

KnowItAll Software Training

LC-MS Databasing using KnowItAll ProcessIt and Minelt

Manual LC-MS Analysis

How to Use KnowItAll ProcessIt to Build User Databases for LC-MS

Purpose

These exercises demonstrate how to use KnowItAll ProcessIt to manually analyze and extract MS spectra from LC-MS data.

Objectives

These exercises will teach you:

- How to use KnowItAll ProcessIt for LC-MS data.
 - How to store LC-MS data in Minelt using user databases.
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Background

The KnowItAll ProcessIt software displays the LC-MS data and allows a user to review and extract their MS scans. Selected scans can be transferred into a Minelt user database for future review and searching.



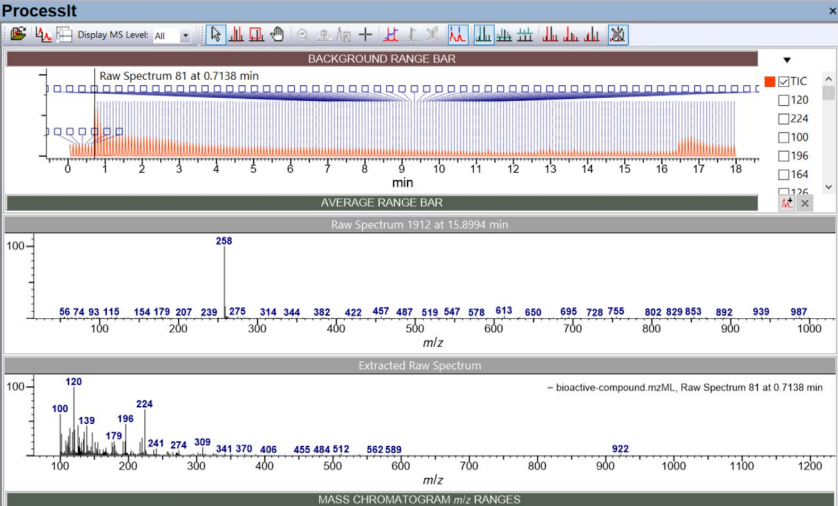
Training Files Used in This Lesson


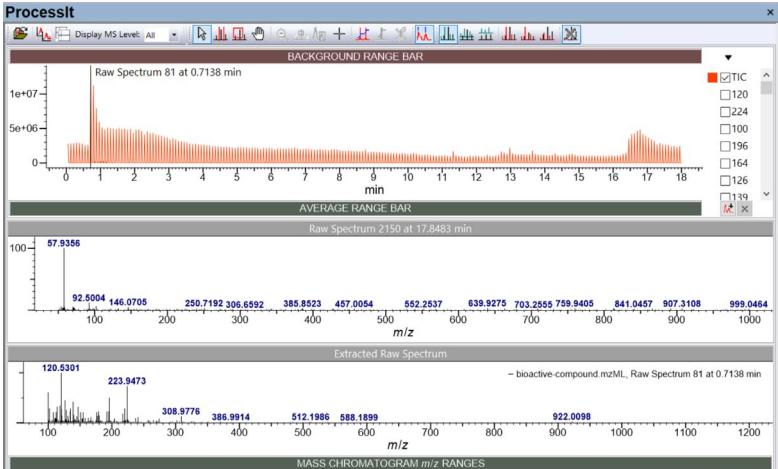
- C:\Users\Public\Documents\Wiley\KnowItAll\Samples\LC-MS\bioactive-compound.mzML

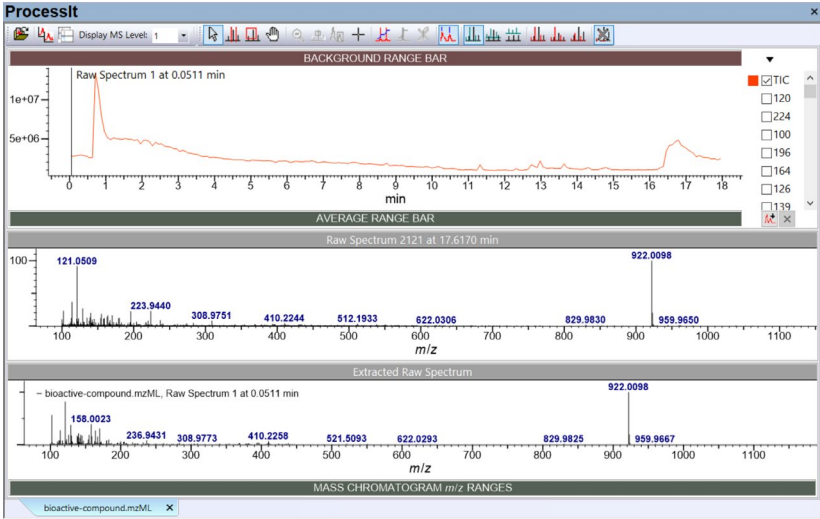
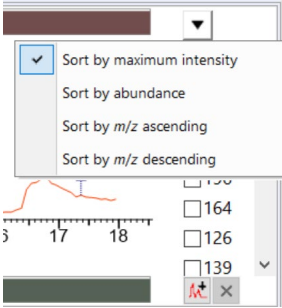
KnowItAll Applications Used


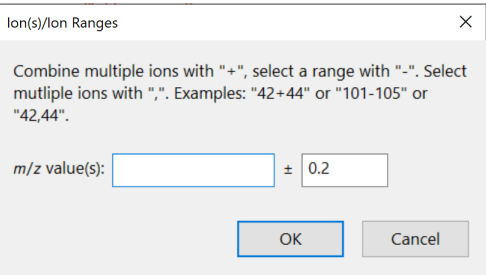
- KnowItAll ProcessIt
- KnowItAll Minelt

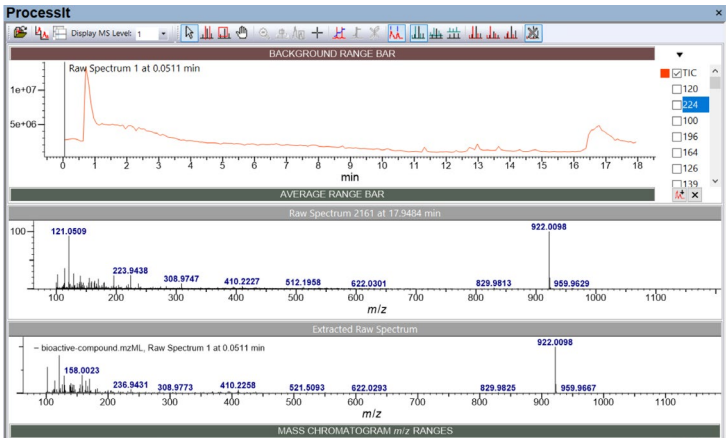
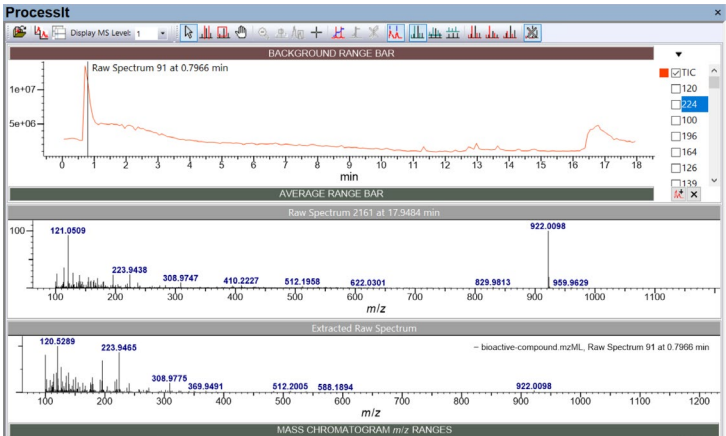
Example: Reviewing MS1 data in ProcessIt

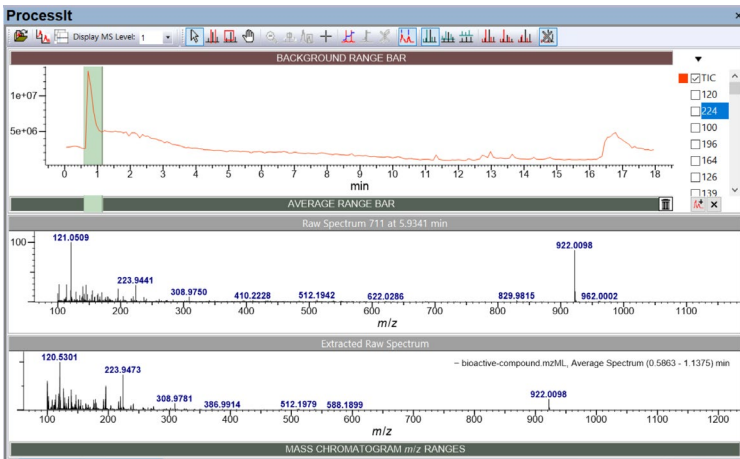
	Action	Result
1	Open ProcessIt application by clicking its icon, typically found in the Data toolbox. 	ProcessIt application is displayed: 
2	Click Open Data File button. Navigate to “C:\Users\Public\Public Documents\Wiley\KnowItAll\Samples\LC-MS”. Select “bioactive-compound.mzML”	The file opens in ProcessIt application:  <ul style="list-style-type: none"> • The top panel displays the chromatogram. • To the right of the chromatogram is the Mass Chromatogram List Pane which displays the extracted total ion chromatograms (TICs). • The middle display is the Raw Spectrum panel. • The bottom display is the Extracted Raw Spectrum panel.

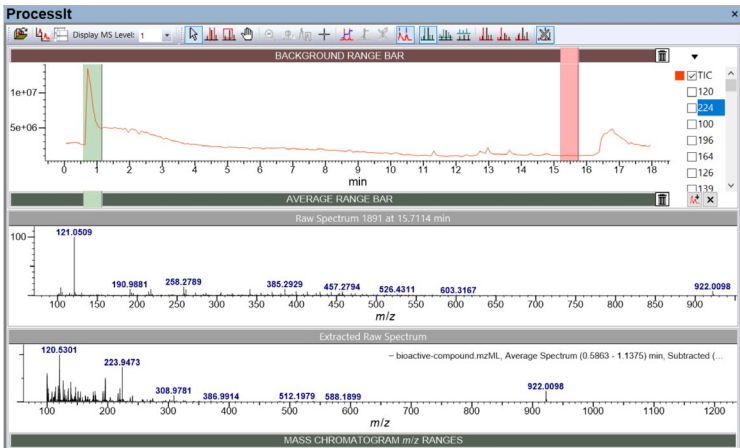

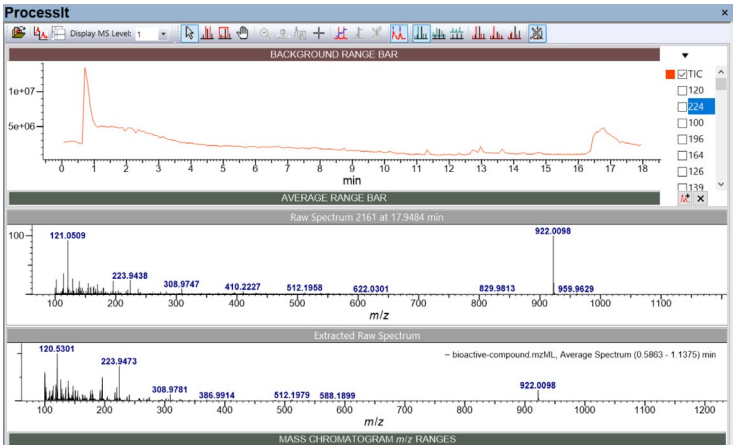
	Action	Result
3	Choose View > Show Accurate Mass.	<p>The masses are now displayed to the full precision:</p>  <p>The screenshot shows the ProcessIt interface with three panels. The top panel is a Total Ion Chromatogram (TIC) showing a peak at 0.7138 min. The middle panel is a mass spectrum for 'Raw Spectrum 932 at 7.7790 min' with a base peak at m/z 219.1735 and other significant peaks at 100.1110, 51.3730, 143.0439, 262.2796, 318.2717, 393.0086, 462.1290, 517.1704, 577.7227, 641.2135, 699.1877, 776.9183, 837.4559, 901.3936, and 967.6191. The bottom panel is an 'Extracted Raw Spectrum' for 'bioactive-compound.mzML, Raw Spectrum 81 at 0.7138 min' with peaks at 120.5301, 223.9473, 308.9776, 386.9914, 512.1986, 588.1899, and 922.0098.</p>
4	Peak boxes can be hidden by unchecking the option View > Peaks on Chromatograms.	<p>The peak boxes are no longer displayed on the chromatogram:</p>  <p>The screenshot shows the ProcessIt interface with three panels. The top panel is a TIC showing a peak at 0.7138 min. The middle panel is a mass spectrum for 'Raw Spectrum 2150 at 17.8483 min' with a base peak at m/z 57.9356 and other significant peaks at 92.5004, 146.0705, 250.7192, 306.6592, 385.8523, 467.0054, 552.2537, 639.9275, 703.2655, 759.9405, 841.0457, 907.3108, and 999.0454. The bottom panel is an 'Extracted Raw Spectrum' for 'bioactive-compound.mzML, Raw Spectrum 81 at 0.7138 min' with peaks at 120.5301, 223.9473, 308.9776, 386.9914, 512.1986, 588.1899, and 922.0098.</p>

	Action	Result
5	<p>Change the value for Display MS Level to 1.</p> <p>Note: The MS Level is filtered on the Standard Toolbar.</p>	<p>The chromatogram display changes to MS Level 1:</p>  <p>Using this filter, only MS Level 1 mass spectra will be displayed.</p>
6	<p>The extracted ion chromatograms (EICs) appear on the Mass Chromatogram List Pane. Use the arrow on the top of the Mass Chromatogram List Pane to adjust the m/z sorting.</p>	<p>By default, "Sort by maximum intensity" will be selected:</p>  <p>Chromatograms can also be sorted by abundance, m/z ascending and m/z descending.</p>


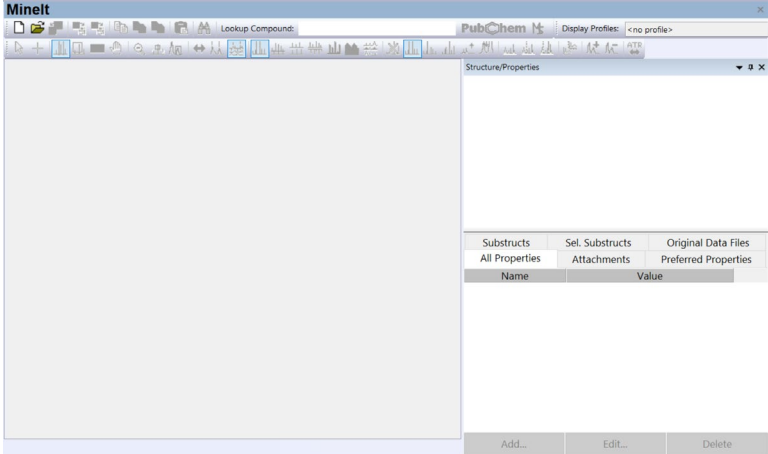
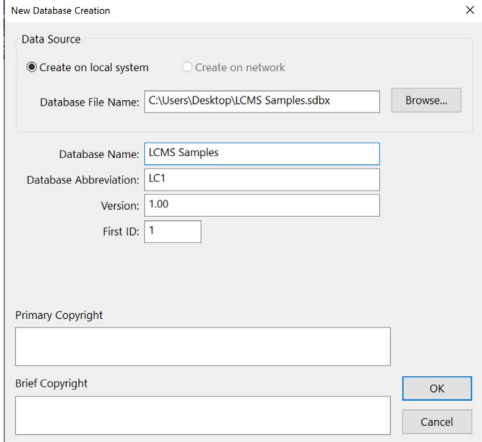
	Action	Result
7	<p>Click to select the boxes next to "120" and "224" on the Mass Chromatogram List Pane. Uncheck the box next to "TIC".</p>	<p>The EICs for m/z 120 (black) and 224 (blue) are displayed, and the TIC is no longer displayed:</p>
8	<p>Click on the + icon on the Mass Chromatogram List Pane.</p> 	<p>The Ion(s)/Ion Ranges dialog opens:</p>  <p>This dialog can be used to manually extract an EIC.</p>

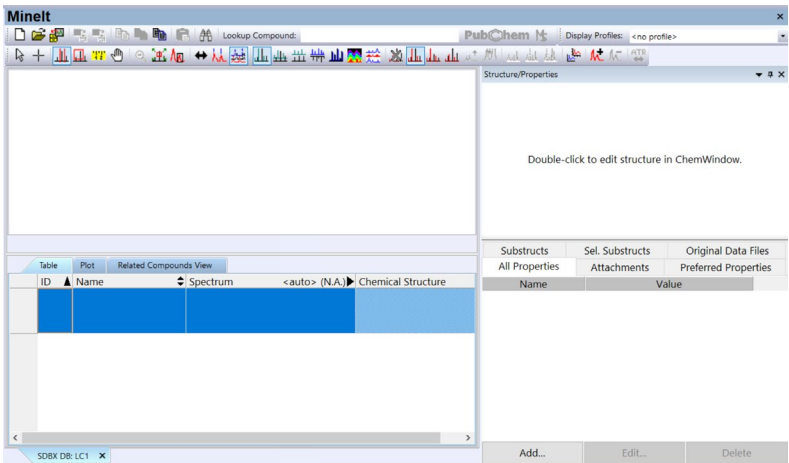

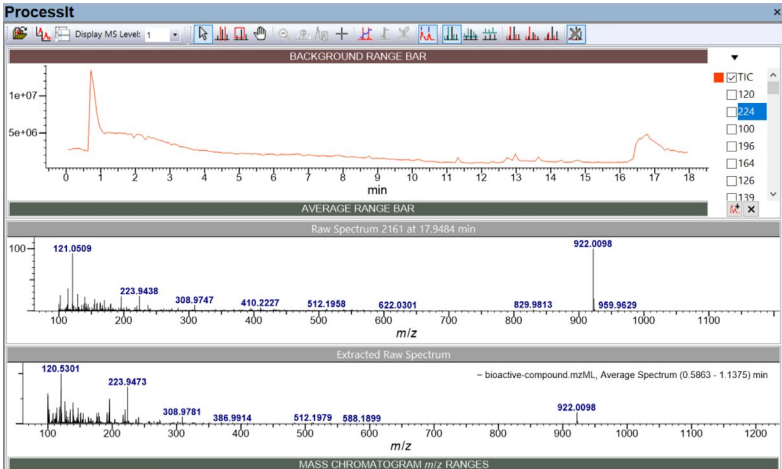
	Action	Result
9	<p>Click Cancel to close the dialog. Click to re-select the box next to "TIC" on the Mass Chromatogram List Pane and unselect the boxes next to the ions 120 and 224.</p>	<p>The Ion(s)/Ion Ranges dialog is closed. The TIC is displayed:</p> 
10	<p>Move the mouse cursor across the chromatogram without clicking the mouse button</p>	<p>As the mouse moves across the spectrum, the raw MS spectrum is displayed for the corresponding timepoint on the Raw Spectrum panel. The raw MS spectrum dynamically updates with the mouse cursor position.</p>
11	<p>Choose a point on the chromatogram at any timepoint and click the left mouse button.</p>	<p>The Extracted Raw Spectrum panel displays the selected timepoint mass spectrum:</p> 

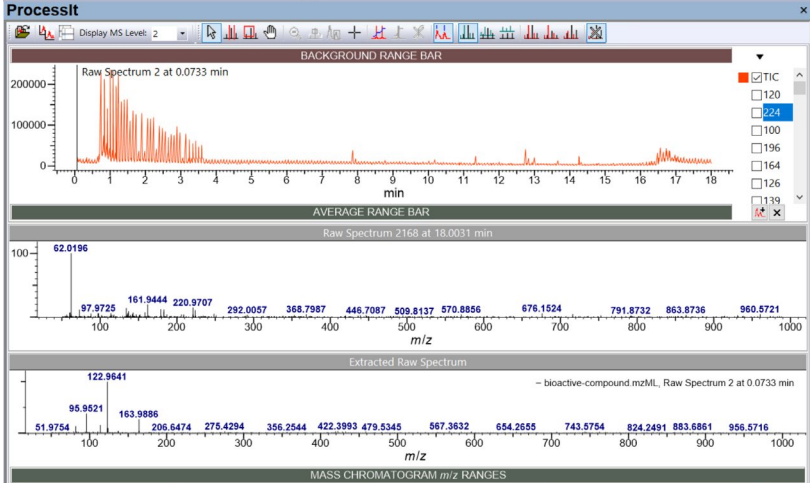
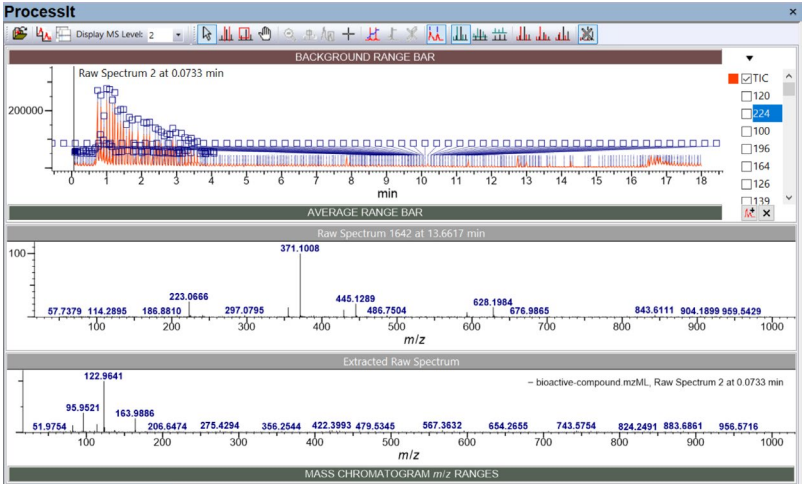
	Action	Result
12	<p>Click on the Average Range Bar and drag the left mouse button across the width of a peak. Release the mouse button.</p> <p>Note: More than one range can be selected.</p>	<p>The selected average range is displayed with a green bar on the chromatogram:</p>  <p>The screenshot displays the ProcessIt software interface. At the top, there is a toolbar with various icons. Below the toolbar, the main window is divided into three panels. The top panel shows a chromatogram with a green bar indicating the selected average range. The middle panel shows the Raw Spectrum for the selected peak, with the x-axis labeled 'm/z' and the y-axis labeled '100'. The bottom panel shows the Extracted Raw Spectrum, also with the x-axis labeled 'm/z' and the y-axis labeled '100'. The chromatogram shows a peak at approximately 1.5 minutes. The Raw Spectrum shows a base peak at m/z 121.0509 and other significant peaks at m/z 223.9441, 308.9750, 410.2228, 512.1942, 622.0286, 829.9815, 922.0098, and 962.0002. The Extracted Raw Spectrum shows a base peak at m/z 120.5301 and other significant peaks at m/z 223.9473, 308.9781, 386.9914, 512.1979, 588.1899, and 922.0098.</p> <p>The Average Range Bar is used to create an averaged mass spectrum of the selected region and is displayed in the Extracted Raw Spectrum panel.</p>

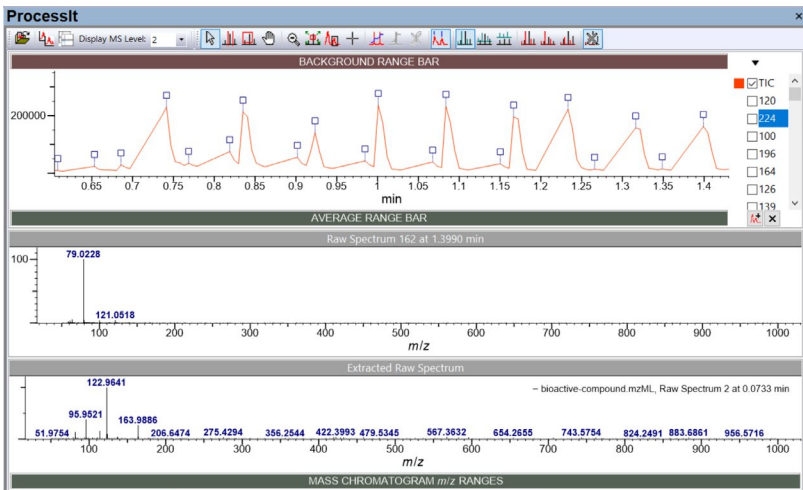
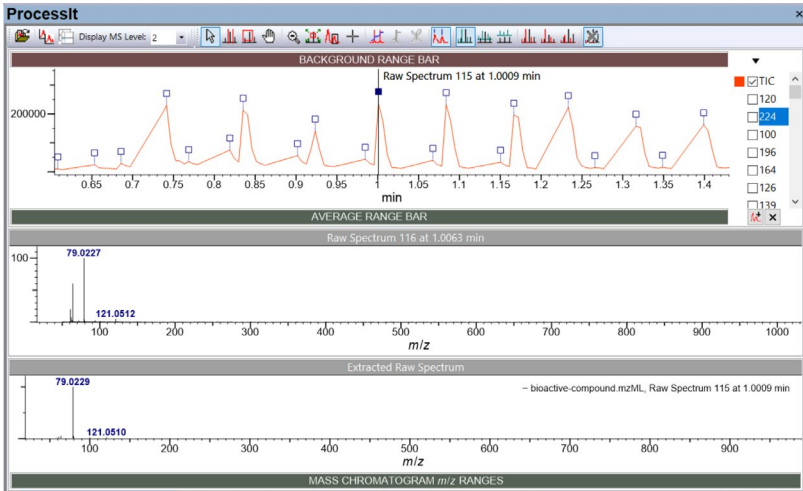
	Action	Result
13	<p>Click on the Background Range Bar and left drag the mouse button across a section of the baseline. Release the mouse button.</p>	<p>The selected background range is displayed with a red bar on the chromatogram:</p>  <p>The Background Range Bar is used to select a background mass spectrum to subtract from the averaged mass spectrum in the Extracted Raw Spectrum panel.</p>
14	<p>Click the trash can icons on the Average Range Bar and Background Range Bar to remove the selected average range.</p> 	<p>The selected ranges are removed:</p> 


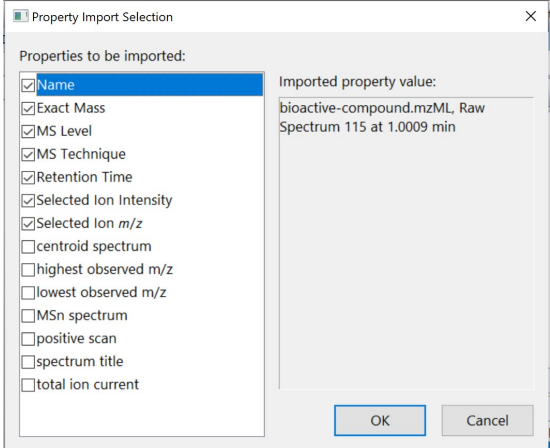
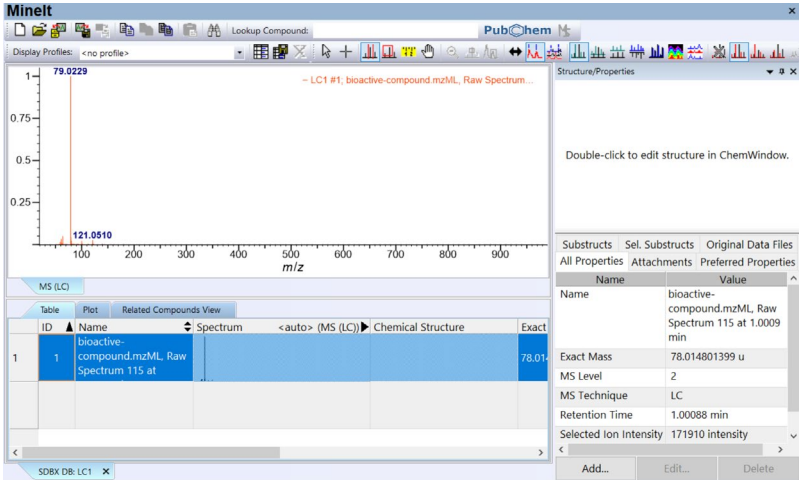
Example: Extracting MS2 data into a user database

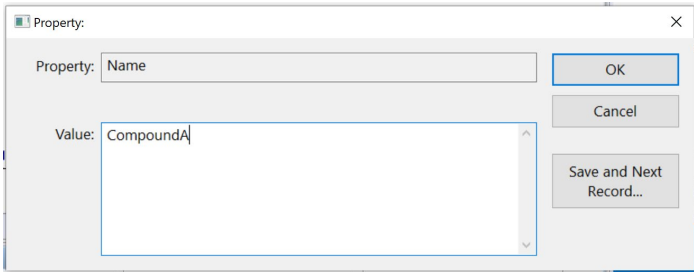
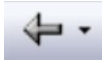
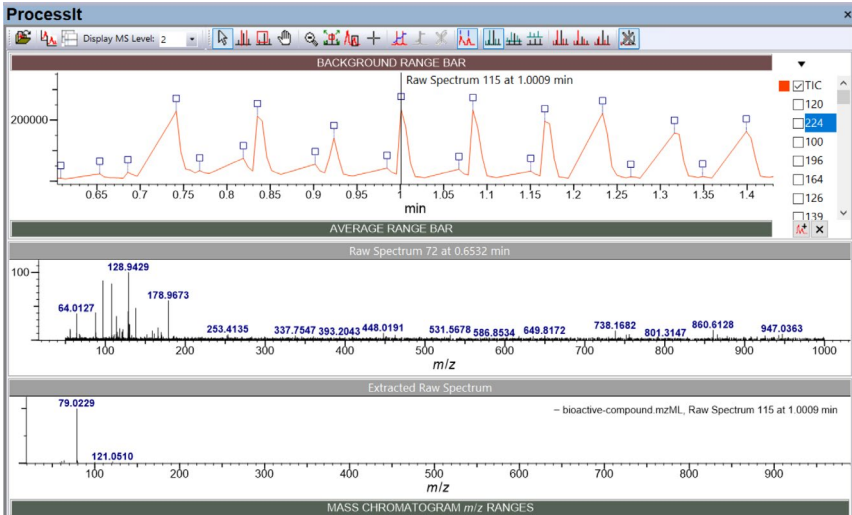
	Action	Result
15	<p>Open the Minelt application by clicking its icon, typically found in the Data toolbox.</p> 	<p>Minelt application is displayed.</p> 
16	<p>Choose Database > New, then click Browse on the New Database Creation dialog. Give the file a name (e.g., "LCMS Samples") and choose a location to save the user database and click Save. In the New Database Creation dialog, write LC1 in Database Abbreviation.</p> <p>Note: The Database Abbreviation is any abbreviation at least 3 characters long.</p>	<p>The New Database Creation dialog is opened. The database is given a name and abbreviation:</p> 

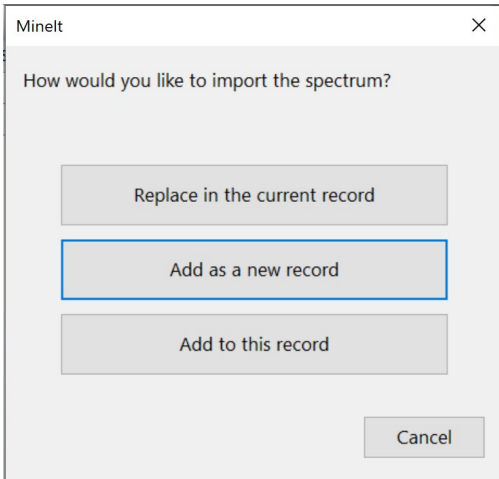
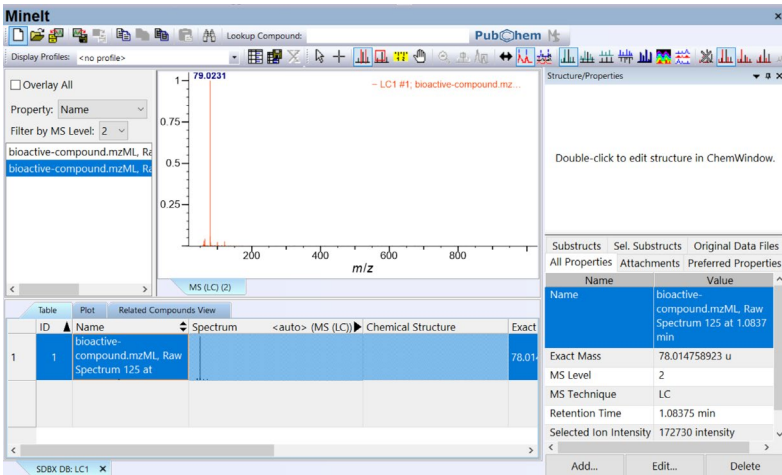
	Action	Result
17	Click OK to save the blank user database.	<p>The New Database Creation dialog closes and a blank database in Minelt is displayed:</p> 
18	Click the Previous Application button to return to ProcessIt .  <p>Note: The Previous and Next Application buttons allow for quick movement between recently used applications.</p>	<p>The chromatogram from the ProcessIt is visible:</p> 

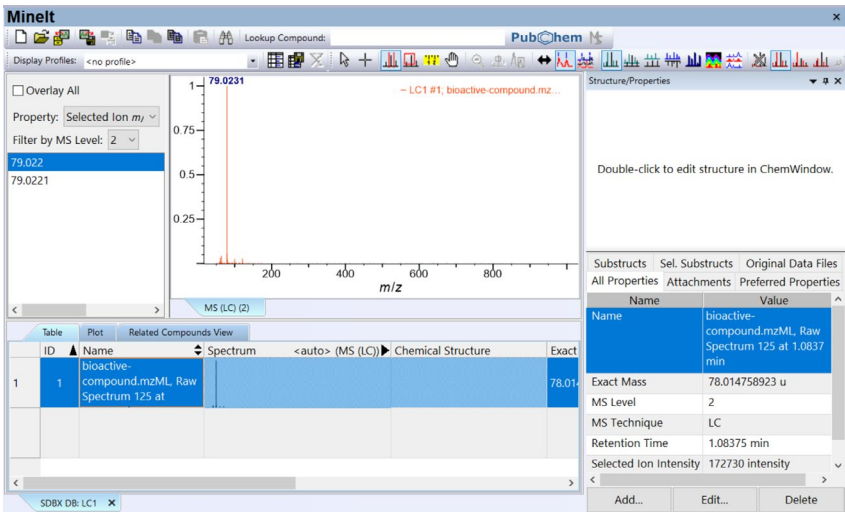
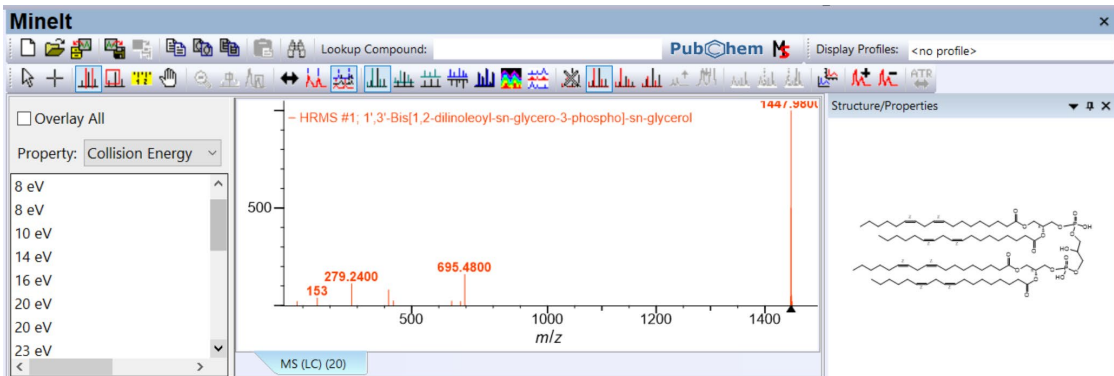
	Action	Result
19	Change the Display MS Level value to 2.	<p>The mass spectra for MS Level 2 are now available for display within the chromatogram:</p>  <p>Using this filter, only MS Level 2 mass spectra will be displayed.</p>
20	Choose View > Peaks on Chromatogram to turn the display of peak labels back on.	<p>The peak labels are displayed on the chromatogram:</p> 

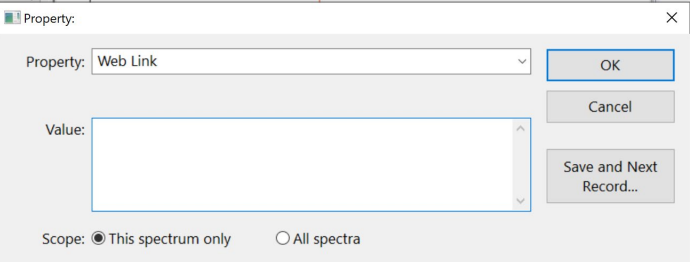
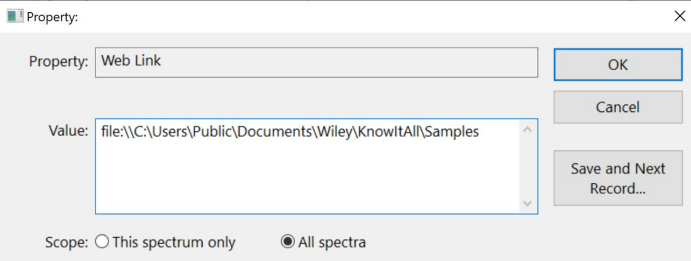
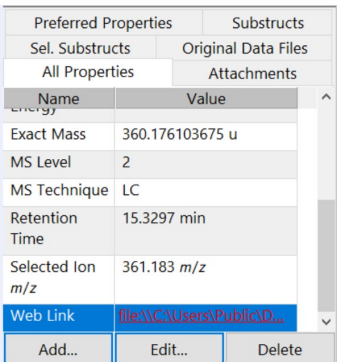
	Action	Result
21	Right click on the spectrum and select Horizontal Zoom Mode . Click the left mouse button and drag across the region around ~ 1 minute. Release the mouse button. Right click on the chromatogram and select Selection Mode .	<p>The display is zoomed in around 1 minute:</p>  <p>The screenshot displays the ProcessIt software interface. The top panel shows a chromatogram with a peak at 1.3990 min. The middle panel shows the raw spectrum for this peak, with peaks at m/z 79.0228 and 121.0518. The bottom panel shows the extracted raw spectrum, with a peak at m/z 122.9641. The x-axis for the chromatogram is time in minutes (0.65 to 1.4), and the x-axis for the mass spectra is m/z (100 to 1000).</p>
22	Click on any peak box on the chromatogram.	<p>The selected MS spectrum is displayed in the Extracted Raw Spectrum panel:</p>  <p>The screenshot displays the ProcessIt software interface. The top panel shows a chromatogram with a peak at 1.0009 min. The middle panel shows the raw spectrum for this peak, with peaks at m/z 79.0227 and 121.0512. The bottom panel shows the extracted raw spectrum, with a peak at m/z 79.0229. The x-axis for the chromatogram is time in minutes (0.65 to 1.4), and the x-axis for the mass spectra is m/z (100 to 1000).</p>

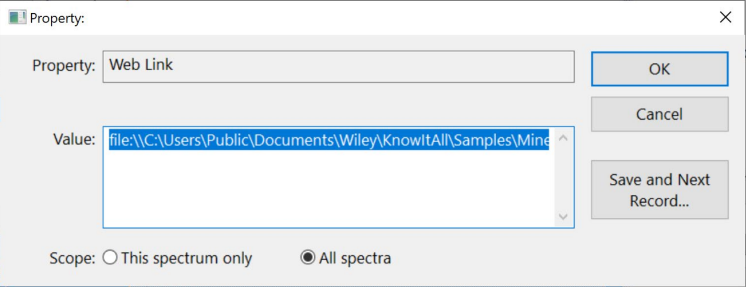
	Action	Result														
23	Click Minelt Database using the Transfer to bar :	<p>The transfer to bar is displayed:</p>  <p>The Property Import Selection dialog appears:</p> 														
24	Click OK on the Property Import Selection dialog.	<p>The MS2 spectrum is added to the user database:</p>  <table border="1" data-bbox="1255 1175 1503 1419"> <thead> <tr> <th>Name</th> <th>Value</th> </tr> </thead> <tbody> <tr> <td>Name</td> <td>bioactive-compound.mzML, Raw Spectrum 115 at 1.0009 min</td> </tr> <tr> <td>Exact Mass</td> <td>78.014801399 u</td> </tr> <tr> <td>MS Level</td> <td>2</td> </tr> <tr> <td>MS Technique</td> <td>LC</td> </tr> <tr> <td>Retention Time</td> <td>1.00088 min</td> </tr> <tr> <td>Selected Ion Intensity</td> <td>171910 intensity</td> </tr> </tbody> </table>	Name	Value	Name	bioactive-compound.mzML, Raw Spectrum 115 at 1.0009 min	Exact Mass	78.014801399 u	MS Level	2	MS Technique	LC	Retention Time	1.00088 min	Selected Ion Intensity	171910 intensity
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	Action	Result
25	<p>Note: Any property for the record can be modified by clicking Edit in the Structure/Properties Table, or double clicking on the cell in the Table.</p> <p>Note: If the structure is known, it can be added by double clicking in the Structure/Properties panel “Double-click to edit structure in Chem Window”.</p>	<p>Upon selecting “Name” property and clicking Edit, the Property dialog is displayed which allows for the record name to be changed:</p> 
26	<p>To continue to add MS2 spectra to the user database, return to ProcessIt using the Previous Application button.</p> 	<p>The chromatogram in ProcessIt is displayed:</p> 

	Action	Result
27	<p>Click a different peak box on the chromatogram. Select Minelt Database on the Transfer to bar.</p> <p>Note: If a record was previously selected in the Minelt user database before transferring the MS2 spectrum, the Minelt import dialog will appear before the Property Import Selection dialog.</p>	<p>Upon sending another record to the database, the Minelt dialog will be launched:</p> 
28	<p>Choose "Add to this record" on the Minelt import dialog. Click OK on the Property Import Selection dialog.</p>	<p>A multiple spectrum record is created. The Property Selection menu appears next to the active spectrum. The total number of LC-MS spectra is summarized on the tab (MS (LC) = 2):</p> 

	Action	Result
29	<p>On the Property Selection menu, use the Property Selection dropdown menu to choose Selected Ion m/z.</p> <p>Note: The Property Selection dropdown menu will display the list of Properties attached to the entire record. If a selected spectrum does not have a value for the selected Property, the spectrum number will be listed in place of the value.</p>	<p>The multiple MS spectra now display in the Property Selection menu by Selected Ion m/z:</p>  <p>The screenshot shows the Minelt software interface. The main plot displays a mass spectrum with a prominent peak at m/z 79.0231. The x-axis is labeled 'm/z' and ranges from 0 to 1000. The y-axis represents relative intensity. The 'Property Selection' dropdown is set to 'Selected Ion m/z'. Below the plot, a table lists spectra with columns for ID, Name, Spectrum, and Exact Mass. The 'Structure/Properties' panel on the right shows the chemical structure and various properties such as Exact Mass (78.014758923 u), MS Level (2), MS Technique (LC), Retention Time (1.08375 min), and Selected Ion Intensity (172730 intensity).</p>
30	<p>Note: The Property Selection dropdown menu can be used to assist in sorting through records with multiple spectra. For example, the example database record shown has 20 LC-MS spectra for the same compound acquired at different collision energies.</p>	<p>Example record with 20 LC-MS spectra measured at different collision energies for 1',3'-Bis[1,2-dilinoleoyl-sn-glycero-3-phospho]-sn-glycerol:</p>  <p>The screenshot shows the Minelt software interface for a specific compound. The main plot displays a mass spectrum with several peaks labeled with their m/z values: 153, 279.2400, 695.4800, and 1447.9801. The x-axis is labeled 'm/z' and ranges from 0 to 1400. The y-axis represents relative intensity. The 'Property Selection' dropdown is set to 'Collision Energy'. Below the plot, a table lists spectra with columns for ID, Name, Spectrum, and Exact Mass. The 'Structure/Properties' panel on the right shows the chemical structure of 1',3'-Bis[1,2-dilinoleoyl-sn-glycero-3-phospho]-sn-glycerol.</p>

	Action	Result
31	<p>With the Minelt record selected, click Add on the Structure/Properties Table. Navigate to Web Link using the Property dialog.</p> <p>Note: The raw chromatogram file or folder can be attached to the Minelt record using the Web Link property for convenient file organization.</p>	<p>The Property dialog opens. Web Link is selected:</p> 
32	<p>In the Value box, write file:\\ and copy+paste a folder pathway after the text: file:\\<i>pathway here</i>. Choose All spectra for the Scope of the property.</p> <p>Note: Make sure there are no extra characters in the folder pathway (such as extra spaces).</p>	<p>Example using text: file:\\C:\\Users\\Public\\Documents\\Wiley\\KnowItAll\\Samples\\</p> 
33	<p>Click OK on the Property dialog.</p>	<p>The Web Link is displayed in the Properties Table for the record:</p> 

	Action	Result
34	Click the Web Link for the record.	The folder described by the pathway opens in File Explorer .
35	<p>Note: The Web Link property can be used to organize various files related to the LC-MS experiment (e.g., .xlsx, .csv, .docx, etc.). These can also be attached to the record using the Attachments tab and clicking Add button.</p>	<p>Example attaching a csv file using Web Link: file:\\C:\Users\Public\Documents\Wiley\KnowItAll\Samples\MineIt\Import.csv</p>  <p>Example attaching a csv using Attachments:</p> 