KnowItAll® Informatics Training

NMR Processing Tools

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WILEY

Process NMR

How to Import and Process 1D NMR Files

Purpose

This exercise demonstrates how to use the KnowItAll Informatics System's ProcessIt NMR application to import and process 1D NMR files.

Objectives

This exercise will teach you:

- How to use ProcessIt NMR tools to improve spectral appearance and correct experimental artifacts
- How to create and use processing macros

Background

You can use the ProcessIt application to import raw data files from major NMR instrument vendors and processed data formats. The application can then be used to process the files and is useful for improving spectral appearance and correcting experimental artifacts.

Training Files Used in This Lesson

- C:\Users\Public\Public Documents\Wiley\KnowItAll\ Samples\NMR\Bruker TopSpin\ C14H10ClNO3\ C14H10ClNO3 H1\fid

KnowItAll Applications Used

- ProcessIt

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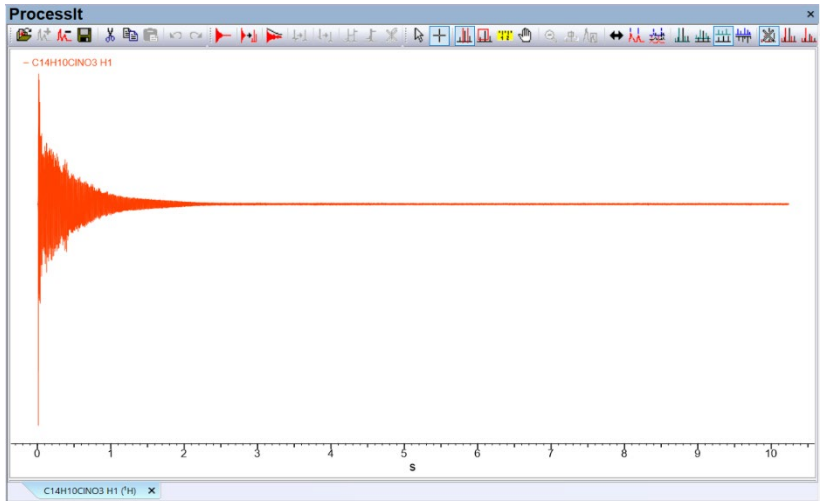
Open a raw NMR file in ProcessIt

	Action	Result
1	Open the **ProcessIt** application by clicking its icon, typically found in the **Spectral Processing** toolbox. 	
2	In **ProcessIt**, click **Open Spectroscopy Data File** button. Navigate to "**C:\Users\Public\Public Documents\Wiley\KnowItAll\ Samples\NMR\Bruker TopSpin\ C14H10ClNO3\ C14H10ClNO3 H1\fid**". **Note**: **ProcessIt** application can import many different NMR formats and nuclei, and can open either the raw (*e.g.,* fid) or processed file.	The file is displayed in the main ProcessIt window: 

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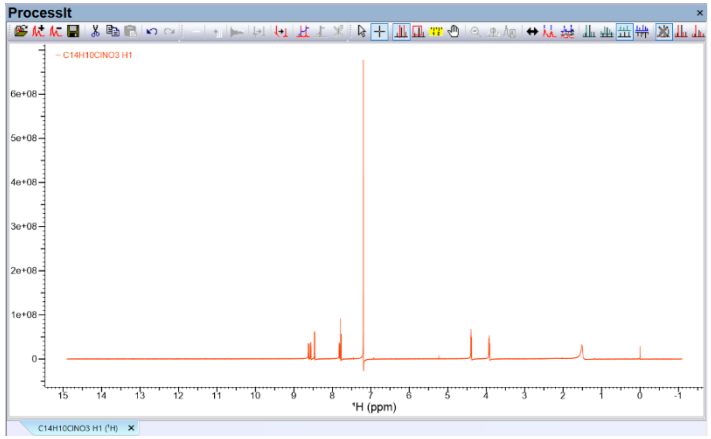
Methods to Transform the File

	Action	Result
1	**<u>Fourier Transform:</u>** If you wish to apply a **Fourier Transform (FT)** without additional preprocessing, begin here (*otherwise move to step 4*). Click the **Fourier Transform** icon on the **Process Toolbar**. 	The **Fourier Transform** dialog appears with a preview of the transformed proton spectrum behind it: 
2	**Note:** The next steps describe how to apply pre-processing to a raw file before performing the transformation. If you are ready to process your transformed file, move to the next section “Methods to Process the Spectrum”.	

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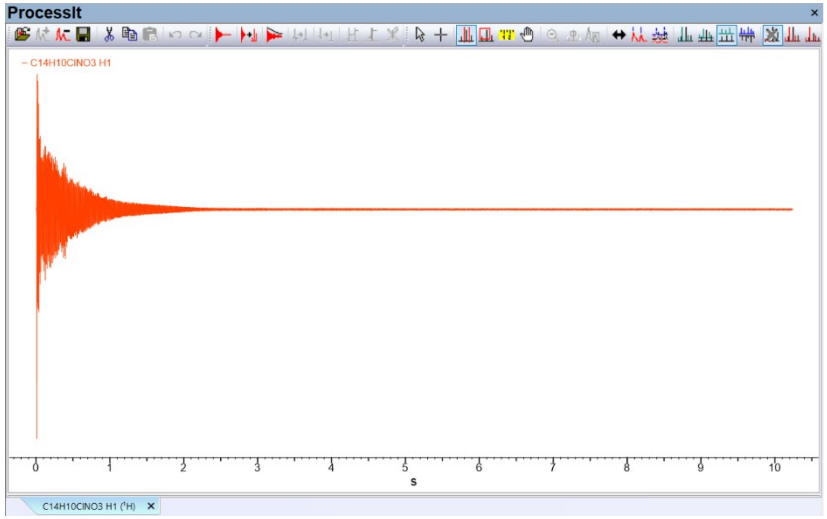
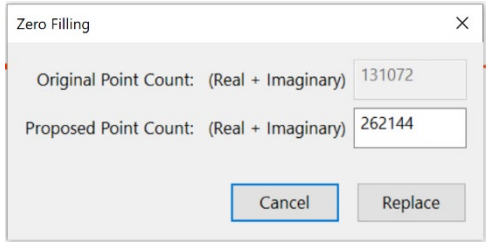
	Action	Result
3	Begin by reopening the file in Step 1. Choose **File > Open Data File** or click the **Open Spectroscopy Data File** icon on the Standard Toolbar.	The file is displayed in the main **ProcessIt** window: 
4	**<u>Zero Filling</u>:** Choose **Process > Zero Fill**, or use the **Zero Fill** icon.	The **Zero Filling** dialog box opens:  The software proposes a new number of points for the spectrum based on the original (experimental) count.

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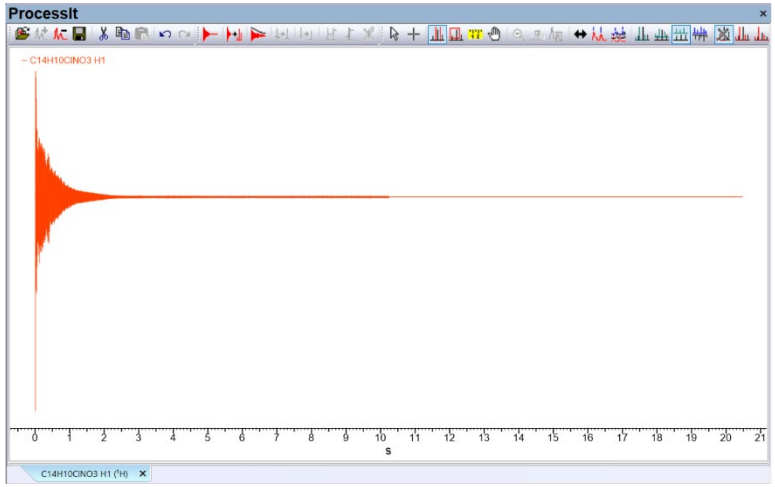
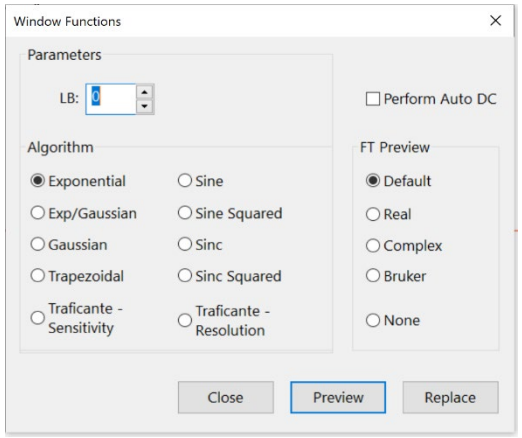
	Action	Result
5	Click **Replace**.	The software executes the **Zero Filling** command: 
6	**<u>Window Functions</u>:** Choose **Process > Window Functions**, or use **the Window Functions** icon.	The **Window Functions** dialog box opens:  **Window Functions** are used extensively to increase resolution and decrease noise in the spectrum.

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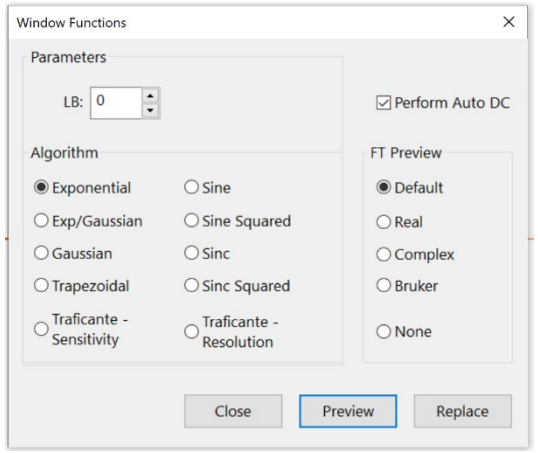
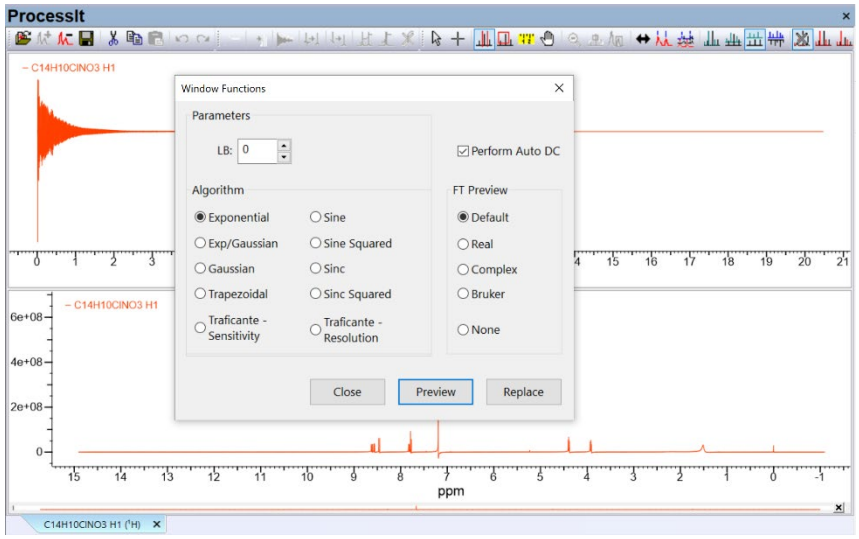
	Action	Result
7	Select **Exponential** under **Algorithm.** Check **Perform Auto DC** to apply a DC Offset correction to the spectrum. Select **Default** under **FT Preview** options to apply the default Fourier Transform algorithm to the processed spectrum. **Note**: The line broadening parameter (**LB**) can also be modified if desired.	
8	Click **Preview** to view the resulting transformed spectrum.	A preview of the transformed spectrum according to the options in the **Window Functions** dialog is displayed: 

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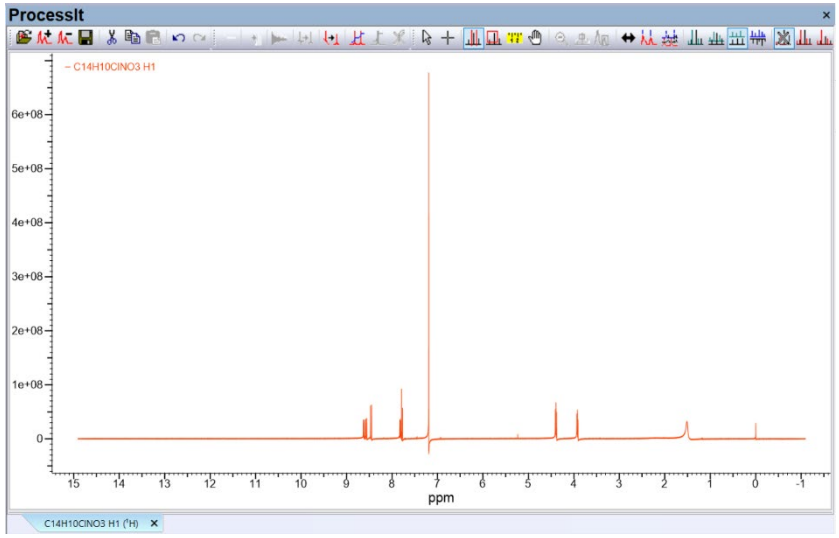
	Action	Result
9	Click **Replace** to accept the previewed Fourier Transform spectrum.	The transformed spectrum is displayed in **ProcessIt**: 

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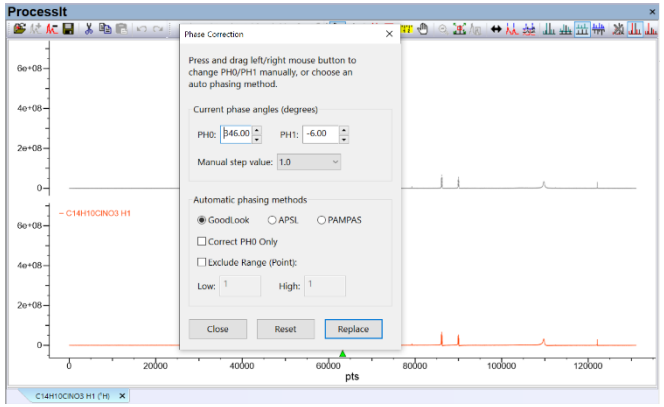
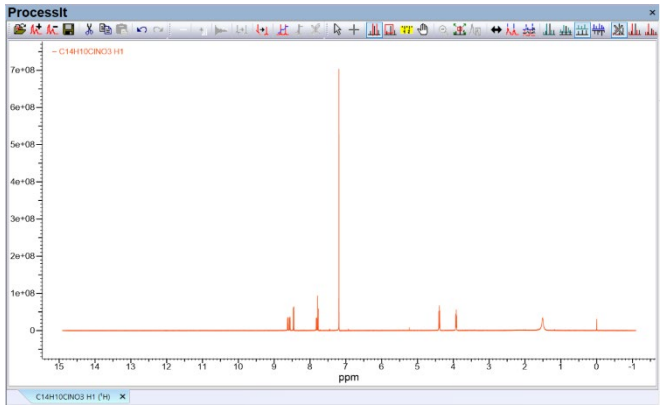
Methods to process the spectrum

	Action	Result
1	**<u>Phasing</u>:** Choose **Process > Phasing**, or select the **Auto Phase** icon. **Note**: Manual phasing can be performed by clicking and dragging with either the left (**PH0**) or right (**PH1**) mouse button.	The **Phase Correction** dialog box opens with a preview of the phased spectrum applying the phase angles in **Current phase angles (degrees)**.  Three different automatic phasing methods are available in the **Phase Correction** dialog: **GoodLook**, **APSL** (Automatic 1D Phase Correction), and **PAMPAS** (Phase Angle Measurement from Peak Area).
2	Retain **GoodLook** as the automatic phasing method. Ensure the checkbox for **Correct PH0 Only** is deselected. Click **Replace**.	The phasing for the spectrum is improved upon applying the **Phase Correction** function: 

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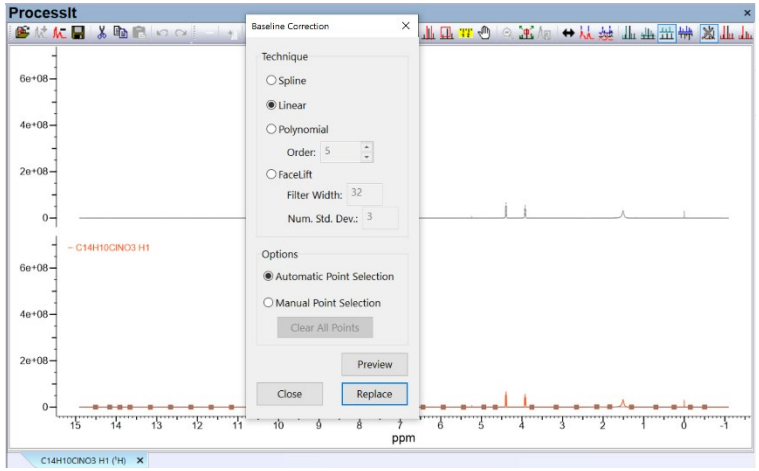
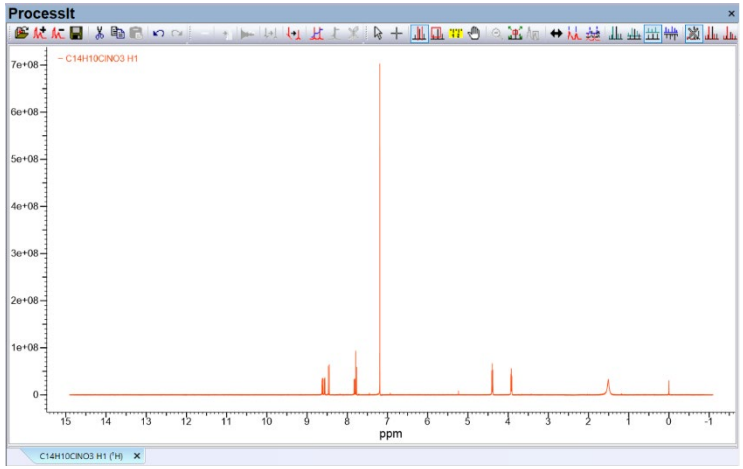
	Action	Result
3	**<u>Baseline Correction</u>:** Choose **Process > Baseline Correction**, or select the **Baseline Correction** icon. 	The **Baseline Correction** dialog box opens and a preview of the processed spectrum is displayed:  **Spline**, **linear**, **polynomial** and **FaceLift** algorithms can be applied to baseline points selected either automatically or manually. As a technique is selected, the resulting spectrum is previewed.
4	Choose **Linear** as the baseline technique. Click **Replace**.	The **Baseline Correction** function is applied: 

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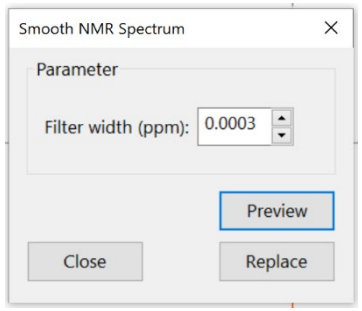
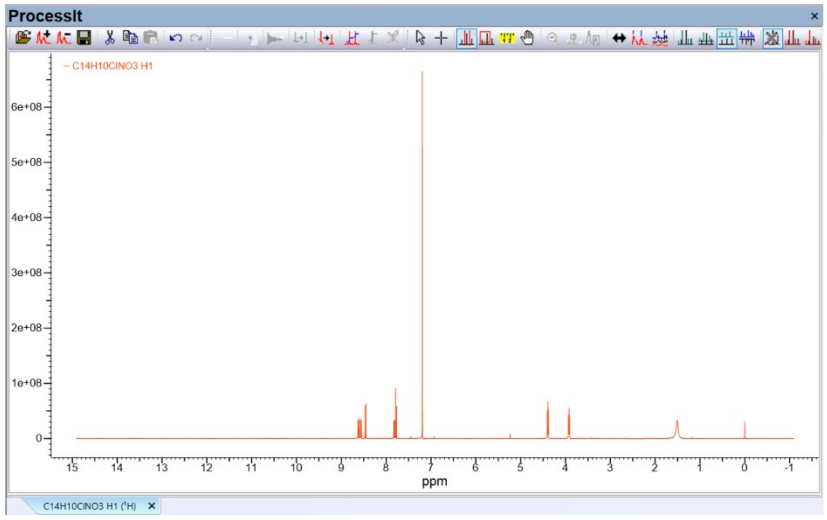
	Action	Result
5	**<u>Smoothing</u>:** Choose **Analysis > Smooth Spectrum**.	The **Smooth NMR Spectrum** popup window opens: 
6	Enter 0.0003 as value in the **Filter width (ppm)** box, then click **Replace**.	The **Smooth NMR Spectrum** popup closes and the smoothed spectrum is displayed: 

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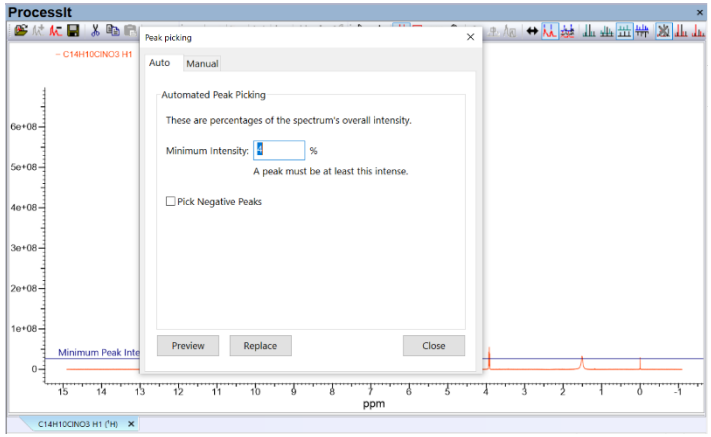
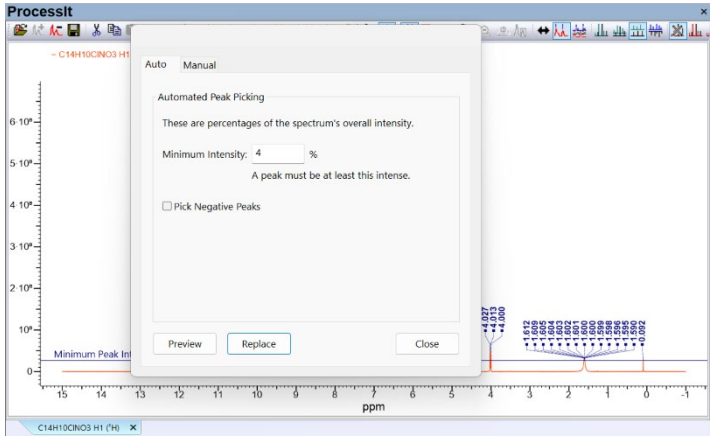
	Action	Result
7	**<u>Peak Picking</u>:** Choose **Process > Peak Picking**.	The **Peak picking** dialogue appears. On the spectrum, a preview for the peaks are displayed as vertical lines using **Automatic Peak Picking** method: 
8	Enter 4 as the value for **Minimum Intensity (%)** and select **Preview** to preview the result of the peak picking. Choose **Replace** to accept the changes.	The peaks appear on the spectrum: 

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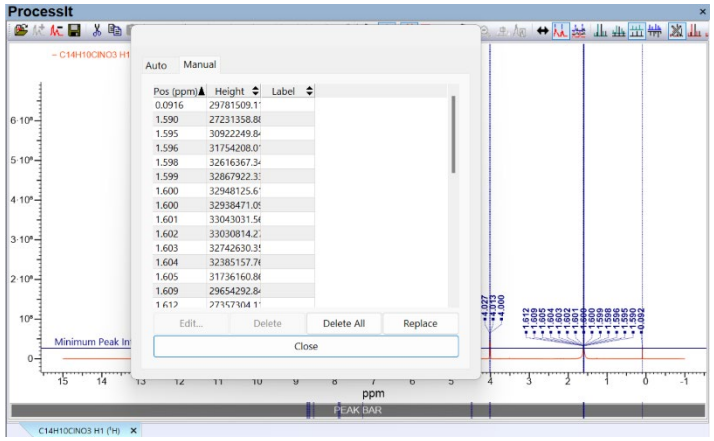
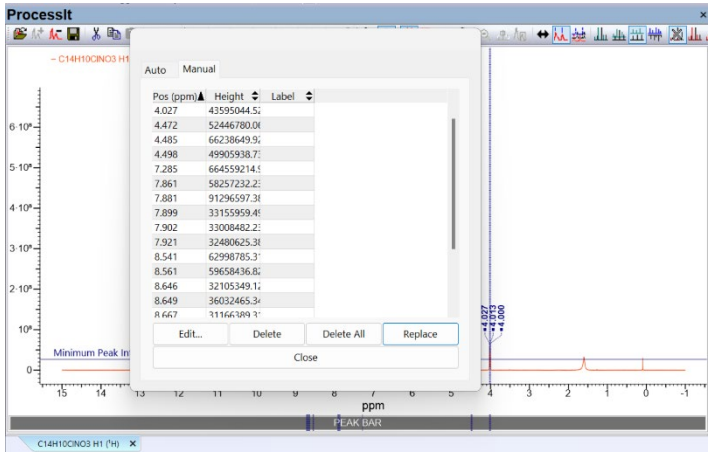
	Action	Result
9	On the **Peak picking** dialog, select the **Manual** tab.	The **Manual Peak Picking** table is visible and the **Peak Bar** is displayed on the spectrum: 
10	Click on the first row in the **Manual Peak Picking** table. Press **CTRL** on the keyboard and click each row with ppm value less than 4.00 ppm so that they become highlighted. Press **Delete** button on keyboard, followed by clicking **Replace** on the dialog. **Note**: Any row can be deleted one at a time by pressing **Delete** button on keyboard.	The peaks less than 4.00 ppm are removed from the **Manual Peak Picking** table and spectrum: 

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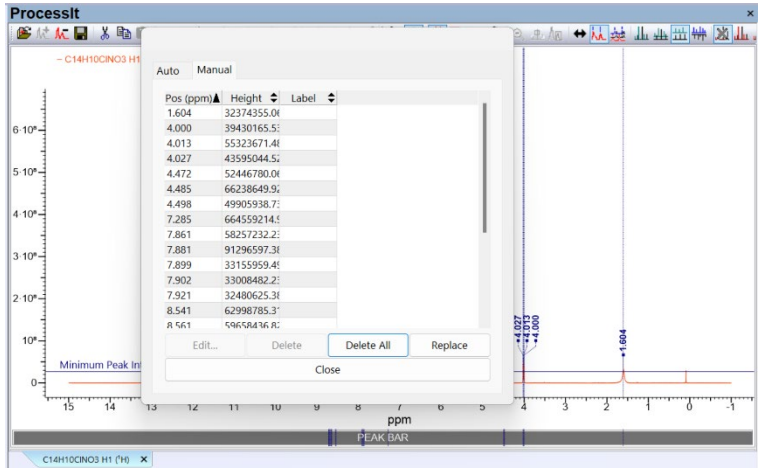
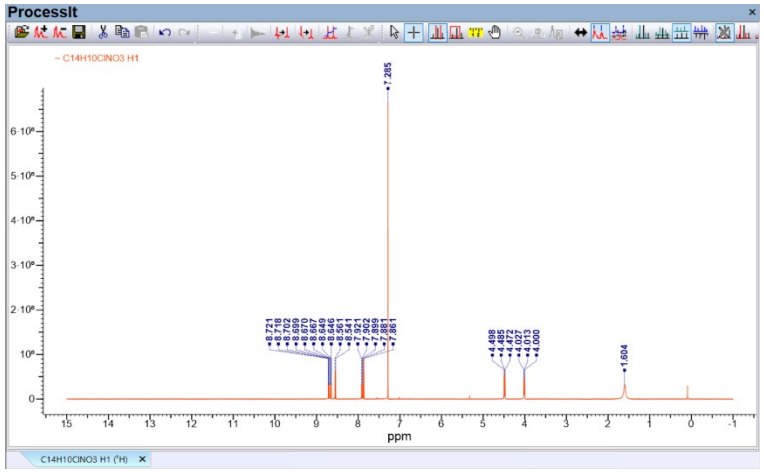
	Action	Result
11	On the spectrum, click on the **Peak Bar** at ~1.60 ppm**.** Then click **Replace** on the **Peak picking** dialog.	A new peak is added to the **Manual Peak Picking** table. A vertical line representing the new peak is displayed on the spectrum with a peak label of the corresponding ppm: 
12	Choose **Close** to close the **Peak picking** dialog.	The spectrum is displayed with peak labels for the picked peaks: 

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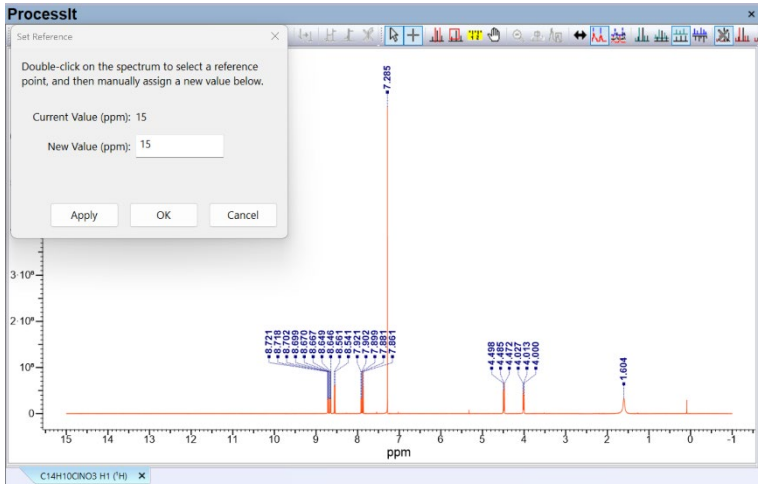
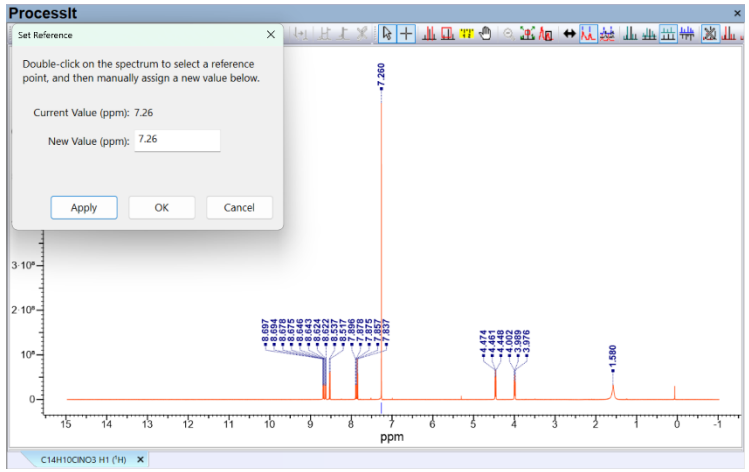
	Action	Result
13	**<u>Reference Spectrum</u>:** Use this tool to reference the spectrum to a solvent or internal standard. Choose **Analysis > Set Reference**.	The **Set Reference** popup window appears: 
14	On the spectrum, double click on the peak near 7.3 ppm to set it as the reference point. Change **New Value (ppm)** to 7.26. Click **Apply**.	Upon clicking **Apply**, the **Current Value (ppm)** becomes 7.26. On the spectrum, all of the peaks move according to the reference value. The spectrum reference point is shifted to 7.26 ppm: 

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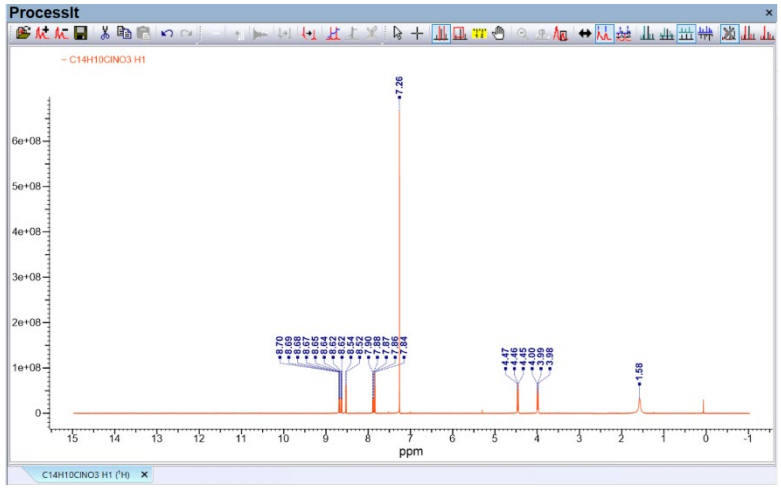
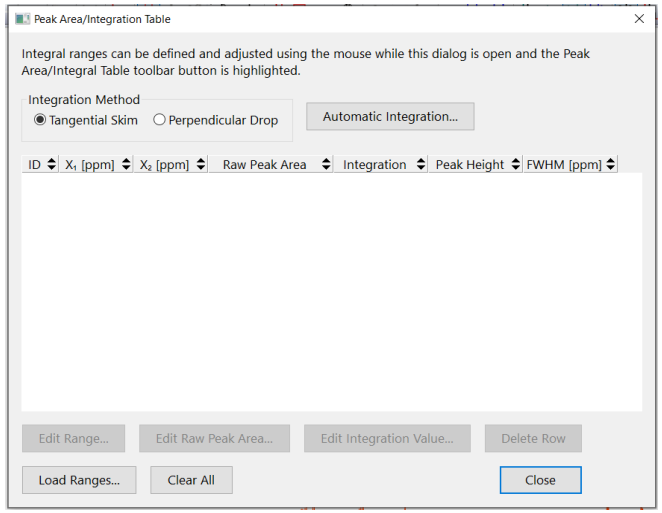
	Action	Result
15	Click **OK** to close the dialog.	The **Set Reference** dialog closes: 
16	**<u>Automatic Integration</u>:** Choose **Analysis > Peak Area/Integration**. **Note:** Two methods for automatic integration are available: **Perpendicular Drop** and **Tangential Skim**. By default, **Perpendicular Drop** is preselected, and is recommended for NMR.	The **Peak Area/Integration Table** popup appears: 

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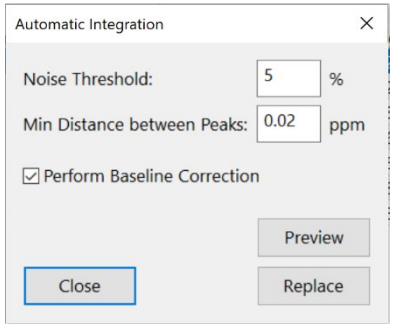
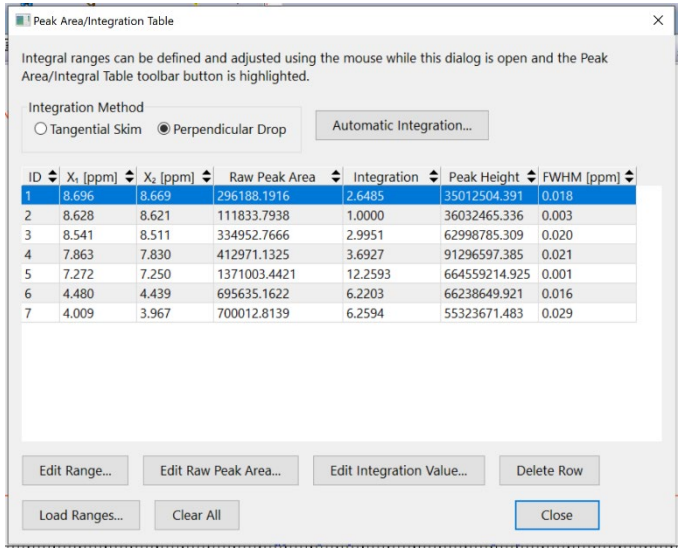
	Action	Result
17	Click **Automatic Integration**. Set the **Noise Threshold** to 5% and **Min Distance between Peaks** as 0.02 ppm. Retain checkbox selection for **Perform Baseline Correction**. The **Automatic Integration** dialog box allows you to specify **Noise Threshold** and **Minimum Distance between Peaks**, and to **Perform Baseline Correction** before determining the integration.	The **Automatic Integration** dialog opens:  If baseline correction is applied, an automatic baseline correction will be performed prior to peak integration. If it is unchecked, the raw data will be integrated.
18	Click **Preview** then **Replace**. **Note:** Any cell in the **Peak Area/Integration Table** can be modified by double clicking directly on the cell, or by selecting a row and clicking **Edit Range**, **Edit Raw Peak Area**, or **Edit Integration Value**. **Note:** Any row can be deleted by clicking **Delete Row**, or all rows can be deleted by clicking **Clear All**.	The **Peak Area/Integration Table** becomes populated with integral information: left x-value (**X_1**), right x-value (**X_2**), **Raw Peak Area**, **Integration**, **Peak Height**, and full width at half maximum (**FWHM**). The integrals are visible on the spectrum:  ID | X_1 [ppm] | X_2 [ppm] | Raw Peak Area | Integration | Peak Height | FWHM [ppm] 1 | 8.696 | 8.669 | 296188.1916 | 2.6485 | 35012504.391 | 0.018 2 | 8.628 | 8.621 | 111833.7938 | 1.0000 | 36032465.336 | 0.003 3 | 8.541 | 8.511 | 334952.7666 | 2.9951 | 62998785.309 | 0.020 4 | 7.863 | 7.830 | 412971.1325 | 3.6927 | 91296597.385 | 0.021 5 | 7.272 | 7.250 | 1371003.4421 | 12.2593 | 664559214.925 | 0.001 6 | 4.480 | 4.439 | 695635.1622 | 6.2203 | 66238649.921 | 0.016 7 | 4.009 | 3.967 | 700012.8139 | 6.2594 | 55323671.483 | 0.029

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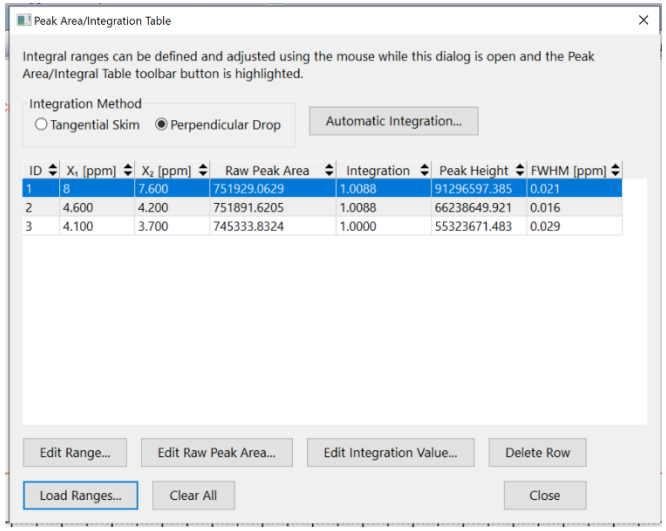
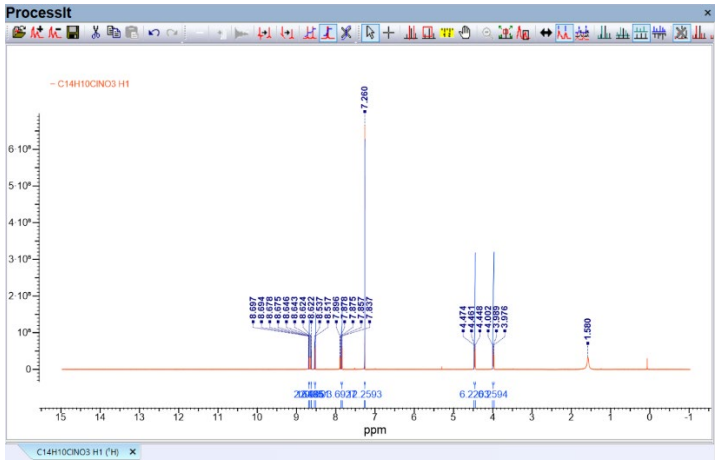
	Action	Result
19	**Note:** Integral ranges can be preloaded into the **Peak Area/Integration Table** dialog. To use this feature, click **Load Ranges** and import a CSV file that has the format X1, X2. For example: X1,X2 8.0,7.6 4.6,4.2 4.1,3.7	The integrals are calculated for the imported regions: 
20	Click **Close** to exit the **Peak Area/Integration Table** dialog.	The **Peak Area/Integration Table** dialog is closed: 

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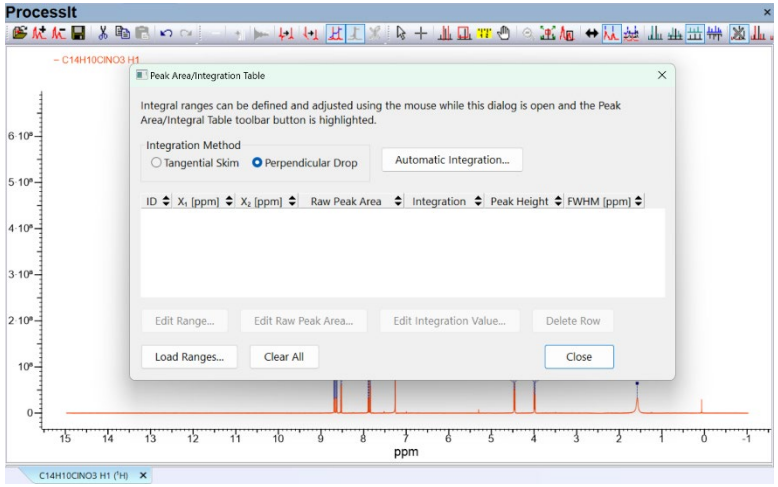
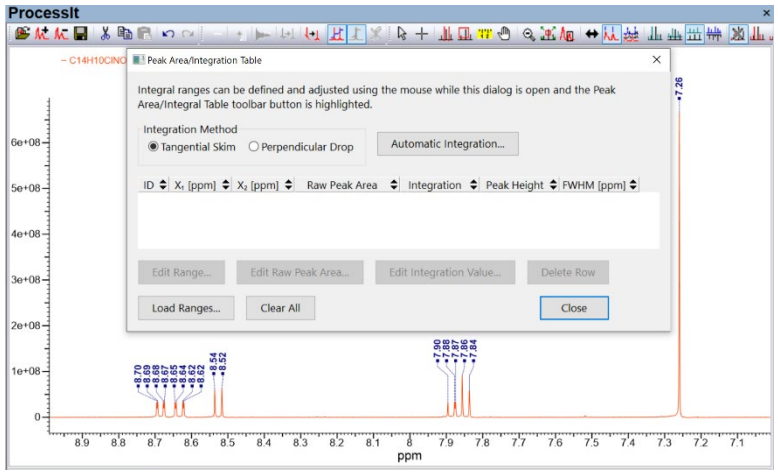
	Action	Result
21	**<u>Manual Integration</u>:** Choose **Analysis > Peak Area/Integration**. To clear any previous integrals, click **Clear All**.	The **Peak Area/Integration Table** popup launches. The integrals are cleared from the table and spectrum: 
22	Move or resize the **Peak/Area Integration Table** popup so that the peaks on the spectrum become visible. Right click on the spectrum and select **Horizontal Zoom Mode**. Click and drag the mouse cursor from ~ 7 to 9 ppm. Let go of the mouse cursor.	The mouse cursor changes to a zoom tool ( ) and the spectrum is focused on the region from ~ 7 to 9 ppm. 

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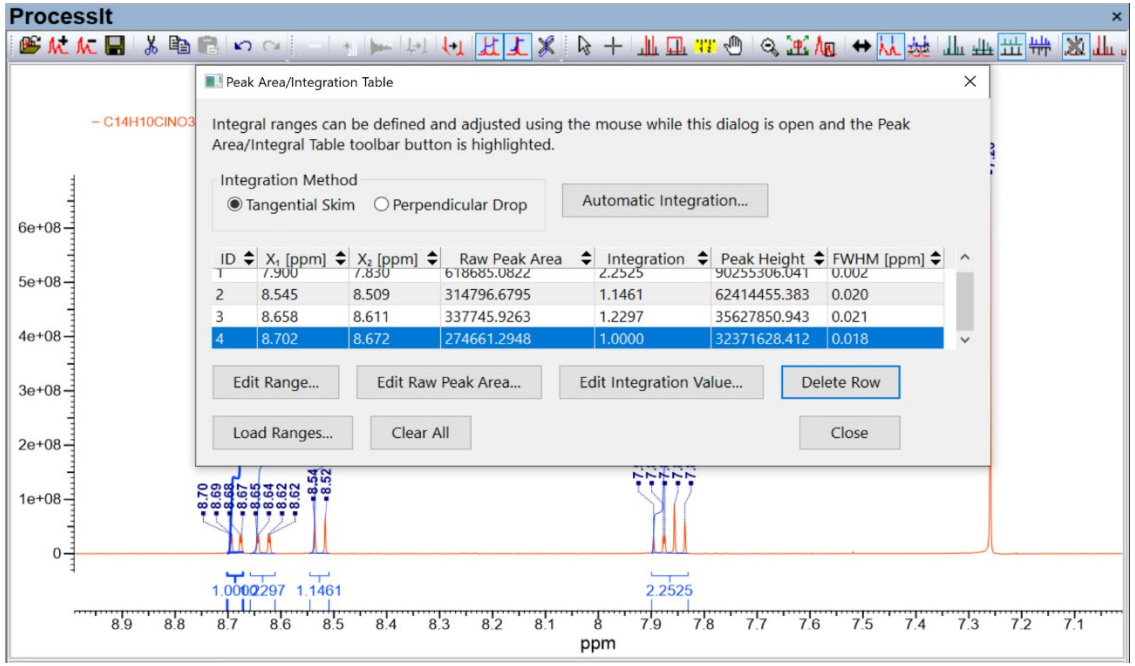
	Action	Result
23	Select the **Peak Area/Integration Table** icon located on the **Process** toolbar to reactivate the integration tool.	Clicking the **Peak Area/Integration Table** icon changes the mouse cursor from the zoom tool ( ) to the integration tool ( ):
24	Click and drag the left mouse button across a multiplet. Release the mouse button at the end of the multiplet. Repeat for each multiplet in visible in the displayed region. The multiplets are: (1) 8.67 – 8.70 ppm, (2) 8.62 – 8.65 ppm, (3) 8.51 – 8.54, (4) 7.83 – 7.91 ppm. **Note:** Dragging functionality is bidirectional (left to right, or right to left). **Note:** Do not integrate the peak at 7.26 ppm which represents the ^{1}H-NMR solvent signal.	The integration value for each multiplet is added as a new row in the **Peak Area/Integration Table**. The integral also display on the spectrum.  By default, the integral values are normalized to 1.0 for the smallest integral. To change the normalization to a different value (*e.g.,* to 2.0), select a row in the table and click **Edit Integration Value**. Change the value in the proceeding **Integration Value** popup and click **OK**. The other integrals will adjust accordingly.

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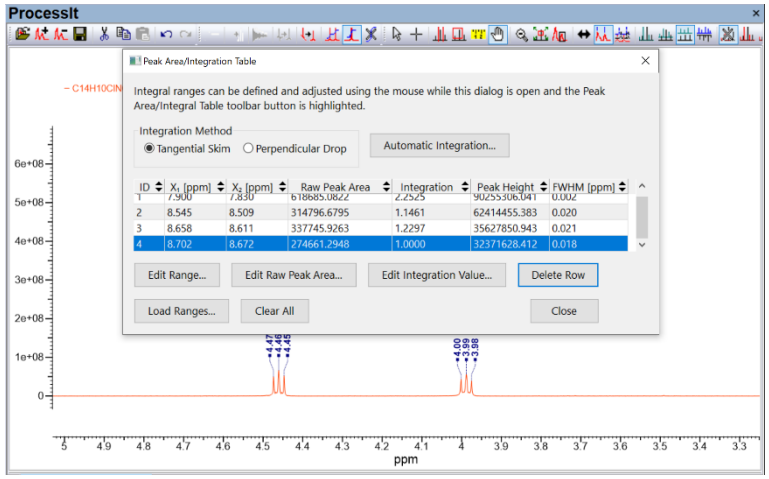
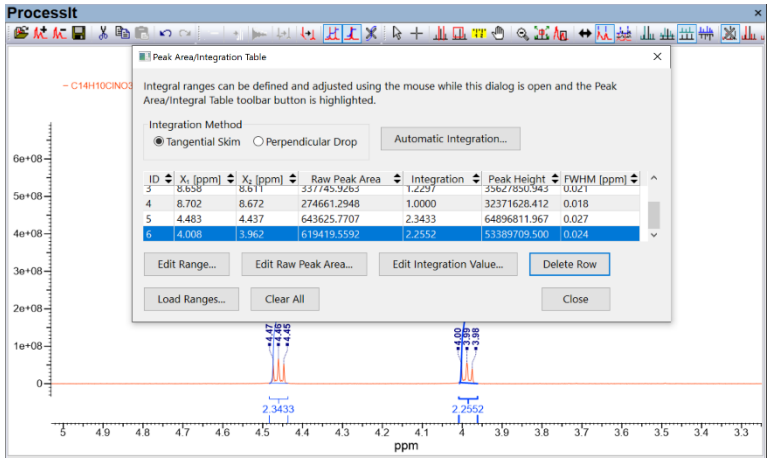
	Action	Result
25	Right click on the spectrum and click **pan mode.** Click and hold the left mouse button. Drag the spectrum to the left to move the spectrum to a lower ppm region. Release the mouse button when more multiplets are visible (~3.9 ppm to ~4.5 ppm).	Upon selecting **pan mode**, the corresponding mouse cursor is displayed ( ) which allows the spectrum to move to the right or left. 
26	To deactivate pan mode and activate the integration tool, reselect the **Peak Area/Integration Table** icon located on the **Process** toolbar. Integrate the two multiplets in this region by dragging the mouse button across each multiplet.	Selecting the **Peak Area/Integration Table** icon changes the mouse cursor back to the integration tool ( ),changing the functionality of the mouse cursor. Two new integrals are added to the spectrum: 

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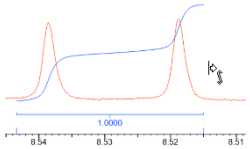
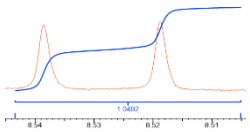
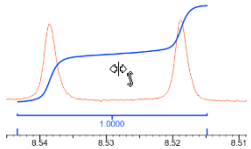
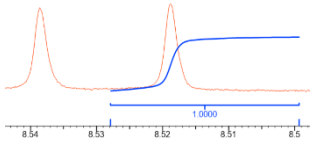
	Action	Result
27	**Note:** On the spectrum, any integral can be resized. To resize an integral, click on the edge of the integral region and drag its edge to left or right along the x-axis.	When the mouse cursor is positioned on the edge of the integral region, the cursor changes from the integral cursor ( ) to display an arrow pointing right ( ) or left ( ). The example integral below becomes wider upon dragging the integral ege to the right: Before dragging right integral edge:  After dragging integral edge to the right: 
28	**Note:** On the spectrum, any integral can be shifted. To relocate an integral, click inside of the integral region and drag it to the left or right on the x-axis.	When the mouse cursor is positioned inside the integral region, the cursor changes from the integral cursor ( ) to display two arrows pointing in either direction ( ). The example integral below moves by clicking and dragging the integral center to the right: Before dragging from integral center:  After dragging to the right from integral center: 

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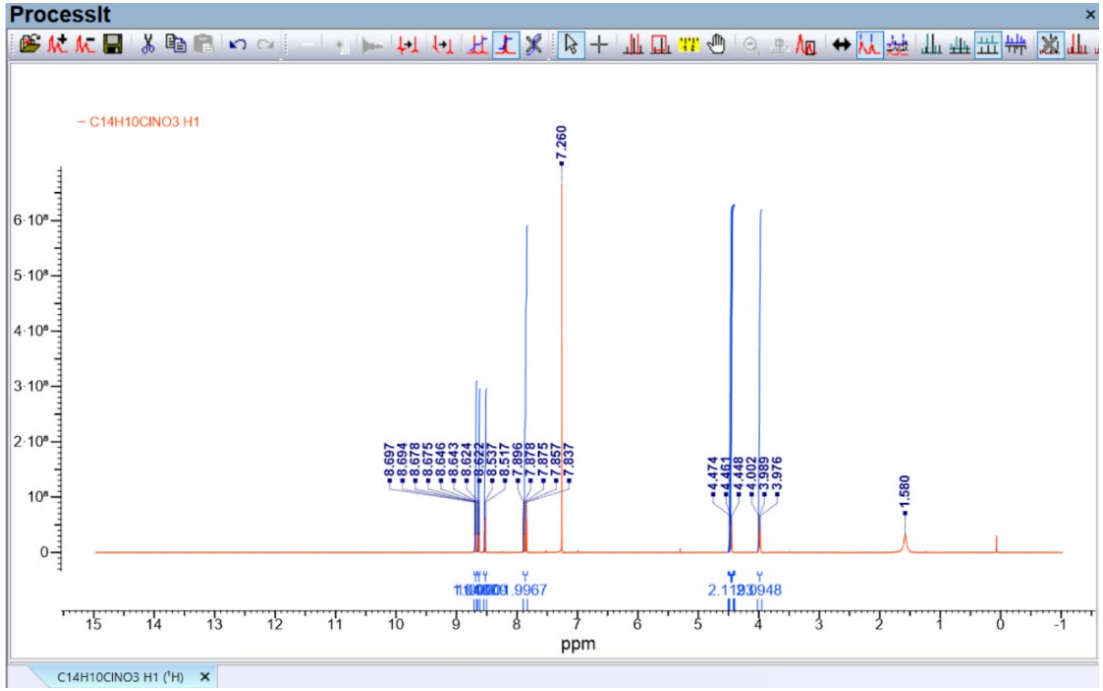
	Action	Result
29	Click **Close** on the **Peak Area/Integration Table** when the integration is complete. To zoom back out, right click and click **View Entire Spectrum**.	The entire spectrum is displayed with integrals: 

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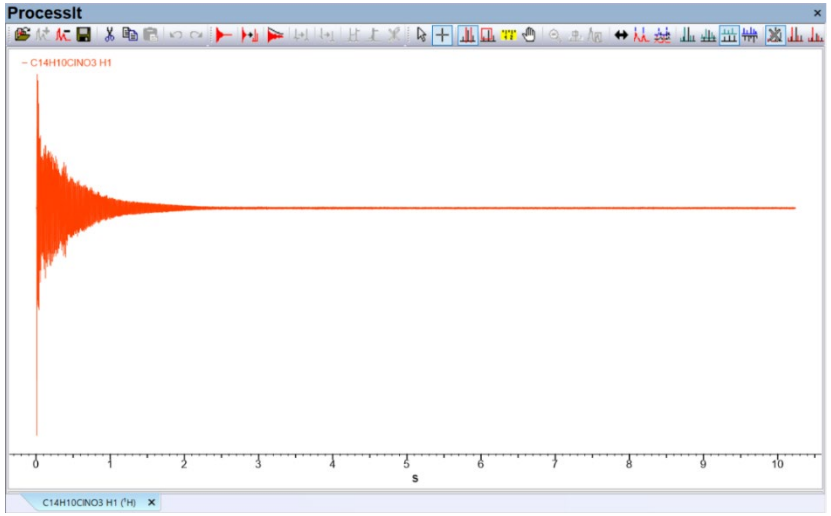
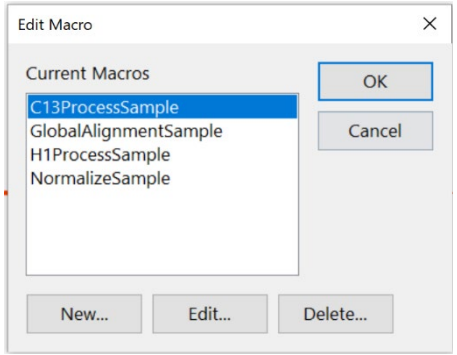
Apply a macro

	Action	Result
1	In **ProcessIt**, click **Open Data File** icon. Navigate to “C:\Users\Public\Public Documents\Wiley\KnowItAll\ Samples\NMR\Bruker TopSpin\ C14H10ClNO3\ C14H10ClNO3 H1\fid”.	The file is displayed in the main **ProcessIt** window: 
2	Choose **Macro > New/Edit**.	The **Edit Macro** dialog box opens: 

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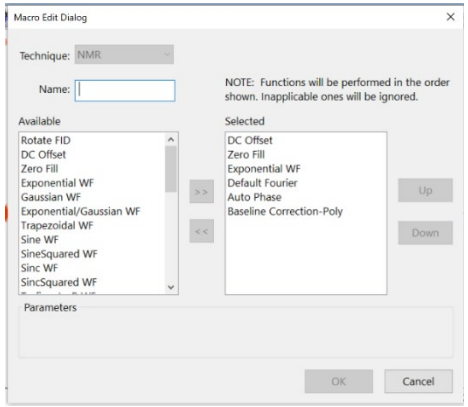
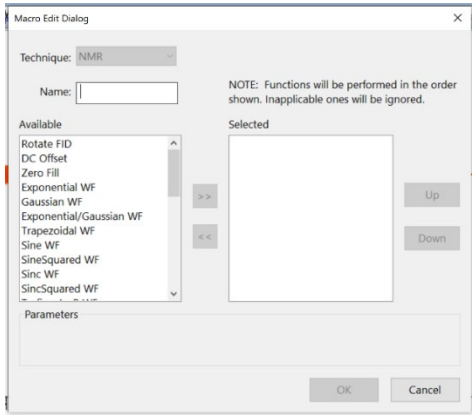
	Action	Result
3	Select **H1ProcessSample** in the list of **Current Macros**, then click **New**.	The **Macro Edit Dialog** opens:  The steps that are part of the **H1ProcessSample** macro are displayed on the under **Selected** functions. The remaining available functions are listed under **Available** functions.
4	**Note**: A new macro can be created by using the arrows to remove **(<<)** or add **(>>)** items between the two lists of functions in the **Macro Edit Dialog**. Remove all of the functions from the **Selected** functions by selecting the item and clicking the left arrow button (**<<**).	The **Selected** functions for the **Macro Edit Dialog** is emptied: 

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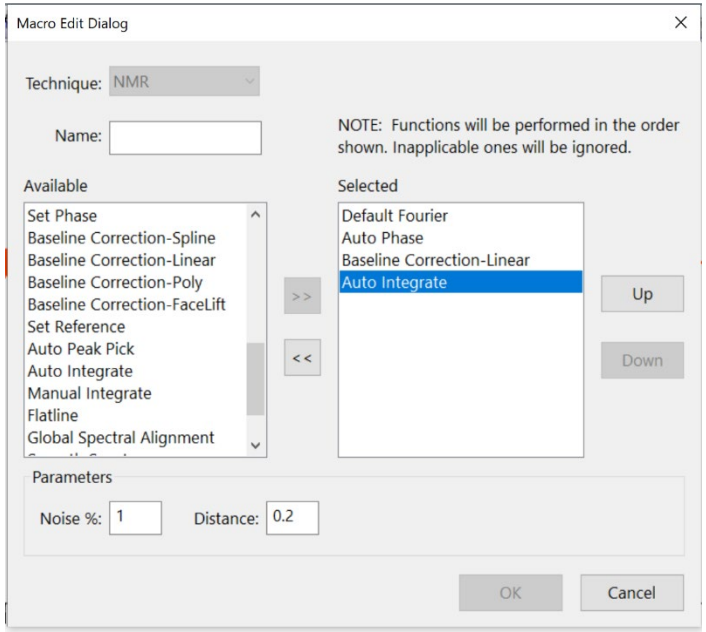
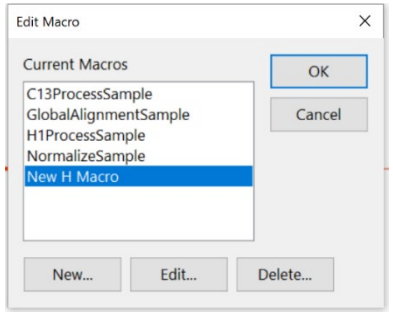
	Action	Result
5	Move the following functions from **Available** to **Selected** using the right arrow button (**>>**): **Default Fourier**, **Auto Phase**, **Baseline Correction-Linear**, and **Auto Integrate**. Multiple functions can be chosen by holding the **CTRL (**control) button down and selecting them, For the **Auto Phase** function, retain the default parameters (Method = GoodLook). For the **Auto Integrate** function, change the **Noise %** value to 1. **Note:** The macro will execute the functions in the order they are displayed in the **Selected** section in the Macro Edit Dialog. Items can be reordered using the **Up** and **Down** buttons.	The added functions are displayed in order under **Selected** functions section in the **Macro Edit Dialog**. 
6	In the **Name** box in the **Macro Edit Dialog**, write “New H Macro”. Click **OK**.	The **Macro Edit Dialog** is closed. “New H Macro” appears on the **Edit Macro** dialog: 

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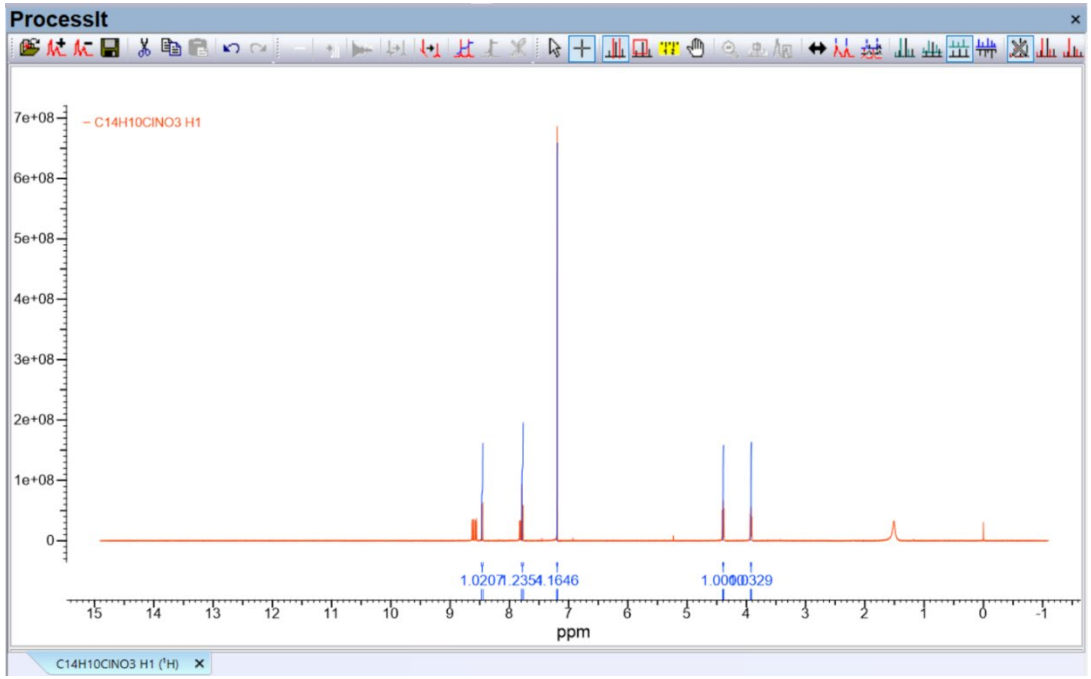
	Action	Result
7	Click **OK** on the **Edit Macro** dialog to close the window. Choose **Macro > New H Macro**. **Note**: To view the Integral values, you may have to select **View > Integral > Values** and **View > Integral > Curves.**	The spectrum is processed according to the steps in the applied macro “New H Macro”: 

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