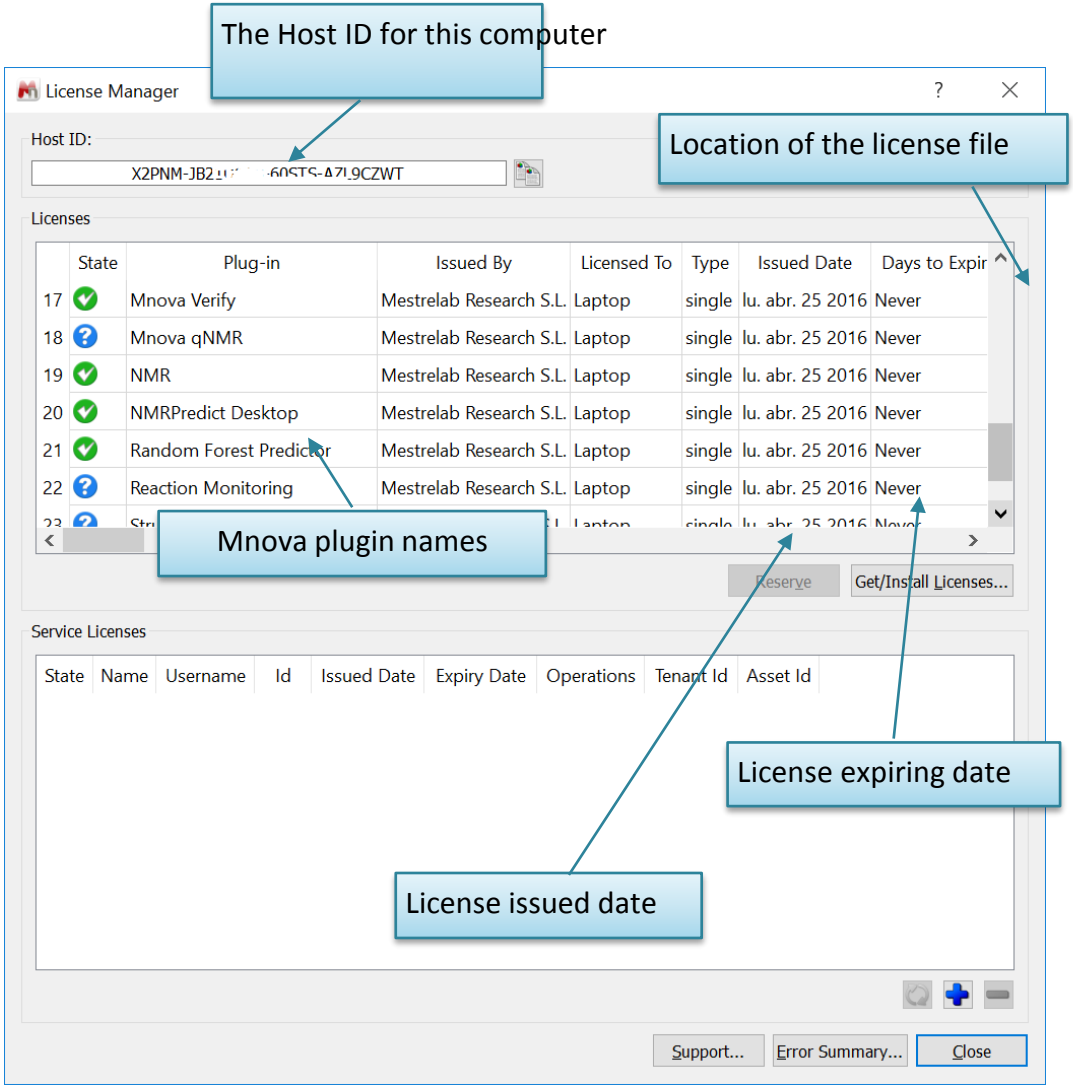
- The instructions for downloading, installing and activating Mnova:
  - <Link to instruction page>

- The Mnova licenses that <XXXX> University has:
  - Mnova Suite (NMR, NMRPredict & MS), unlimited
  - Etc.

- The sample data used in this tutorial are located at:
  - <Link to data folder>.

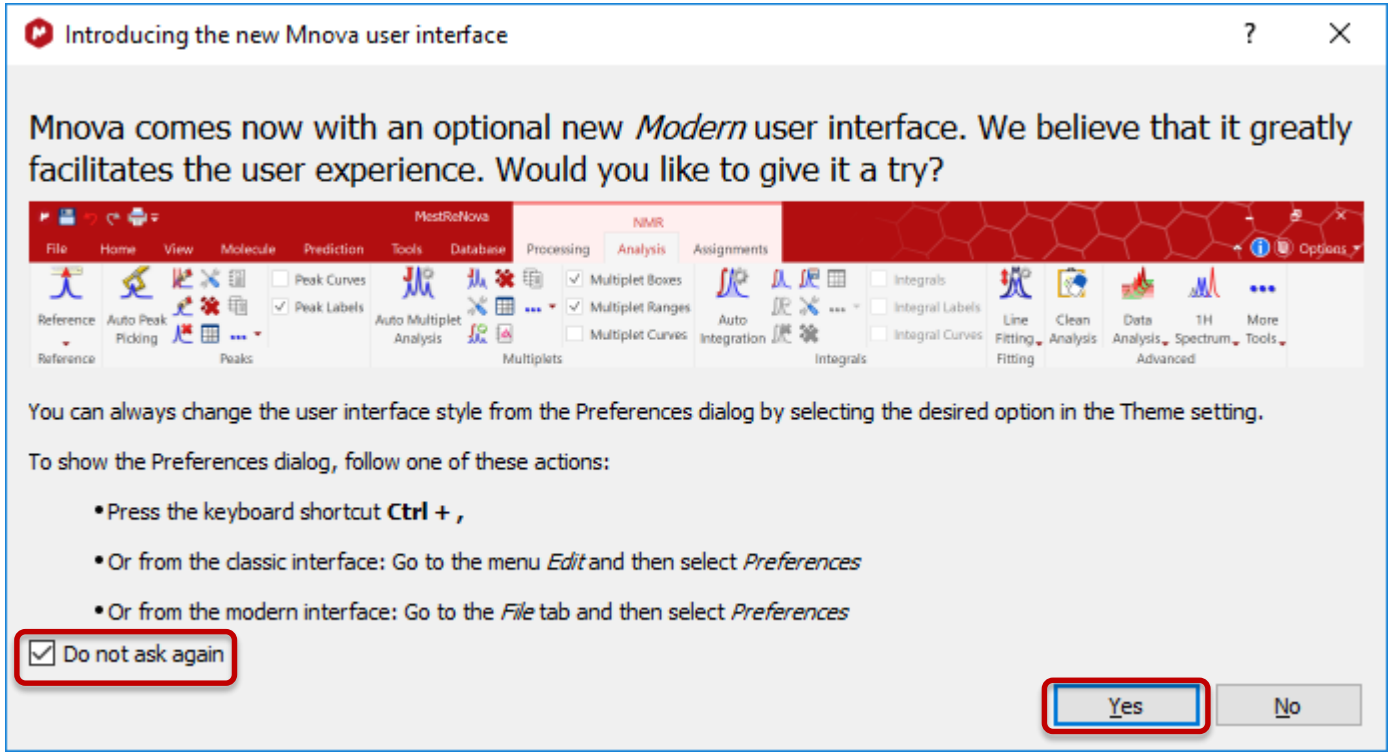
Install and activate Mnova in General

- Download and install Mnova from www.mestrelab.com. Choose ***File > Help > License Manager*** to open the License Manager dialog.
- Activate Mnova using your purchased license files, or apply for 45 day free trial licenses (Click ***Get/Install Licenses***)
- Make sure that there are green checkmarks for NMR and other plugins that are supposed to be activated
- For managing campus/site/ concurrent licenses, see http://www.mestrelab.com/mlicserver



Use the New Graphical User Interface (GUI)

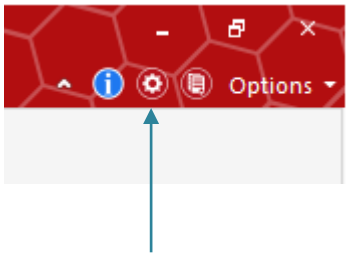
The first time you start Mnova, it asks if you want to use the “Modern” GUI. Choose it and check “Do not ask again”. Restart Mnova, you will enjoy the modular ribbon GUI introduced since version 12.



*Note: This instruction is based on the Modern GUI. You can always switch back to the “Classic” GUI from **”File/Preferences”.***

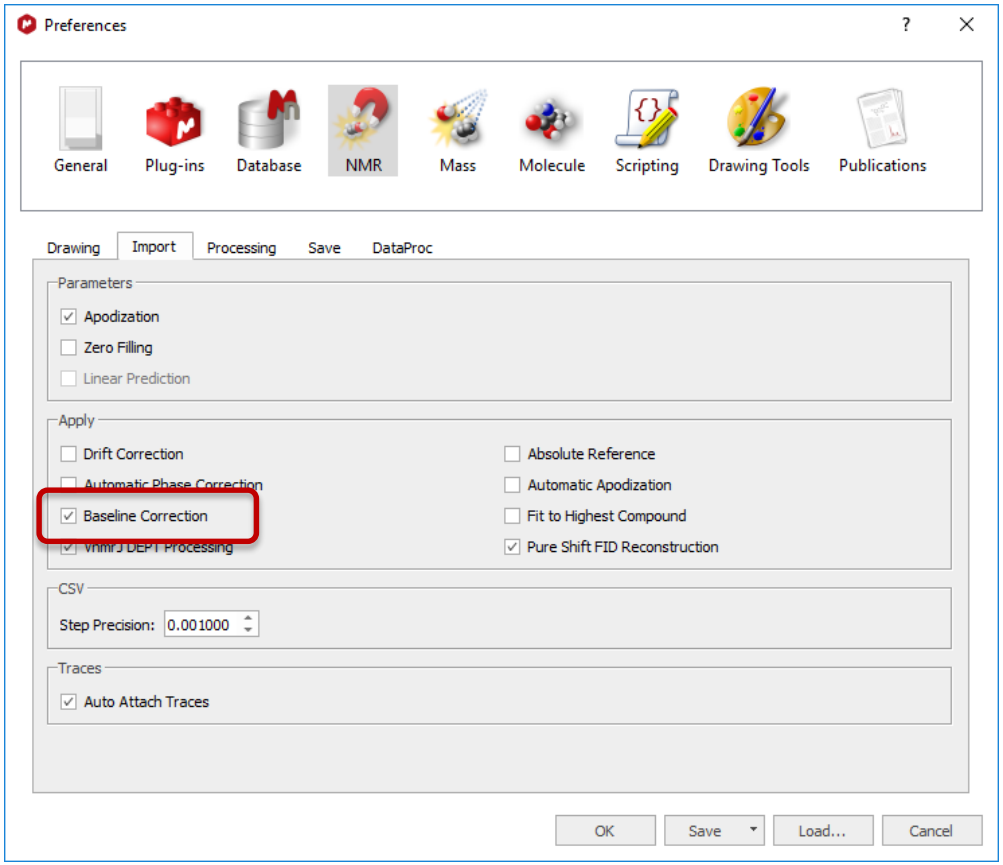
Turn on Auto Baseline Correction

Choose File/Preferences. In the NMR> Import Tab, check Baseline Correction so that baseline is automatically done when you open an NMR spectrum.



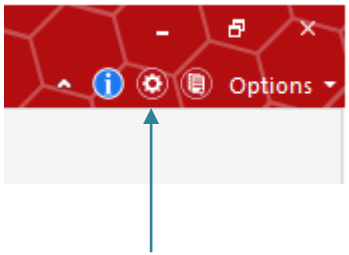
Note: Automatic Baseline Correction use the default algorithm of "Bernstein Polynomial with order of 3". This option may make manual phasing of 2D NMR sluggish. In that case, turn baseline off from Processing .

Tip: There are many options and settings that you may change in the Preferences Dialog, especially the resolution of image copying and image exporting in the Drawing Tab.



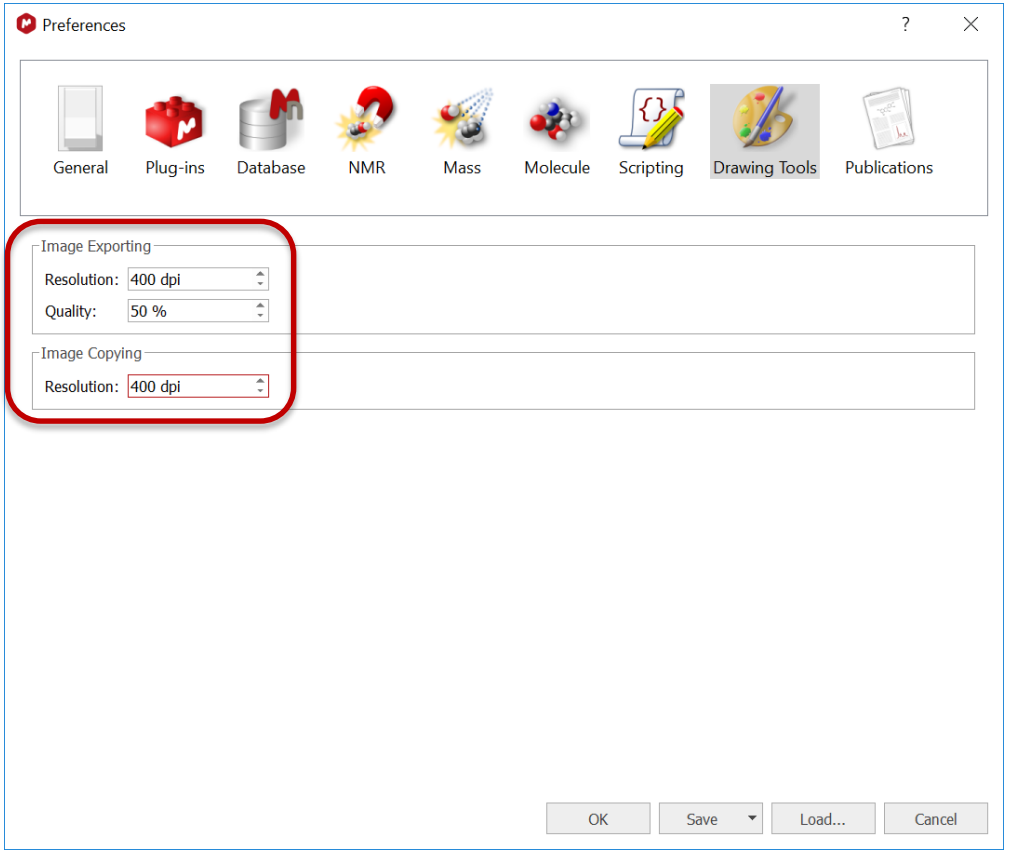
Setup the resolution for publishing spectra

Choose File/Preferences. In the Drawing Tools tab, change the resolutions for Image Exporting and Image Copying to numbers similar to something shown below.



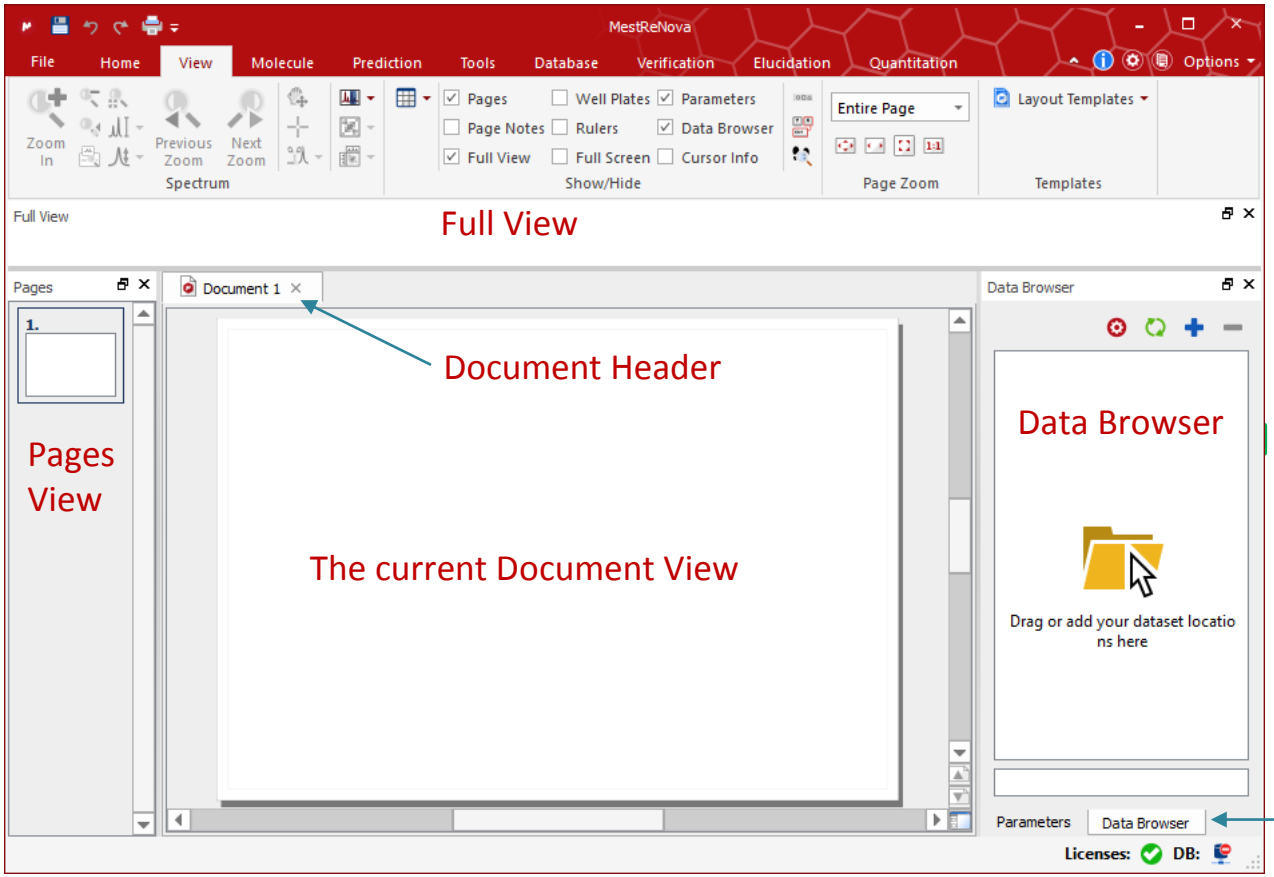
The resolution for Image Exporting is used when you choose File > Save As and save the selected objects in Mnova as a graphical image file.

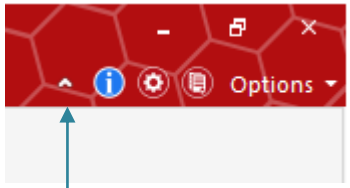
The resolution for Image Copying is used when you copy selected objects in Mnova and paste them to another application.



Setup the Workspace

- In the View Ribbon, check the Pages, Full View, Parameters, and Data Browser Views
- Dock and arrange them as shown below





Click here to minimize the ribbon

Click here to switch the panels or tables

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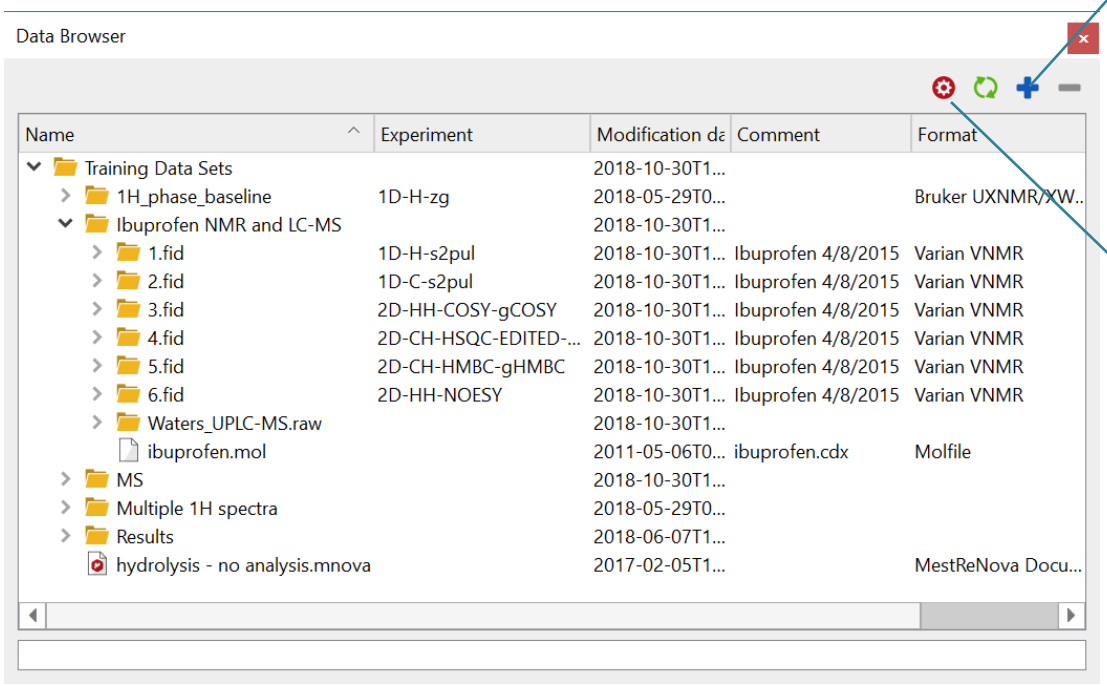
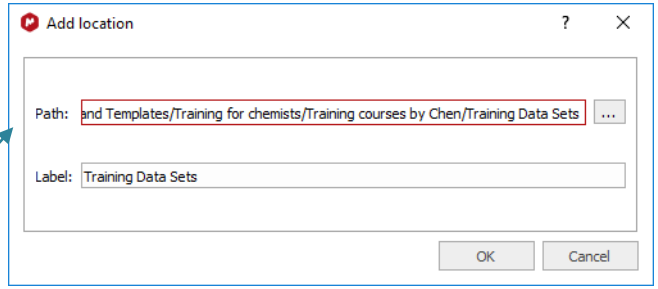
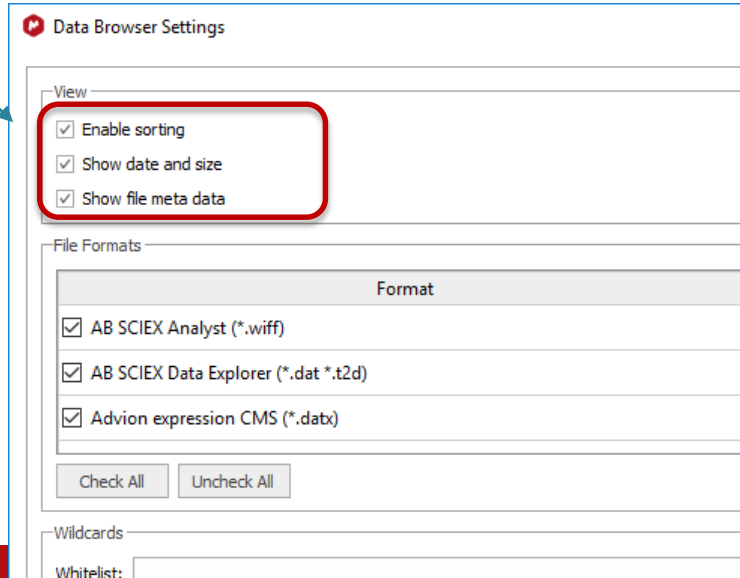
SETUP

Setup Data Browser

- Click “+” in the Data Browser, navigate to the directory where the sample NMR data are located and click OK to add it.
- Click the Settings button to turn on the display of the meta data, date and time, and enable sorting
- Make sure you see the data files similar to those shown below







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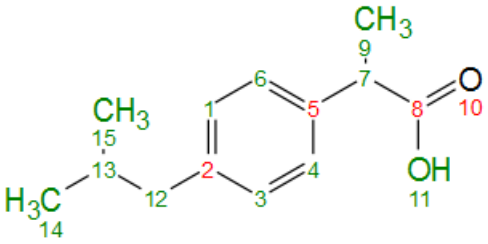
PROCEDURE

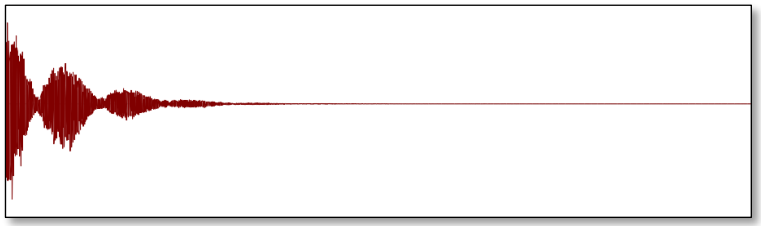
^{1}H processing and analysis: general procedure

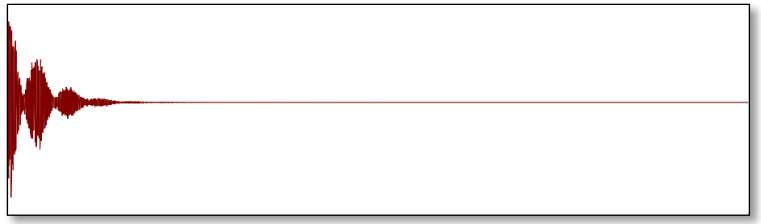
- Open the raw data
- Pre-process the FID: drift correct, apodize, zero fill, linear predict, etc.
- Fourier transform
- Phase correct and baseline correct
- Chemical shift reference
- Peak-pick, integrate, multiplet analysis
- Structure verification and peak assignment
- Report and publish

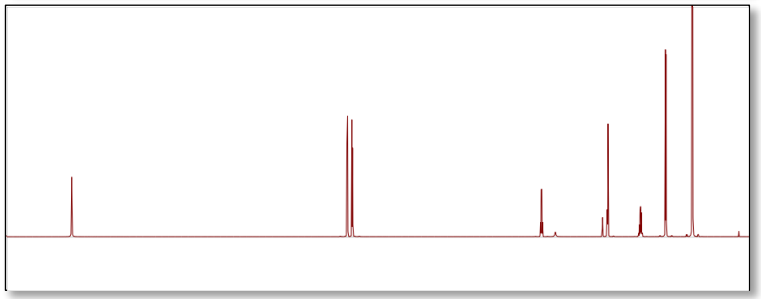


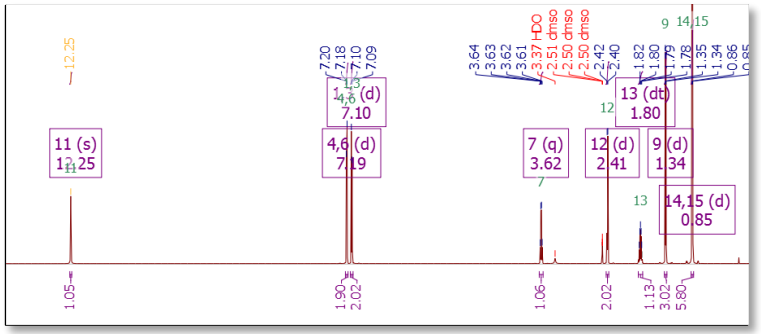
^{1}H NMR (600 MHz, DMSO-d_6) δ 12.25 (s, 1H), 7.19 (d, J = 7.8 Hz, 2H), 7.10 (d, J = 7.9 Hz, 2H), 3.62 (q, J = 7.1 Hz, 1H), 2.41 (d, J = 7.2 Hz, 2H), 1.80 (dt, J = 13.5, 6.8 Hz, 1H), 1.34 (d, J = 7.1 Hz, 3H), 0.85 (d, J = 6.7 Hz, 6H).

Note: Most of these steps are done automatically by Mnova. However, you retain full control at all times



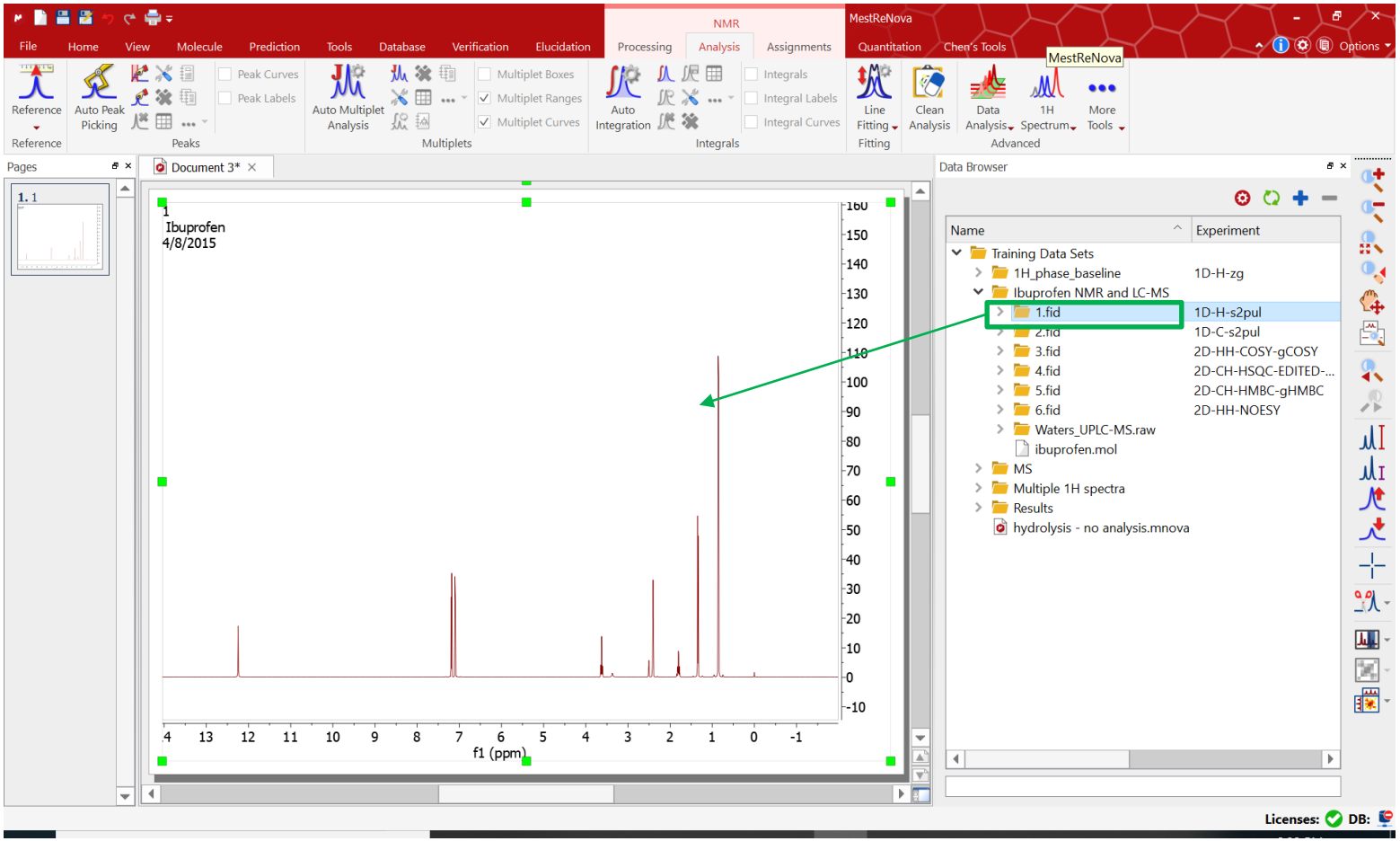






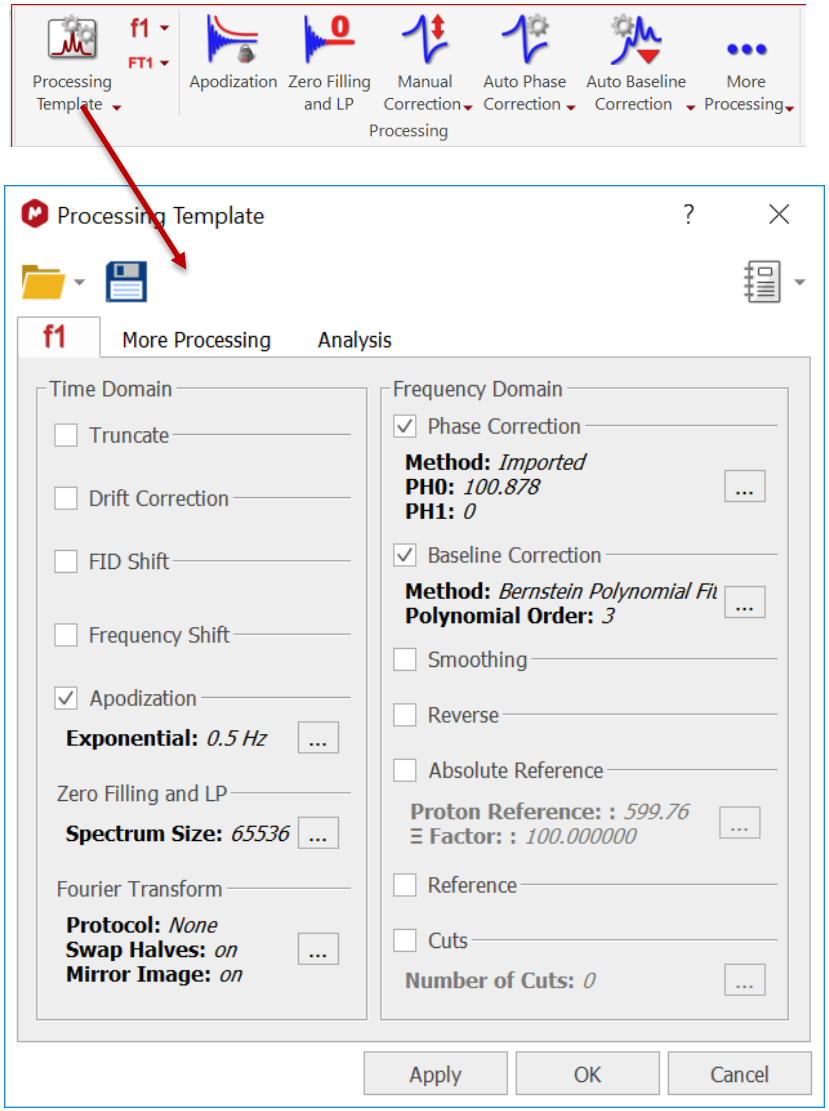
Open a H-1 spectrum

- In Data Browser, expand the folders Training Data Sets > Ibuprofen NMR and LC-MS, and drag the “1.fid” folder (1D H-1 spectrum) to the main window.
- Notice the H-1 spectrum is automatically processed and displayed.



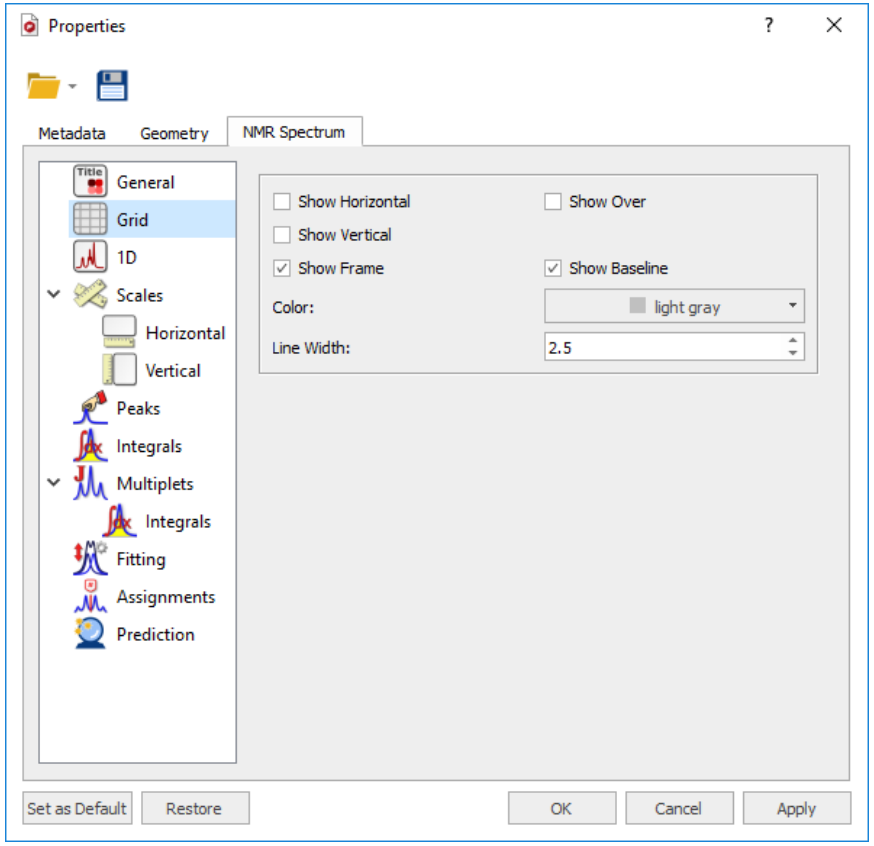
Verify the processing parameters

- In most cases, Mnova processes the spectrum automatically using the parameters from the instrument. The spectrum should be well-processed if the original processing parameters were well set. The Processing Tab is for you to re-process the spectrum when needed.
- Choose Processing > Processing Template to verify the processing parameters. Make sure they look the same as displayed on the right.
- Click OK or Apply to re-process the spectrum.



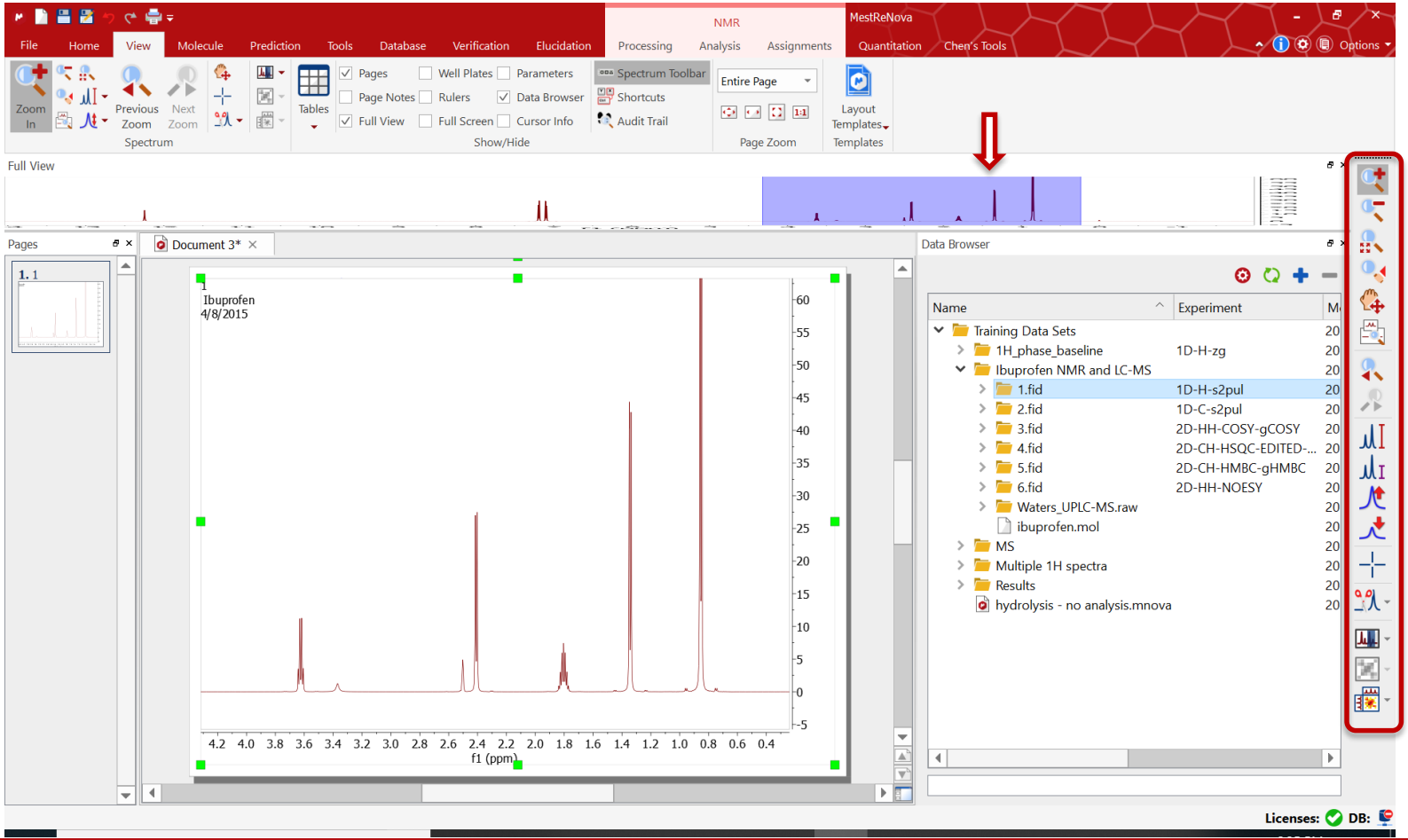
Change the Display Properties

- Double click on the spectrum to open the Properties Dialog, view the properties that can be changed.
- In the Grid Category, uncheck Show Horizontal, and Show Vertical, check Show Baseline
- Click Apply, and then Set as Default to apply the settings to 1D spectra opened in the future



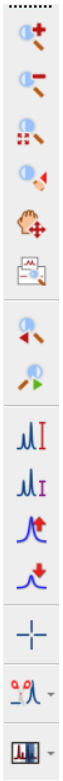
Navigate in the H-1 Spectrum

- Use the Spectrum Toolbar to zoom in/out, pan, and change the Y scale (see next slide for details)
- Use the Full View to move to different zoom in area (click or drag)

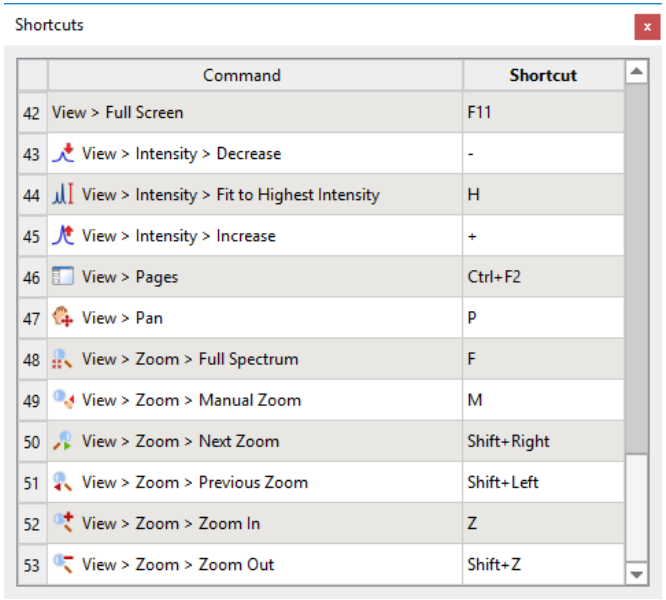


Spectrum visualization tools

- The Spectrum Toolbar is visible only after you open a spectrum.
- Learn some short-cut keys by choosing View > Shortcuts



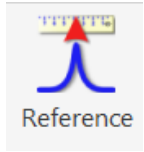
Zoom in/Zoom out (or press Z) *
Zoom out
Full spectrum (or press F)
Manual Zoom in to defined ppm range
Pan spectrum (or press P) **
Expansion – click&drag to draw an inset (or press E)
Previous Zoom level
Next Zoom level
Fit to Highest Intensity (or press H)
Fit to highest compound peak
Increase Intensity (or rotate mouse wheel)
Decrease Intensity (or rotate mouse wheel)
Crosshair Cursor (or press C) for measuring *J*-couplings
Cut (or press X) to hide parts of the spectrum
Edit Blind regions



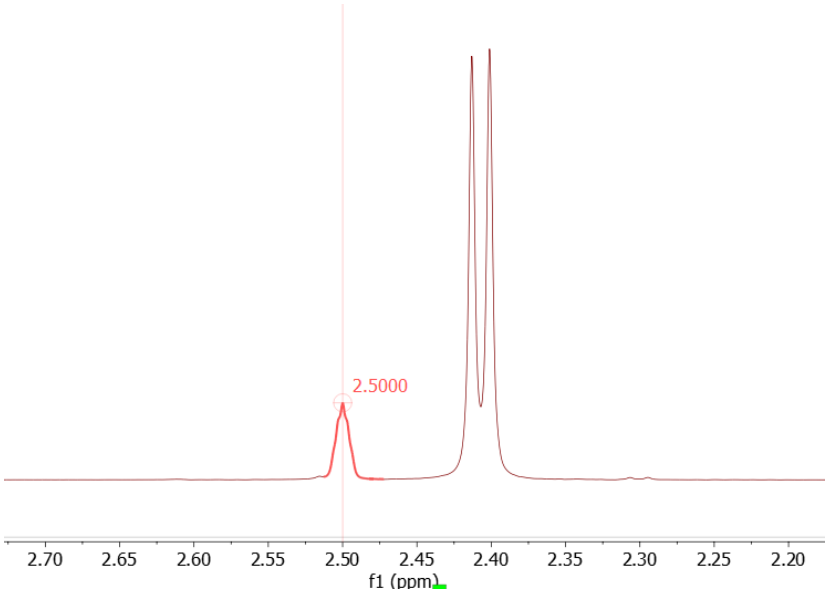
	Command	Shortcut
42	View > Full Screen	F11
43	View > Intensity > Decrease	-
44	View > Intensity > Fit to Highest Intensity	H
45	View > Intensity > Increase	+
46	View > Pages	Ctrl+F2
47	View > Pan	P
48	View > Zoom > Full Spectrum	F
49	View > Zoom > Manual Zoom	M
50	View > Zoom > Next Zoom	Shift+Right
51	View > Zoom > Previous Zoom	Shift+Left
52	View > Zoom > Zoom In	Z
53	View > Zoom > Zoom Out	Shift+Z

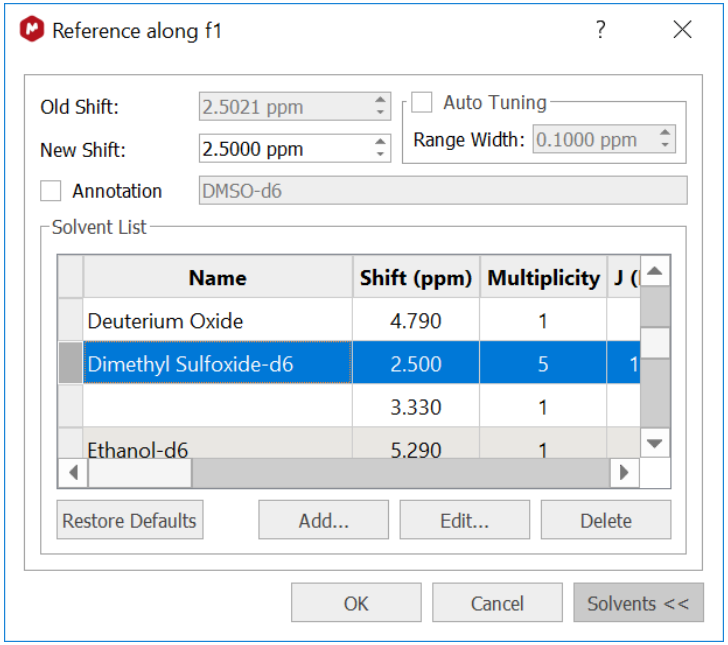
** Press **Z** several times to toggle between horizontal/vertical/box zoom*
*** Press **P** several times to toggle between free/horizontal/vertical panning*

Chemical Shift Referencing



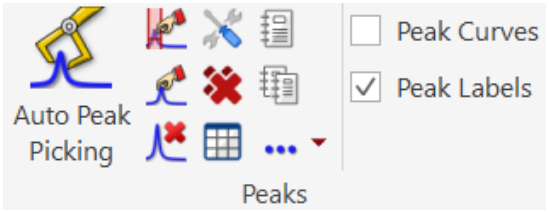
- This spectrum uses DMSO-d6 as the solvent. We can reference the chemical shifts by setting its middle peak to 2.5 ppm.
- Zoom to the DMSO peak at around 2.5 ppm. Choose Analysis > Reference, and click on the top of the middle peak.
- Set it to 2.5 ppm either manually or from the Solvent List.

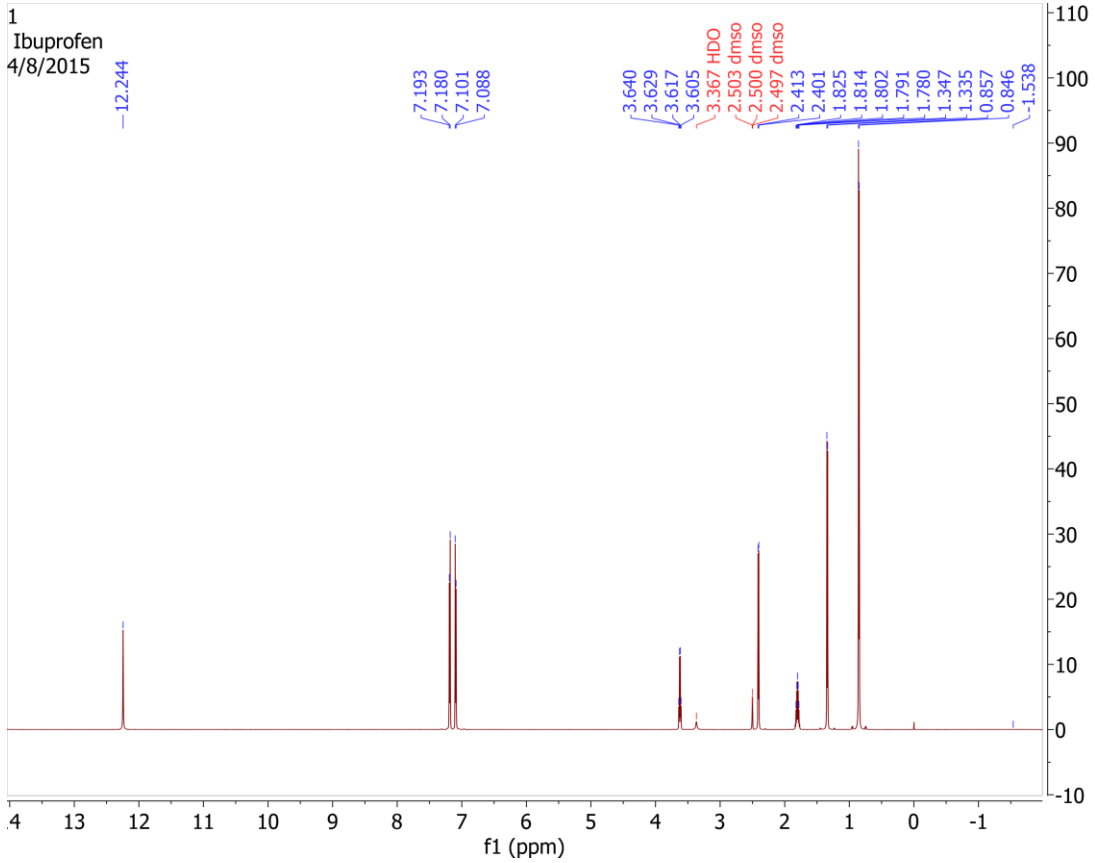


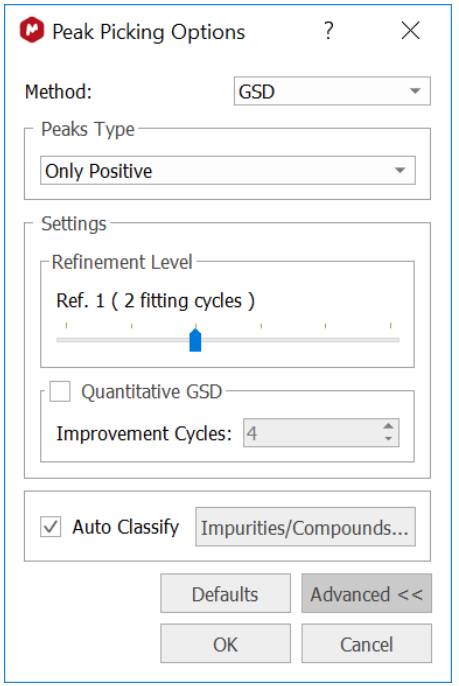


Peak picking

- Click the Peaks > Options  to verify the peak picking options. Default settings are used here as shown to the right.
- Click the Auto Peak Picking tool to pick all the peaks
- Using other peak picking tools to display/delete/add/change peaks as needed.

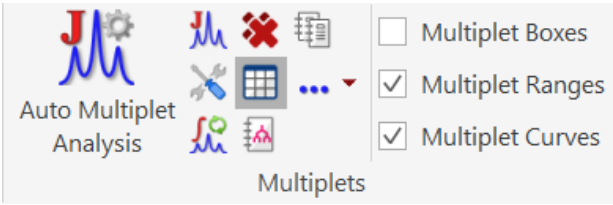


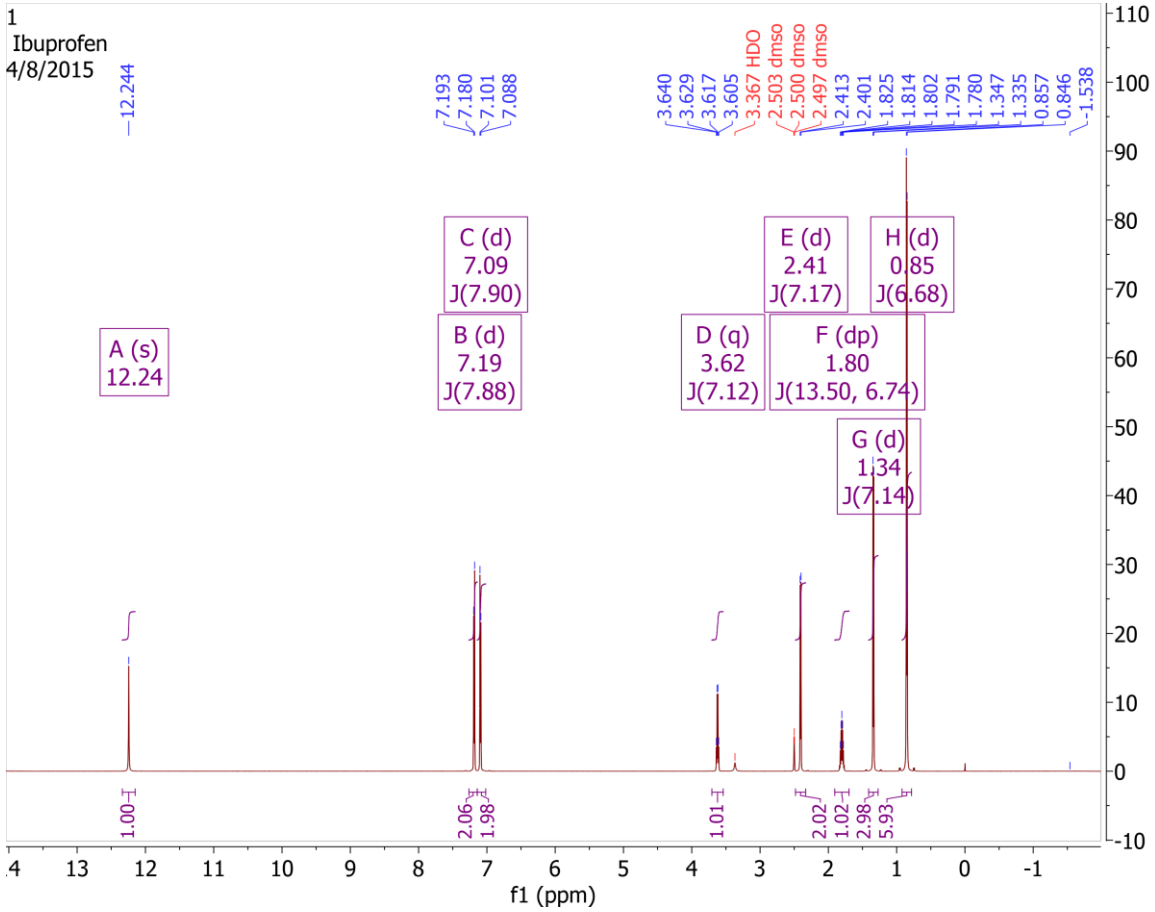


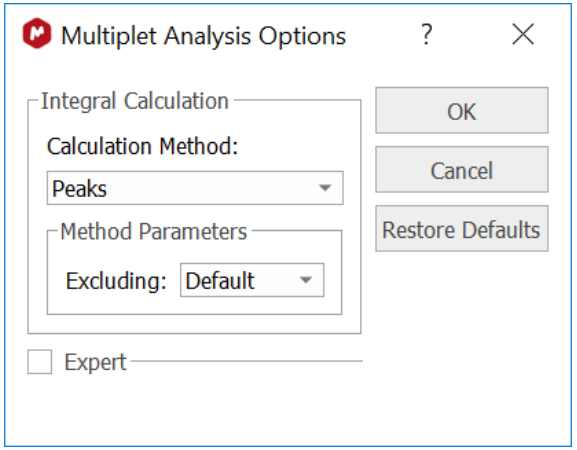


Multiplet analysis

- Click the Multiplets > Options  to verify the multiplet analysis options. Default settings are used here as shown to the right.
- Click the Auto Multiplet Analysis tool to do the multiplet analysis based on the picked peaks

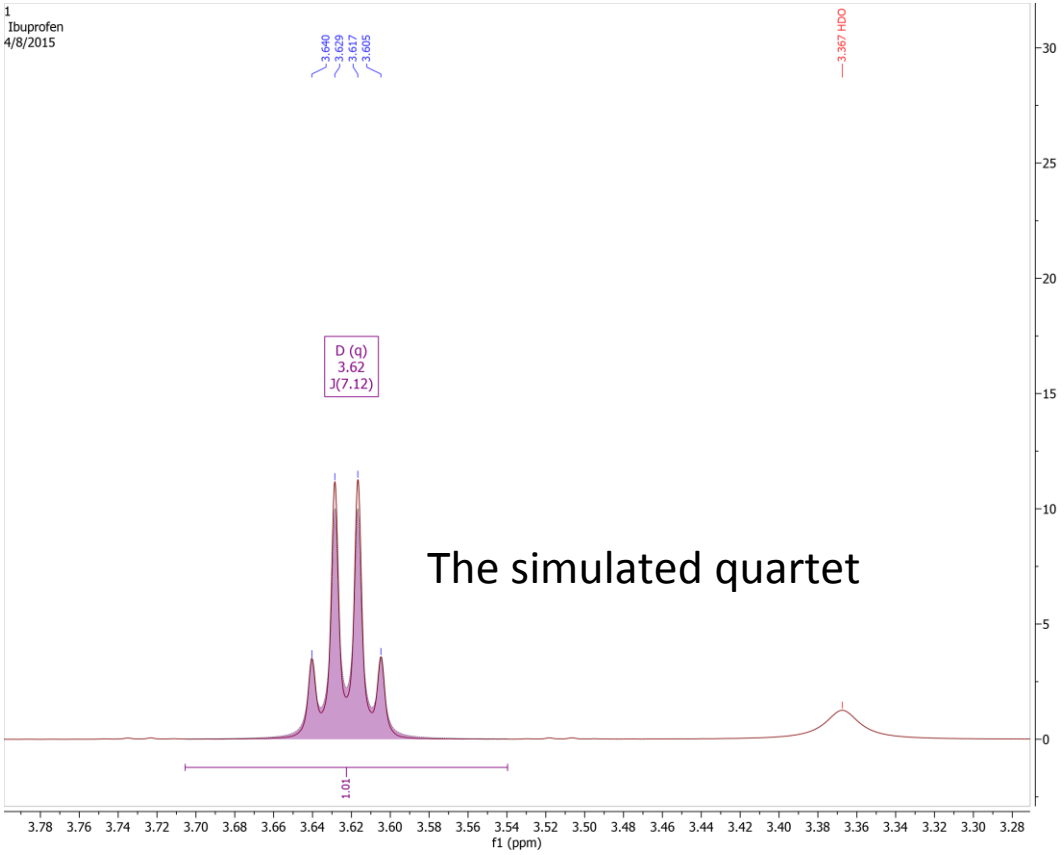


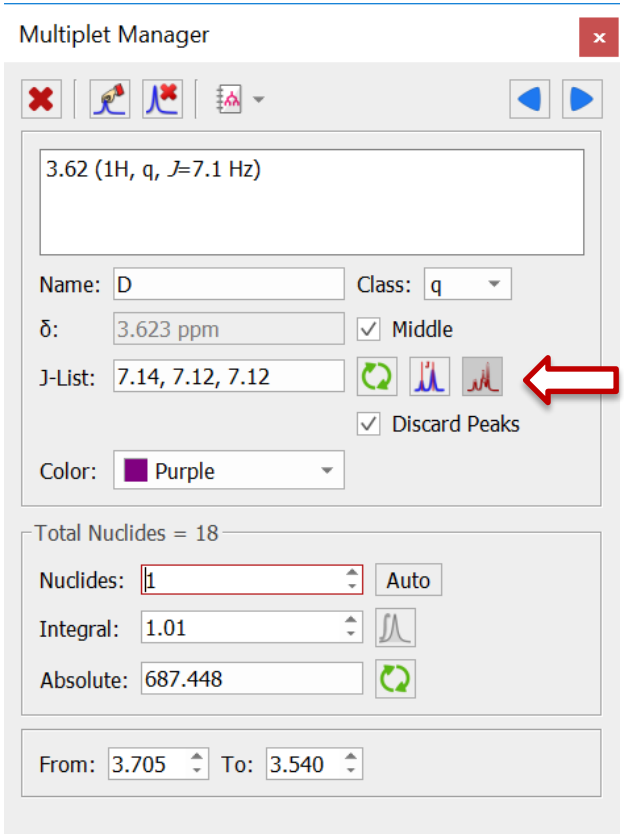




Multiplet Manager

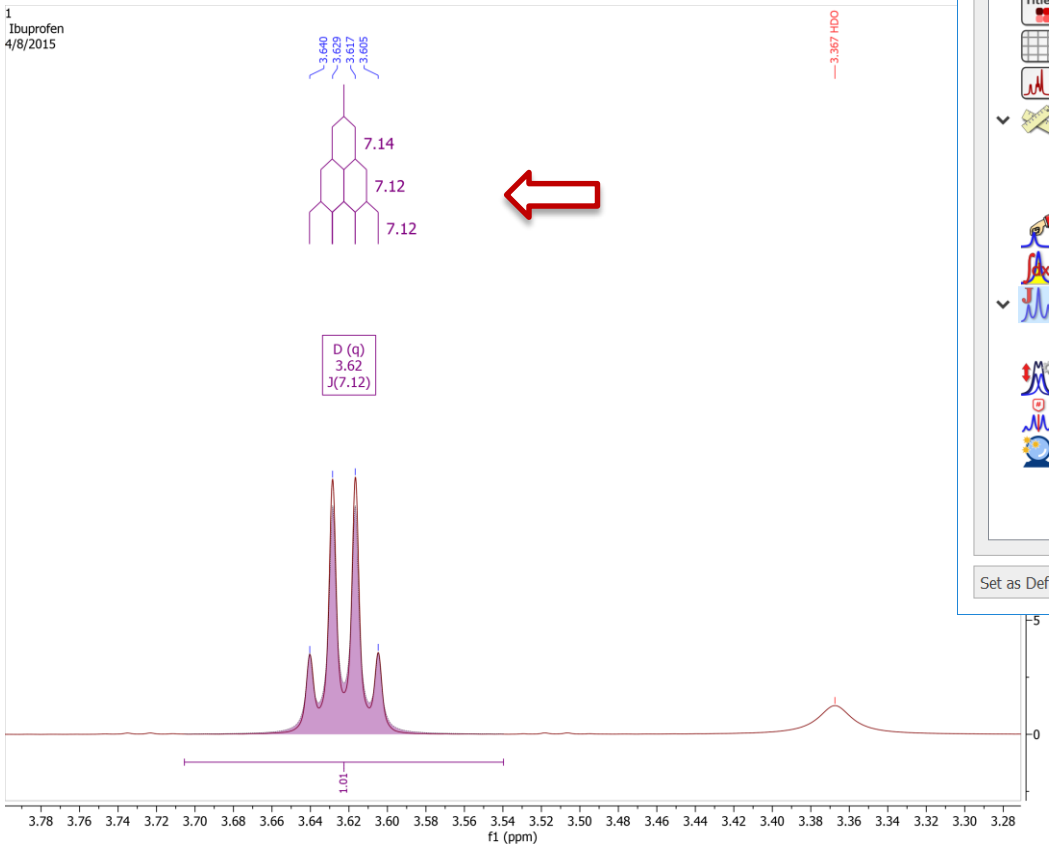
- Double click on a multiplet label to open the Multiplet Manager.
- Use the tools there to verify and change multiplet analysis results if needed.

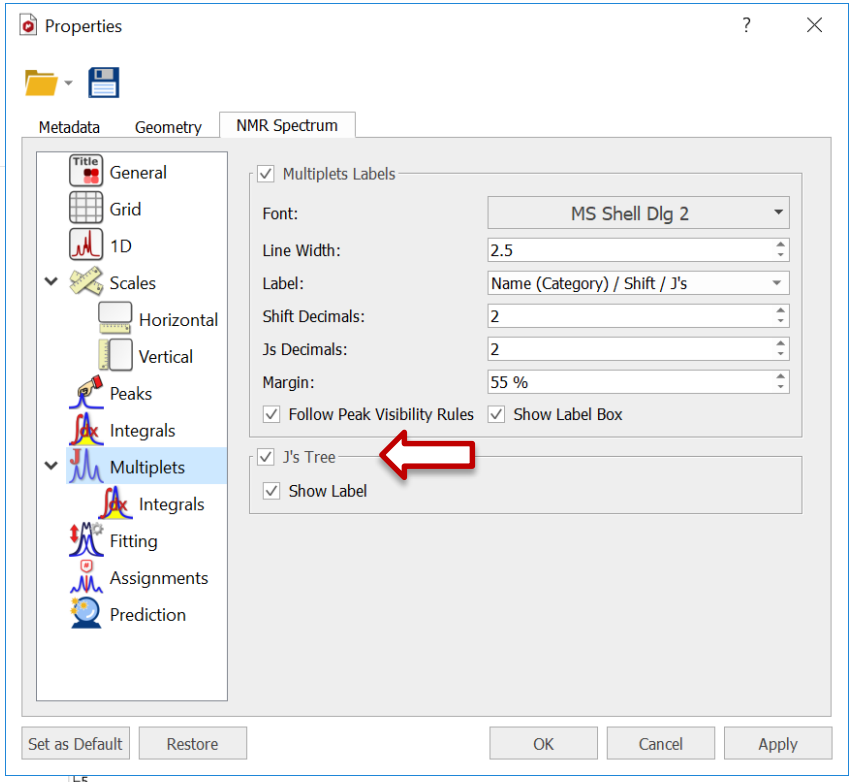




Multiplet Manager

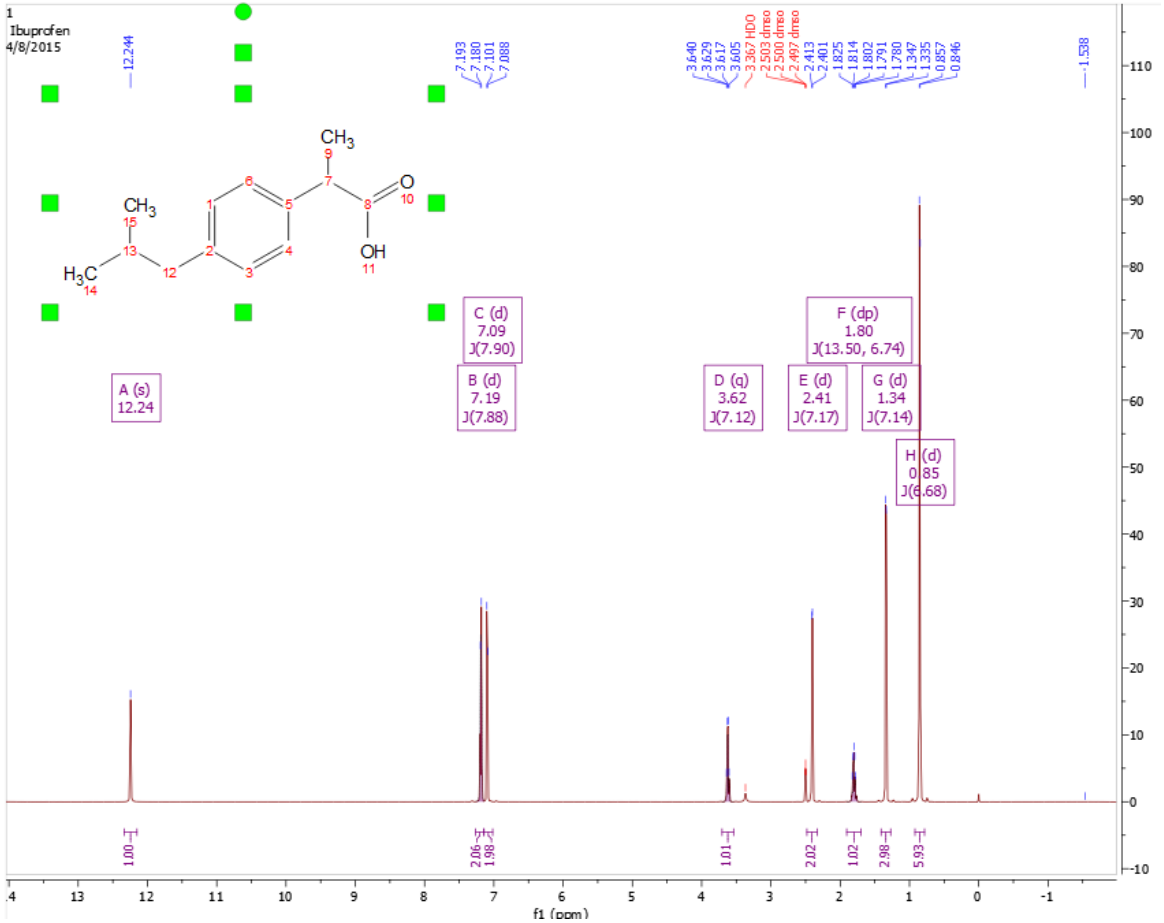
- Double click on the spectrum to open the Properties dialog.
- Choose Multiplets, and check J's Tree to display the J-coupling tree for visual verification of the multiplet analysis results.

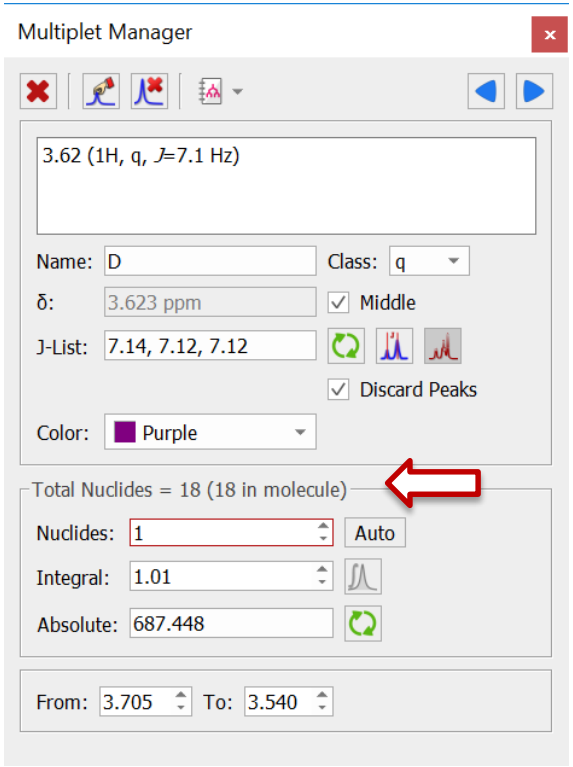




Verify the number of Hs

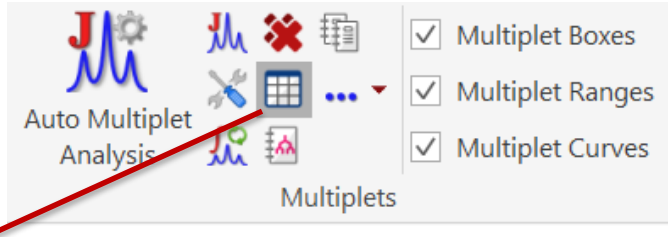
- Open the Ibuprofen.mol file from the Data Browser.
- Note the number of protons from multiplet analysis vs. that from the structure

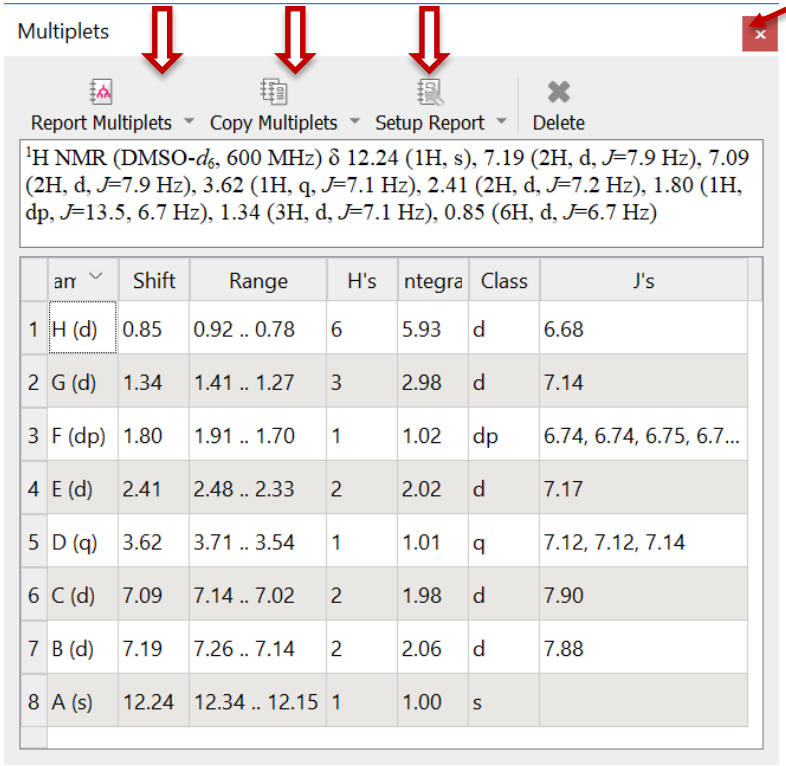




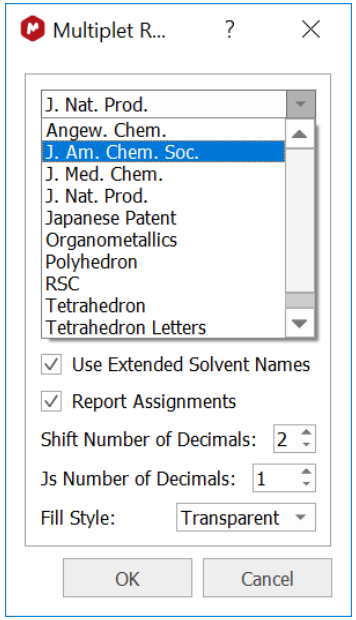
Report the multiplets

- Use the Multiplet Table tool to display the Multiplets Table.
- Click Setup Report to change the reporting format
- Click Report to report the multiplets texts





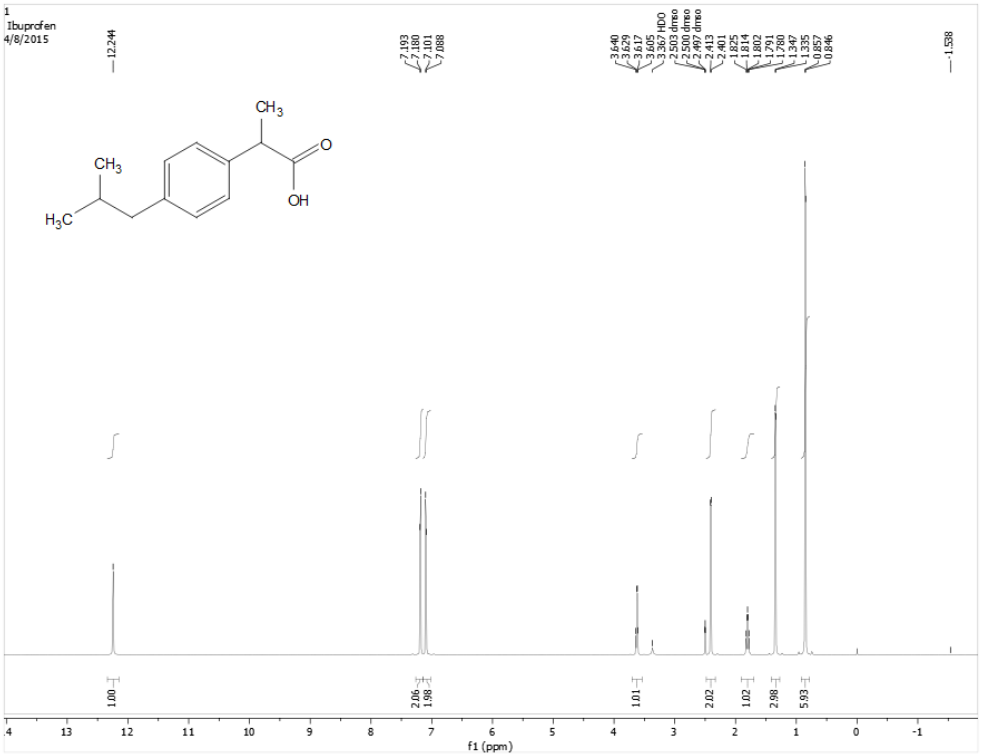
	arr	Shift	Range	H's	ntegra	Class	J's
1	H (d)	0.85	0.92 .. 0.78	6	5.93	d	6.68
2	G (d)	1.34	1.41 .. 1.27	3	2.98	d	7.14
3	F (dp)	1.80	1.91 .. 1.70	1	1.02	dp	6.74, 6.74, 6.75, 6.7...
4	E (d)	2.41	2.48 .. 2.33	2	2.02	d	7.17
5	D (q)	3.62	3.71 .. 3.54	1	1.01	q	7.12, 7.12, 7.14
6	C (d)	7.09	7.14 .. 7.02	2	1.98	d	7.90
7	B (d)	7.19	7.26 .. 7.14	2	2.06	d	7.88
8	A (s)	12.24	12.34 .. 12.15	1	1.00	s	

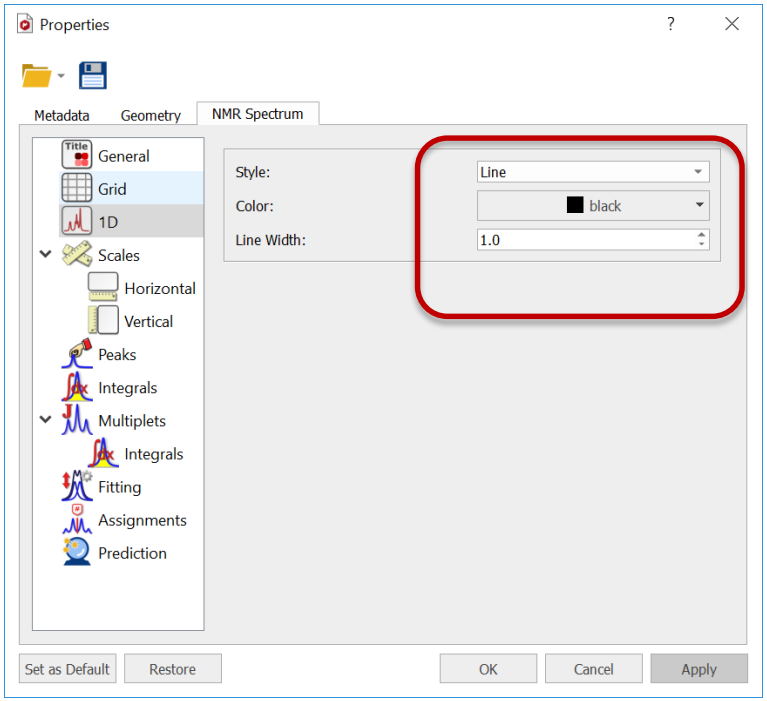


¹H NMR (DMSO-d_6, 600 MHz) δ 12.24 (1H, s), 7.19 (2H, d, J=7.9 Hz), 7.09 (2H, d, J=7.9 Hz), 3.62 (1H, q, J=7.1 Hz), 2.41 (2H, d, J=7.2 Hz), 1.80 (1H, dp, J=13.5, 6.7 Hz), 1.34 (3H, d, J=7.1 Hz), 0.85 (6H, d, J=6.7 Hz)

Publishing a spectrum

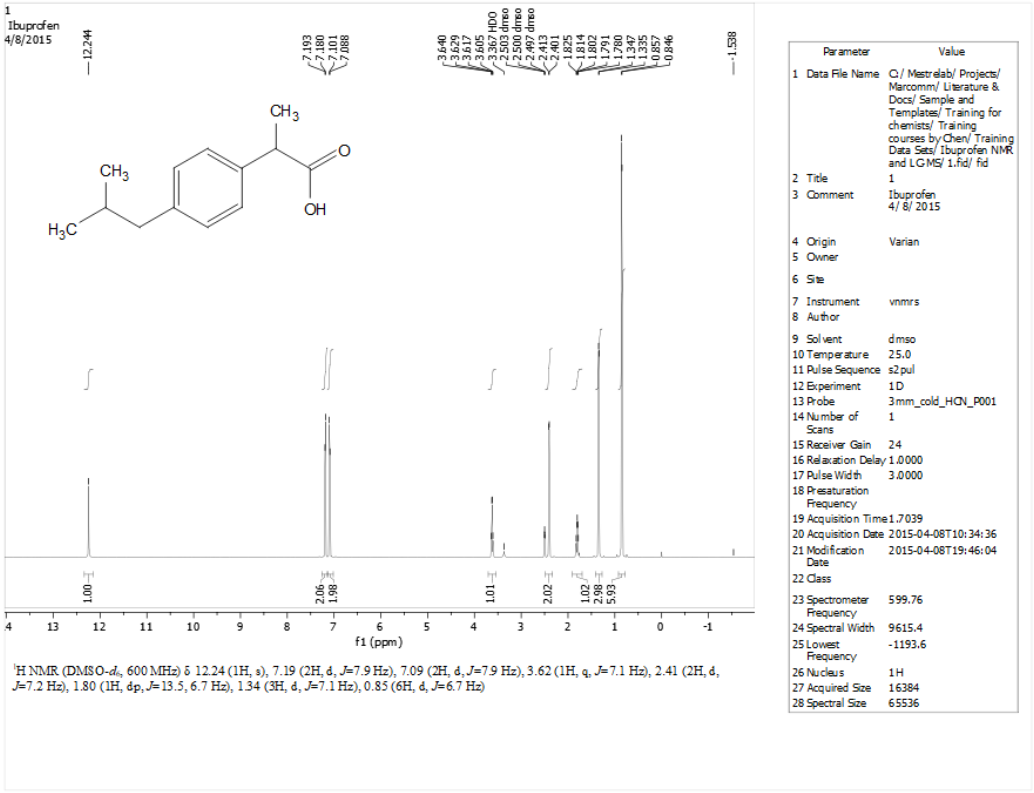
- To publish the spectrum on a black and white journal, double click the spectrum to open the Properties Dialog, and set the 1D properties to as shown on the right.
- Choose other properties to display, such as the peak labels, multiplet labels, integrals, etc.
- Copy the spectrum and structure objects and paste them to other documents, such as MicroSoft Word or PPT.





Display the parameters

- Check View > Parameters Table to display the Parameters Table, and report the parameters on the spectrum. Manually resize the text box to similar to as shown below.
- Report the multiplets and resize the box to as shown below.



	Parameter	Value
1	Data File Name	C:/ Mestrelab/ Projects/ Marcomm/ Literature & Docs/ Sample and Templates/ Training for chemists/ Training courses by Chen/ Training Data Sets/ Ibuprofen NMR and LCMS/ 1.fid/ fid
2	Title	1
3	Comment	Ibuprofen 4/ 8/ 2015
4	Origin	Varian
5	Owner	
6	Site	
7	Instrument	vnmrs
8	Author	
9	Solvent	dmso
10	Temperature	25.0
11	Pulse Sequence	s2pul
12	Experiment	1D
13	Probe	3mm_cold_HCN_P001
14	Number of Scans	1
15	Receiver Gain	24
16	Relaxation Delay	1.0000
17	Pulse Width	3.0000
18	Presaturation Frequency	
19	Acquisition Time	1.7039
20	Acquisition Date	2015-04-08T10:34:36
21	Modification Date	2015-04-08T19:46:04
22	Class	
23	Spectrometer Frequency	599.76
24	Spectral Width	9615.4
25	Lowest Frequency	-1193.6
26	Nucleus	1H
27	Acquired Size	16384
28	Spectral Size	65536

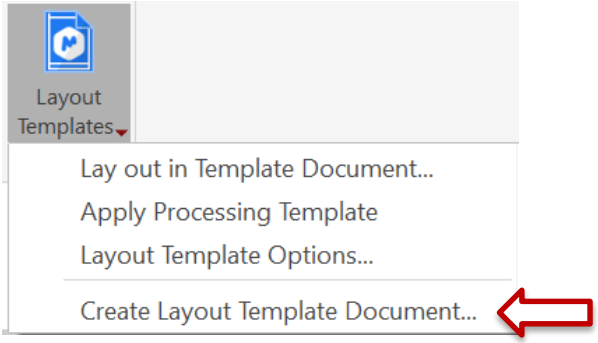
Parameters

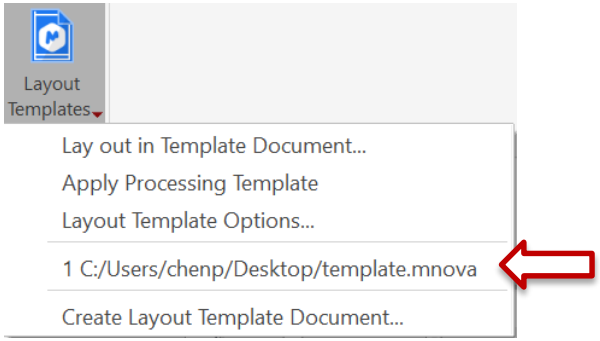
Report Copy Setup Customize

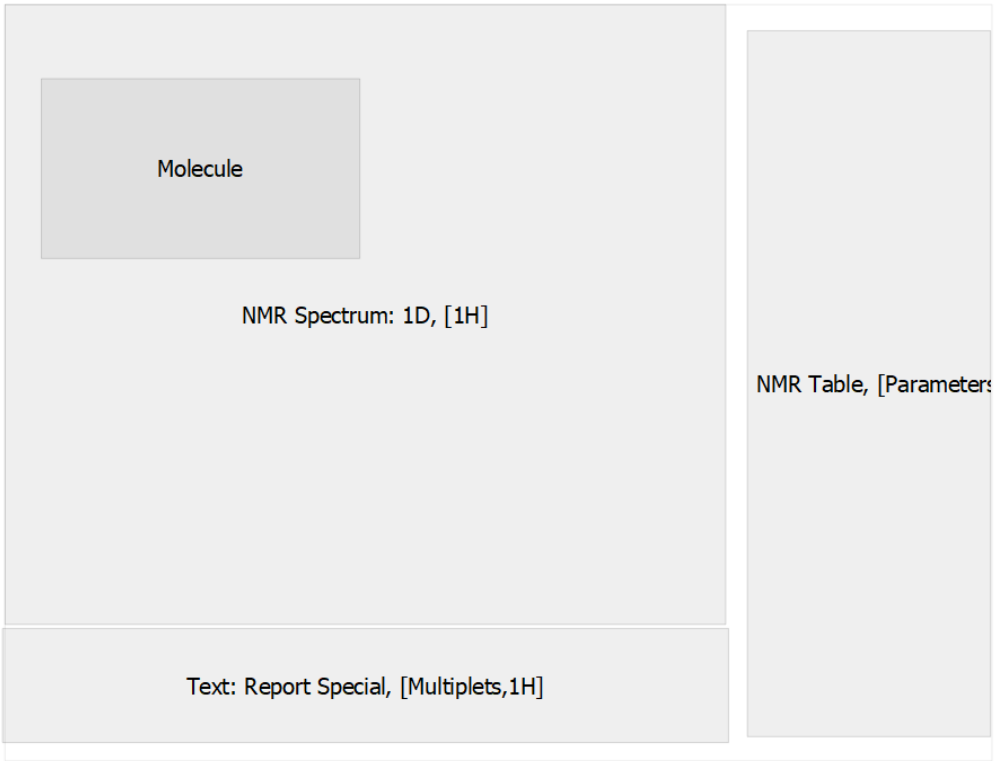
	Parameter	Value
1	Data File Name	C:/Mestrelab/Projects/Marcomm/Liter...
2	Title	1
3	Comment	Ibuprofen 4/8/2015
4	Origin	Varian
5	Owner	
6	Site	
7	Instrument	vnmrs
8	Author	
9	Solvent	dmso
10	Temperature	25.0
11	Pulse Sequence	s2pul
12	Experiment	1D
13	Probe	3mm_cold_HCN_P001
14	Number of Scans	1
15	Receiver Gain	24
16	Relaxation Delay	1.0000
17	Pulse Width	3.0000

Create a layout template

- Click on View > Layout Template and choose Create Layout Template to save a layout template. You can edit it.
- Choose File > New and open the H-1 spectrum again, and choose View > Layout Template > [*Saved Template Name*] to apply it.

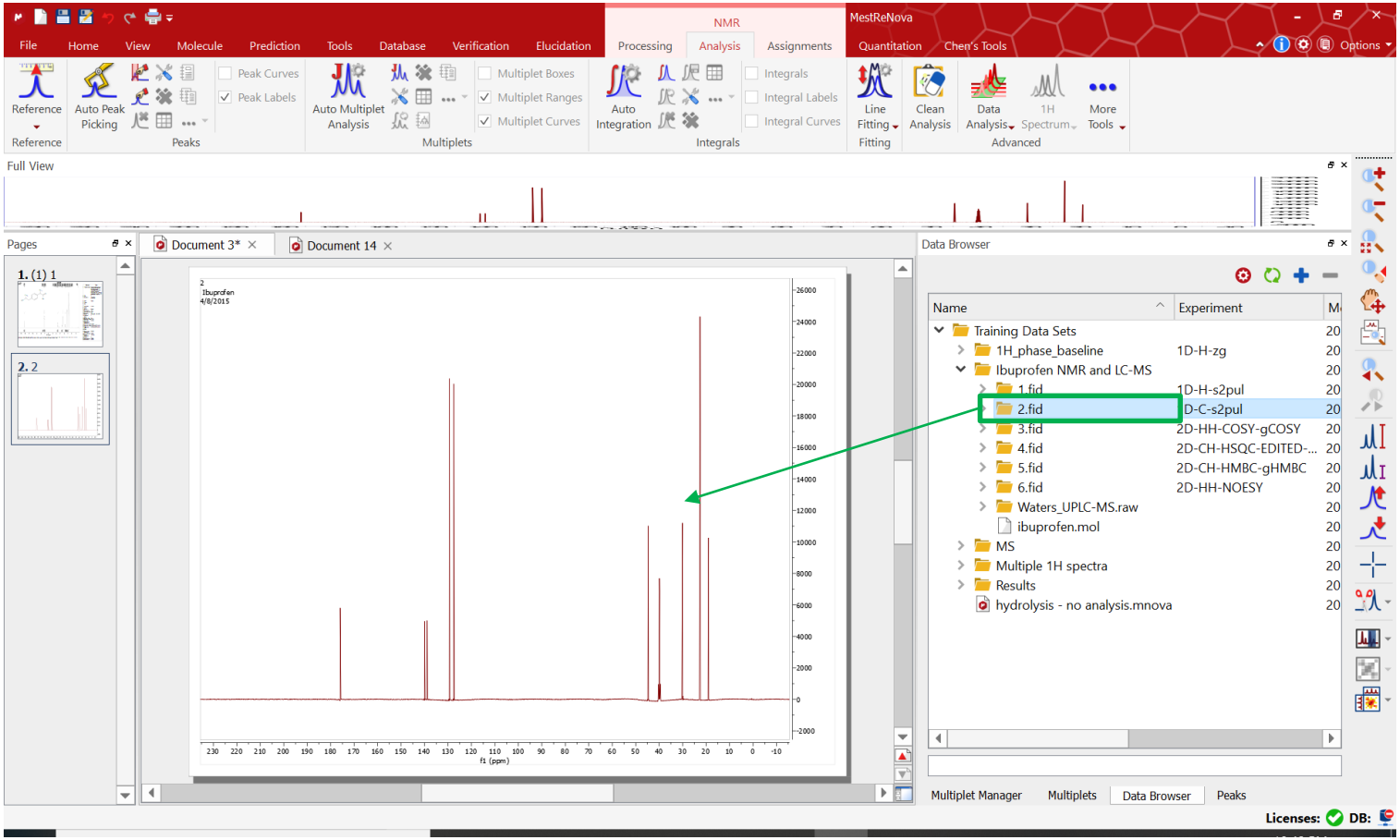






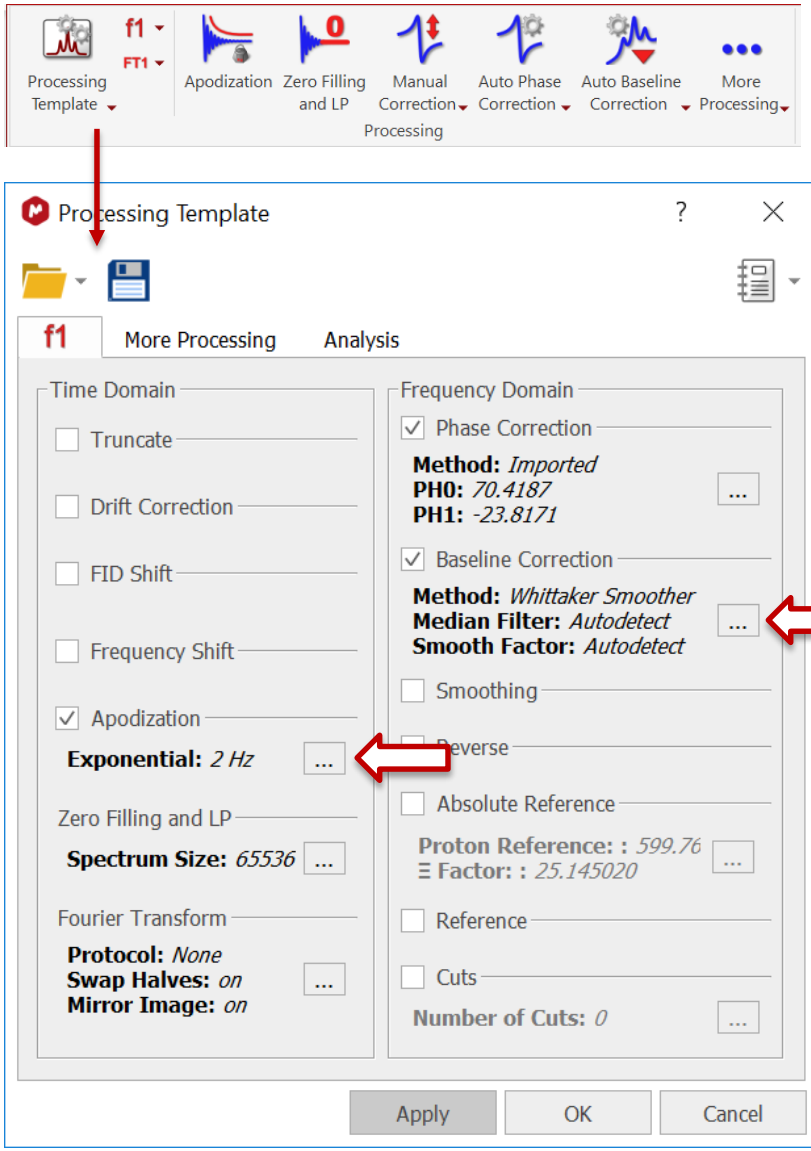
Open a C-13 spectrum

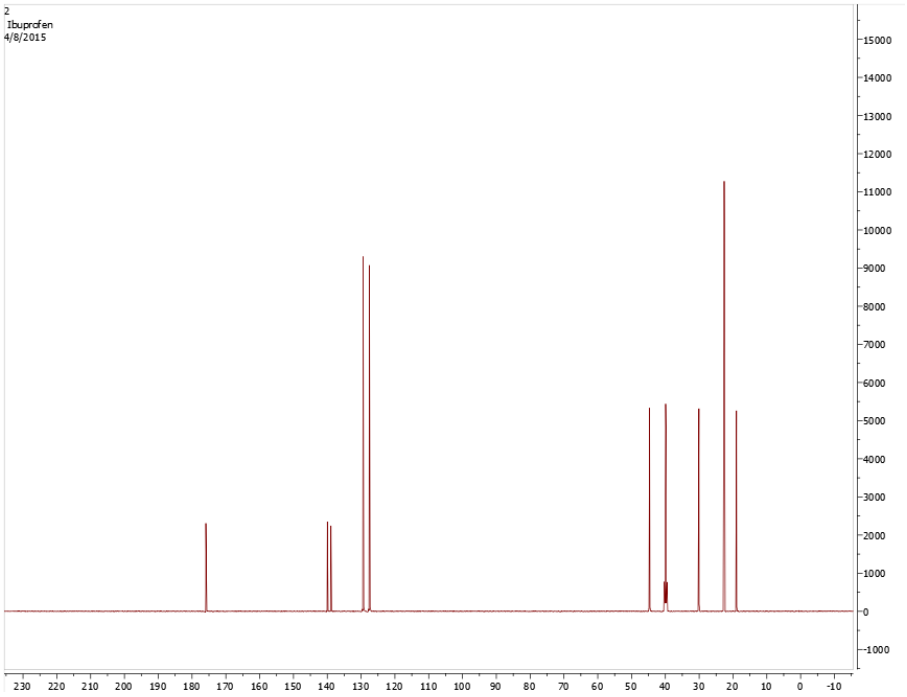
➢ In Data Browser, open the C-13 spectrum of Ibuprofen.



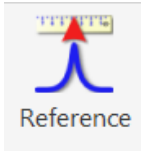
Verify the processing parameters

- Choose Processing > Processing Template, and set the parameters similar to the ones shown to the right.
- Click OK or Apply to re-process the spectrum.

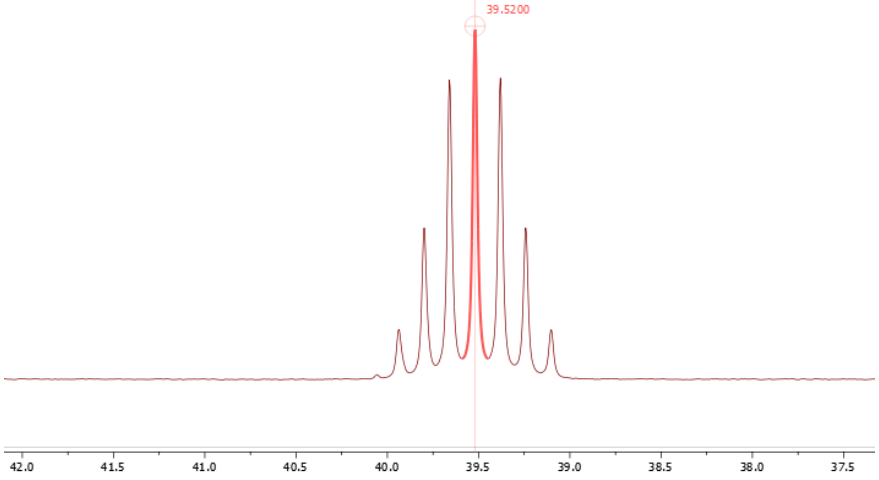


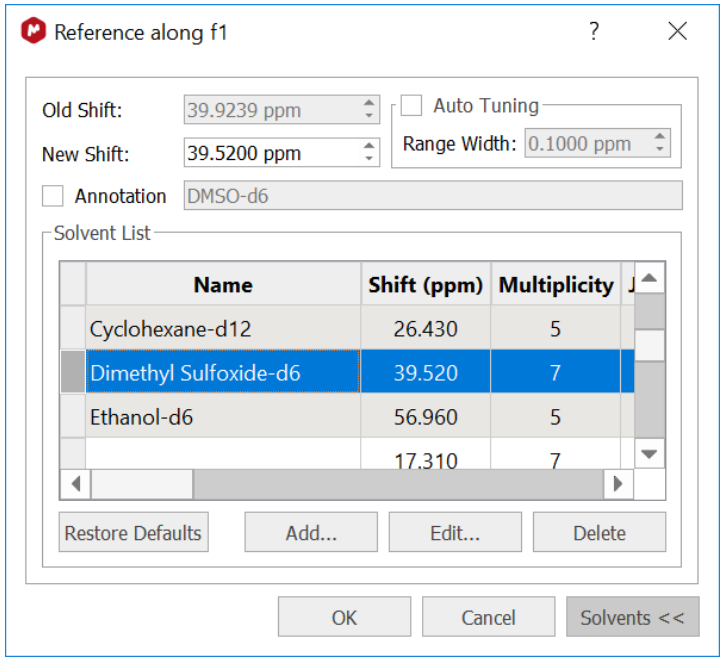


Chemical Shift Referencing

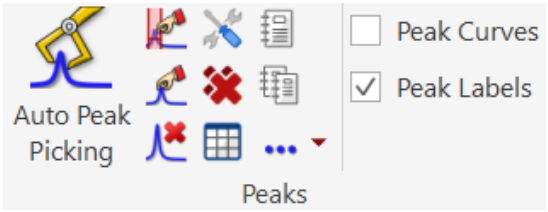


- This spectrum uses DMSO-d6 as the solvent. We can reference the chemical shifts by setting its middle peak to 39.52 ppm.
- Zoom to the DMSO peak at around 39 ppm. Choose Analysis > Reference, and click on the top of the middle peak.
- Set it to 39.52 ppm either manually or from the Solvent List.

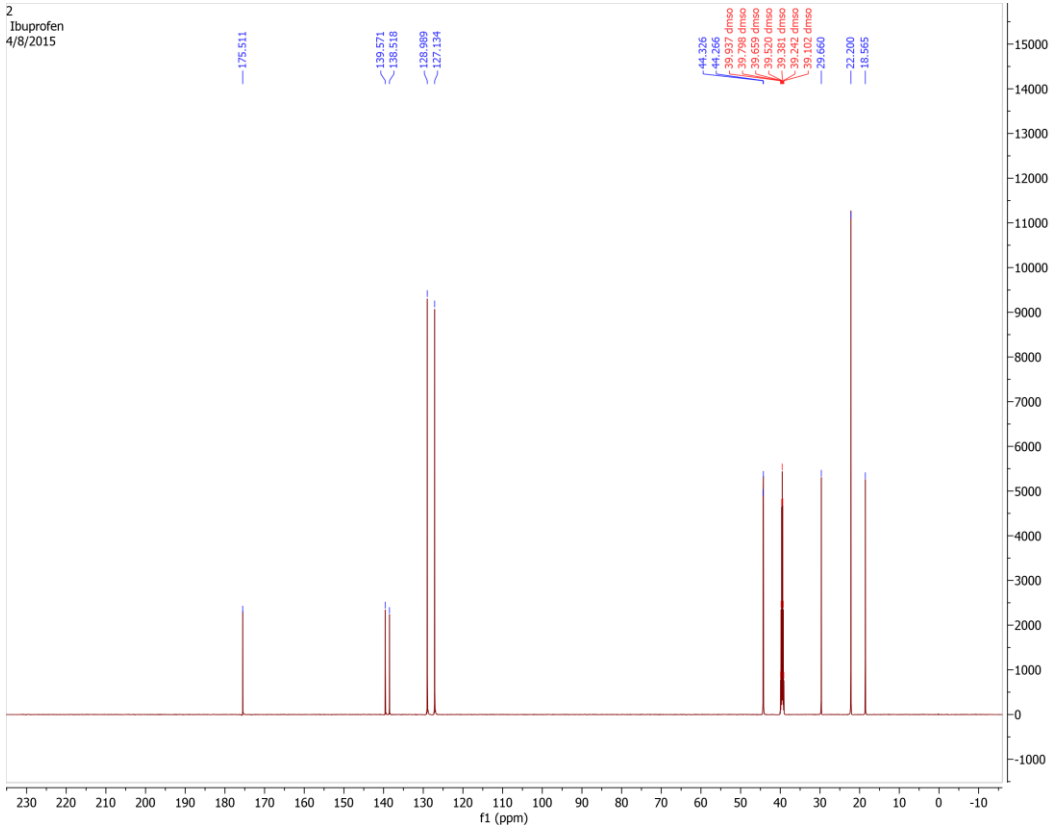


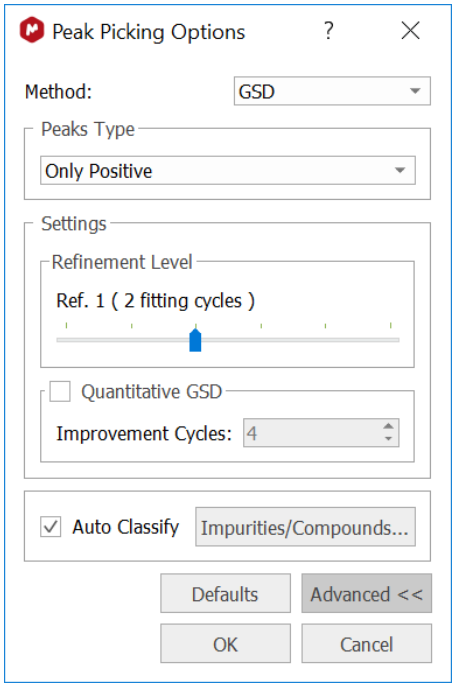


Peak picking



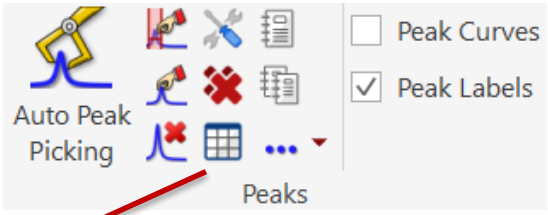
- Click the Peaks > Options  to verify the peak picking options. Default settings are used here as shown to the right.
- Click the Auto Peak Picking tool to pick all the peaks
- Using other peak picking tools to display/delete/add/change peaks as needed.

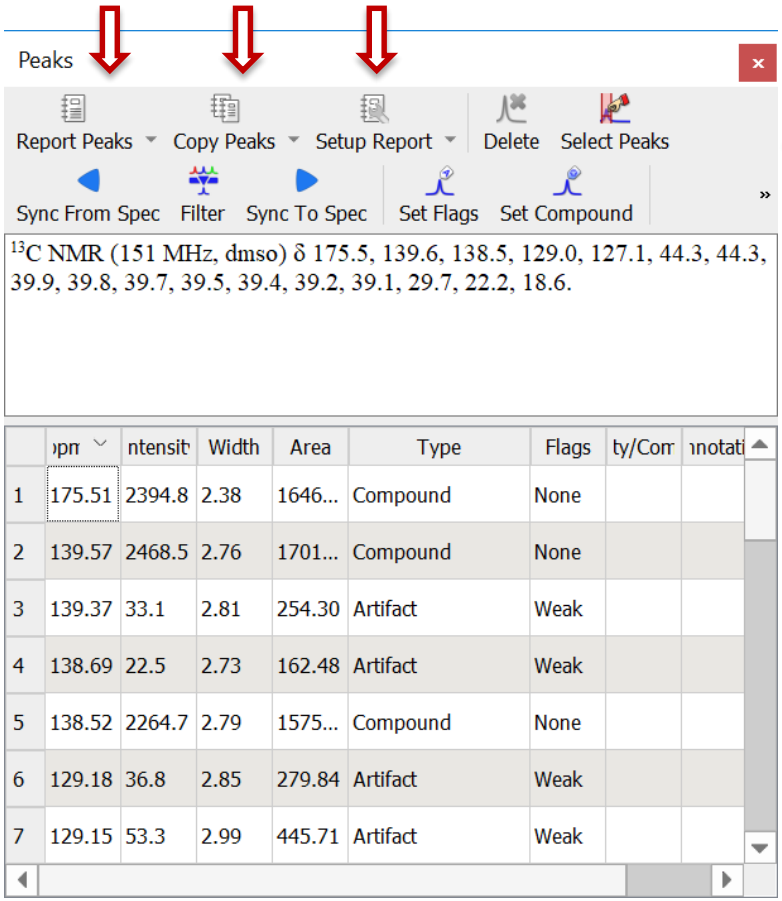




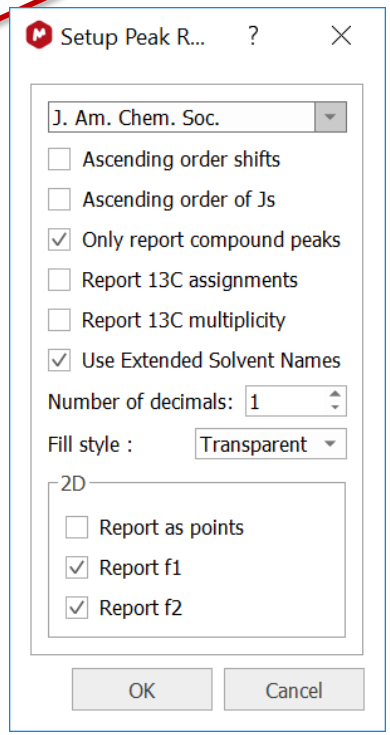
Report the C-13 peaks

- Use the Peak Table tool to display the Peaks Table.
- Click Setup Report to change the reporting format
- Click Report to report the multiplets texts





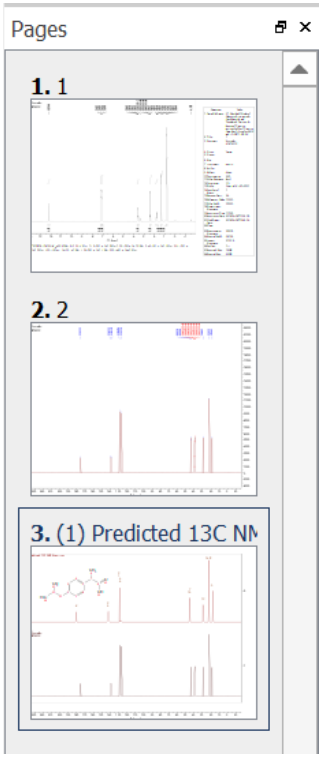
	ppm	Intensity	Width	Area	Type	Flags	Purity/Comp	Annotation
1	175.51	2394.8	2.38	1646...	Compound	None		
2	139.57	2468.5	2.76	1701...	Compound	None		
3	139.37	33.1	2.81	254.30	Artifact	Weak		
4	138.69	22.5	2.73	162.48	Artifact	Weak		
5	138.52	2264.7	2.79	1575...	Compound	None		
6	129.18	36.8	2.85	279.84	Artifact	Weak		
7	129.15	53.3	2.99	445.71	Artifact	Weak		

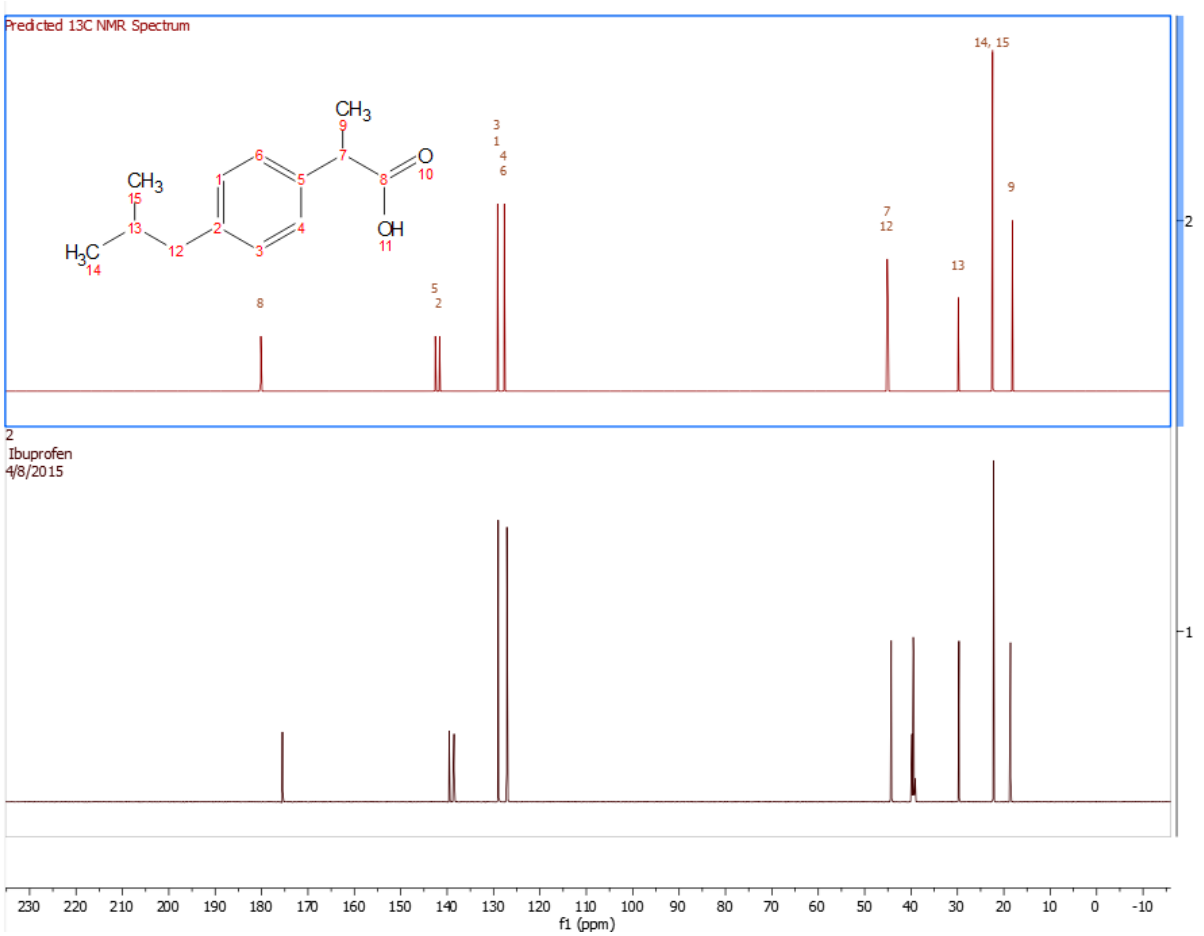


^{13}C NMR (151 MHz, DMSO-d_6) δ 175.5, 139.6, 138.5, 129.0, 127.1, 44.3, 44.3, 29.7, 22.2, 18.6.

Verify the structure by predict and compare

- Make a copy of the C-13 spectrum (Ctrl-C and Ctrl-V in the Pages View).
- Open the Ibuprofen.mol to bring in the structure to the C-13 spectrum.
- Choose Predict > Predict Compare.





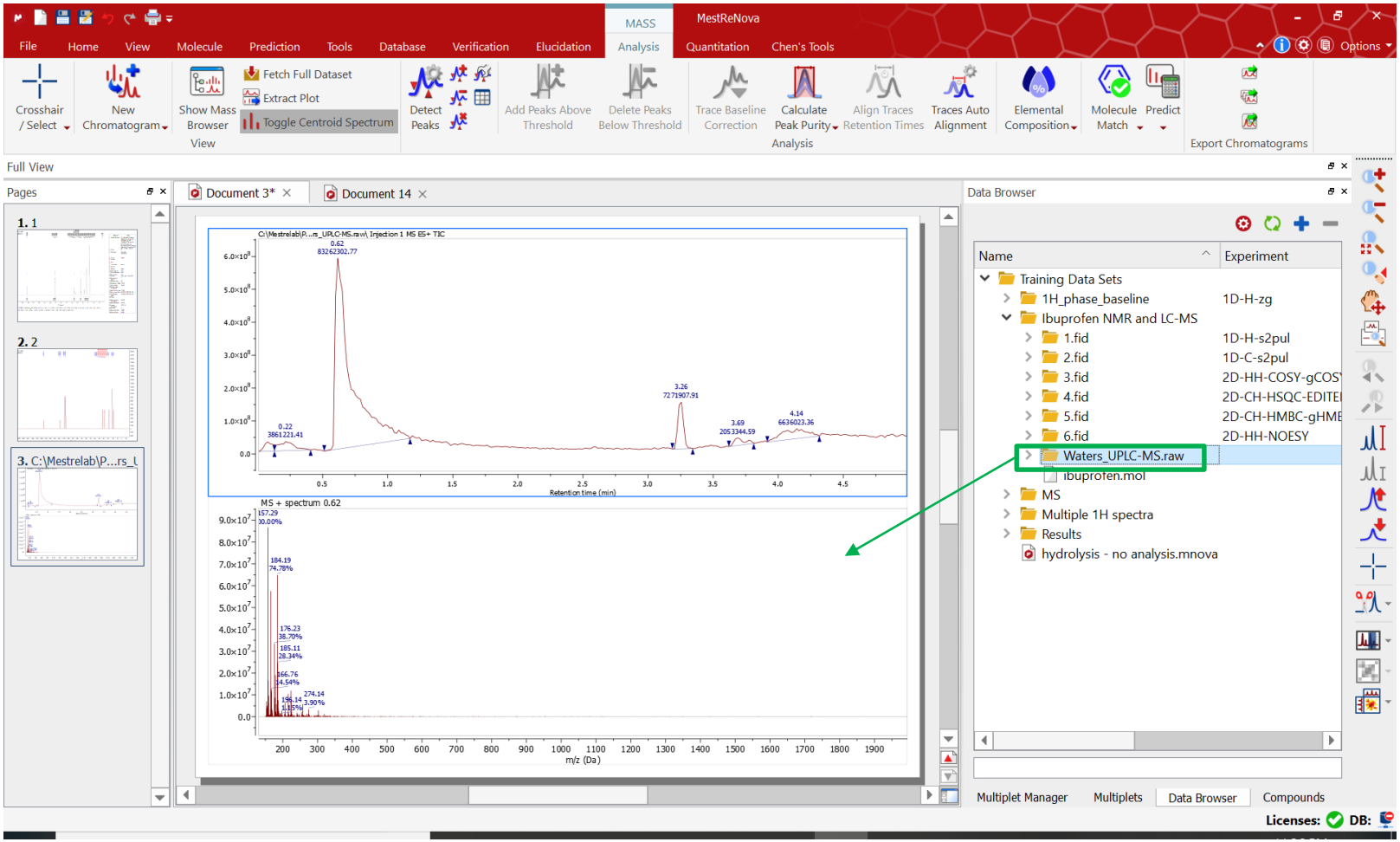
Open the LC-MS data

➢ In Data Browser, open the LC-MS data Ibuprofen (low resolution data acquired on Waters).

H-1

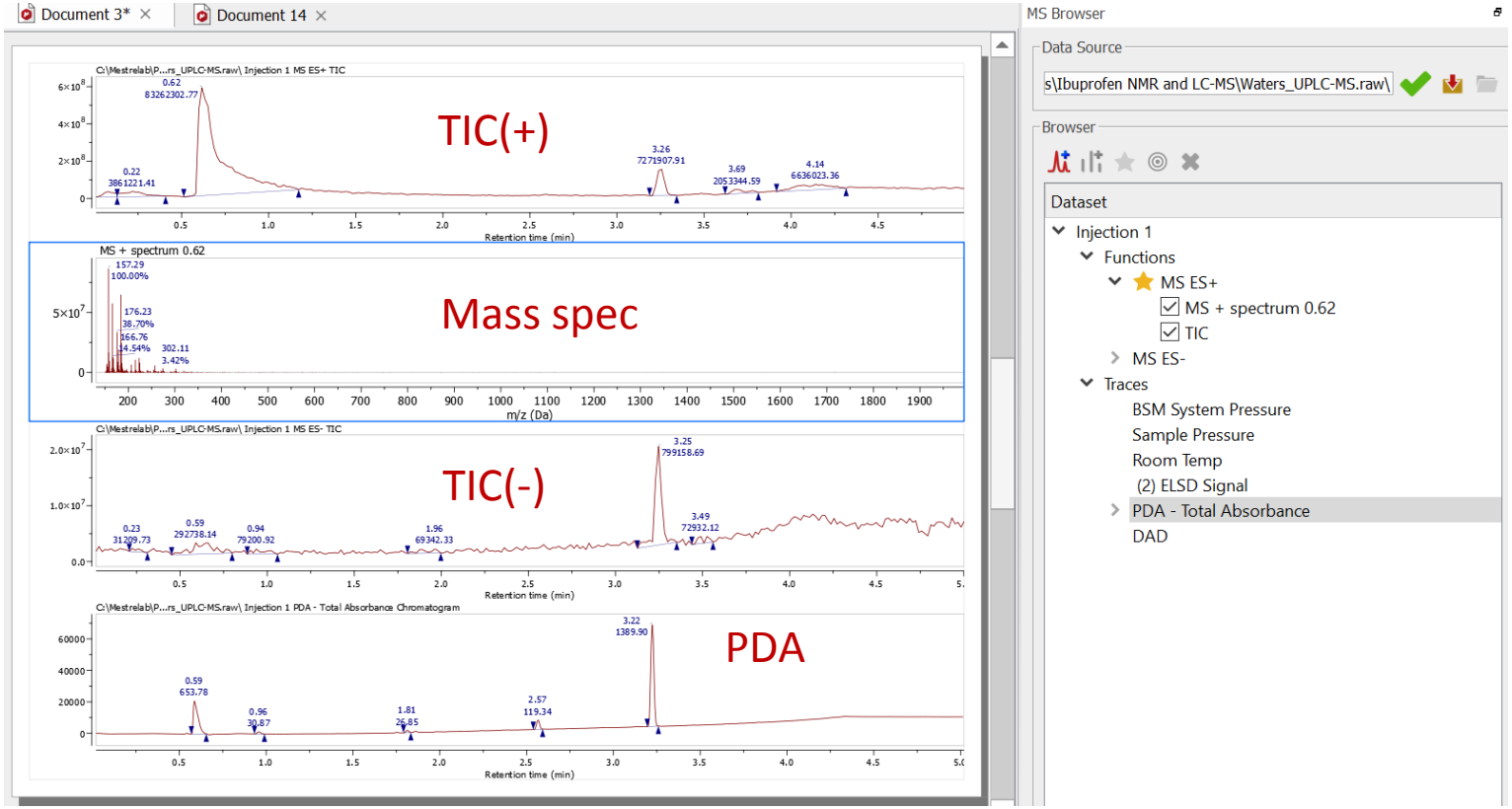
C-13

MS



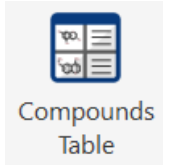
Display chromatograms

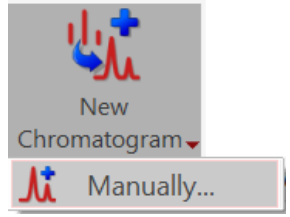
- Choose MASS Analysis > Show Mass Browser to display the Mass Browser.
- Open the negative polarization TIC
- Open the PDA Total Absorbance Chromatogram

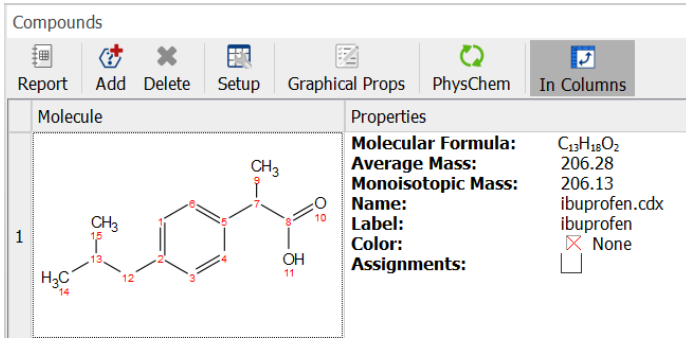


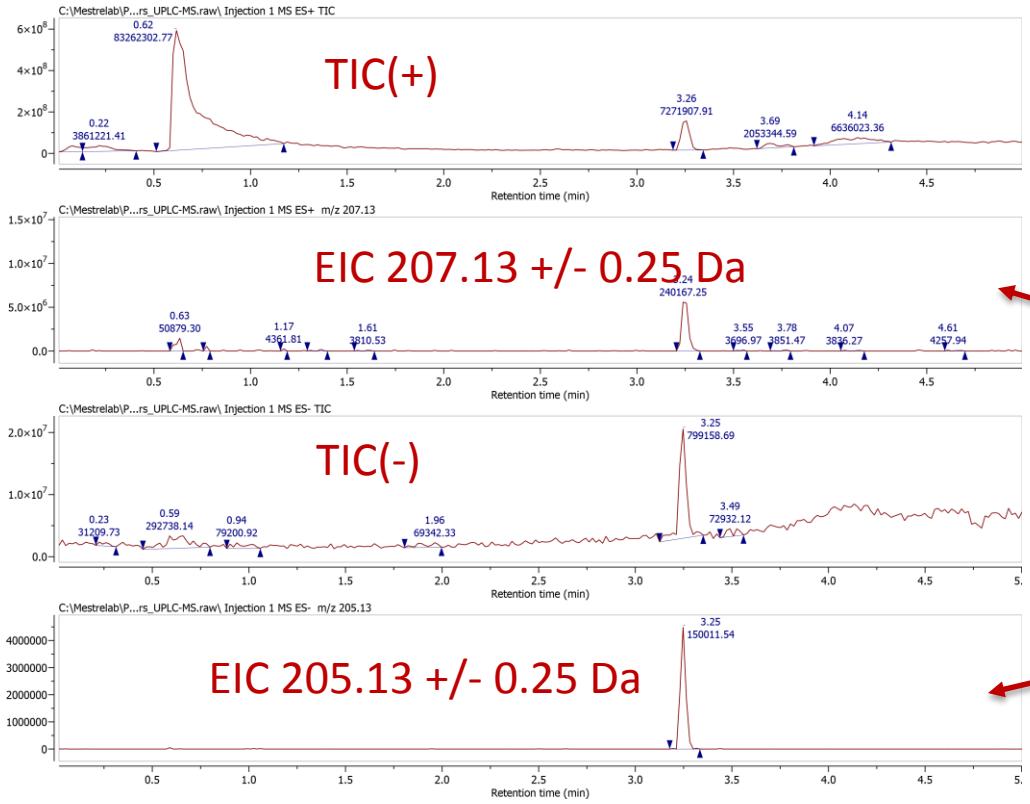
Verify the structure

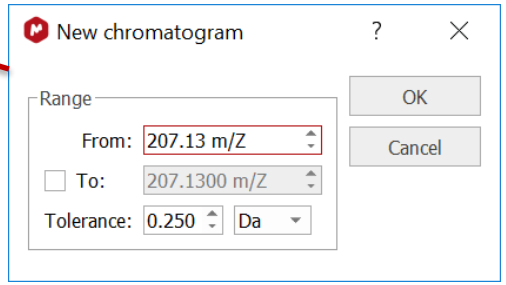
- Open the Ibuprofen.mol file from the Data Browser.
- Choose Molecule > Compound Table to find its monoisotopic mass: 206.13
- Hight the TIC(+), click Mass > New Chromatogram > Manually, and enter a value of 207.13 +/- 0.25 Da to display the new chromatogram (also called Extracted Icon Chrom., EIC)

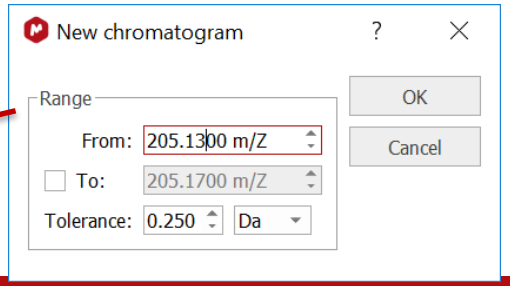






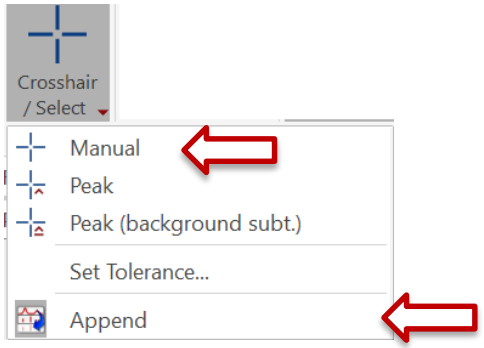


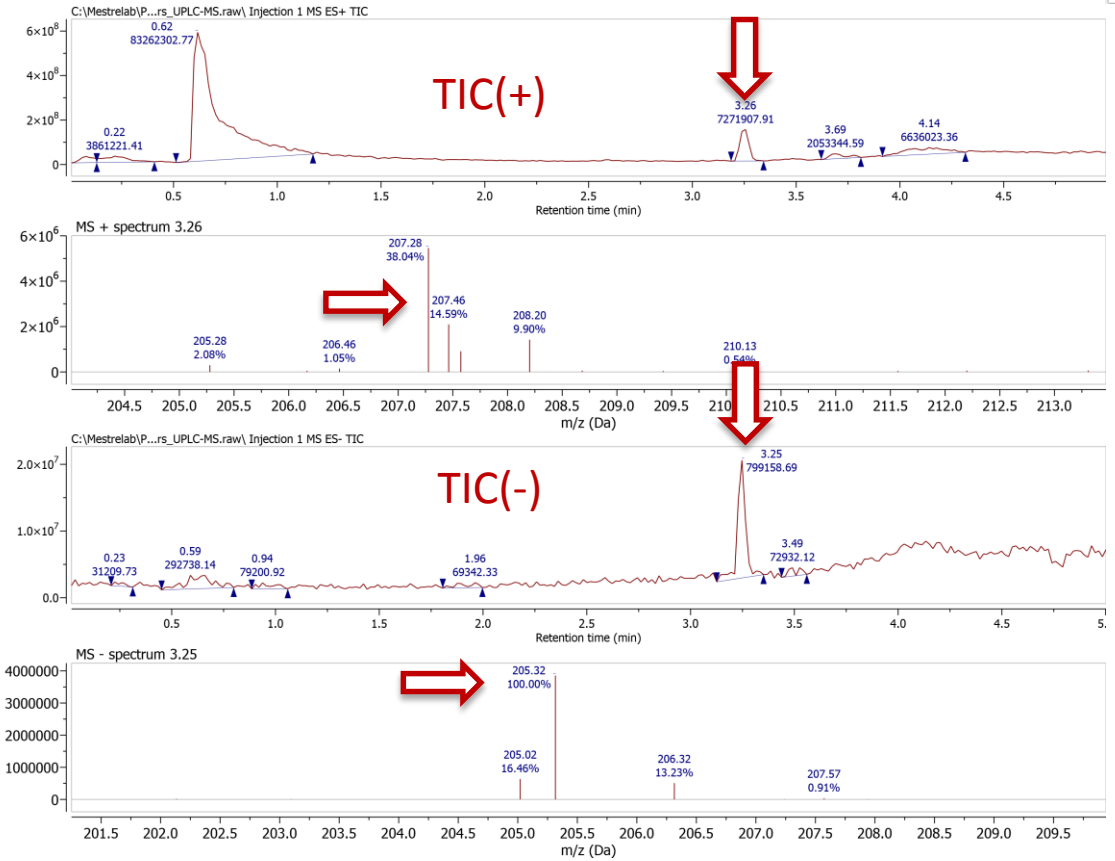




Find the molecule ion peaks

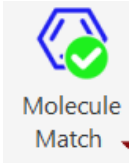
- Click the Crosshair tool, and click on the peak around 3.25 min in both TIC(+) and TIC(-)
- Zoom into the mass spec to find the mol. Ion peaks at around 207.13 and 205.13 Da, respectively.



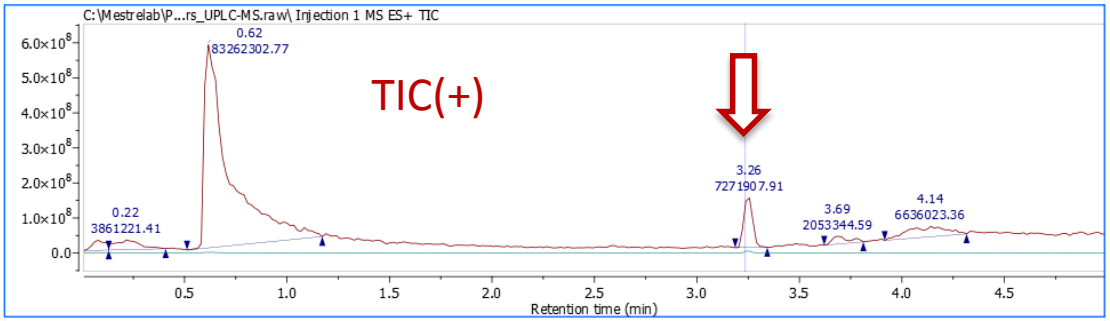


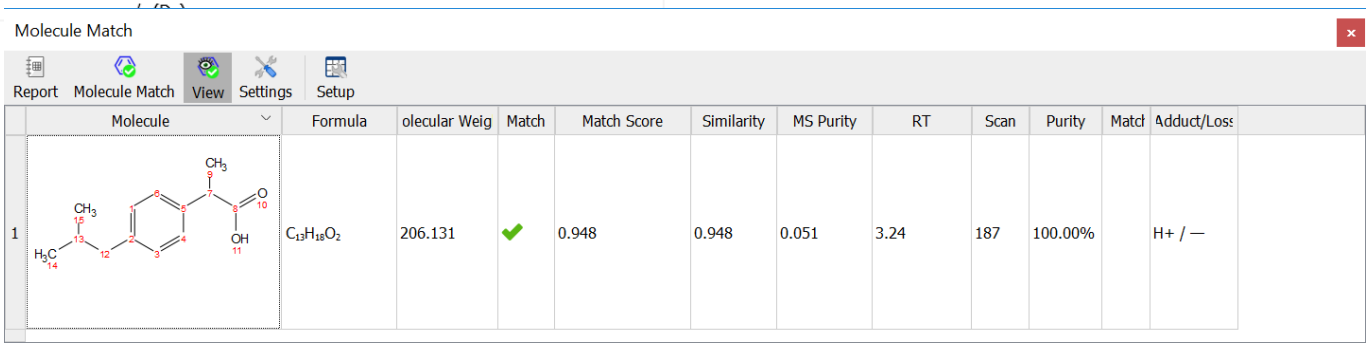
Tip: Use the Mass Browser to hide or delete unwanted plots. Right-click on a plot and choose Move up/Move Down etc. to re-order of the plots

Use Mol Match to verify the elemental composition

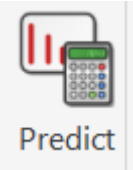


- Open the Ibuprofen.mol. Click Molecule Match.
- The Molecule Match Table shows the matching results.
- Click on the structure in the table to display the mol match results on the spectrum

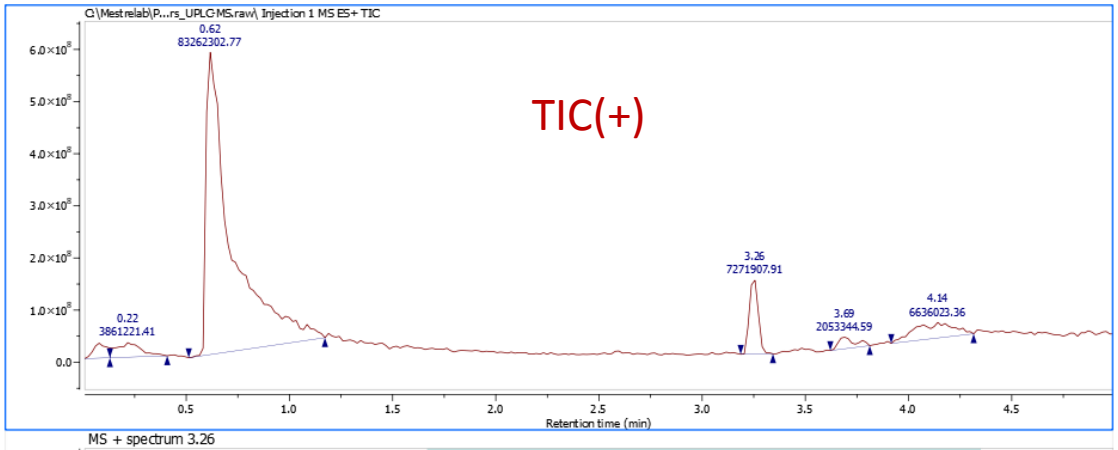


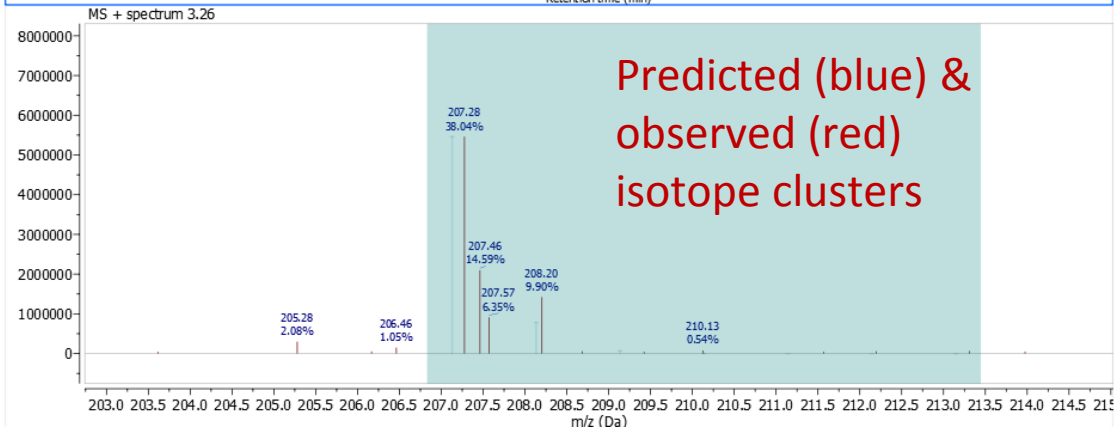


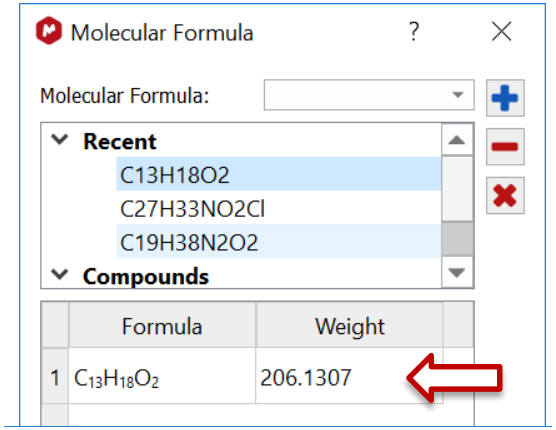
Predict and verify the molecule ion peaks

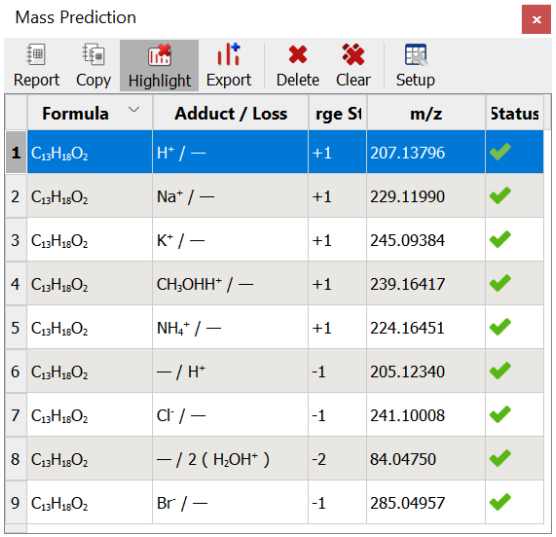


- Click the Predict tool, and choose the MF C13H18O2 and press “+” to use it for prediction
- In the Mass Prediction List, highlight the first row. The predicted molecule ion and isotope peaks are displayed on top of the experiment peak for comparison.





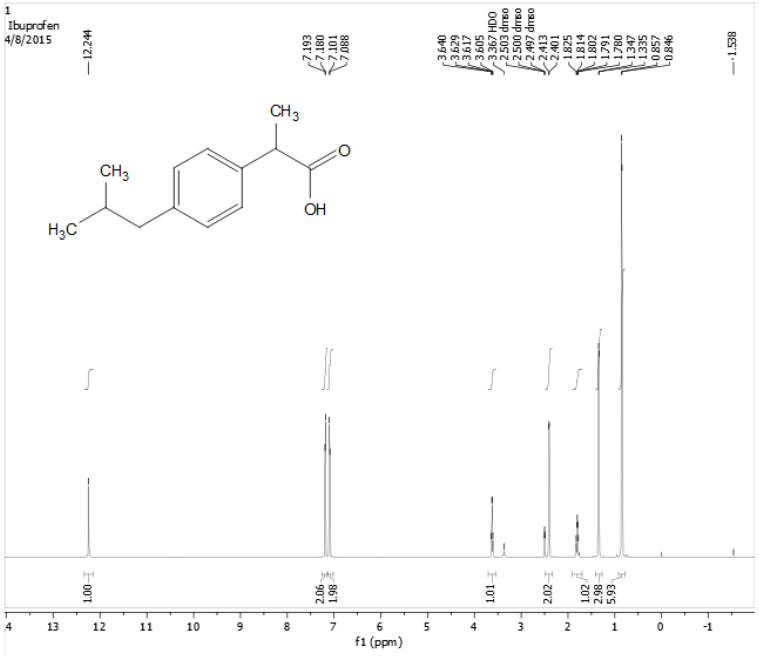




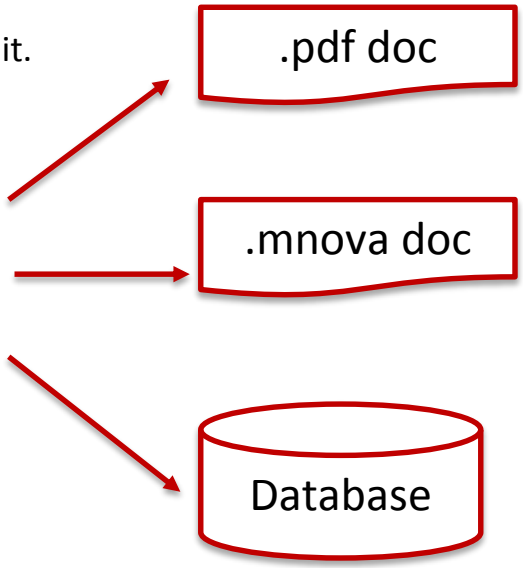
	Formula	Adduct / Loss	rge St	m/z	Status
1	$C_{13}H_{18}O_2$	H^+ / —	+1	207.13796	✓
2	$C_{13}H_{18}O_2$	Na^+ / —	+1	229.11990	✓
3	$C_{13}H_{18}O_2$	K^+ / —	+1	245.09384	✓
4	$C_{13}H_{18}O_2$	CH_3OHH^+ / —	+1	239.16417	✓
5	$C_{13}H_{18}O_2$	NH_4^+ / —	+1	224.16451	✓
6	$C_{13}H_{18}O_2$	— / H^+	-1	205.12340	✓
7	$C_{13}H_{18}O_2$	Cl^- / —	-1	241.10008	✓
8	$C_{13}H_{18}O_2$	— / 2 (H_2OH^+)	-2	84.04750	✓
9	$C_{13}H_{18}O_2$	Br^- / —	-1	285.04957	✓

Save the results

- Choose File > Export to PDF to save a PDF report of the page.
- Chose File > Save as to save all the results to a .mnova file.
- In the Advanced Tutorial we will learn to save the results to a database.
- Now can close the document or continue to add other spectra to it.

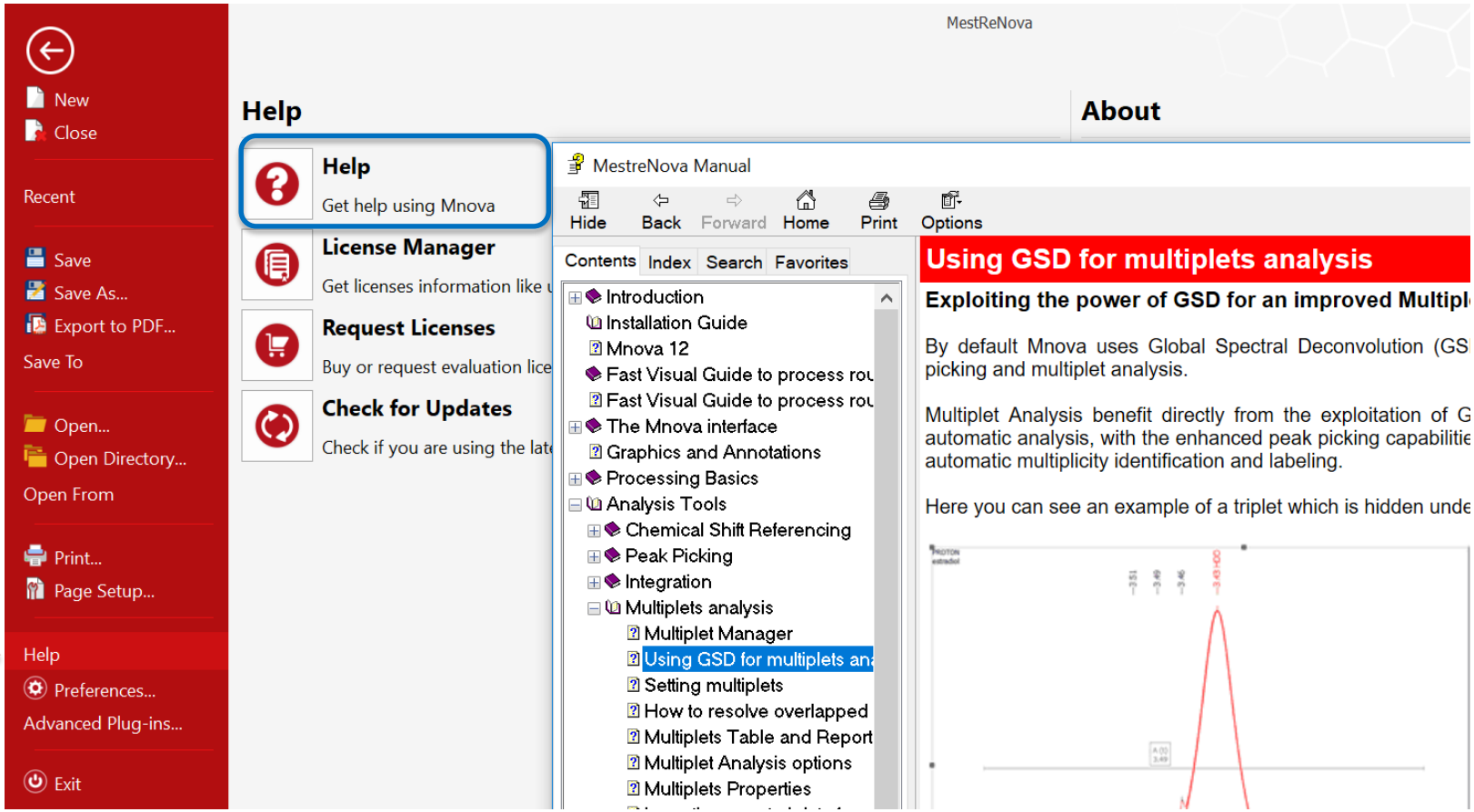


	Parameter	Value
1	Data File Name	C:/ Mestrelab/ Projects/ Marcomm/ Literature & Docs/ Sample and Templates/ Training for chemists/ Training courses by Chen/ Training Data Sets/ Ibuprofen NMR and LCMS/ 1.fid/ fid
2	Title	1
3	Comment	Ibuprofen 4/ 8/ 2015
4	Origin	Varian
5	Owner	
6	Site	
7	Instrument	vnmrs
8	Author	
9	Solvent	dmso
10	Temperature	25.0
11	Pulse Sequence	s2pul
12	Experiment	1D
13	Probe	3mm_cold_HCN_P001
14	Number of Scans	1
15	Receiver Gain	24
16	Relaxation Delay	1.0000
17	Pulse Width	3.0000
18	Presaturation Frequency	
19	Acquisition Time	1.7039
20	Acquisition Date	2015-04-08T10:34:36
21	Modification Date	2015-04-08T19:46:04
22	Class	
23	Spectrometer Frequency	599.76
24	Spectral Width	9615.4
25	Lowest Frequency	-1193.6
26	Nucleus	1H
27	Acquired Size	16384
28	Spectral Size	65536



More help information

- Use the Help Facility of Mnova: Help > Contents
- Visit www.mestrelab.com for manuals, tutorials, videos and publications
- Email support@mestrelab.com for technical questions



Mestrelab Research

chemistry software solutions

Mnova Training - Advanced

Version 12.0.3

Oct. 23, 2018

Chen Peng, PhD,
VP of Business Development, North America & Asia
Mestrelab Research SL
chen.peng@mestrelab.com
858.736.4563

Mestrelab Research
chemistry software solutions

Main Topics

- Opening and processing 2D NMR
- Assigning peaks to atoms
- Reporting assignment results
- Creating a database to save the data
- Searching Wiley C & H databases
- Analyzing arrayed spectra

Note: This tutorial covers the NMR, NMRPredict, MS, DB plugins of Mnova, and Wiley DBs

Specifics for <xxxxxx> University (To be completed by instructor)

- The instructions for downloading, installing and activating Mnova:
  - <Link to instruction page>

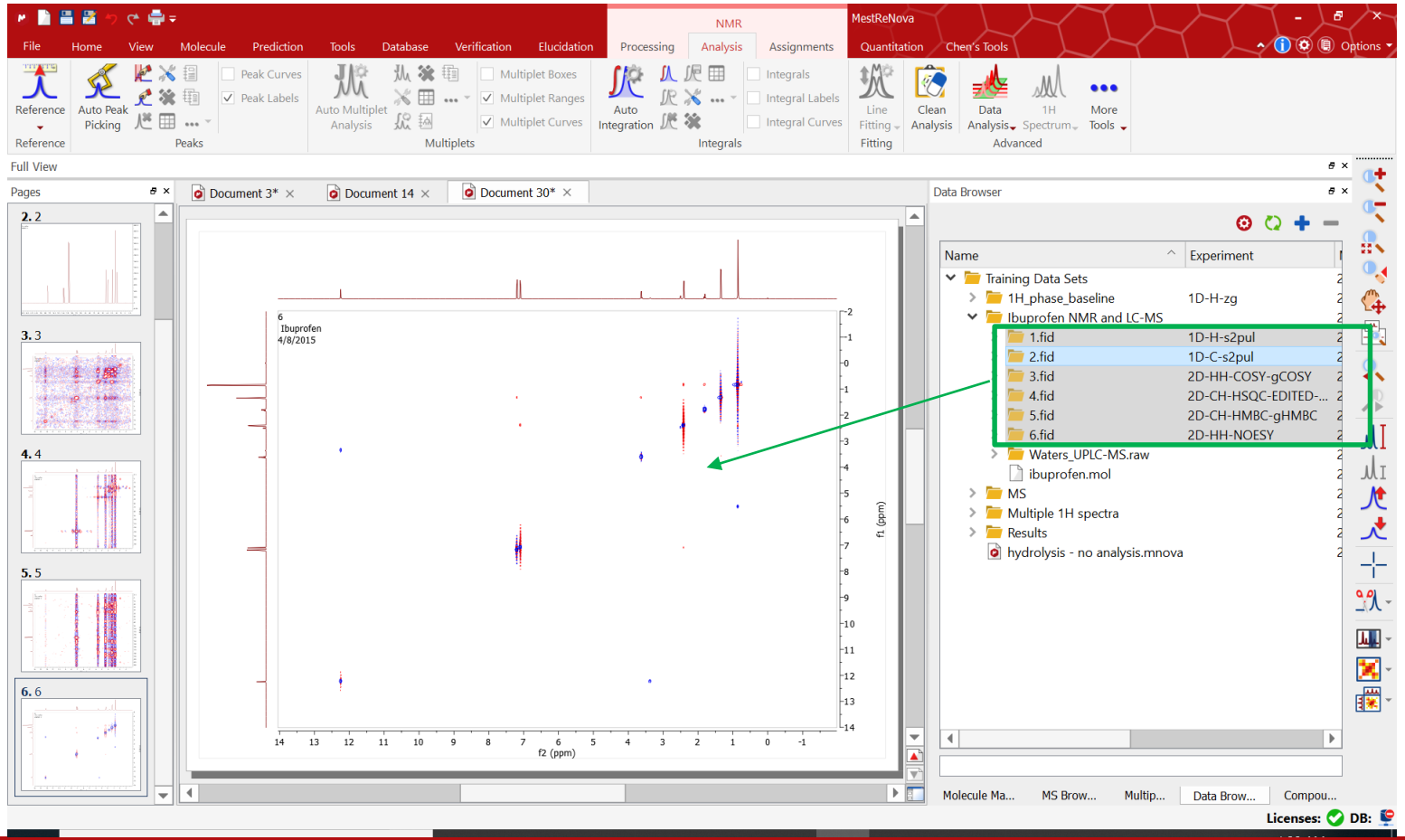
- The Mnova licenses that <XXXX> University has:
  - Mnova Suite (NMR, NMRPredict & MS), unlimited
  - Etc.

- The sample data used in this tutorial are located at:
  - <Link to data folder>.

PROCESSING

Open a 1D and 2D spectra of Ibuprofen

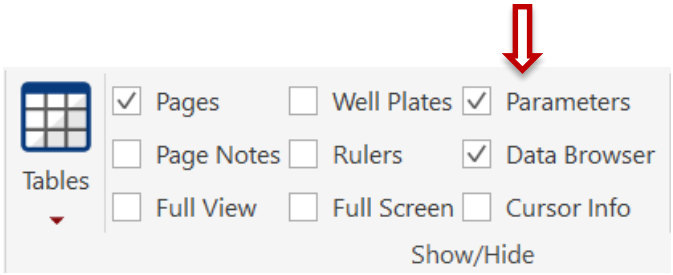
- Choose File > New to open a new blank document.
- In Data Browser, choose the 1D H/C, 2D HSQC, HMBC, COSY, and NOESY spectra and drag all of them to the main window.
- Re-process and analyze the H-1 and C-13 spectra according to the Basic Tutorial.

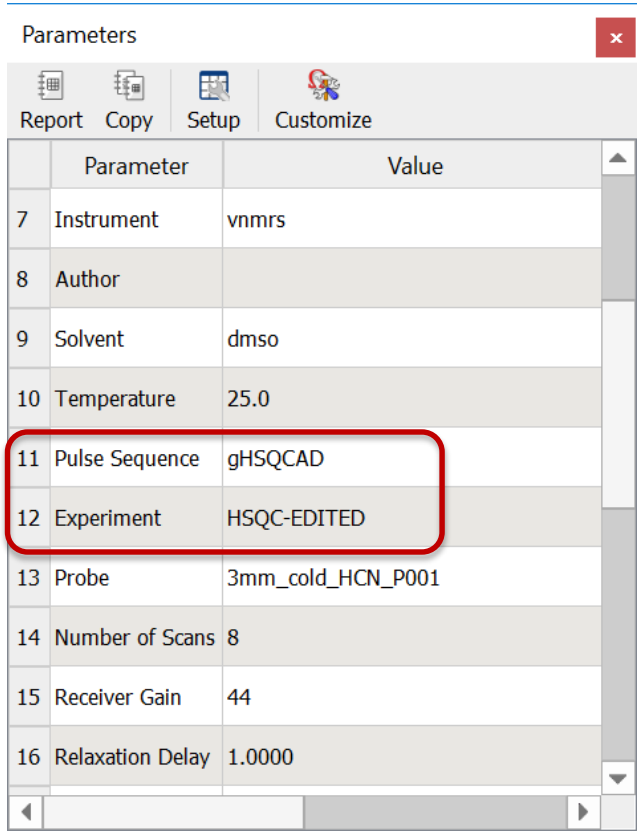


PARAMETERS

Which is which?

- Check View > Parameters Table to display the Parameters Table
- The Experiment and Pulse Sequence parameters usually indicate the type of NMR data

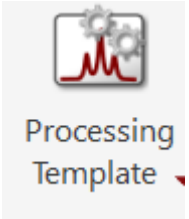




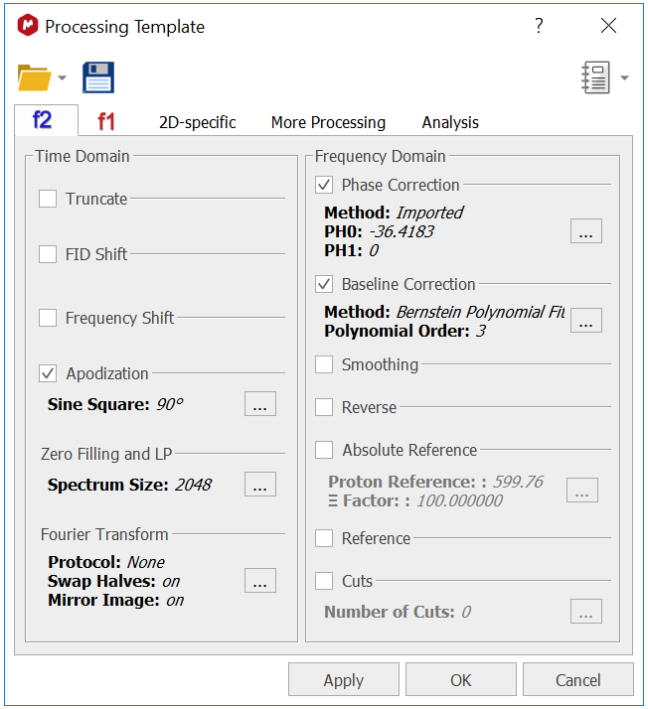
	Parameter	Value
7	Instrument	vnmrs
8	Author	
9	Solvent	dmso
10	Temperature	25.0
11	Pulse Sequence	gHSQCAD
12	Experiment	HSQC-EDITED
13	Probe	3mm_cold_HCN_P001
14	Number of Scans	8
15	Receiver Gain	44
16	Relaxation Delay	1.0000

Tip: You can display the Experiment as part of the spectrum title. Double click on the spectrum and setup the Title in the Properties Dialog.

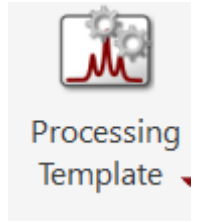
Rules of thumb for 2D processing



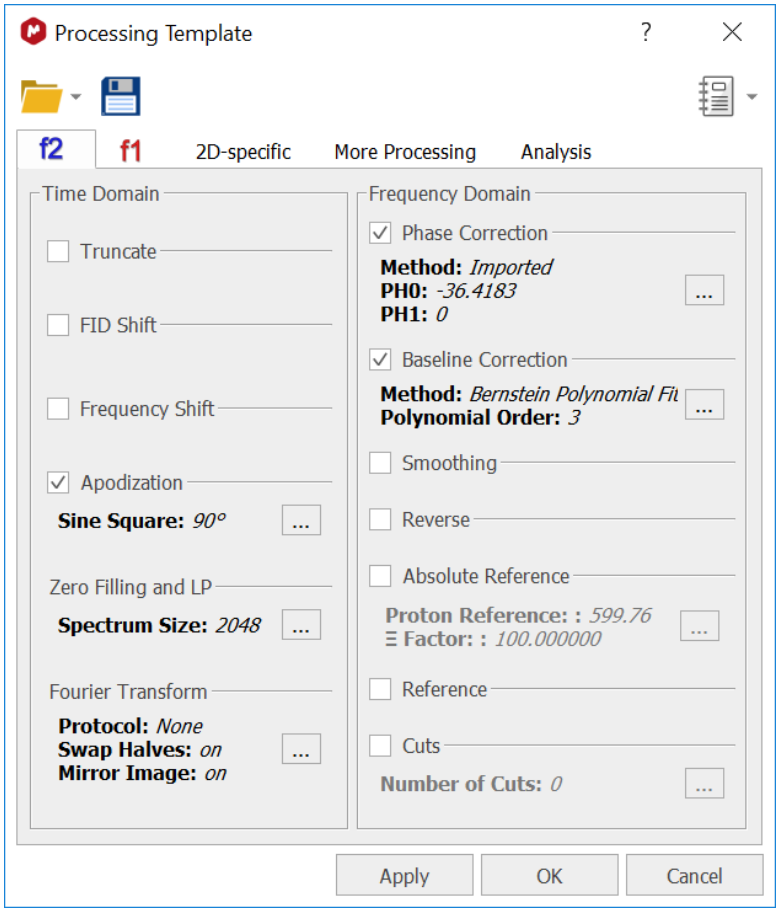
- Apodization: To improves the line shape, resolution and S/N ratio
  - COSY: Use Sine Square 0 for F2 & F1
  - Others: Use Since Square 90 F2 & F1
- Zero Fill and LP (Linear Prediction): To improve resolution
  - F2: at least double of the original datapoints, 2048 or 4096
  - F1: At least double of the original datapoints, 1024 or 2048
  - F1: Do LP if original datapoints <= 128 (optional)
- Phase Correction: To improve line shape
  - Use Imported or Automated (Regions) first
  - Do manual correction if needed.
- Baseline Correction: To reduce noise
  - Use Bernstein Polynomial Fit on either dimension
- Other 2D-Specific parameters (optional):
  - COSY, NOESY: Use Symmetrize with caution
  - HSQC, HMBC: Use Reduce T1 Noise Reduction when needed.

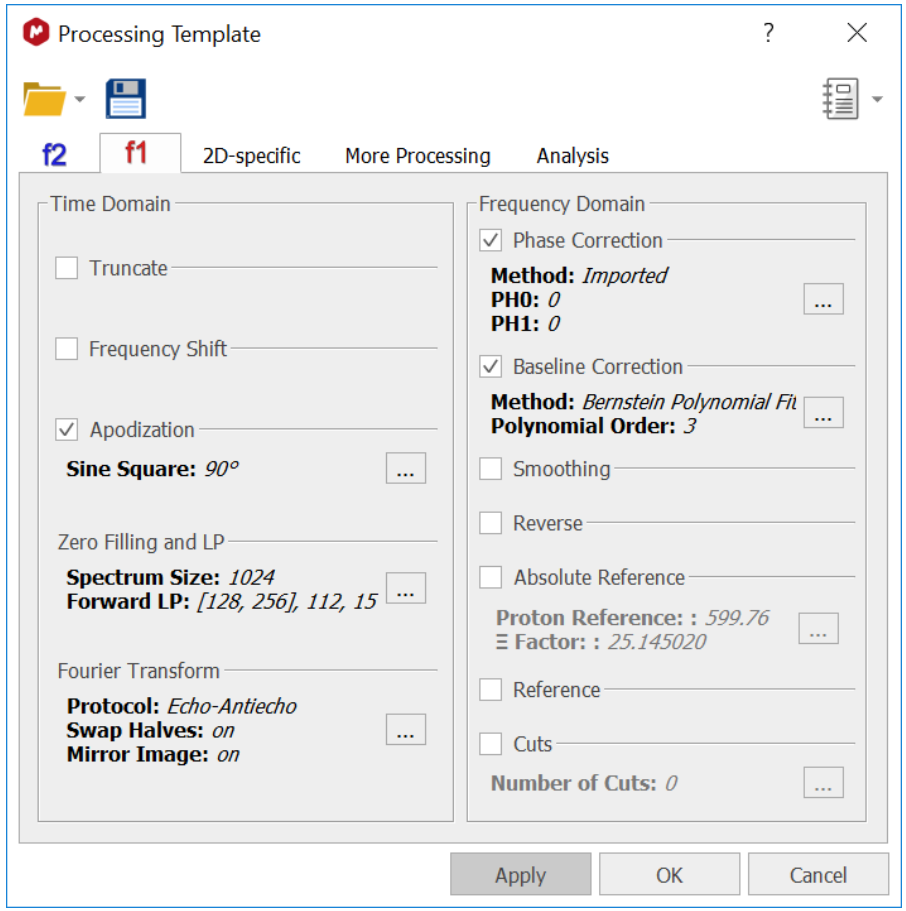


Re-process HSQC spectrum



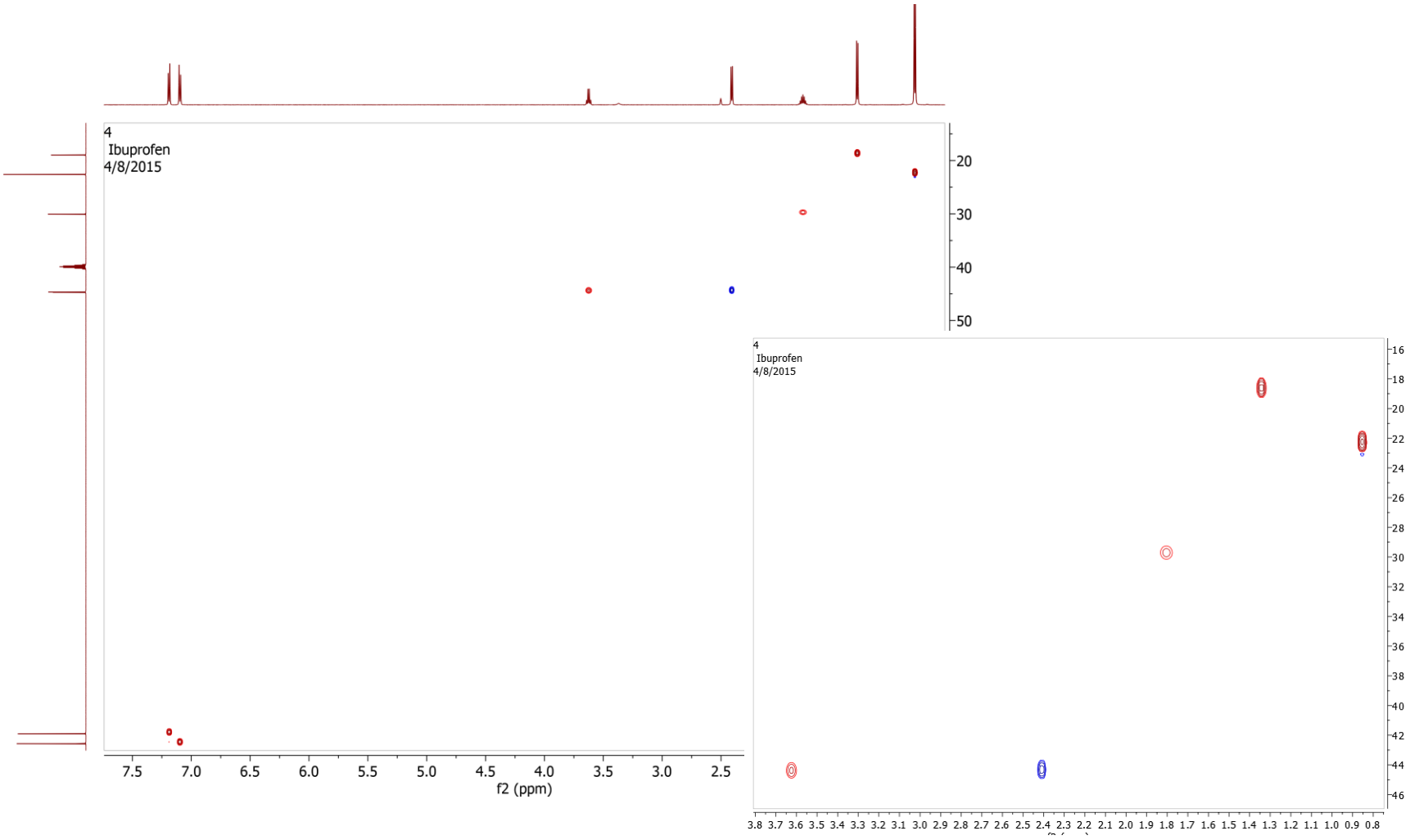
- Reprocess the HSQC spectrum as shown below.
- Note the apodization functions for F2 and F1
- Note the forward linear prediction for F1 applied here



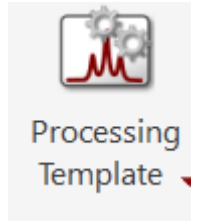


Re-process HSQC spectrum

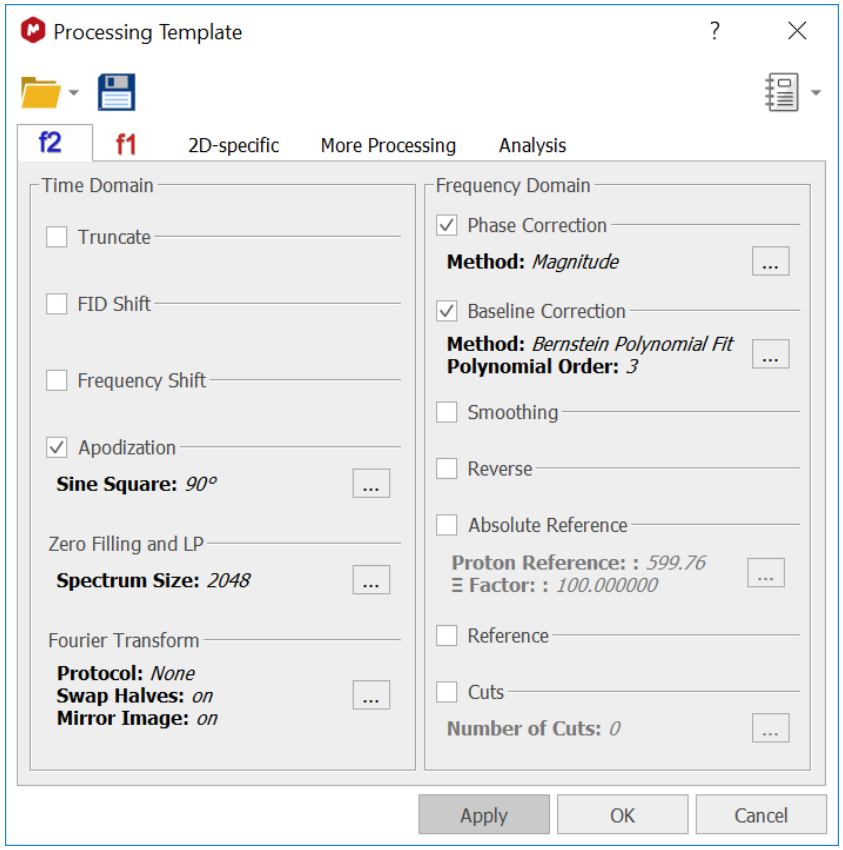
- The re-processed HSQC spectrum shows better line shape, and higher resolution on the F1 dimension

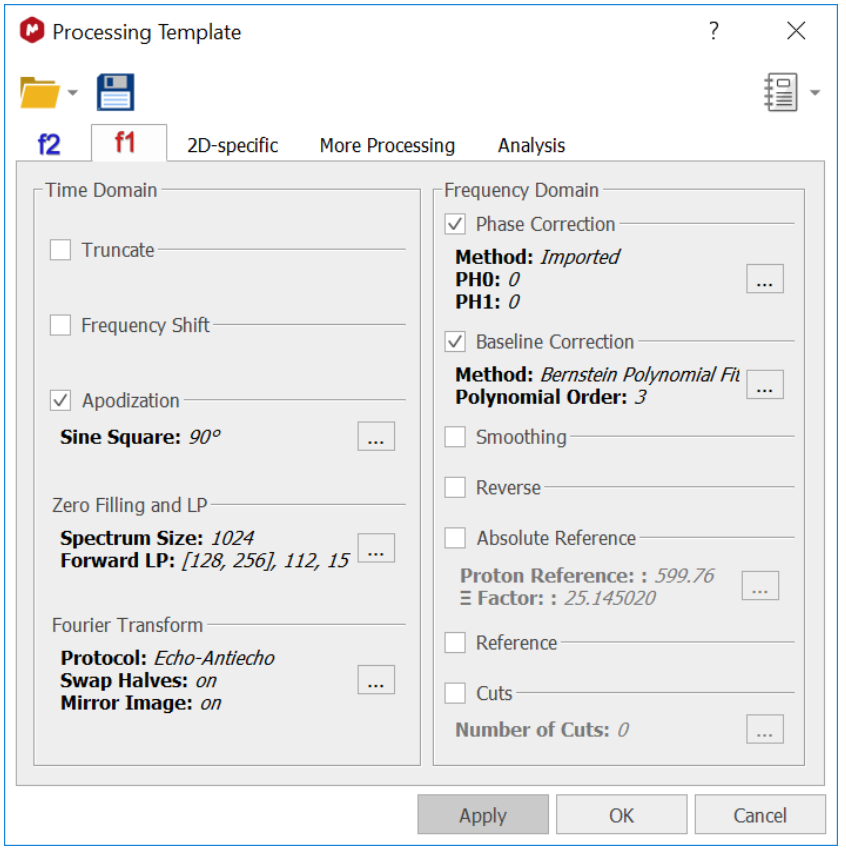


Re-process HMBC spectrum



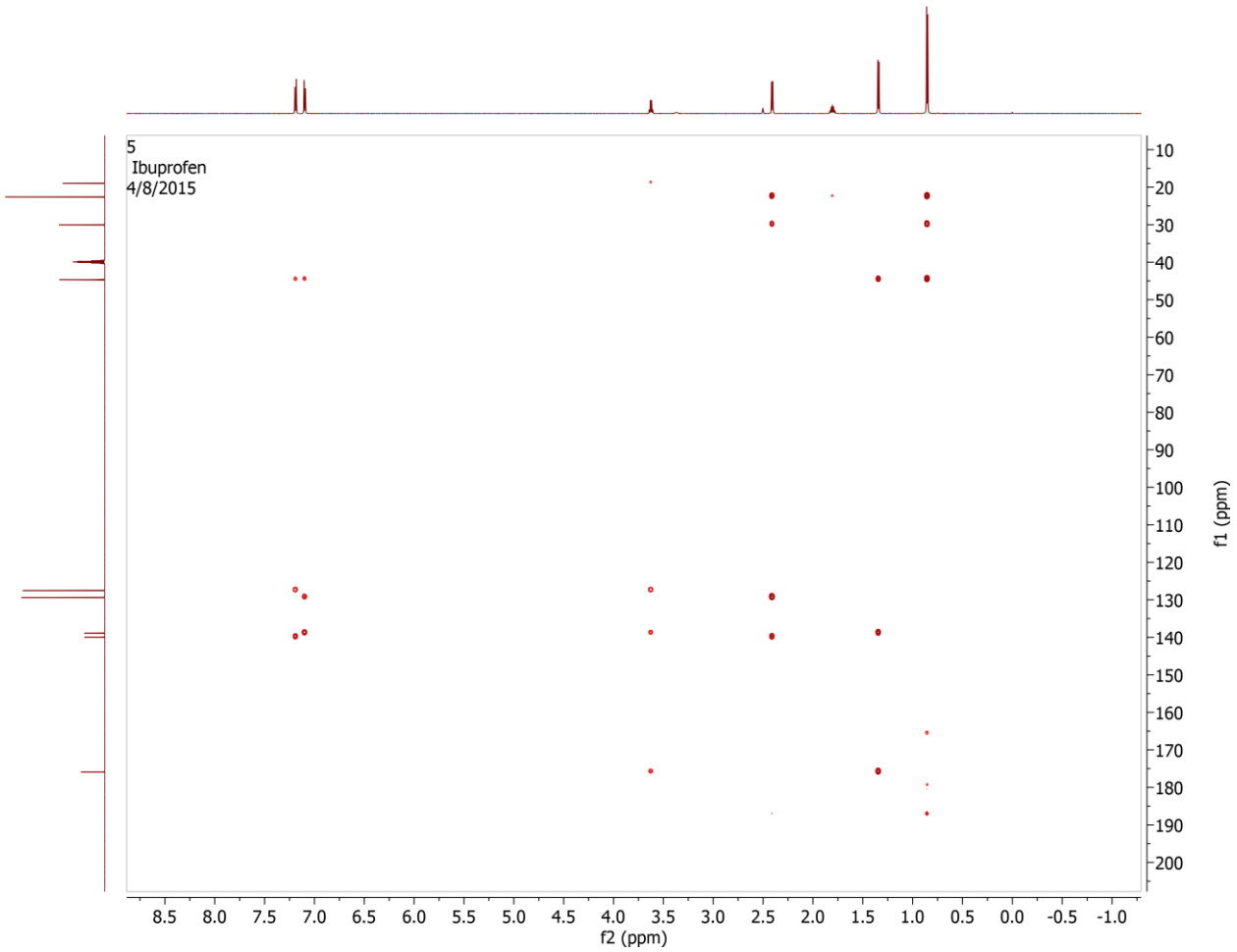
- Reprocess the HMBC spectrum as shown below.
- Note the apodization functions for F2 and F1
- Note the forward linear prediction for F1 applied here





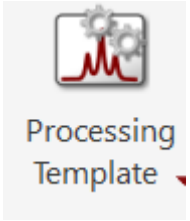
Re-process HMBC spectrum

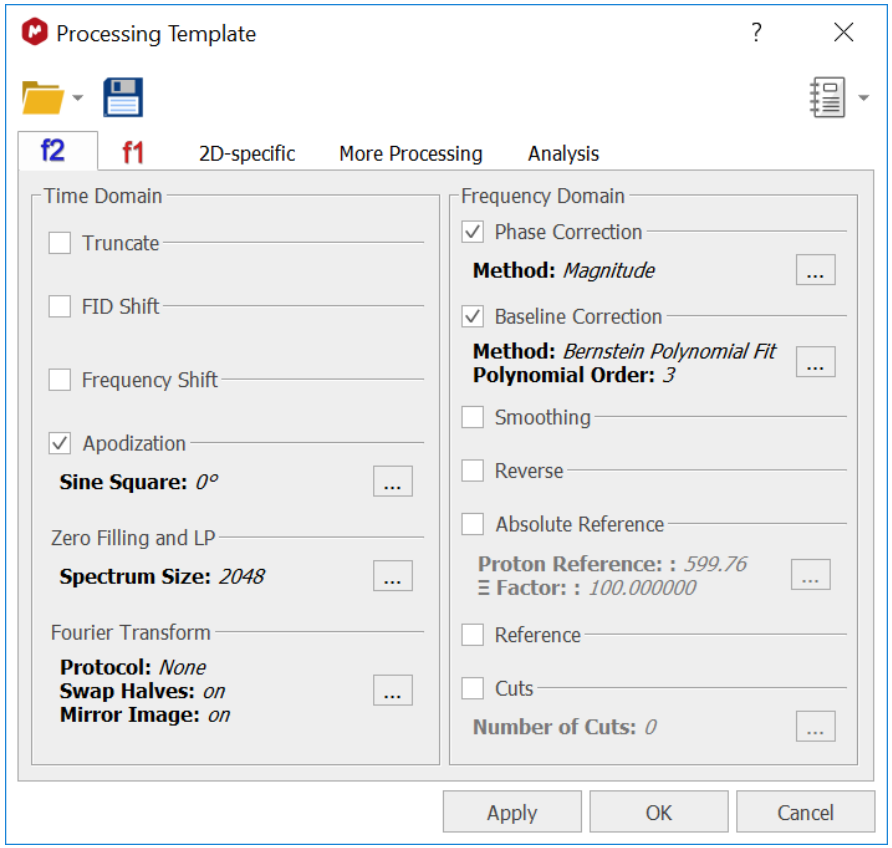
- The re-processed HSQC spectrum shows better line shape, and higher resolution on the F1 dimension

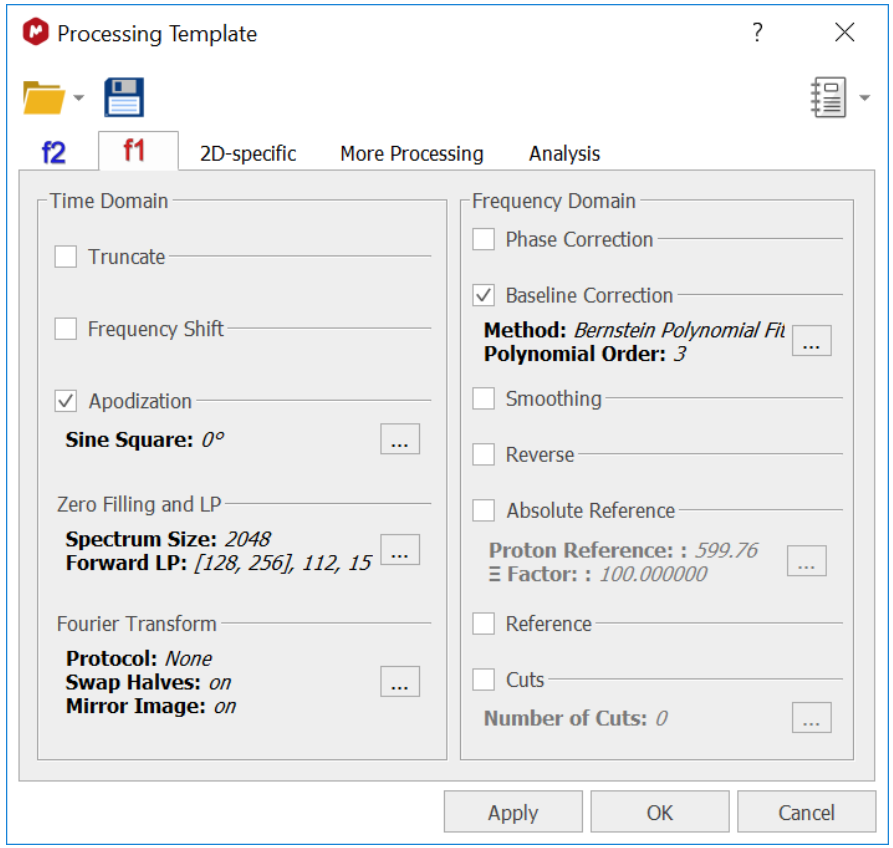


Re-process COSY spectrum

- Reprocess the COSY spectrum as shown below.
- Note the apodization functions for F2 and F1
- Note the forward linear prediction for F1 applied here
- Turn off Symmetrize in the 2D-specific Tab

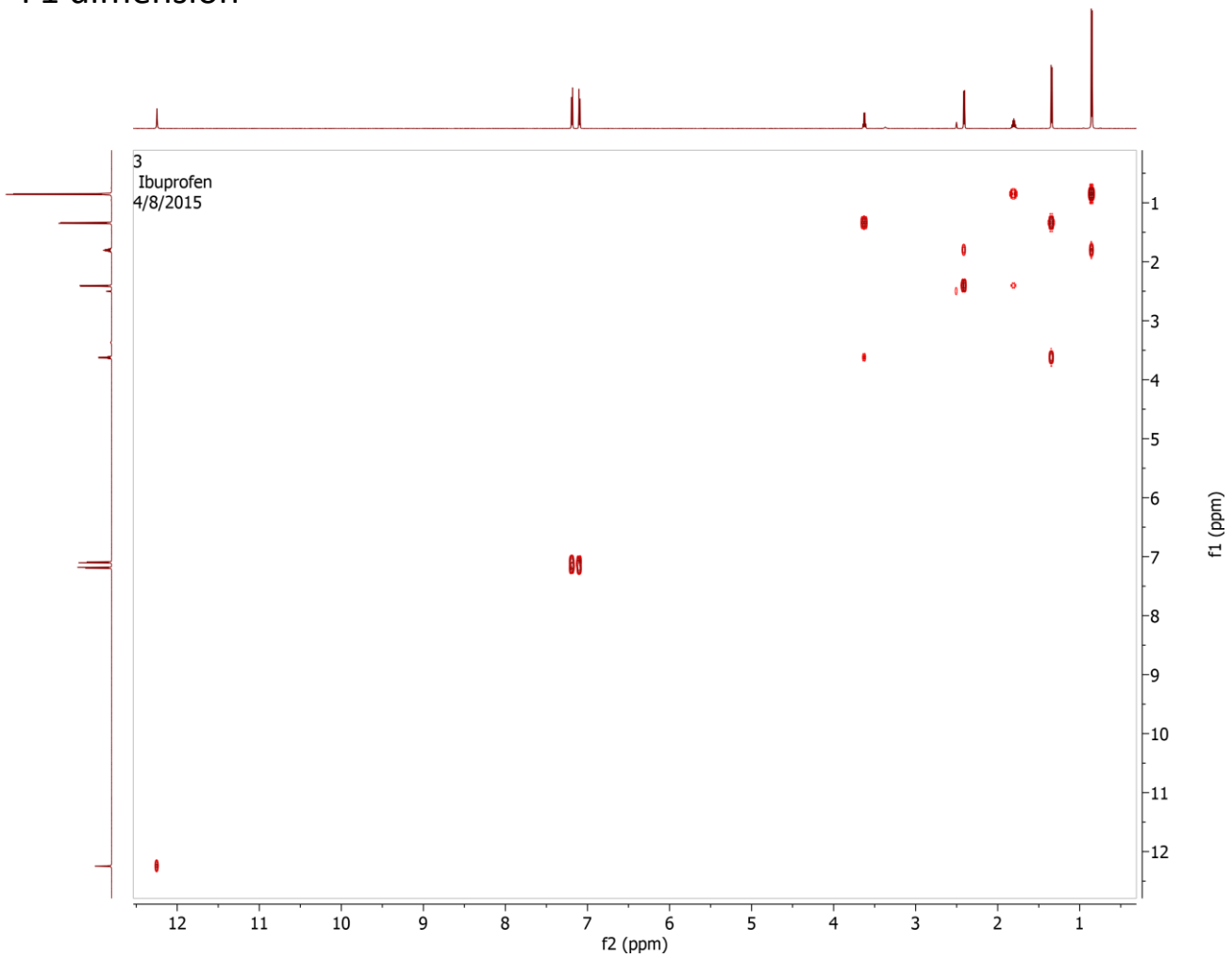




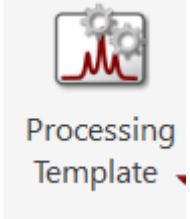


Re-process COSY spectrum

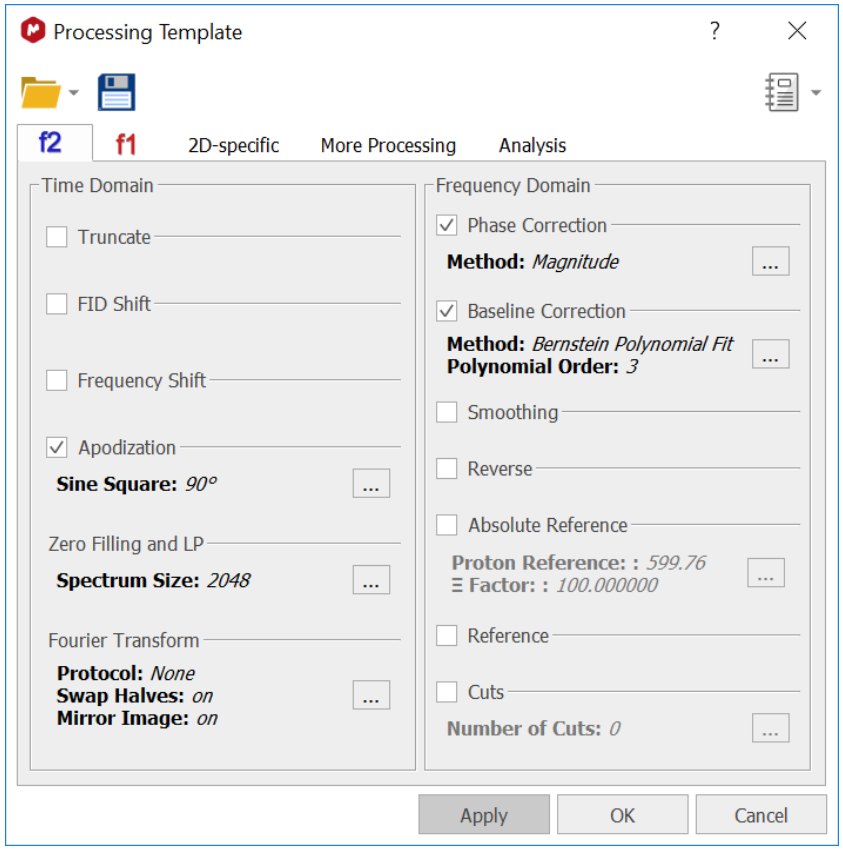
- The re-processed COSY spectrum shows better line shape, and higher resolution on the F1 dimension

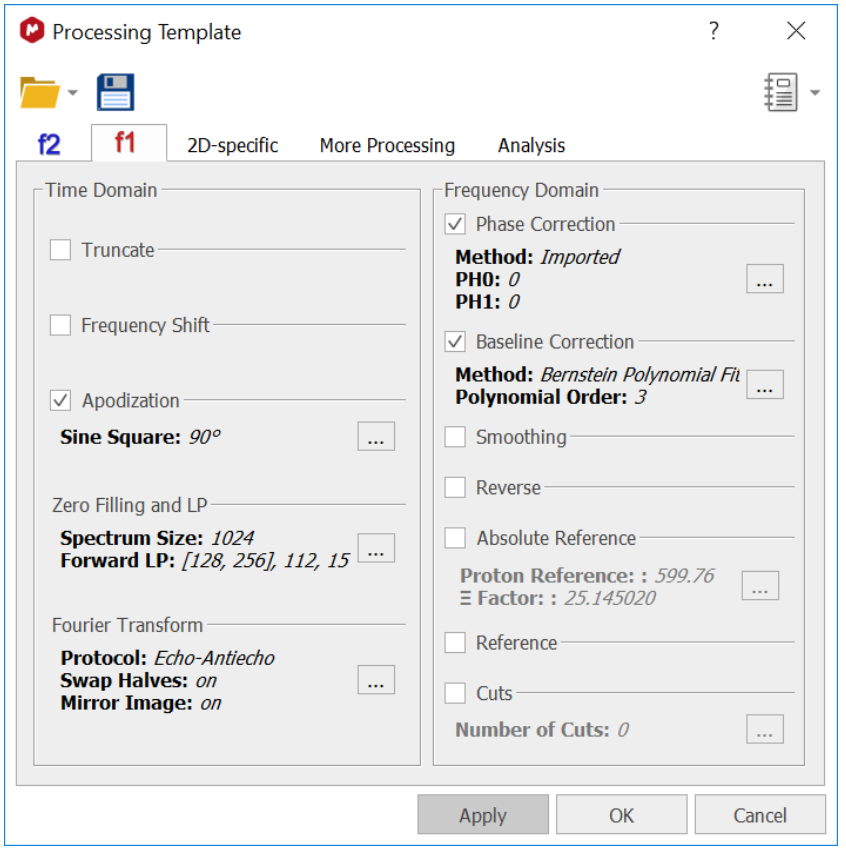


Re-process NOESY spectrum



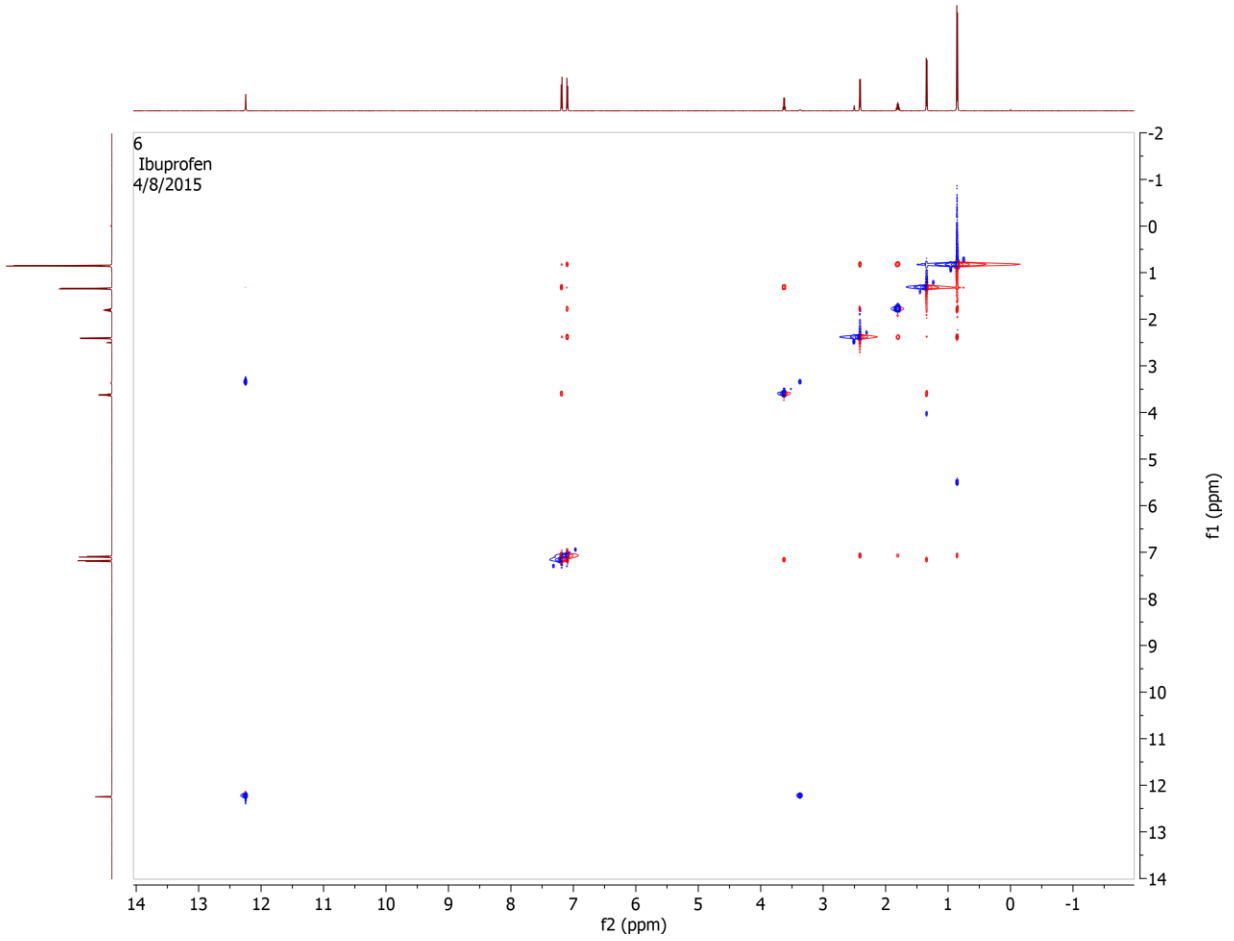
- Reprocess the NOESY spectrum as shown below.
- Note the apodization functions for F2 and F1
- Note the forward linear prediction for F1 applied here





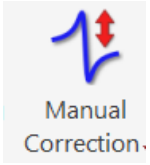
Re-process NOESY spectrum

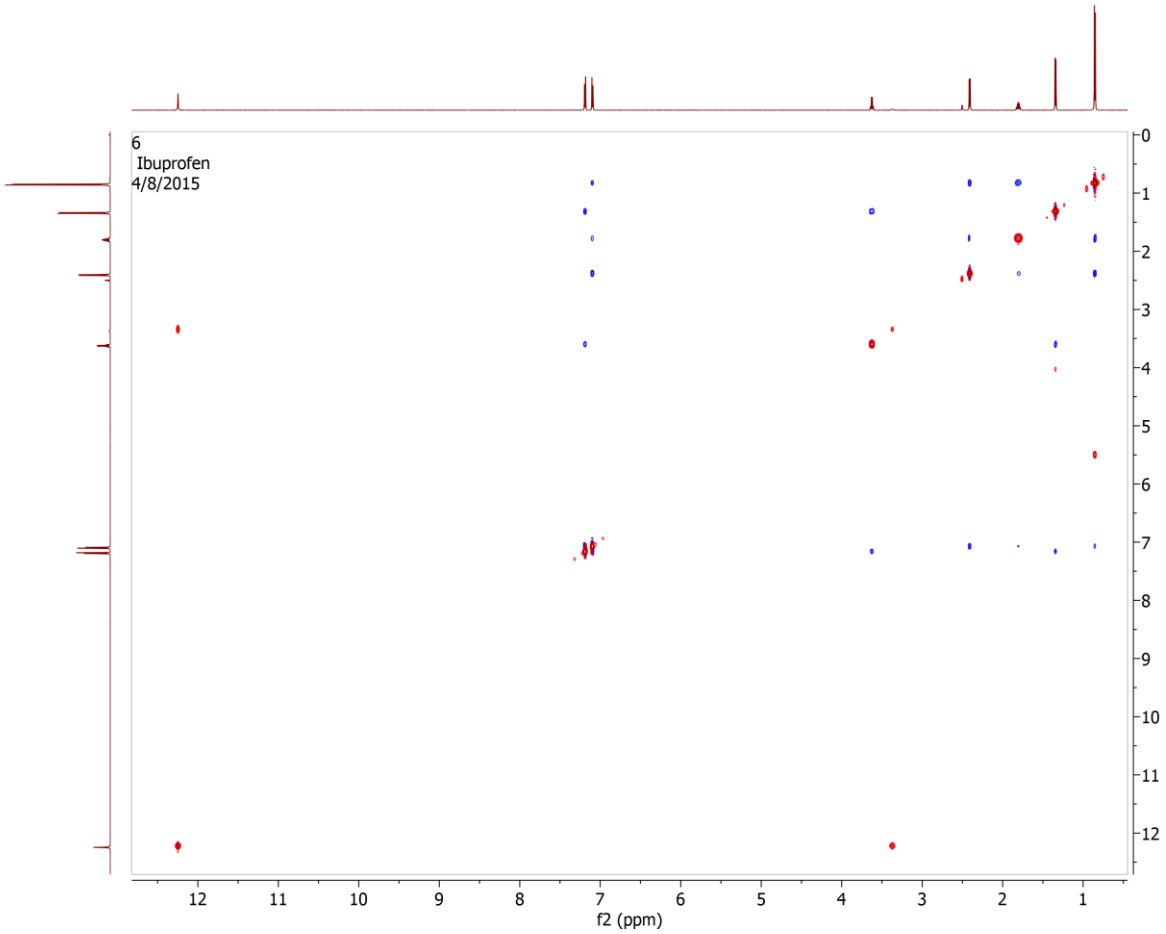
- The re-processed NOESY spectrum shows better line shape, and higher resolution on the F1 dimension, though there still some phase errors

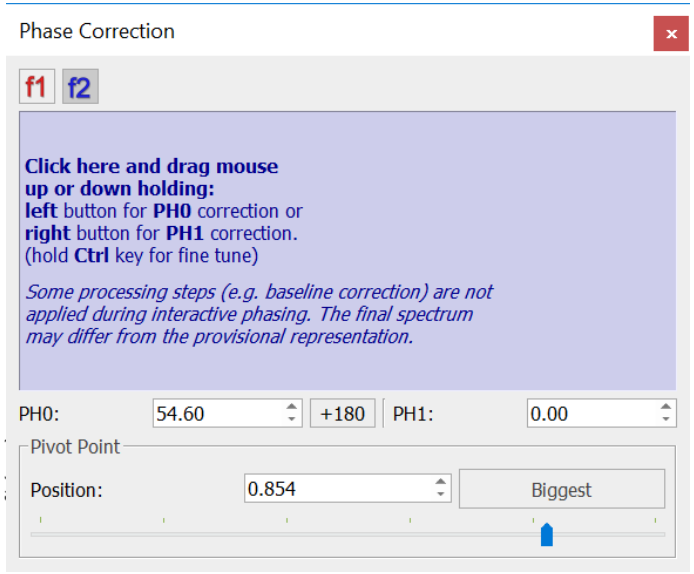


Phase correction for NOESY spectrum

- Do Manual phase correction for either both dimensions.
- Also applied +180 for PH0 to make the cross peaks negative and diagonal peaks positive

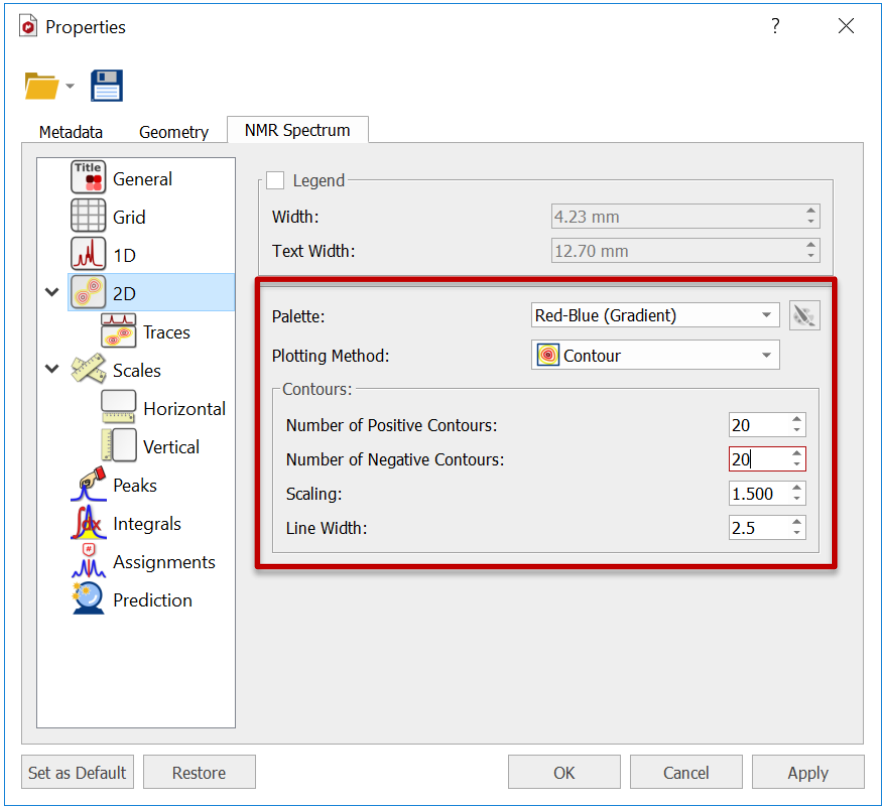


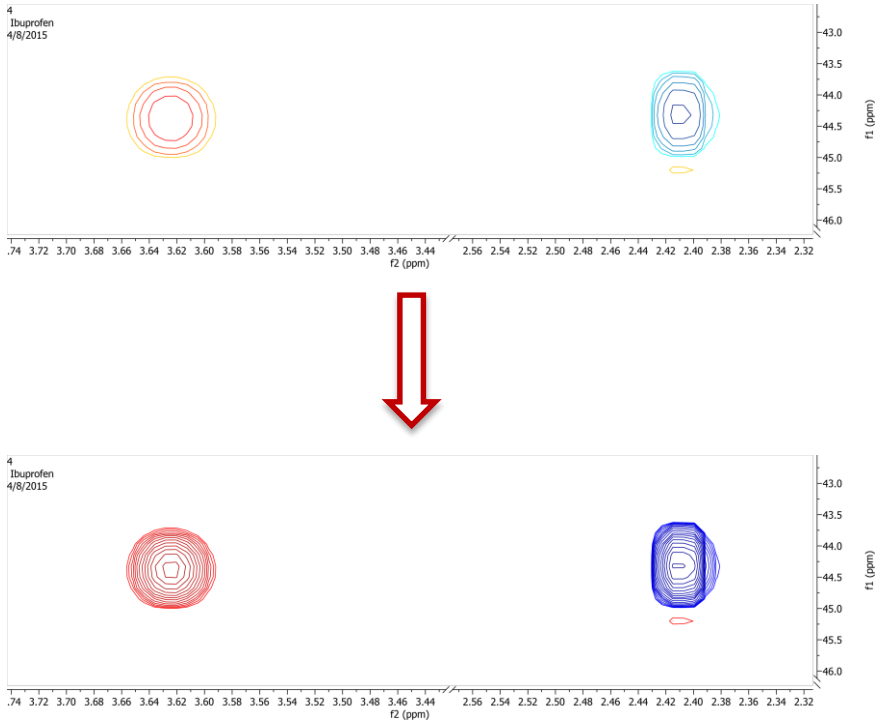




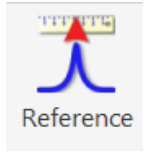
Change the Display Properties

- Double click on the spectrum to open the Properties Dialog, view the properties that can be changed
- In the 2D Category, adjust the highlighted parameters and click Apply to see the effects
- Click Set as Default to retain the settings for 2D spectra display in the future

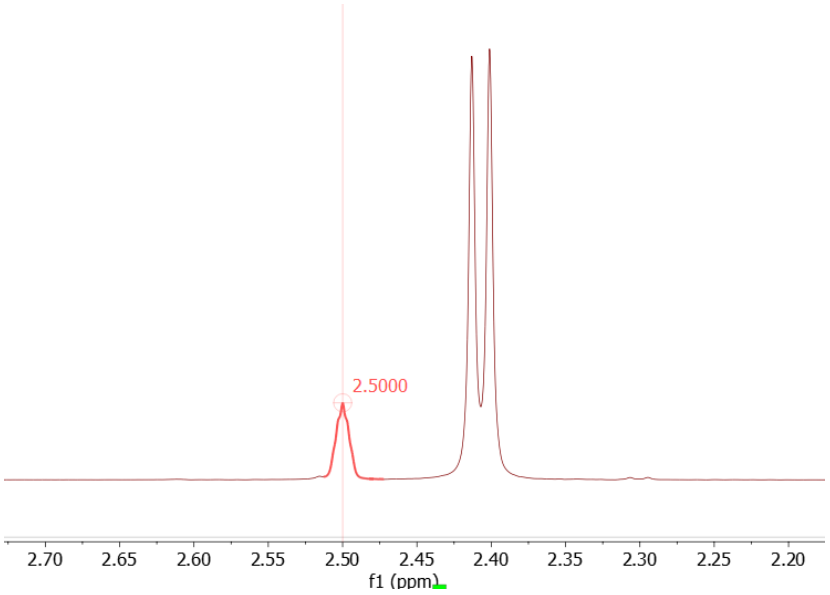


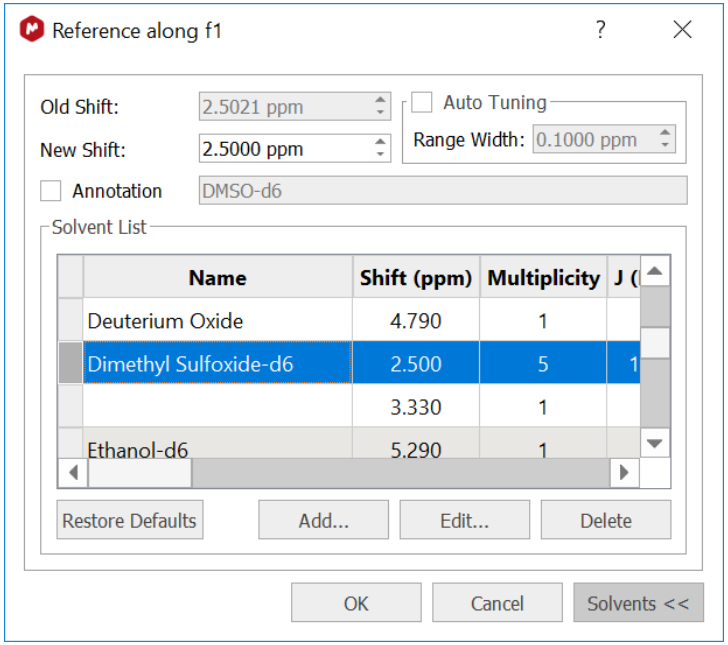


Chemical shift referencing for H-1



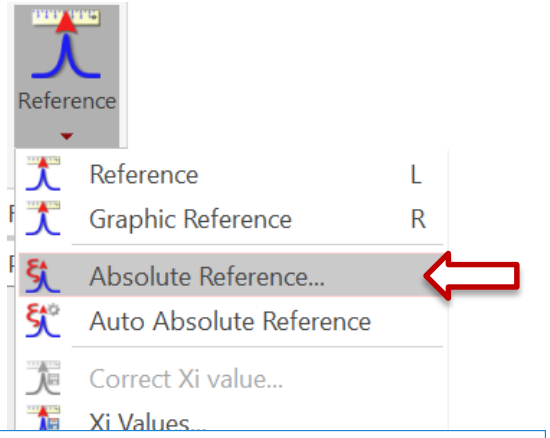
- This spectrum uses DMSO-d6 as the solvent. We can reference the chemical shifts by setting its middle peak to 2.5 ppm.
- Zoom to the DMSO peak at around 2.5 ppm. Choose Analysis > Reference, and click on the top of the middle peak.
- Set it to 2.5 ppm either manually or from the Solvent List.

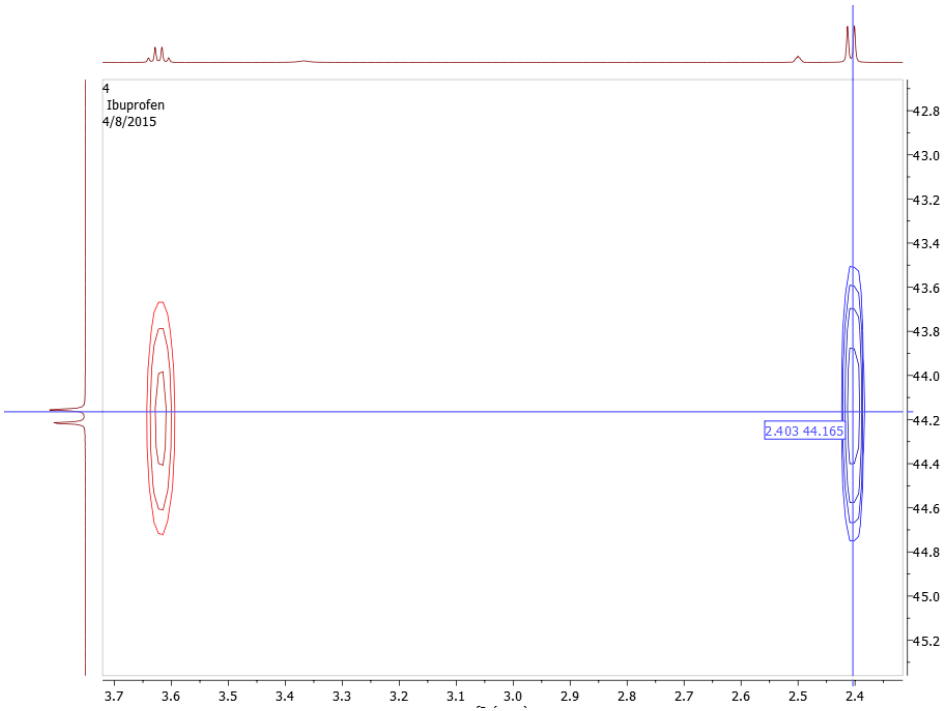


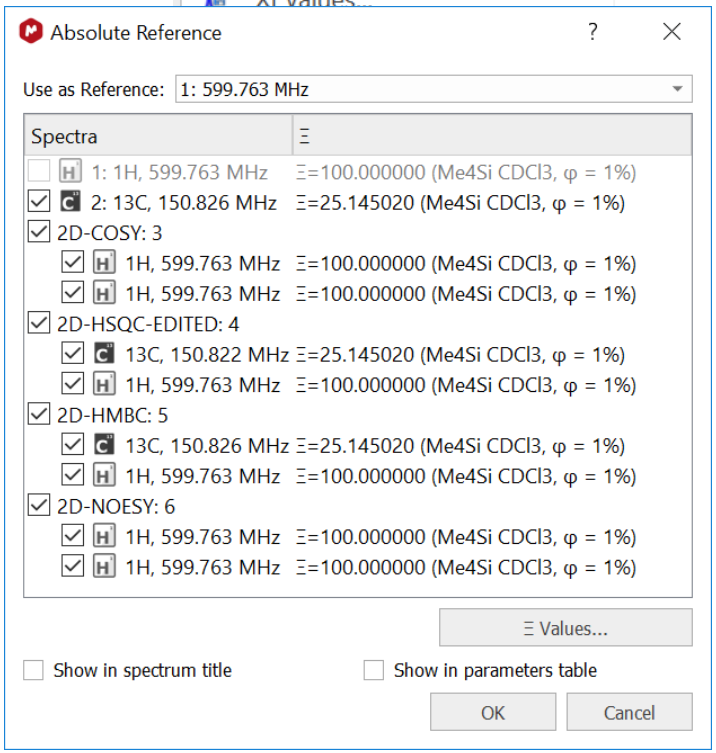


Chemical shift referencing for other spectra

- Choose Analysis > References > Absolute Reference, and click OK to the dialog.
- This applies referencing to all other spectra in the document using the H-1 spectrum as a standard.
- Note this works only if the spectra were from the same sample acquired on the same instrument.

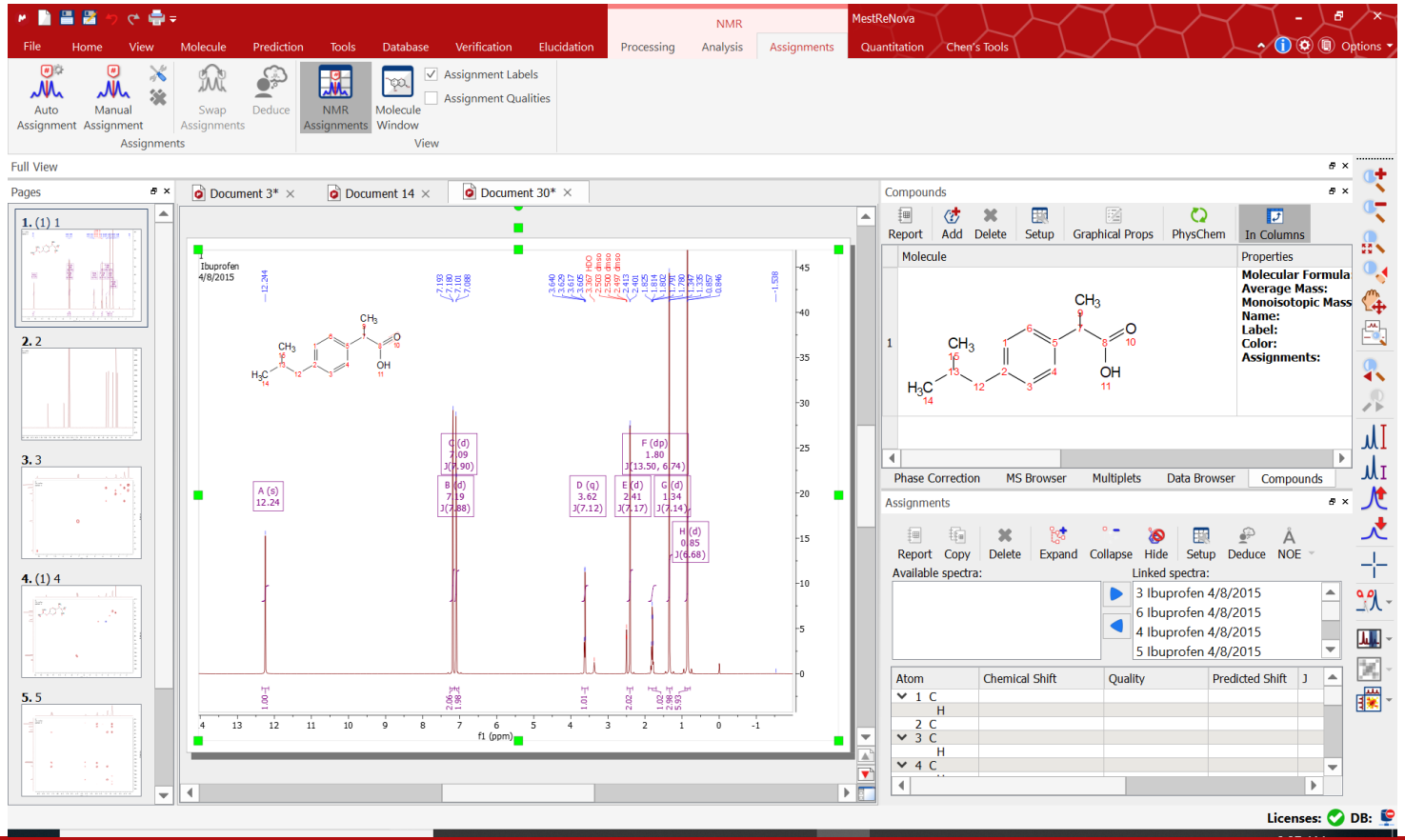






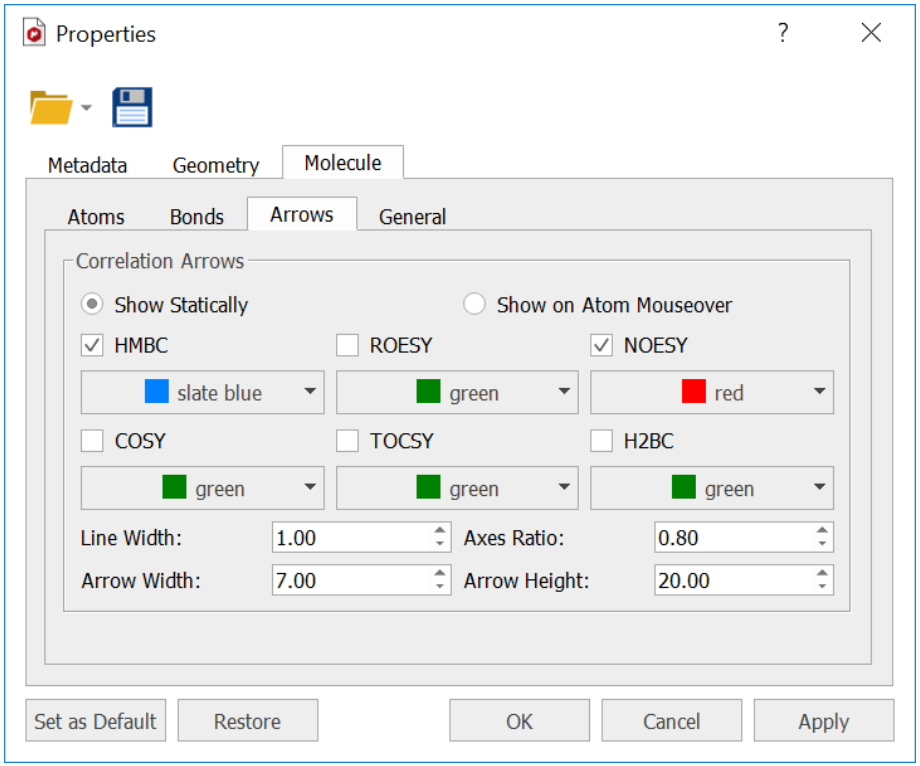
Open the structure for peak assignment

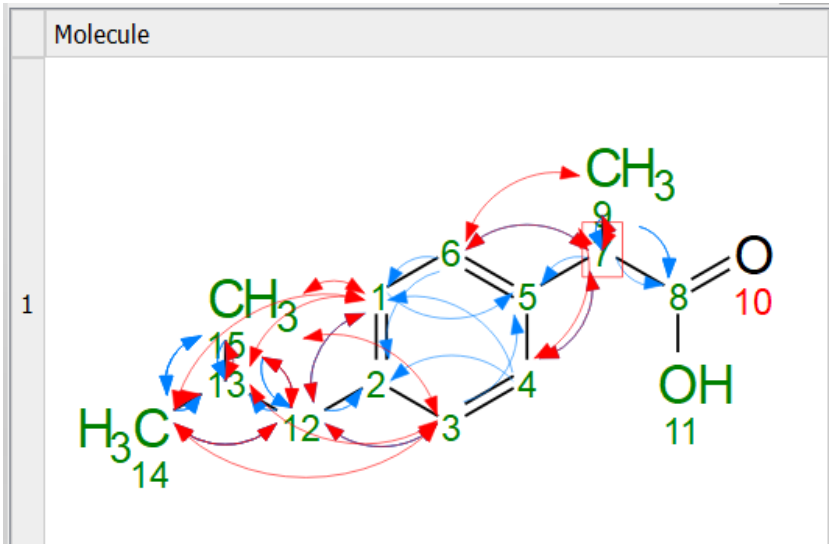
- Open the Ibuprofen.mol file from the Data Browser.
- Choose Molecule > Compound Table to show the structure on the side.
- Note: Open the same molecule only once. If needed, use Report on the Compound Table to report the structure to other pages.
- Choose View > Tables and check Assignment Table. Make sure all spectra are “linked” in it.



Show 2D correlation as arrows

- Double-click on the structure (or click on Graphical Props in the Compounds Table)
- In the Arrows Tab, turn on the display of HMBC and NOESY correlation as different colors
- The correlations will be displayed as the assignments are added

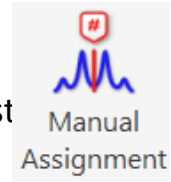




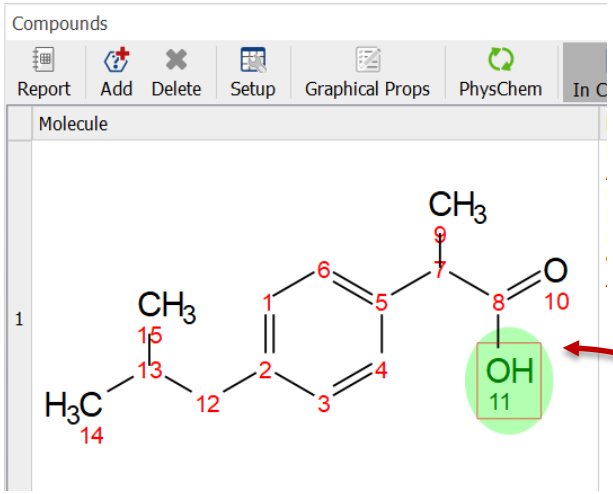
Tip: Click Save as Default button to save the settings

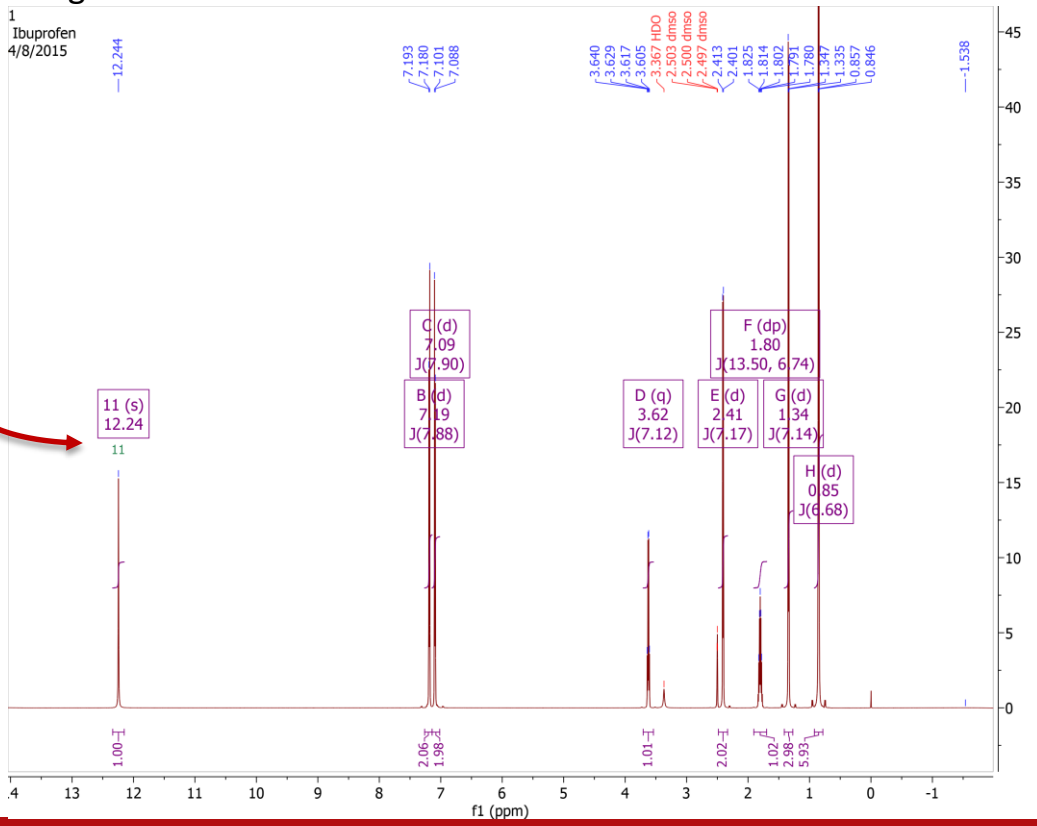
Assign H-1 multiplets

- Click A key to switch to Manual Assignment mode
  - Click on a multiplet lablel and assign it to an atom (This is the most common way to assign H-1 peaks)
  - Click on a peak and assign it to an atom
  - Click and drag on the spectrum, and assign the range to an atom
- View chemical shift assignment grids on the other “linked” spectra
- Optionally, use NMRPredict to assist the assignment



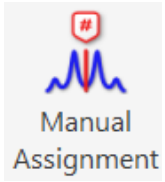
Shortcut = A



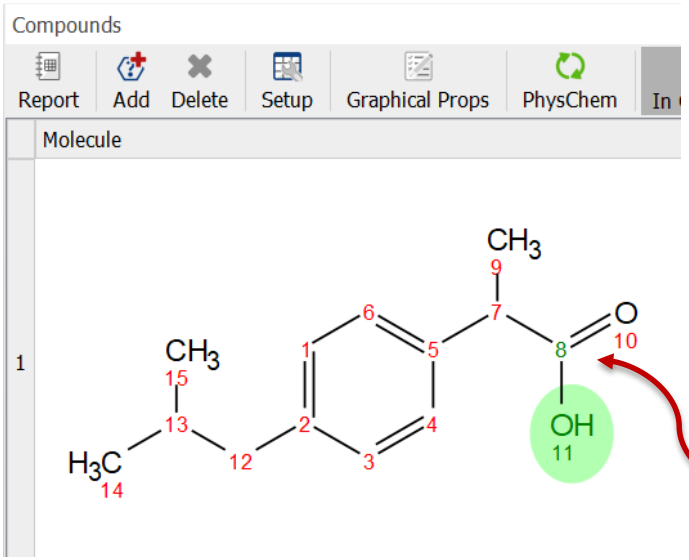


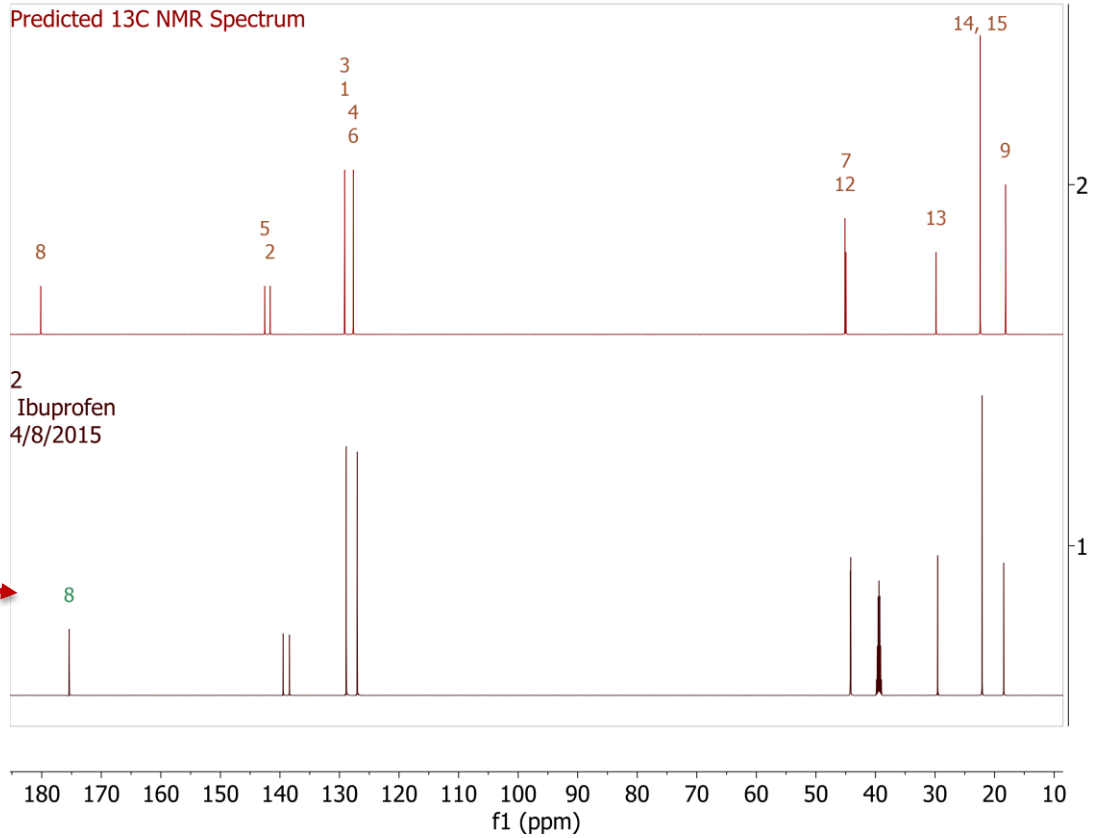
Assign C-13 peaks

- Make a copy of the C-13 spectrum.
- Choose Predict > Predict Compare to predict the 13C spectrum
- Use the prediction to guide the manual assignment



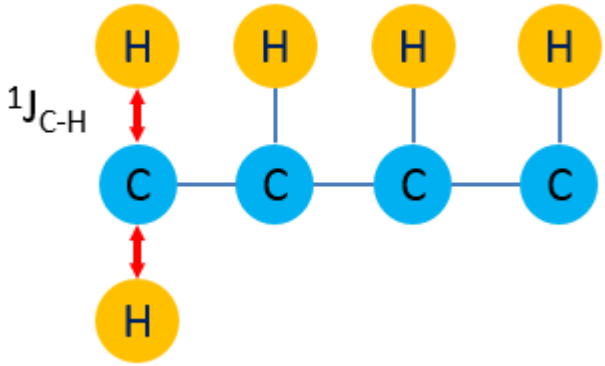
Shortcut = A

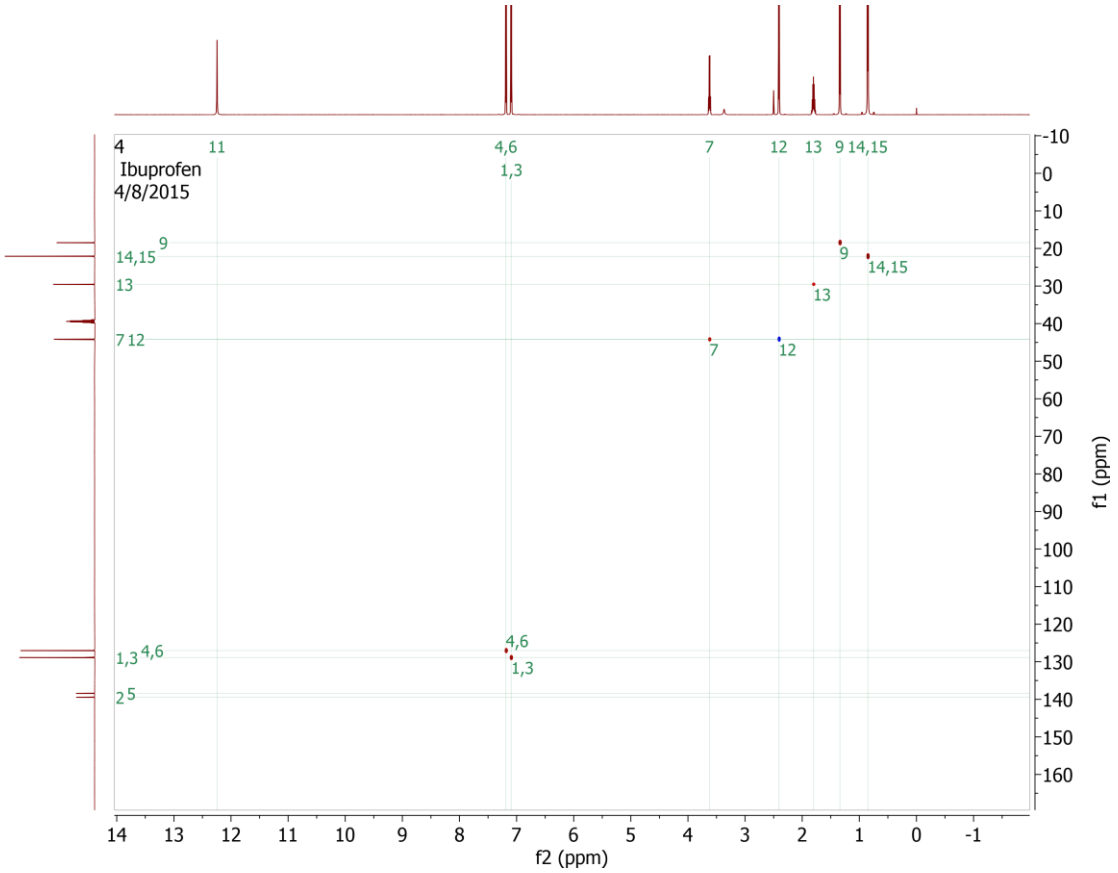


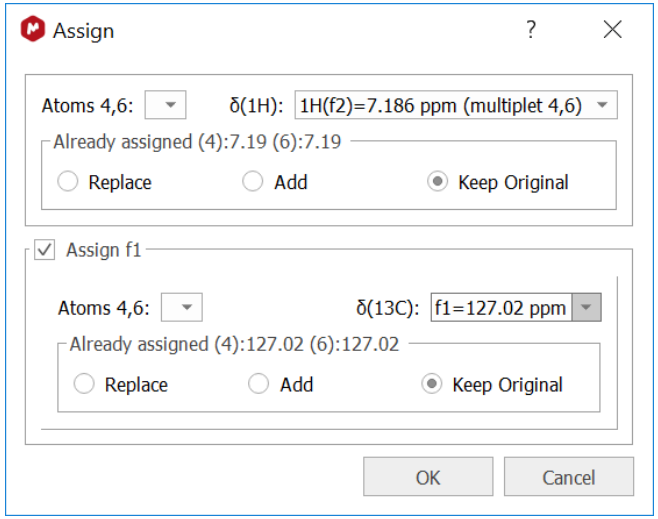


Assign HSQC peaks

- Assign a cross peak to connected H-C atom pair
- Always start from F2 to F1
- Hold Alt key to display the Assign Dialog for more choices
- Always use “original” 1D H/C chemical shifts if available

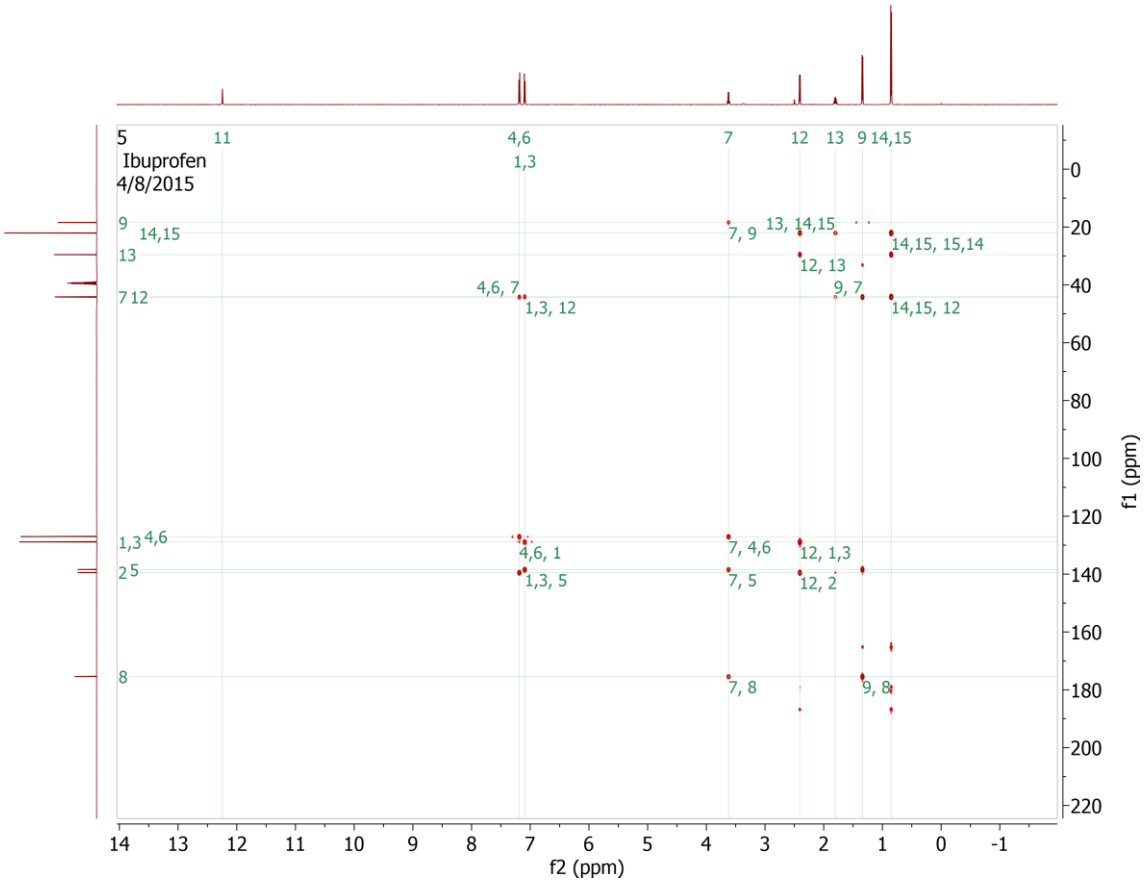


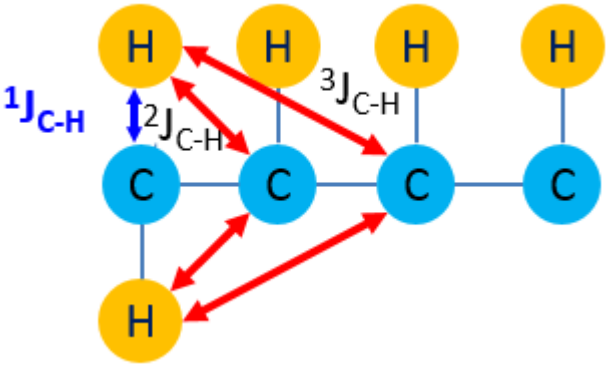


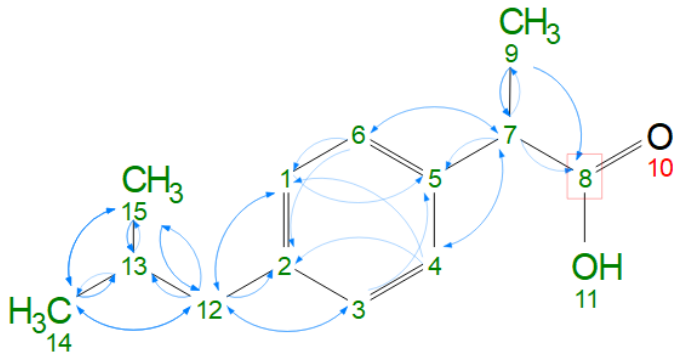


Assign HMBC peaks

- Assign a cross peak to connected H-C with 2 or 3 bonds away
- Always start from F2 to F1
- Always use “original” 1D H/C chemical shifts if available

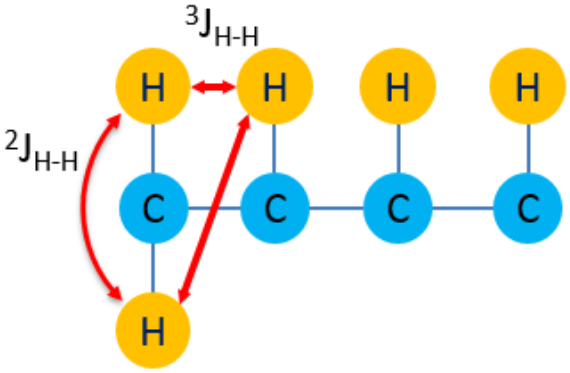


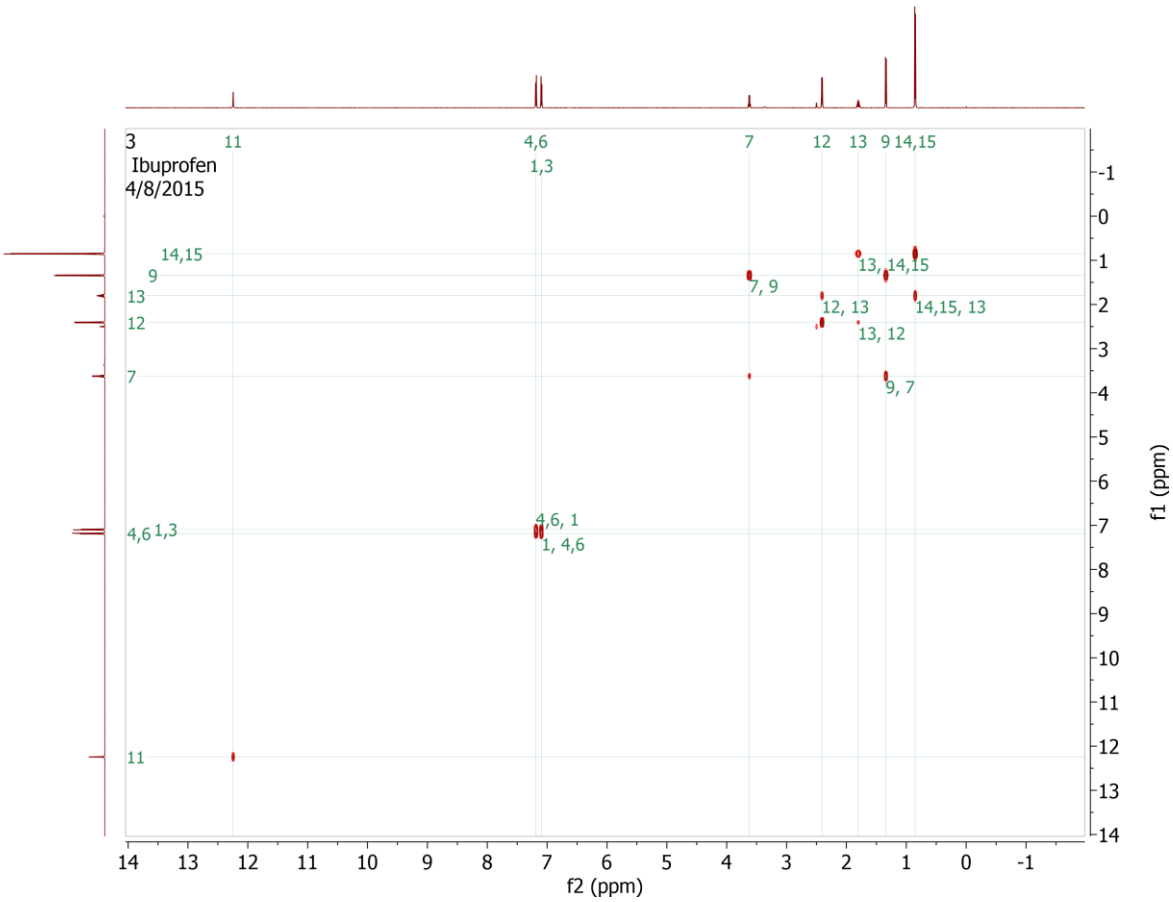


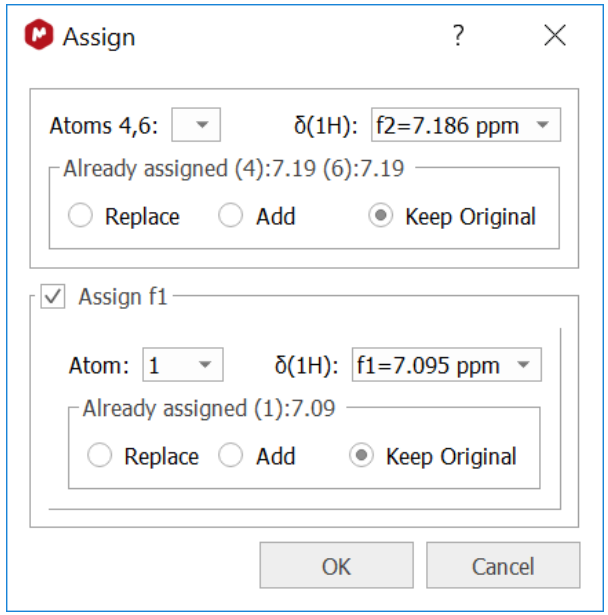


Assign COSY peaks

- Assign a cross peak to connected H-H pair with 2 or 3 bonds away. Weak couplings between 4-5 bonds is also possible
- Always start from F2 to F1
- Always use "original" 1D H-1 chemical shifts if available

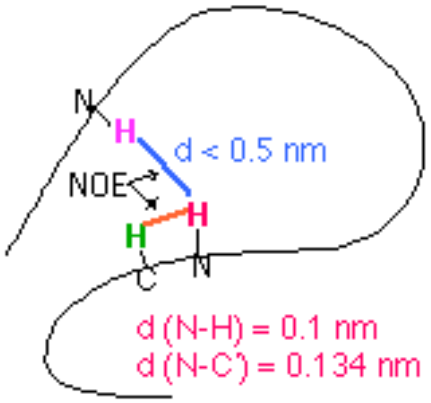


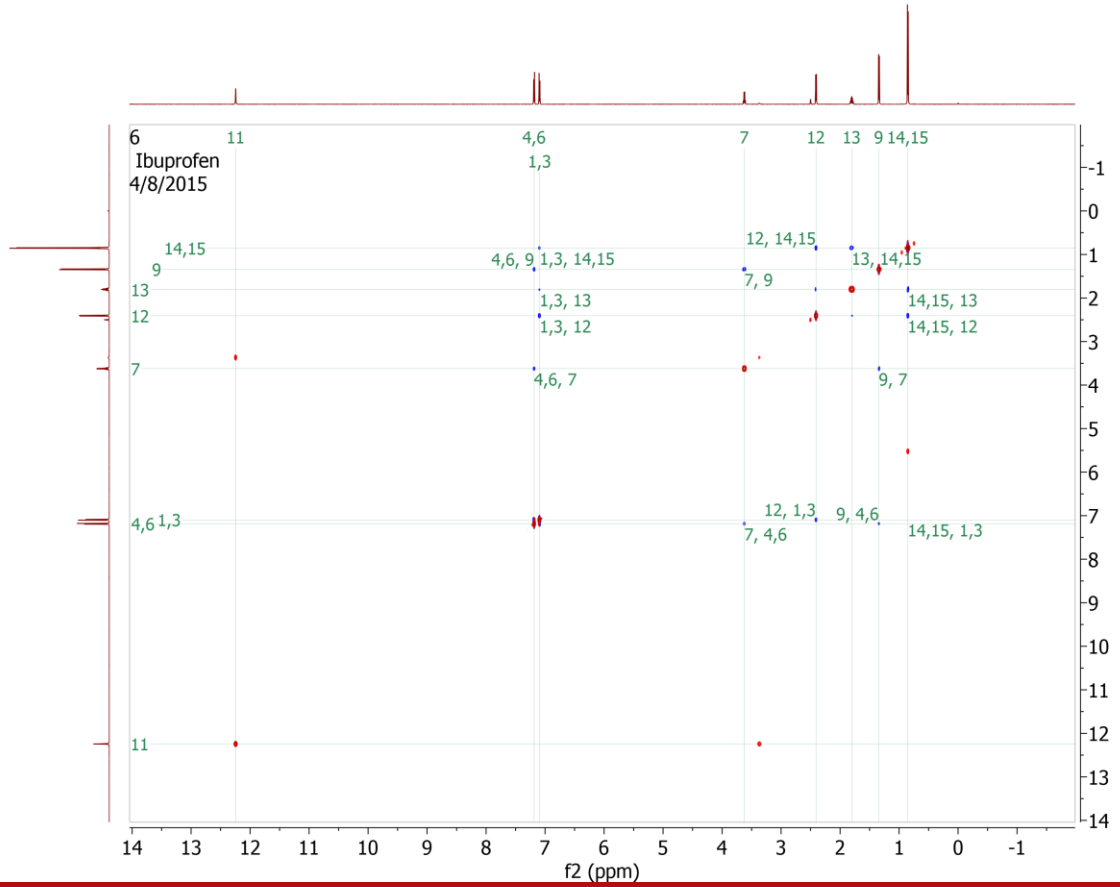


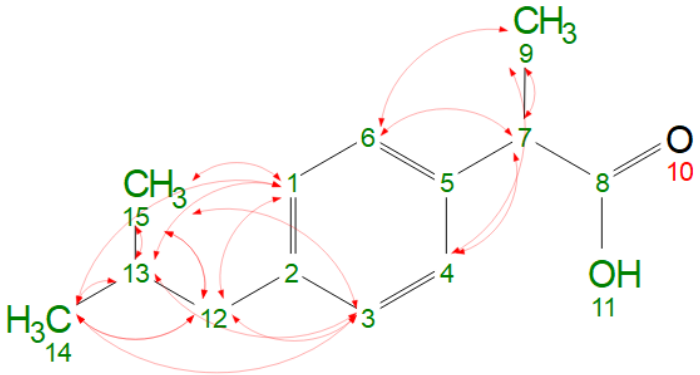


Assign NOESY peaks

- Assign a cross peak to spatially approximate H-H pair (~5A or less).
- For small molecules, NOE cross peaks are usually negative in phase. Positive one may be from J-couplings and should be ignored with caution.
- Always start from F2 to F1
- Always use “original” 1D H-1 chemical shifts if available







Assignments table

- Click Assignment > Assignment Table to display the Assignment Table
- The check boxes can be used to turn on/off the display of individual correlations on the structure

Assignments

Report Copy Delete Expand Collapse Hide Setup Deduce NOE

Atom	Chemical Shift	Quality	Predicted Shift	J	NOE	COSY	HSQC	HMBC	NOESY
1 C	128.88		129.12				1	✓ 4, 6, 12	
H	7.09	0.71				✓ 4, 6	1	✓ 5, 12	✓ 12, 13, 14, 15
2 C	139.46		141.62					✓ 4, 6, 12	
3 C	128.88		129.12				3	✓ 12	
H	7.09	0.71					3	✓ 5, 12	✓ 12, 13, 14, 15
4 C	127.02		127.66				4	✓ 7	
H	7.19	0.71				✓ 1	4	✓ 1, 2, 7	✓ 7, 9
5 C	138.41		142.54					✓ 1, 3, 7	
6 C	127.02		127.66				6	✓ 7	
H	7.19	0.71				✓ 1	6	✓ 1, 2, 7	✓ 7, 9
7 C	44.22		44.96				7	✓ 4, 6, 9	
H	3.62	0.71				✓ 9	7	✓ 4, 5, 6, 8, 9	✓ 4, 6, 9
8 C	175.40		180.15					✓ 7, 9	
9 C	18.45		18.15				9	✓ 7	
H3	1.34	0.71				✓ 7	9	✓ 7, 8	✓ 4, 6, 7
10 O									
11 O									
H	12.24	0.56							
12 C	44.16		45.12				12	✓ 1, 3, 14, 15	
H2	2.41	0.71				✓ 13	12	✓ 1, 2, 3, 13, 14,...	✓ 1, 3, 14, 15
13 C	29.55		29.81				13	✓ 12, 14, 15	
H	1.80	0.25				✓ 12, 14, 15	13	✓ 14, 15	✓ 1, 3, 14, 15
14 C	22.09		22.41				14	✓ 12, 13, 15	
H3	0.85	0.71				✓ 13	14	✓ 12, 13, 15	✓ 1, 3, 12, 13
15 C	22.09		22.41				15	✓ 12, 13, 14	
H3	0.85	0.71				✓ 13	15	✓ 12, 13, 14	✓ 1, 3, 12, 13

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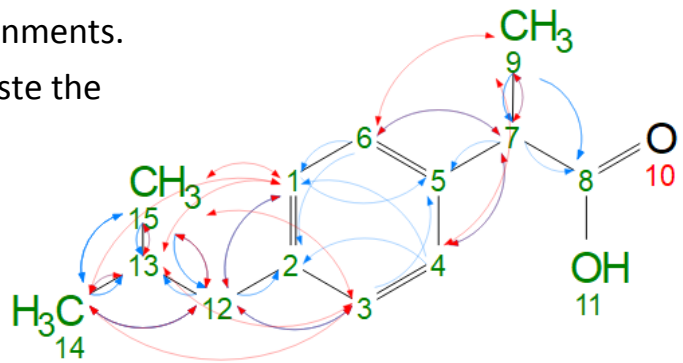
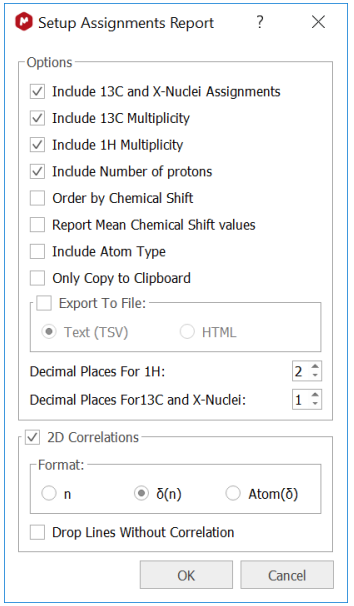
REPORT

Report assignments

- Choose Tools > Loaded Scripts > Report > Assignments.
- Report the assignments on the spectrum or paste the table to another document

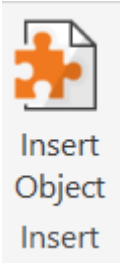


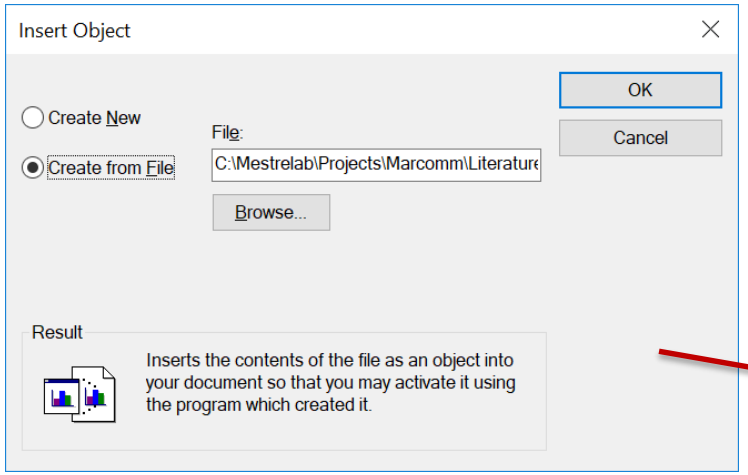


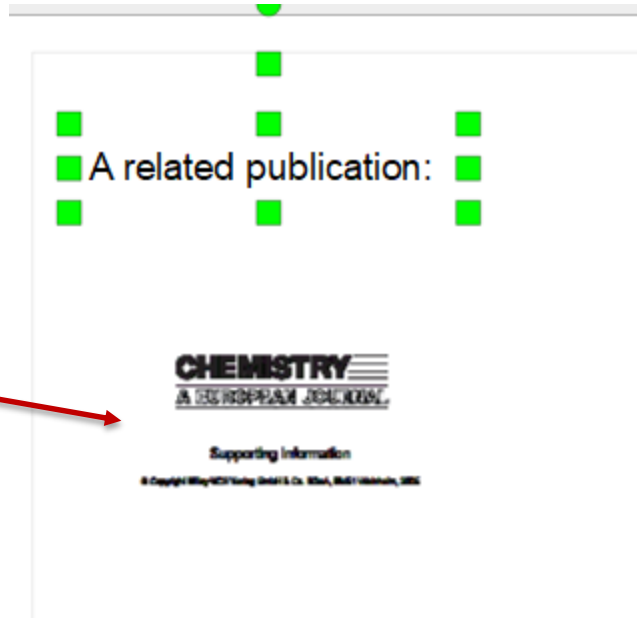
No	δ_H (Multiplicity, *J*, nH)	δ_C (Multiplicity, *J*)	HSQC-EDITED	HMBC	COSY	NOESY
1	7.09 (d, 7.9 Hz, 1H)	128.9(s)	128.9(1)	44.2(12),138.4(5)	7.19(4), 7.19(6)	0.85(14), 0.85(15), 1.80(13),2.41(12)
2	-	139.5(s)	-	-	-	-
3	7.09 (d, 7.9 Hz, 1H)	128.9(s)	128.9(3)	44.2(12),138.4(5)	-	0.85(14), 0.85(15), 1.80(13),2.41(12)
4	7.19 (d, 7.9 Hz, 1H)	127.0(s)	127.0(4)	44.2(7),128.9(1), 139.5(2)	7.09(1)	1.34(9),3.62(7)
5	-	138.4(s)	-	-	-	-
6	7.19 (d, 7.9 Hz, 1H)	127.0(s)	127.0(6)	44.2(7),128.9(1), 139.5(2)	7.09(1)	1.34(9),3.62(7)
7	3.62 (q, 7.1 Hz, 1H)	44.2(s)	44.2(7)	18.5(9),127.0(4), 127.0(6), 138.4(5), 175.4(8)	1.34(9)	1.34(9),7.19(4), 7.19(6)
8	-	175.4(s)	-	-	-	-
9	1.34 (d, 7.1 Hz, 3H)	18.5(s)	18.5(9)	44.2(7), 175.4(8)	3.62(7)	3.62(7),7.19(4), 7.19(6)
11	12.24 (s, 1H)	-	-	-	-	-
12	2.41 (d, 7.2 Hz, 2H)	44.2(s)	44.2(12)	22.1(14), 22.1(15), 29.6(13),128.9(1), 128.9(3), 139.5(2)	1.80(13)	0.85(14), 0.85(15),7.09(1), 7.09(3)
13	1.80 (dp, 13.5,6.7 Hz, 1H)	29.6(s)	29.6(13)	22.1(14), 22.1(15)	0.85(14), 0.85(15),2.41(12)	0.85(14), 0.85(15),7.09(1), 7.09(3)
14	0.85 (d, 6.7 Hz, 3H)	22.1(s)	22.1(14)	22.1(15), 29.6(13),44.2(12)	1.80(13)	1.80(13), 2.41(12),7.09(1), 7.09(3)
15	0.85 (d, 6.7 Hz, 3H)	22.1(s)	22.1(15)	22.1(14), 29.6(13),44.2(12)	1.80(13)	1.80(13), 2.41(12),7.09(1), 7.09(3)

Insert a PDF to the document

- Choose Home > Insert Object, choose Create from File, and insert a PDF to the document
- A preview logo of the document is displayed.
- Add a text box annotation to it
- You can double click on the preview to open it







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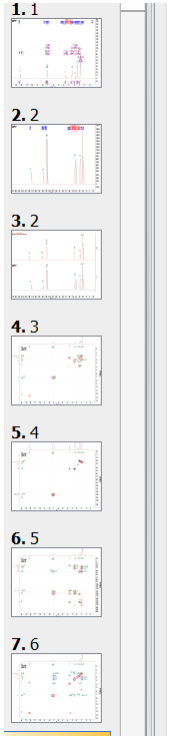
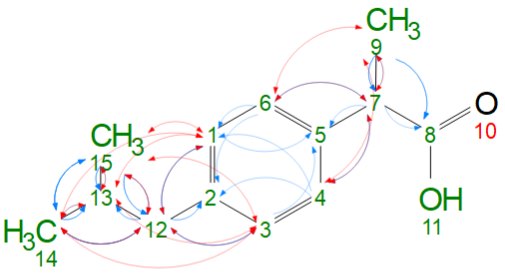
REPORT

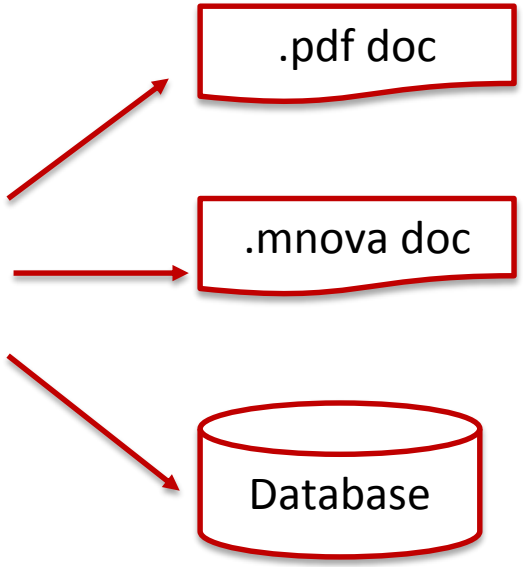
Save the results

- Choose File > Export to PDF to save a PDF report of the page.
- Chose File > Save as to save all the results to a .mnova file.
- Save all the results to a database (see steps later)
- Now you can close the document or continue to add other spectra to it.



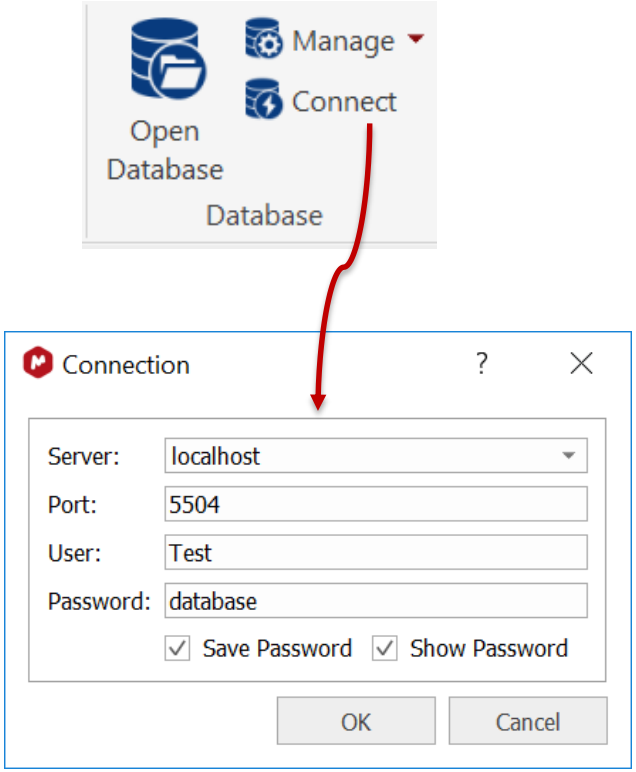


No	δ H (Multiplicity, J, nH)	δ C (Multiplicity, J)	HSQC-EDITED	HMBC	COSY	NOESY
1	7.09 (d, 7.9 Hz, 1H)	128.9(s)	128.9(1)	44.2(12),138.4(5)	7.19(4), 7.19(6)	0.85(14), 0.85(15), 1.80(13),2.41(12)
2	-	139.5(s)	-	-	-	-
3	7.09 (d, 7.9 Hz, 1H)	128.9(s)	128.9(3)	44.2(12),138.4(5)	-	0.85(14), 0.85(15), 1.80(13),2.41(12)
4	7.19 (d, 7.9 Hz, 1H)	127.0(s)	127.0(4)	44.2(7),128.9(1), 139.5(2)	7.09(1)	1.34(9),3.62(7)
5	-	138.4(s)	-	-	-	-
6	7.19 (d, 7.9 Hz, 1H)	127.0(s)	127.0(6)	44.2(7),128.9(1), 139.5(2)	7.09(1)	1.34(9),3.62(7)
7	3.62 (q, 7.1 Hz, 1H)	44.2(s)	44.2(7)	18.5(9),127.0(4), 127.0(6), 138.4(5), 175.4(8)	1.34(9)	1.34(9),7.19(4), 7.19(6)
8	-	175.4(s)	-	-	-	-
9	1.34 (d, 7.1 Hz, 3H)	18.5(s)	18.5(9)	44.2(7), 175.4(8)	3.62(7)	3.62(7),7.19(4), 7.19(6)
11	12.24 (s, 1H)	-	-	-	-	-
12	2.41 (d, 7.2 Hz, 2H)	44.2(s)	44.2(12)	22.1(14), 22.1(15), 29.6(13),128.9(1), 128.9(3), 139.5(2)	1.80(13)	0.85(14), 0.85(15),7.09(1), 7.09(3)
13	1.80 (dp, 13.5,6.7 Hz, 1H)	29.6(s)	29.6(13)	22.1(14), 22.1(15)	0.85(14), 0.85(15),2.41(12)	0.85(14), 0.85(15),7.09(1), 7.09(3)
14	0.85 (d, 6.7 Hz, 3H)	22.1(s)	22.1(14)	22.1(15), 29.6(13),44.2(12)	1.80(13)	1.80(13), 2.41(12),7.09(1), 7.09(3)
15	0.85 (d, 6.7 Hz, 3H)	22.1(s)	22.1(15)	22.1(14), 29.6(13),44.2(12)	1.80(13)	1.80(13), 2.41(12),7.09(1), 7.09(3)



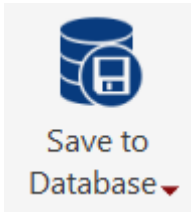
Install MyData DB Server

- Make sure MyData Server is installed and running as a service*
- Connect to MyData Server with the default account setting
- Create and open a new database (Database > Manage > Add)

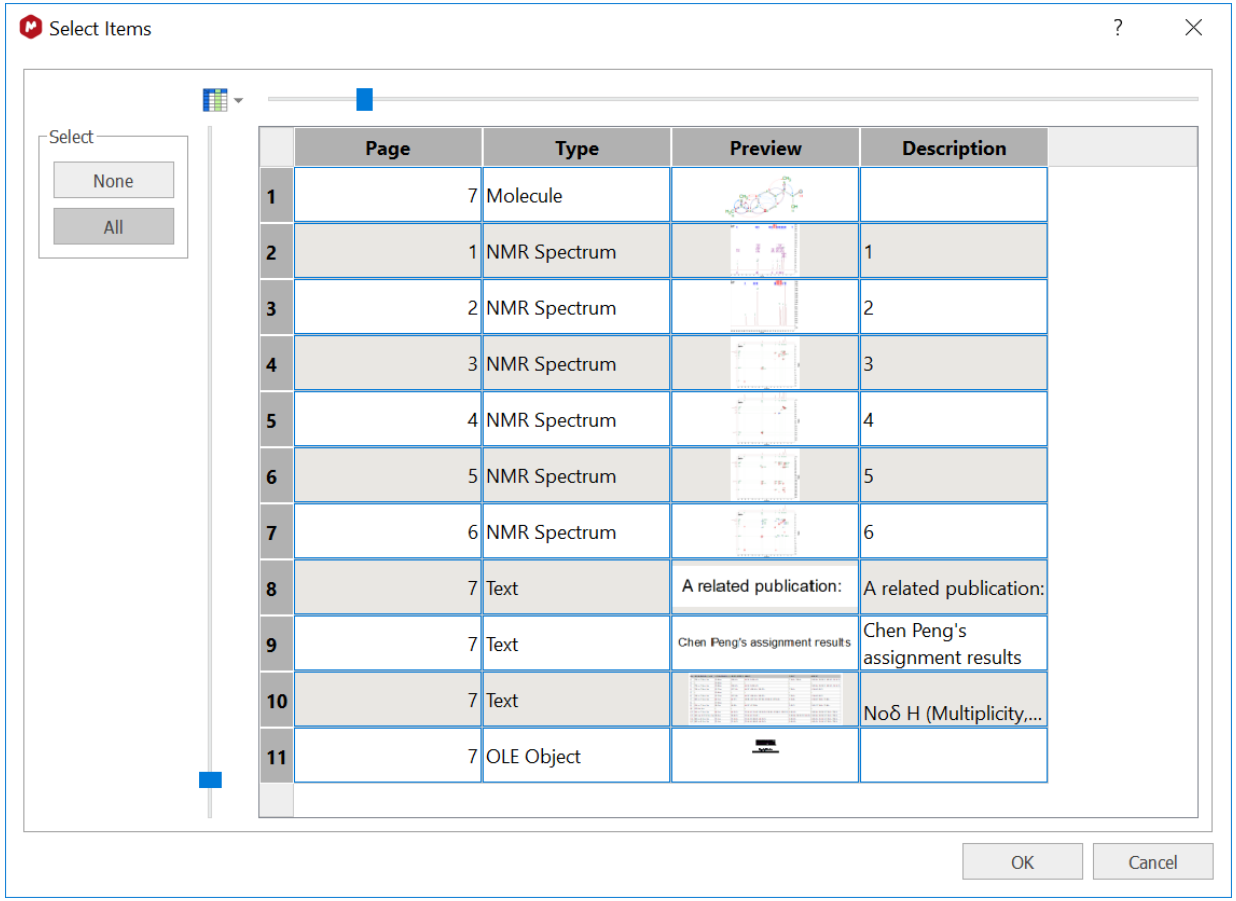


If not yet, download the DB MyData Server Installer from http://mestrelab.com/download/mnova/db/ and install it. You may need to download the latest Java too. No license is needed for MyData DB Server.

Save data to database

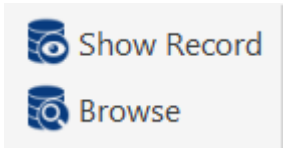


- Choose Database > Save to Database
- Click All in the Select Items dialog
- Click OK to save all items to the new database

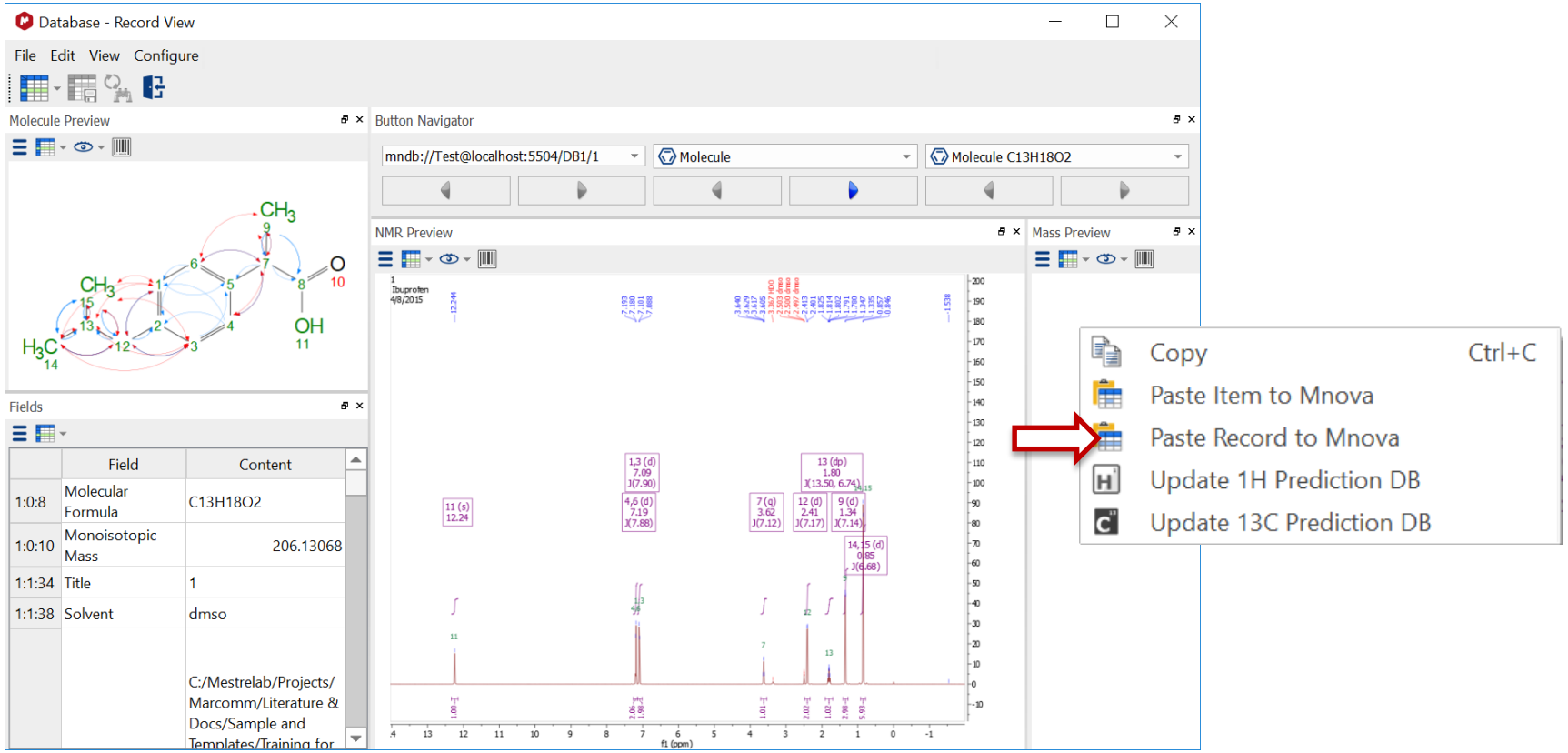


DATABASE

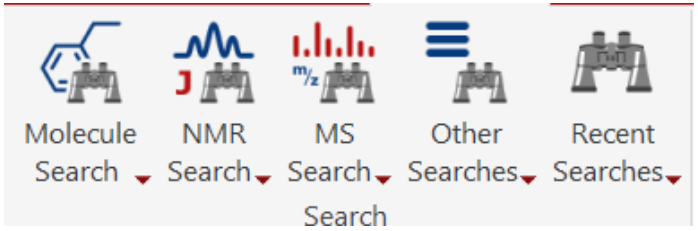
Browse and download from database



- Choose Database > Browse or Show Record
- Display the contents in Record View (or Table View)
- Choose File > New in Mnova to open a new document
- Right click on the spectrum in the Database Dialog and choose Paste Record to Mnova to download the whole record to Mnova



Search your own database



- Search by text
- Search by peaks
- Search by multiplets
- Search by 2D peaks
- Search by structure/substructure

Search Wiley databases

- Wiley C-13 and H-1 databases are collection of published C13 and H-1 spectra of known organic compounds. They are usually installed on a central server (Mnova DB Enterprise Server) for remote access.
- Make sure you have an account to access the Wiley databases. Contact your library administrators if you don't have it.
- Once connected to the server, the searching methods are the same as with MyData DB Server.

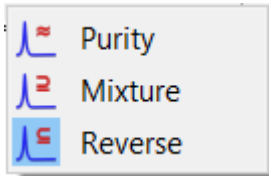
Wiley C-13 database

- 13C NMR Spectra: 268,000
- Structures: 268,000
- Compounds: 228,000
- Replicate Spectra: 40,000
- Collected and reviewed by Wolfgang Robien, with carefully reviewed peak assignments, specific measurement and instrument parameters, where available.
- Using proprietary quality assurance measures developed by the author and Wiley, this is the finest collection of 13C NMR spectra available

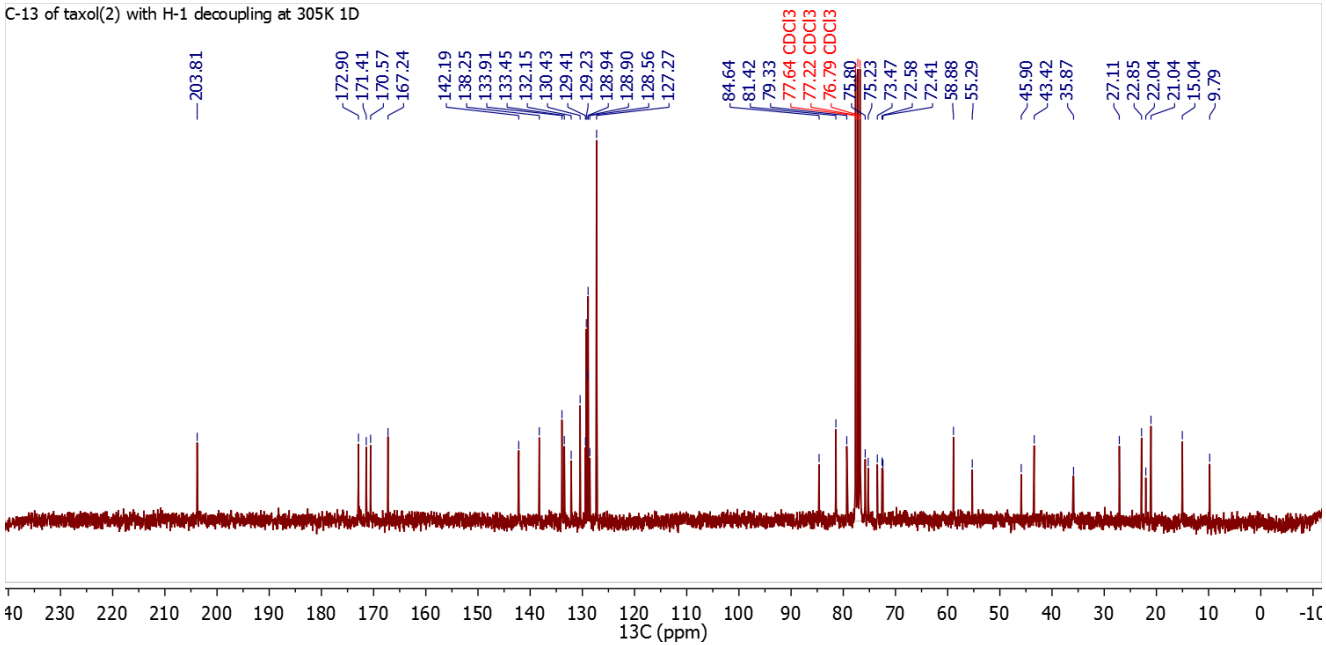
Search Wiley database

- 1H NMR Spectra: 157,000
- Structures: 157,000
- Compounds: 155,000
- Replicate Spectra: 2,000
- Collected and reviewed by Alexander Yarkov, with carefully reviewed peak assignments, and specific measurement and instrument parameters, where available.
- Using proprietary quality assurance measures developed by the author and Wiley, this is the finest and largest collection of 1H NMR spectra available.

Search a C-13 spectrum

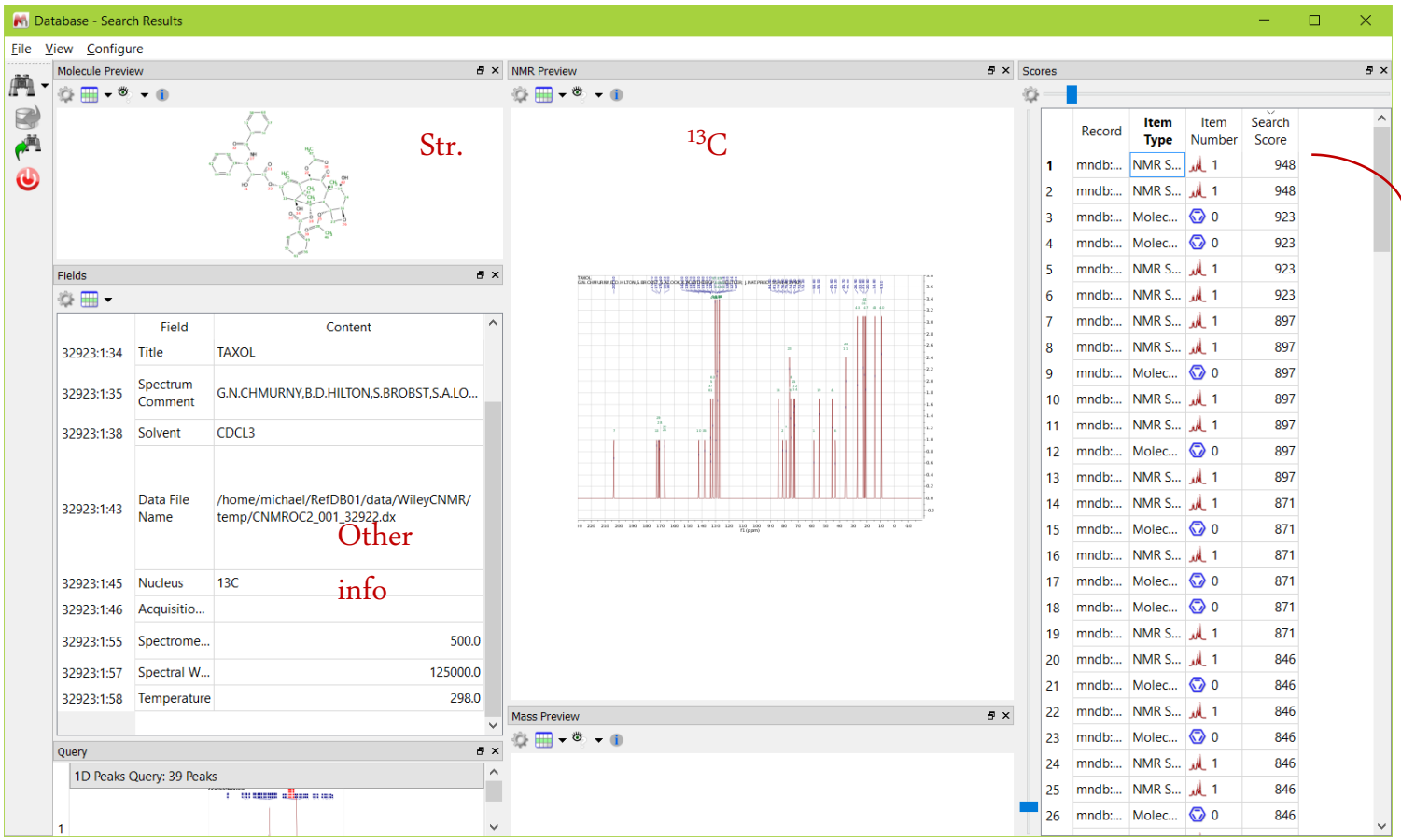


- Open Training Data Sets > Database Search > Taxol > C-13 > fid
- Do Analysis > Auto Peak Picking, right click and choose peak search
- Peak Search Settings:
  - Reverse to penalize only unmatched query peaks.
  - Tolerance = +/- 1.0 ppm
- Choose the top hits with score > 800 (out of 1000)
- Taxol has the highest score 948



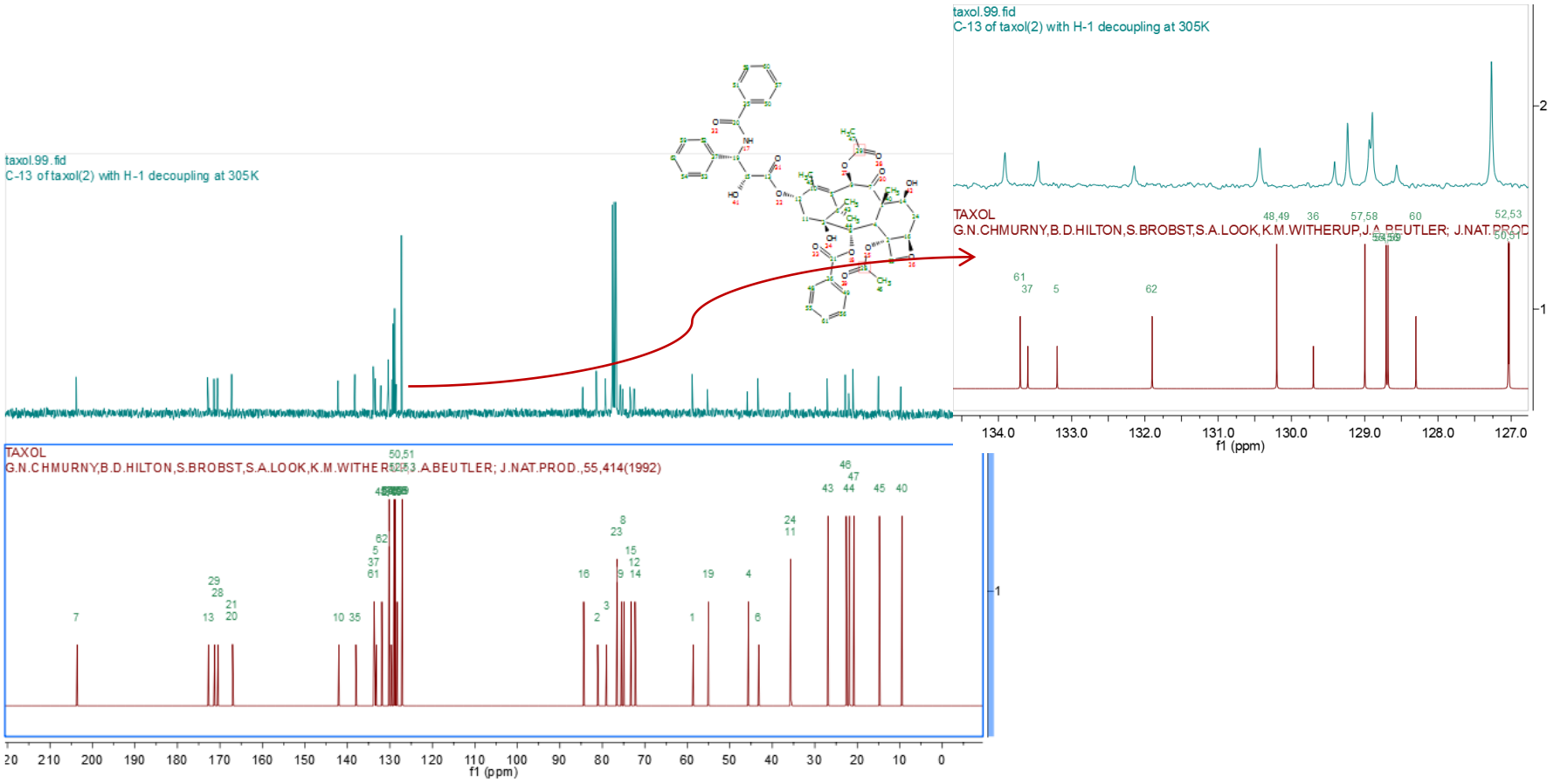
C-13 search results

- Hit list, and preview of hits



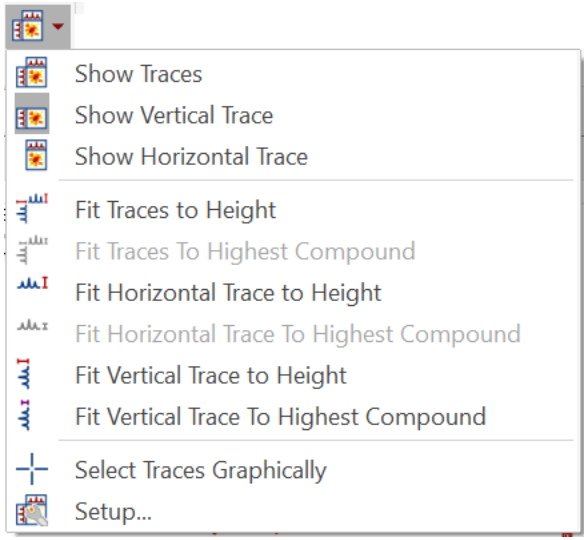
Search a C-13 spectrum

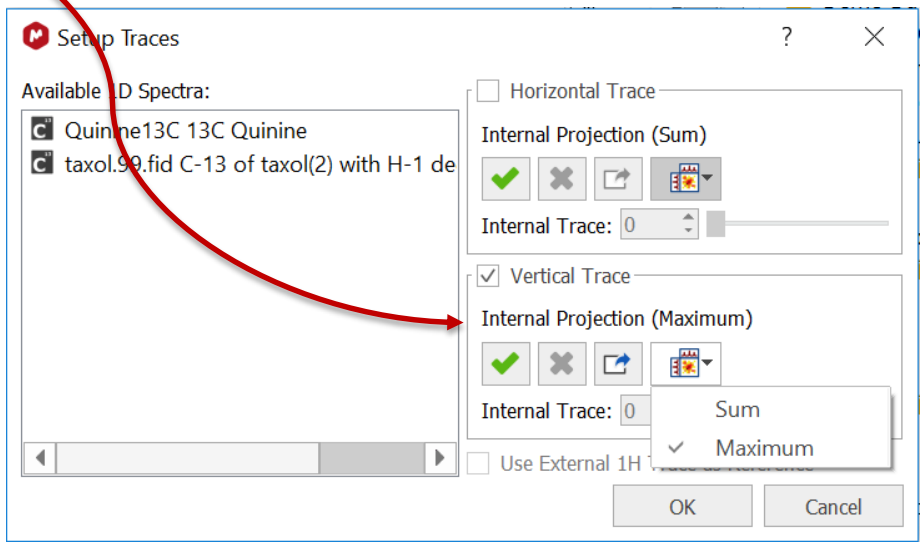
- Right click on the spectrum, choose Paste Record to Mnova to download the hit spectrum and compare with the experimental one



Search C-13 peaks from HMBC

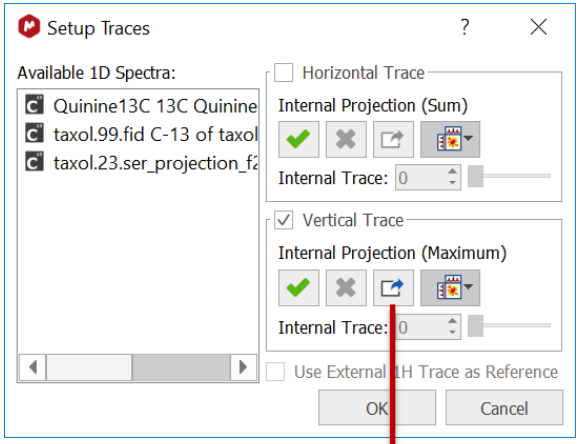
- Open Training Data Sets > Database Search > Taxol > HMBC > ser
- Choose Processing > More Processing > Reduce t1 Noise.
- Choose View > 2D Traces > Setup
- In the Setup Traces dialog, choose to display Internal Projection (Maximum) as Vertical Trace

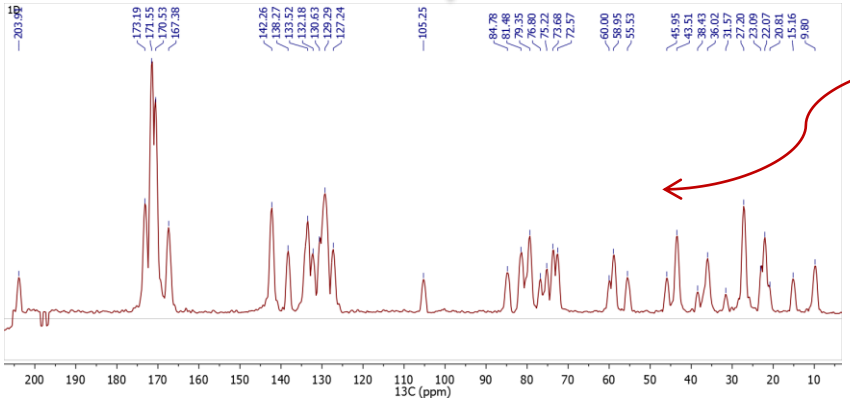


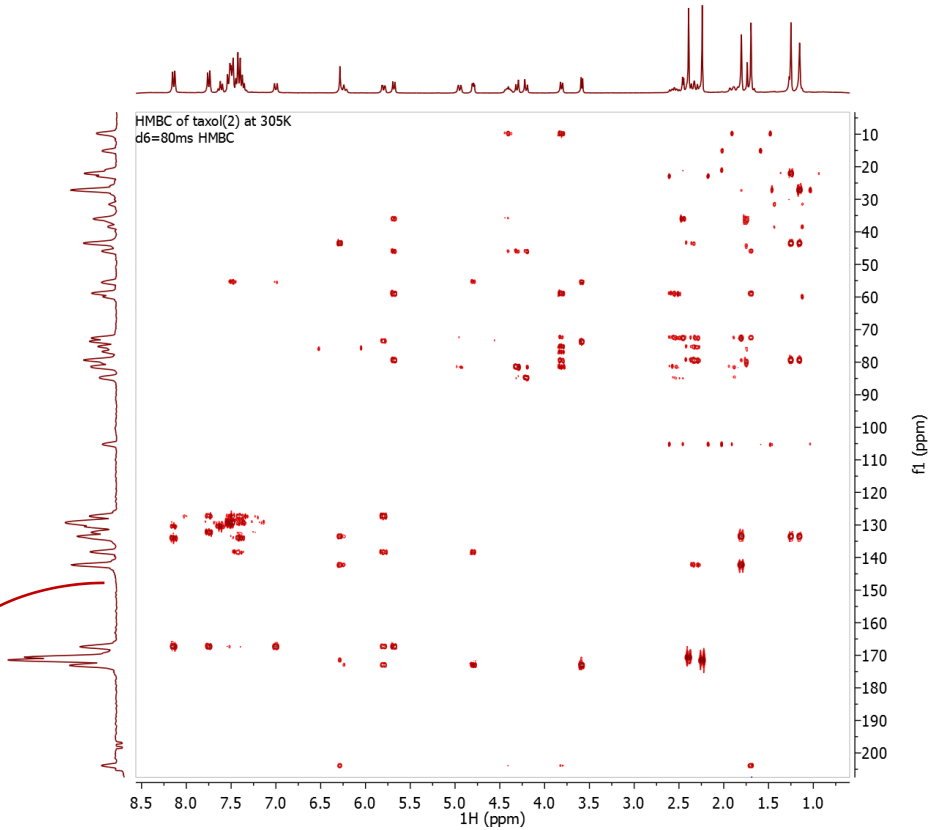


Search C-13 peaks from HMBC

- Click Extract in the Setup Trace Dialog to generate a pseudo 1D C13 spectrum in a new page, and do auto peak picking on the pseudo C13 spectrum.

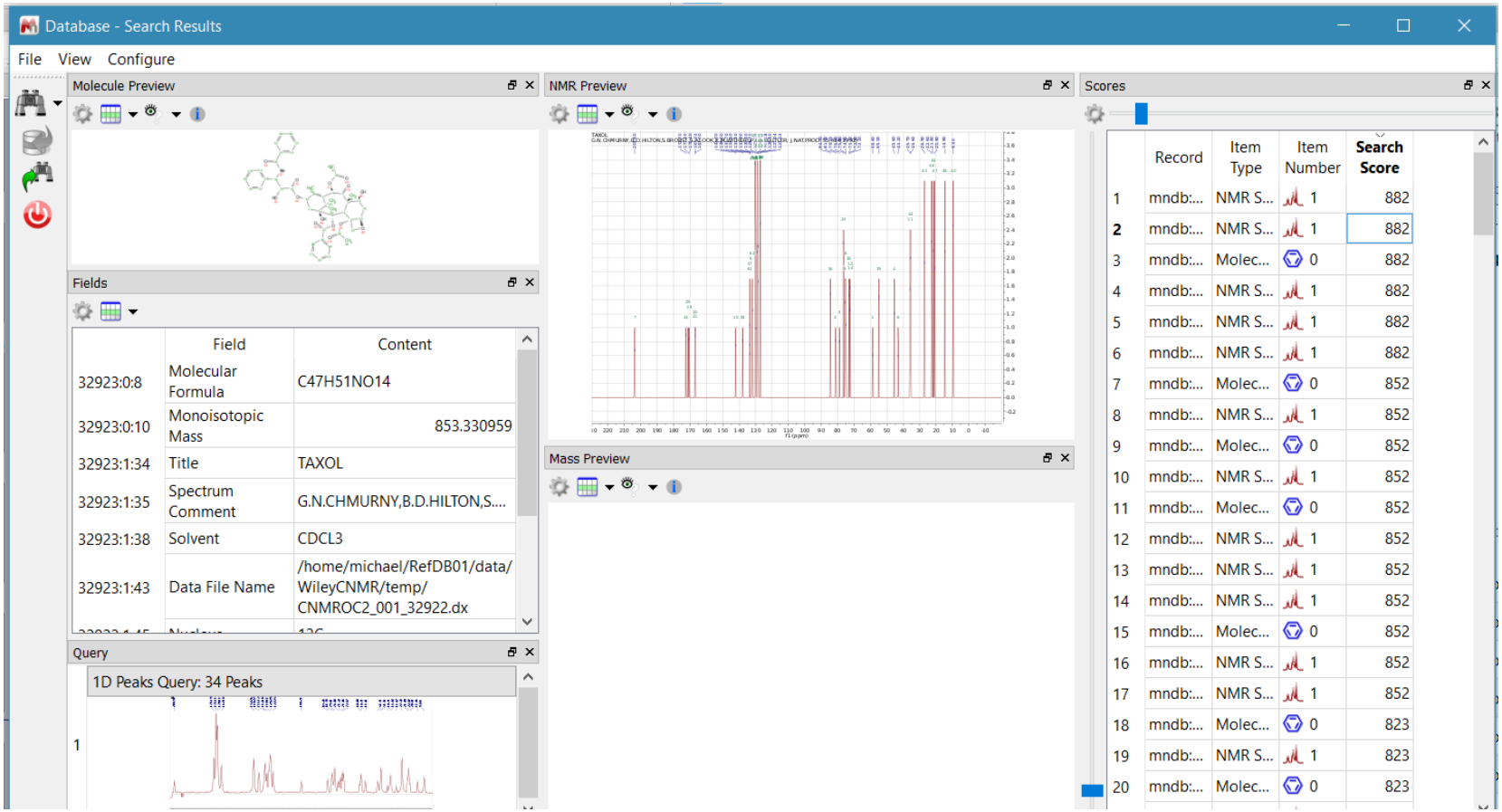






Search C-13 peaks from HMBC

- Right click on the C-13 spectrum and do a peak search again. Taxol is listed as one of the top hits again

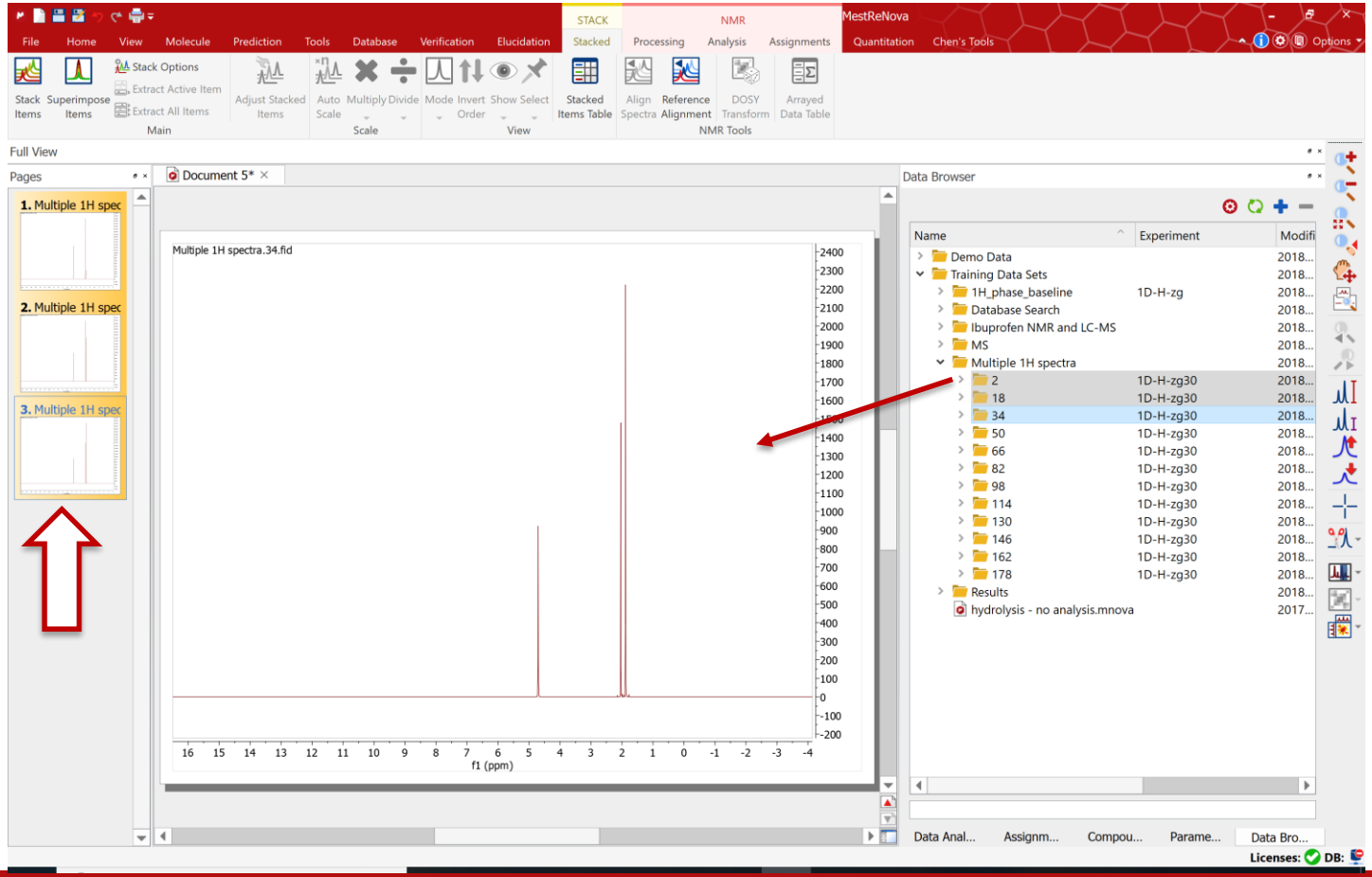


Process arrayed spectra

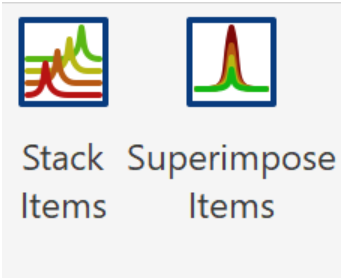
- Open and stack multiple 1D spectra
- Re-process multiple spectra
- Analyze arrayed spectra together

Stack a few spectra

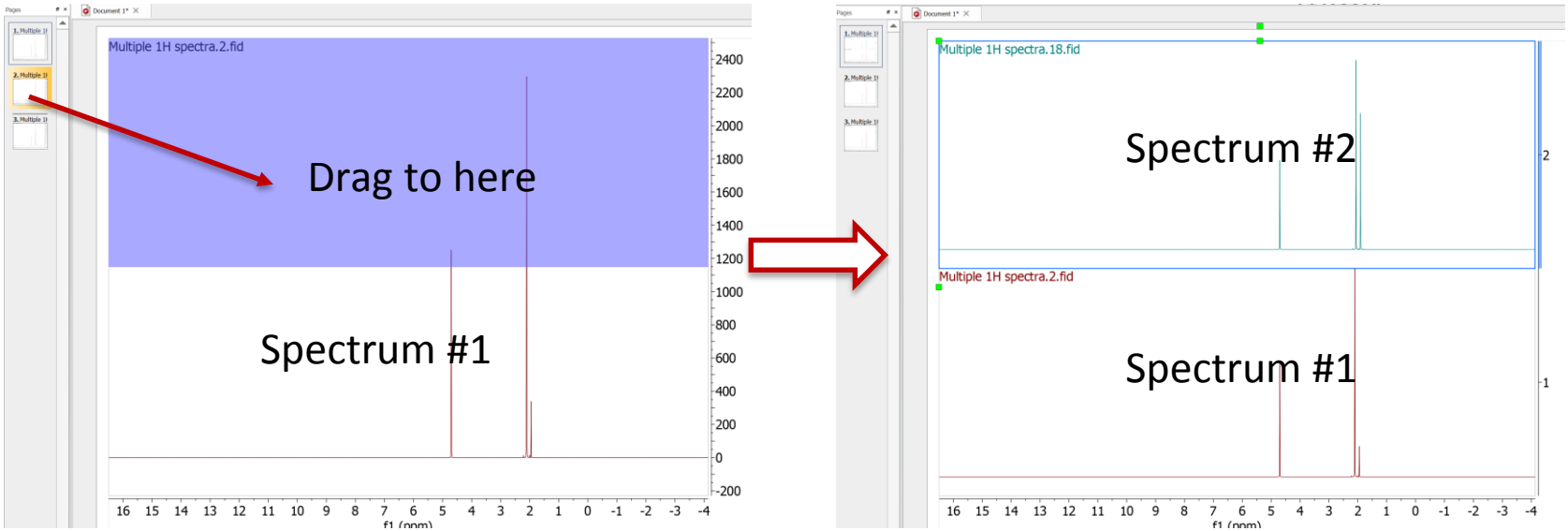
- Open the first 3 spectra from the Multiple 1H spectra folder in Data Browser
- The Stacked Ribbon is visible if you highlight multiple spectra in the Pages View



Stack a few spectra

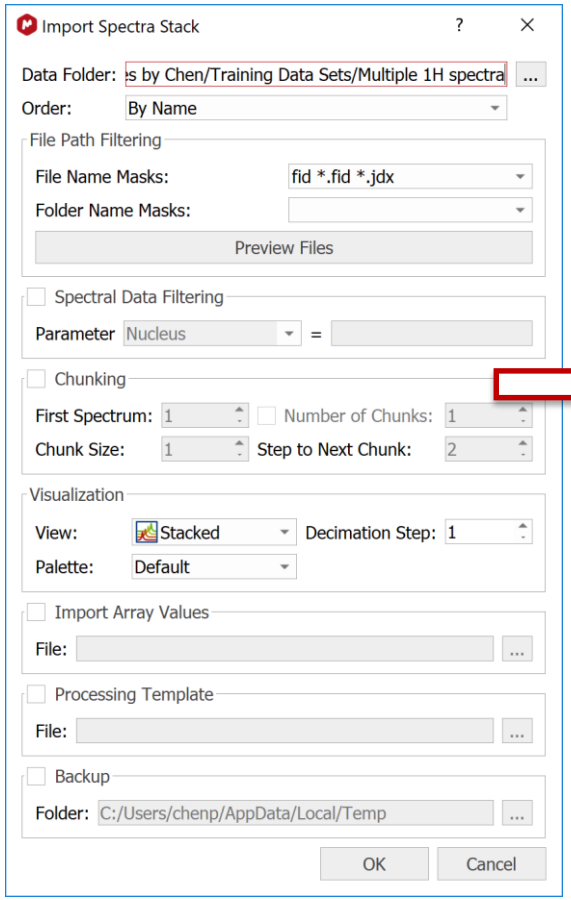


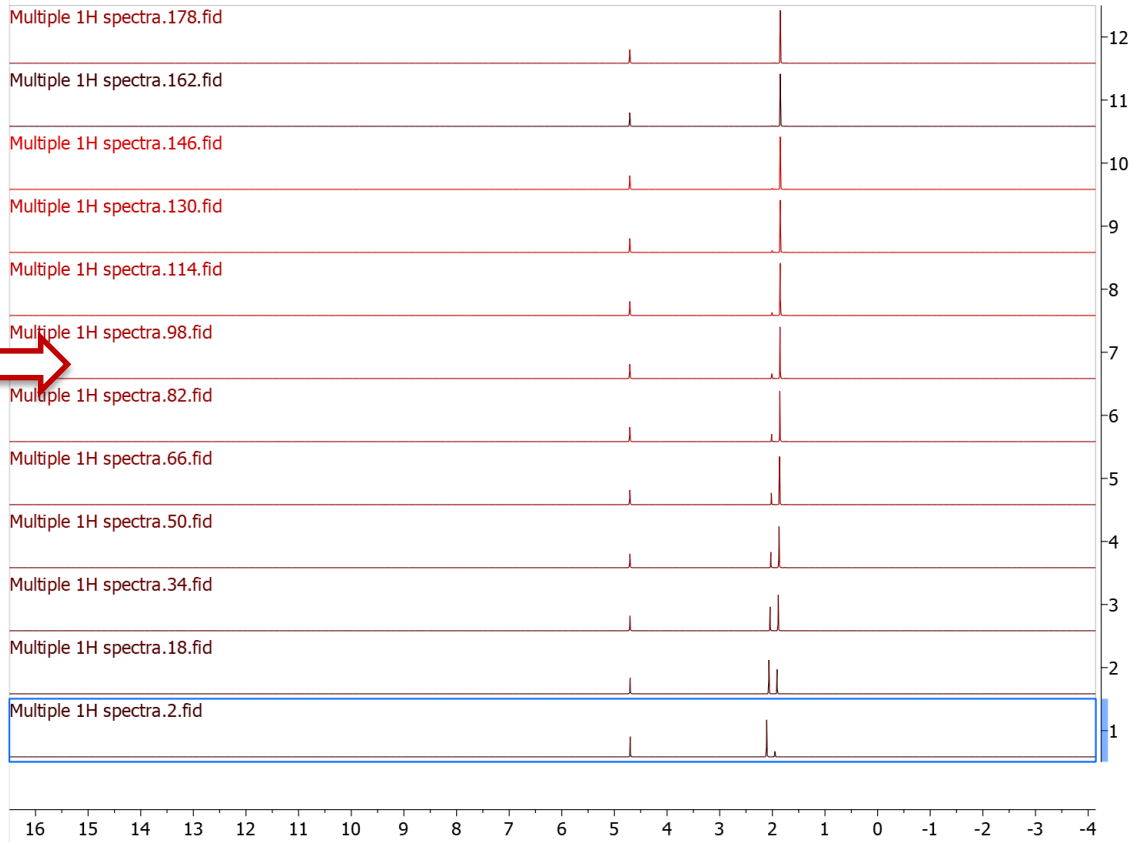
- You can use the Stack Items or Superimpose Items tools to stack or superimpose the highlighted spectra in the Pages View, or:
- Drag the thumbnail of another spectra from the Pages View to the current spectrum to stack them in desired way.
- Continue to drag the 3rd spectrum to the stack. Note you can put the spectrum to the top, middle or bottom, or to replace an existing spectrum in the stack.
- Try the different Stacking Mode, and other tools in the Stacked Ribbon



Stack many spectra

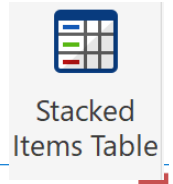
➢ Choose Tools > Loaded Scripts > Directory Spectra Stack, navigate to the directory "Multiple H-1 Spectra" in the training dataset. Click OK to import and stack all of them.

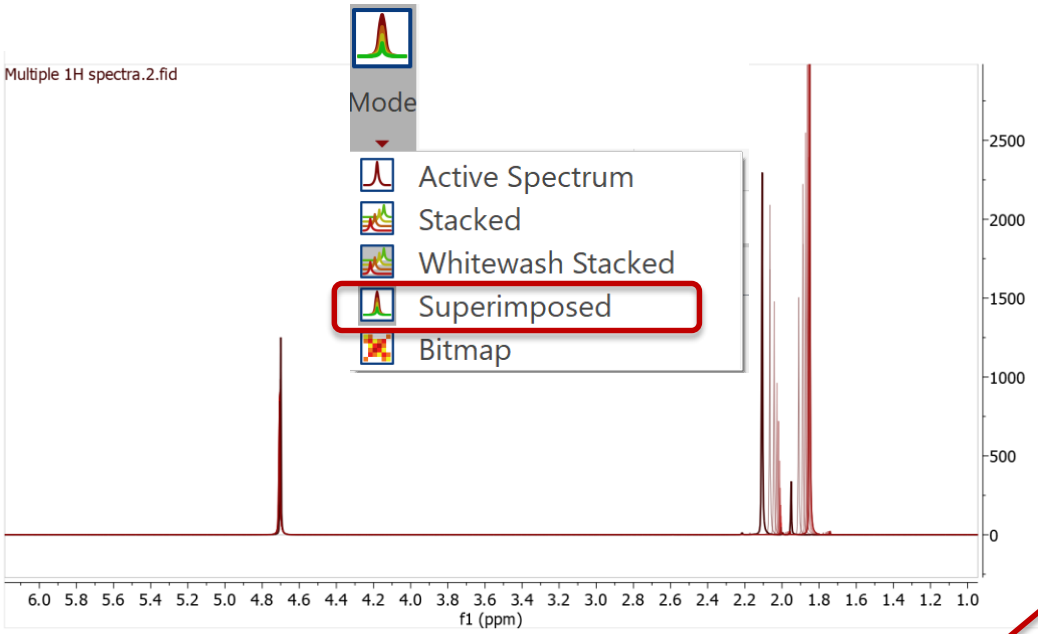


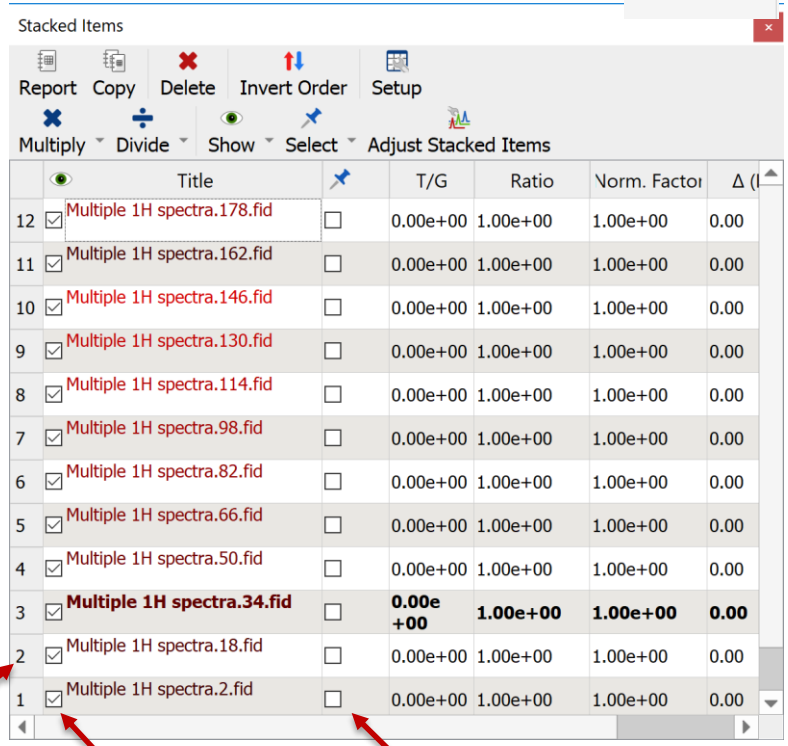


Stacking mode and Stacked Items Table

- Choose Stacked > Mode to try different display modes. Choose Superimposed mode to make sure the baseline and phasing is OK for all spectra.
- Choose Stacked > Stacked Items Table to display the Table. You can manipulate the spectra in many ways using the tools on this Table.
- If needed, you can reprocess all or selected spectra





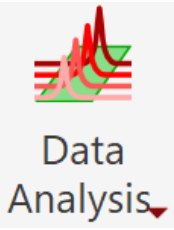


Click and drag here to change the order

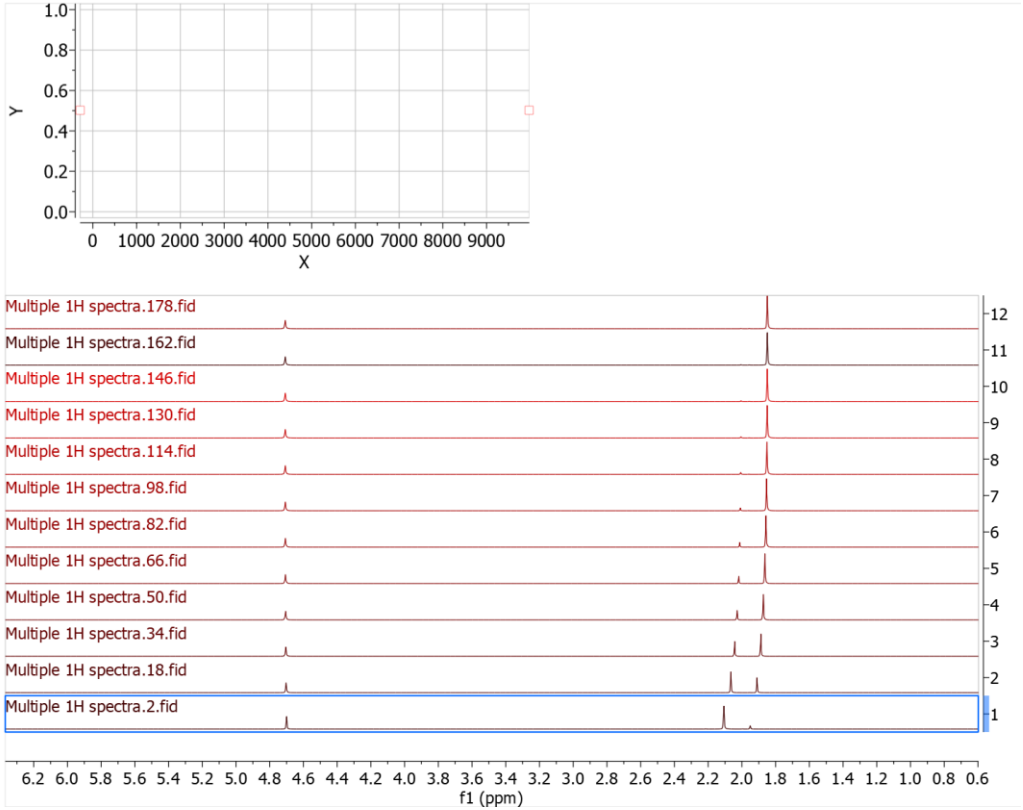
Check/uncheck these to show/hide spectra

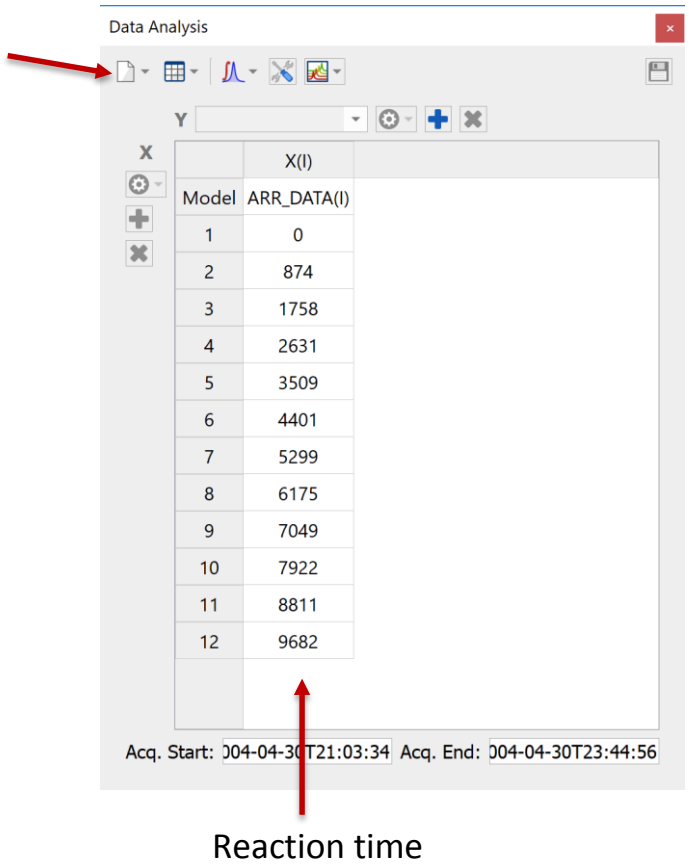
Check these to choose spectra to change

Analyze arrayed spectra

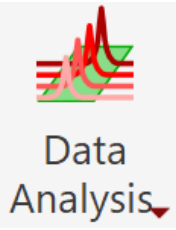


- Choose Analysis > Data Analysis > Show Table to display the Data Analysis Table.
- Click on the Empty Graph to import the X values (reaction time in this case) and display an empty XY graph.

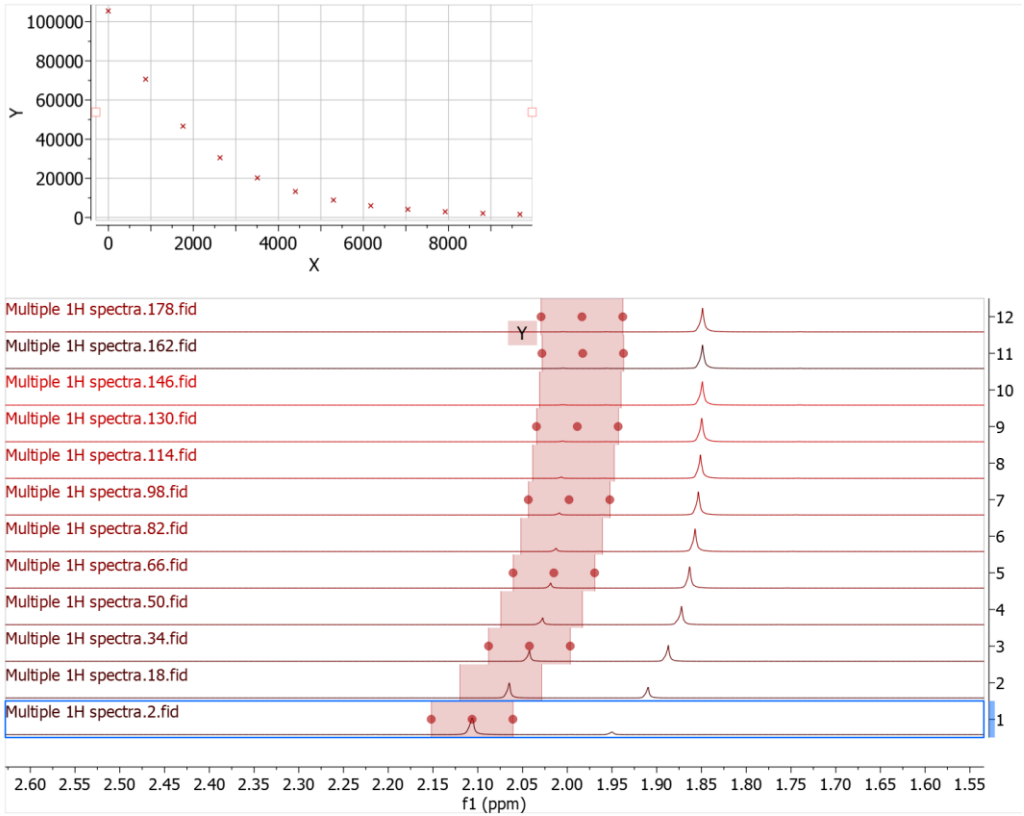


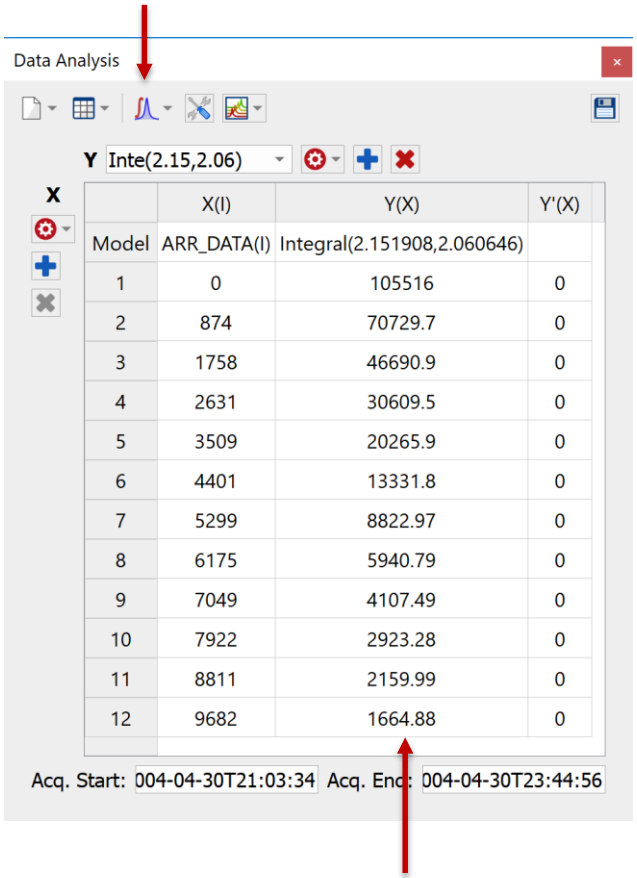


Integrate arrayed spectra



- Click the Pick Integral tool.* Click and drag on first (bottom) spectrum to define the integration range
- If needed, adjust the handlers to change the integration range for other spectra**
- The integrals are displayed on the XY graph and in the Data Analysis Table.

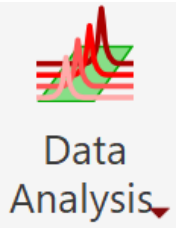




Integrals

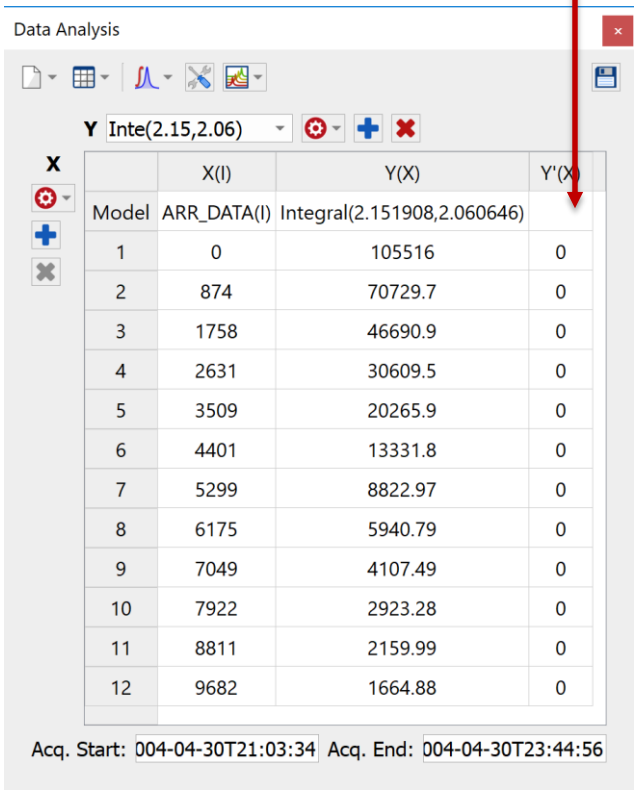
*Use Sum Method (default) for integration unless you are integrating overlapped peaks. Click Options for Integration in the Analysis Ribbon to verify. ** You can increase the # of handlers by using the Edit Model Option Tool.

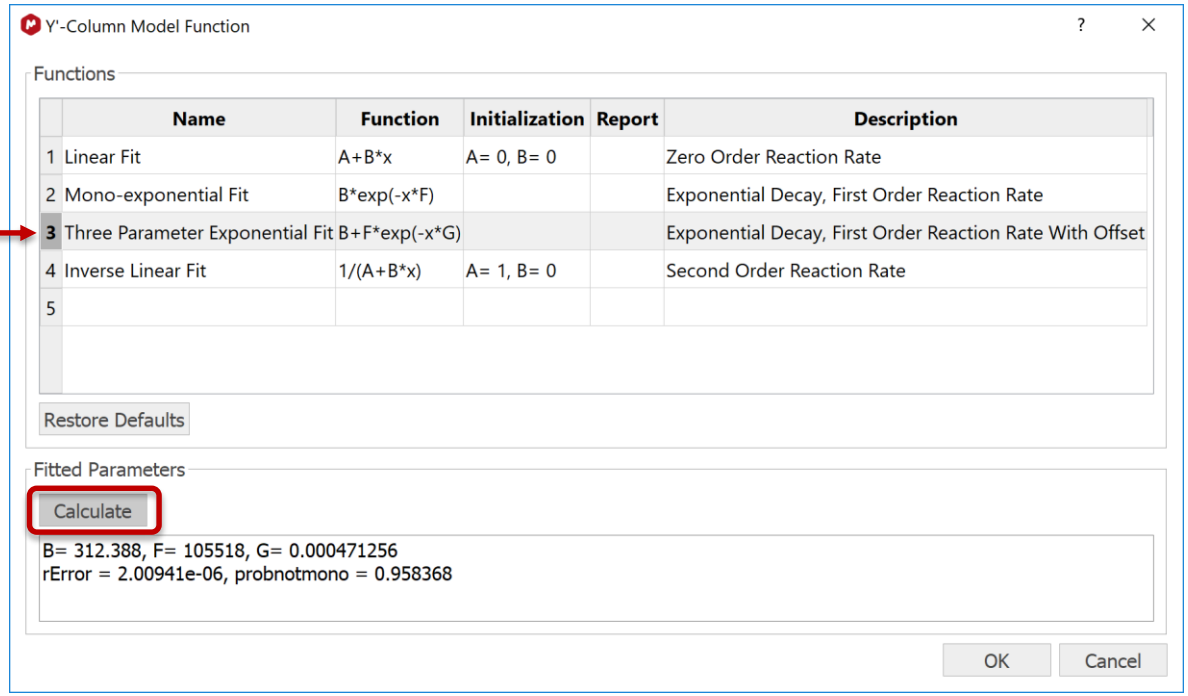
Fit XY to a function



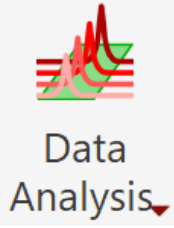
- Click the Model cell under Y'(X) in Data Analysis.
- Choose the 3rd function, and click Calculate to fit the XY values to a first order reaction (with offset)

Double click here

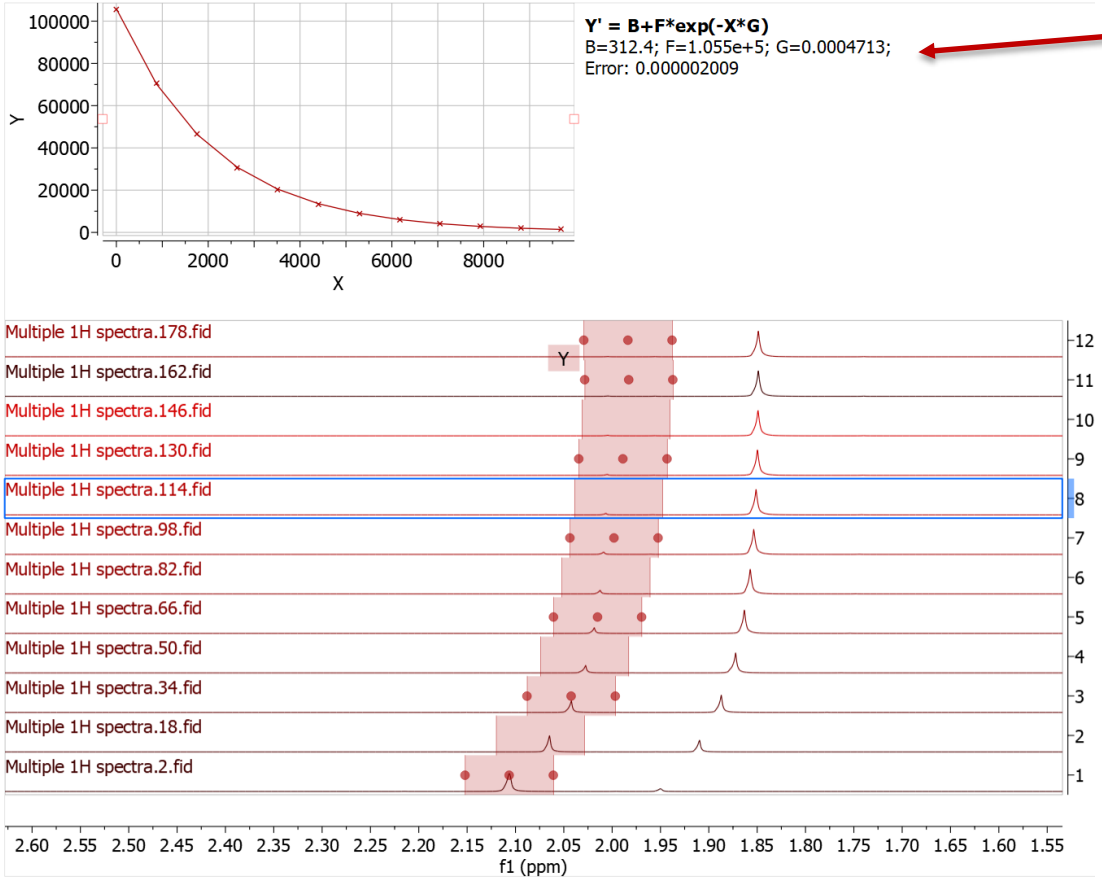


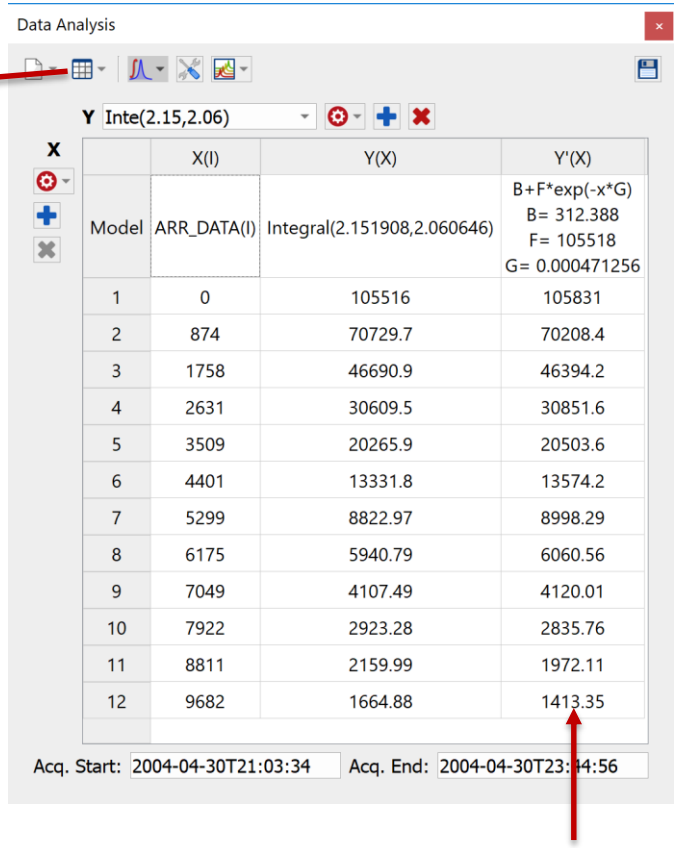


Report kinetic parameters



- Click the Report tools from the Data Analysis Panel to report the results next to the XY Graph
- Choose Report to Clipboard and paste the results to another document
- Repeat these steps for the other peaks around 1.91 ppm.





	X(I)	Y(X)	Y'(X)
Model	ARR_DATA(I)	Integral(2.151908,2.060646)	B+F*exp(-x*G) B= 312.388 F= 105518 G= 0.000471256
1	0	105516	105831
2	874	70729.7	70208.4
3	1758	46690.9	46394.2
4	2631	30609.5	30851.6
5	3509	20265.9	20503.6
6	4401	13331.8	13574.2
7	5299	8822.97	8998.29
8	6175	5940.79	6060.56
9	7049	4107.49	4120.01
10	7922	2923.28	2835.76
11	8811	2159.99	1972.11
12	9682	1664.88	1413.35

Fitting results

HELP INFORMATION

More help information

- Use the Help Facility of Mnova: Help > Contents
- Visit www.mestrelab.com for manuals, tutorials, videos and publications
- Email support@mestrelab.com for technical questions