KnowItAll Software Training

LC-MS Databasing using KnowItAll ProcessIt and MineIt

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 MineIt using user databases.

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 MineIt user database for future review and searching.

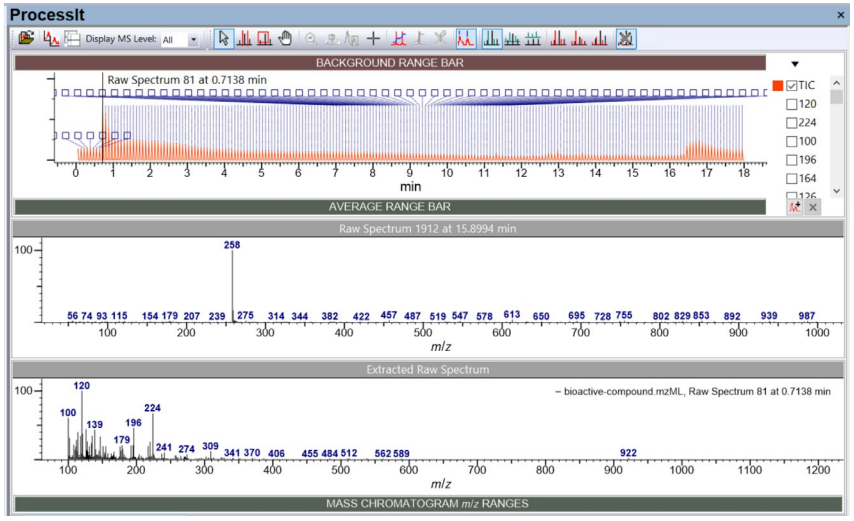
Training Files Used in This Lesson

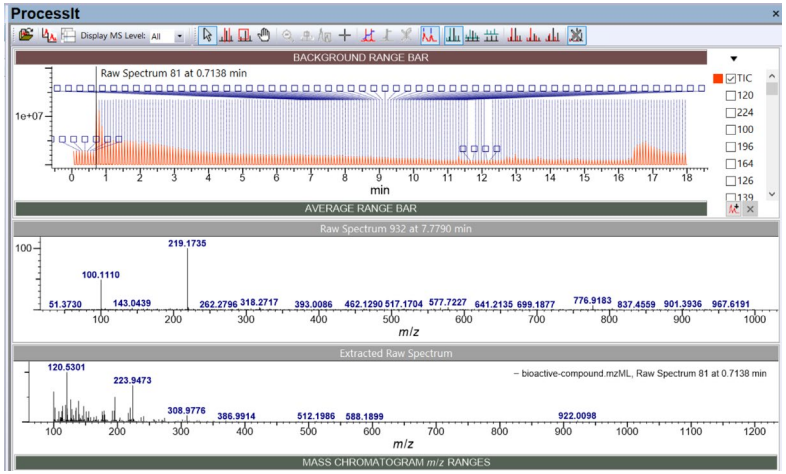
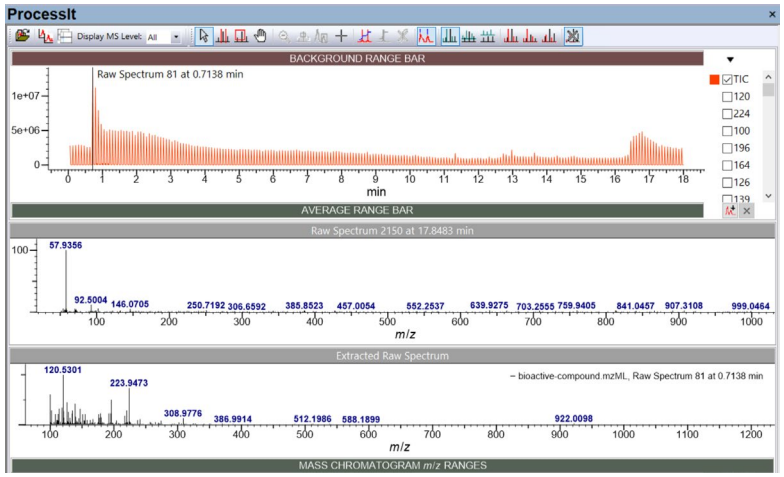
- C:\Users\Public\Documents\Wiley\KnowItAll\Samples\LC-MS\bioactive-compound.mzML

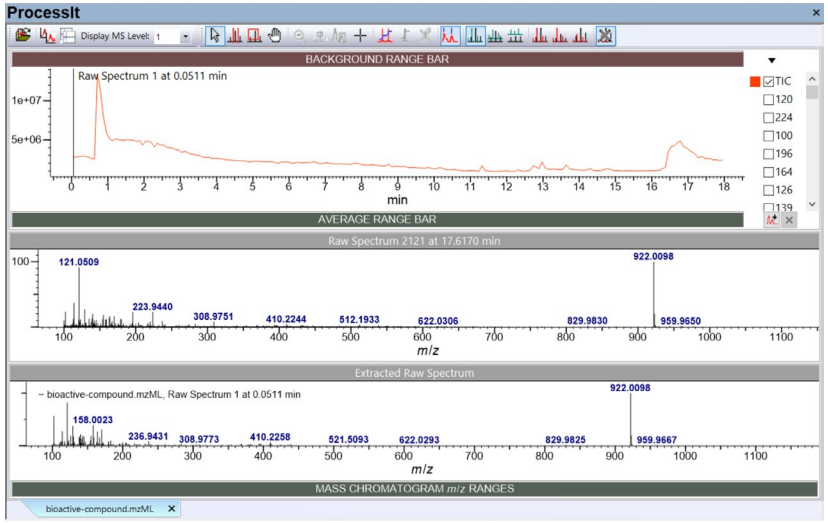
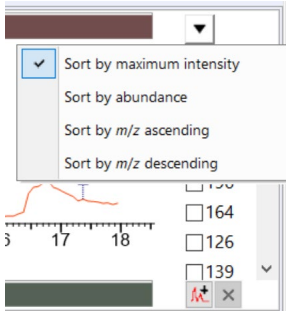
KnowItAll Applications Used

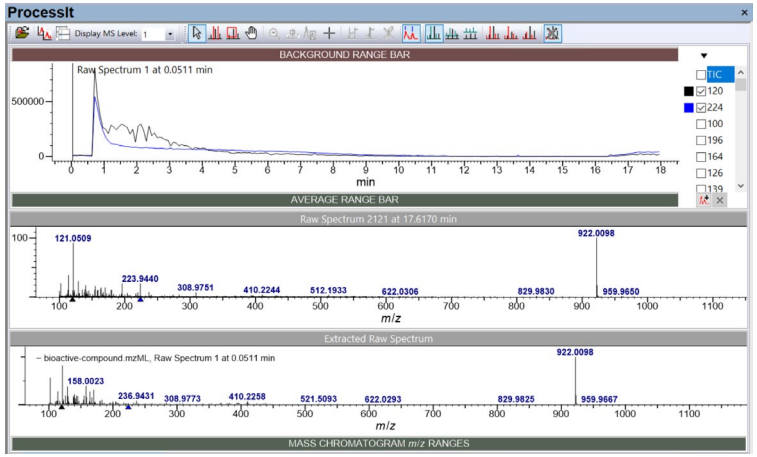
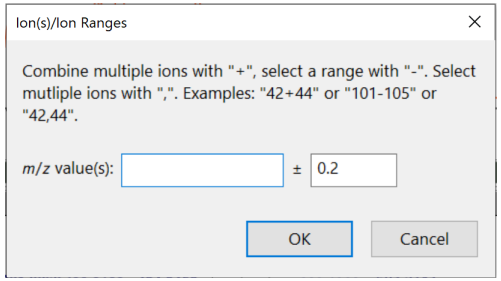
- KnowItAll ProcessIt
- KnowItAll MineIt

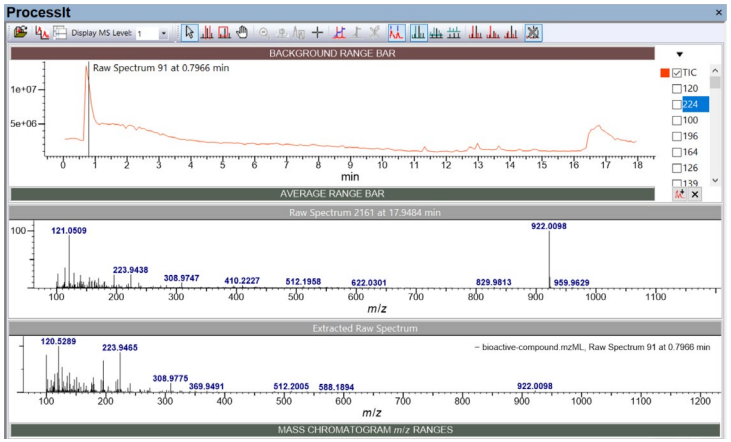
Example: Reviewing MS1 data in ProcessIt

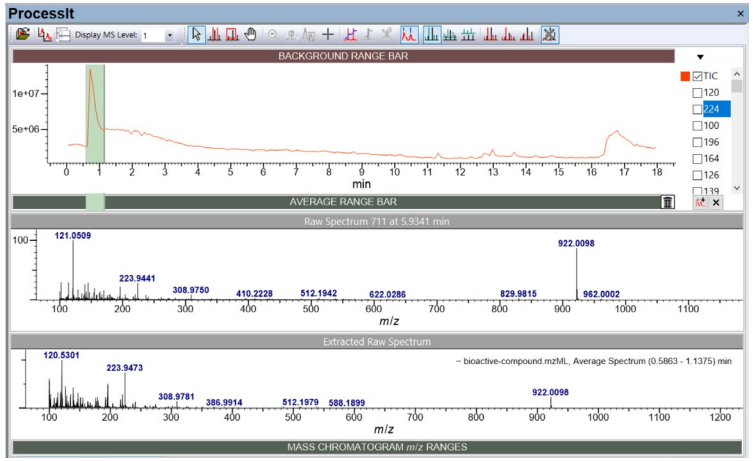
	Action	Result
1	Open **ProcessIt** application by clicking its icon, typically found in the **Data** toolbox. 	**ProcessIt** application is displayed: 
2	Click **Open Data File** button. Navigate to "C:\Users\Public\Public Documents\Wiley\KnowItAll\Samples\LC-MS\". Select "bioactive-compound.mzML"	The file opens in **ProcessIt** application:  • 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.

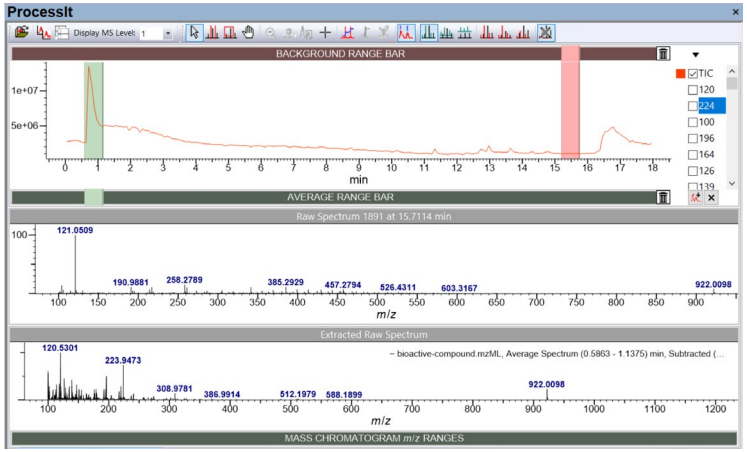
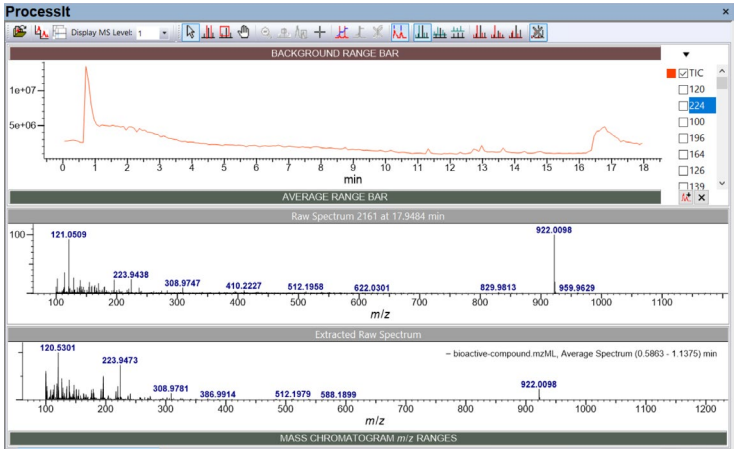
	Action	Result
3	Choose **View > Show Accurate Mass**.	The masses are now displayed to the full precision: 
4	**Peak boxes** can be hidden by unchecking the option **View > Peaks on Chromatograms**.	The **peak boxes** are no longer displayed on the **chromatogram**: 

	Action	Result
5	Change the value for **Display MS Level** to **1**. **Note:** The MS Level is filtered on the **Standard Toolbar**.	The chromatogram display changes to MS Level 1:  Using this filter, only MS Level 1 mass spectra will be displayed.
6	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.	By default, “Sort by maximum intensity” will be selected:  Chromatograms can also be sorted by abundance, m/z ascending and m/z descending.

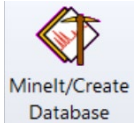
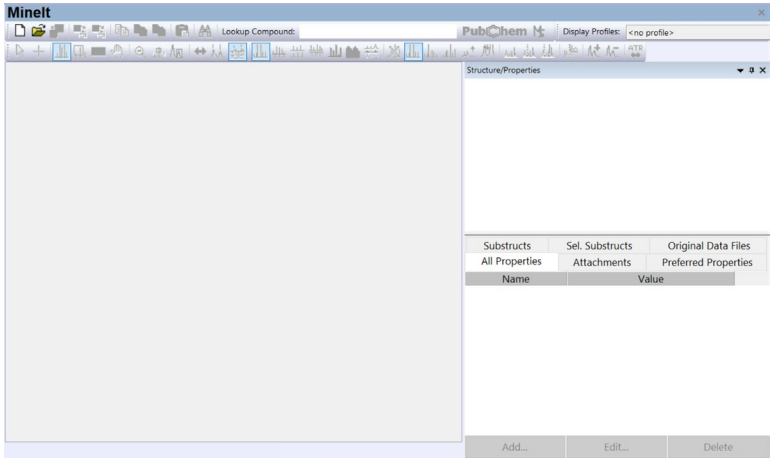
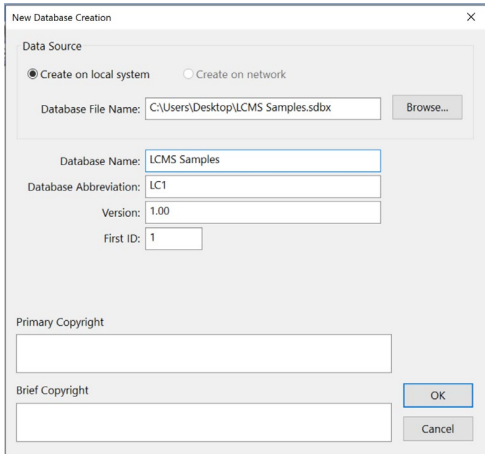
	Action	Result
7	Click to select the boxes next to “120” and “224” on the **Mass Chromatogram List Pane**. Uncheck the box next to "TIC".	The EICs for m/z 120 (black) and 224 (blue) are displayed, and the TIC is no longer displayed: 
8	Click on the **+** icon on the **Mass Chromatogram List Pane**. 	The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

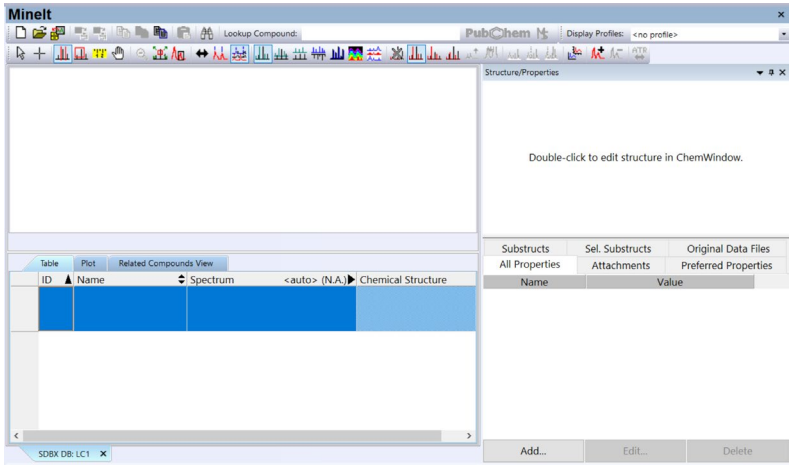
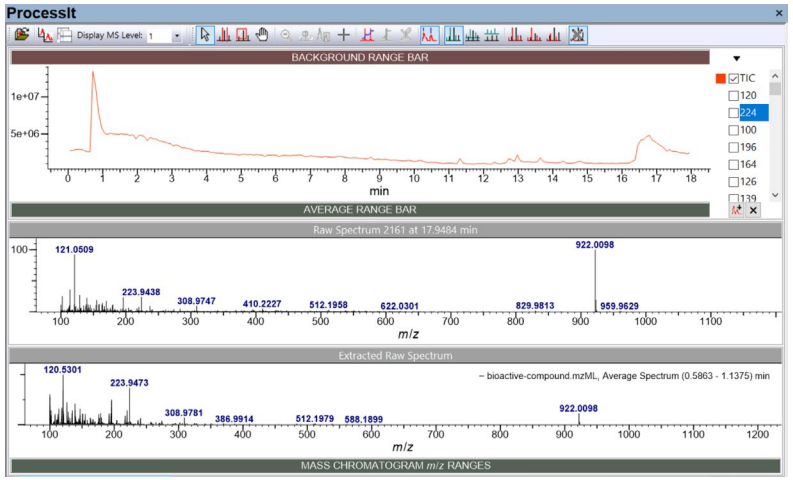
	Action	Result
9	Click **Cancel** to close the dialog. Click to re-select the box next to "TIC" on the **Mass Chromatogram List Pane** and unselect the boxes next to the ions 120 and 224.	The **Ion(s)/Ion Ranges** dialog is closed. The TIC is displayed: 
10	Move the mouse cursor across the chromatogram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

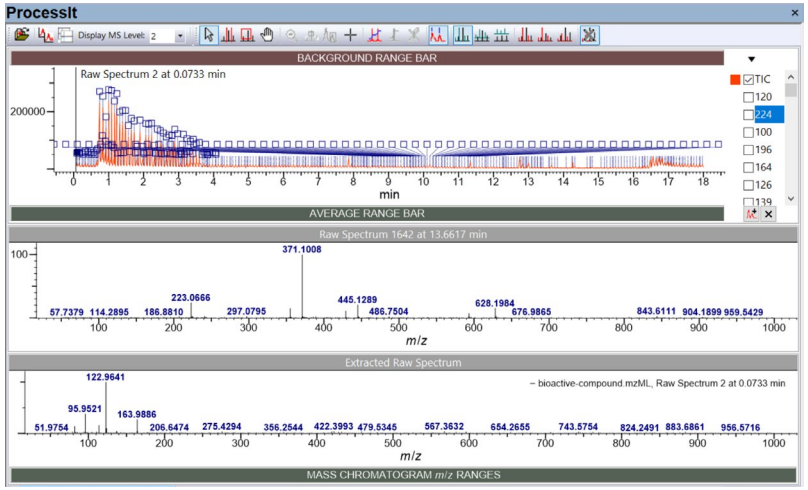
	Action	Result
12	Click on the **Average Range Bar** and drag the left mouse button across the width of a peak. Release the mouse button. **Note:** More than one range can be selected.	The selected average range is displayed with a green bar on the chromatogram:  The **Average Range Bar** is used to create an averaged mass spectrum of the selected region and is displayed in the **Extracted Raw Spectrum** panel.

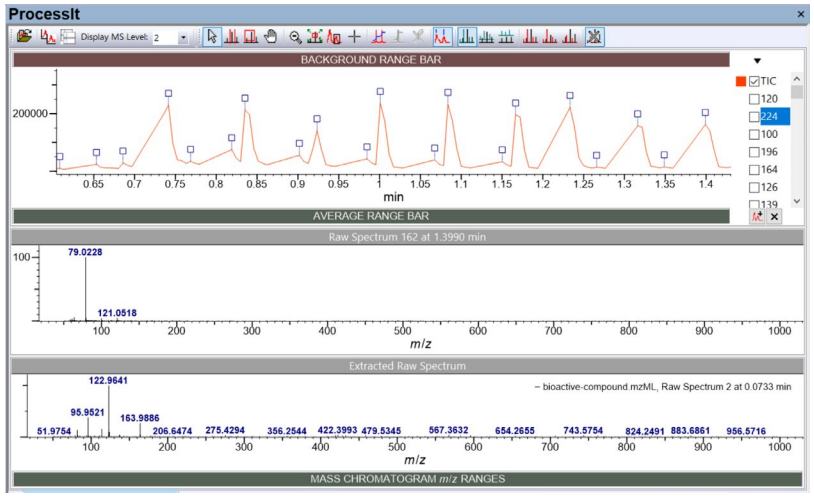
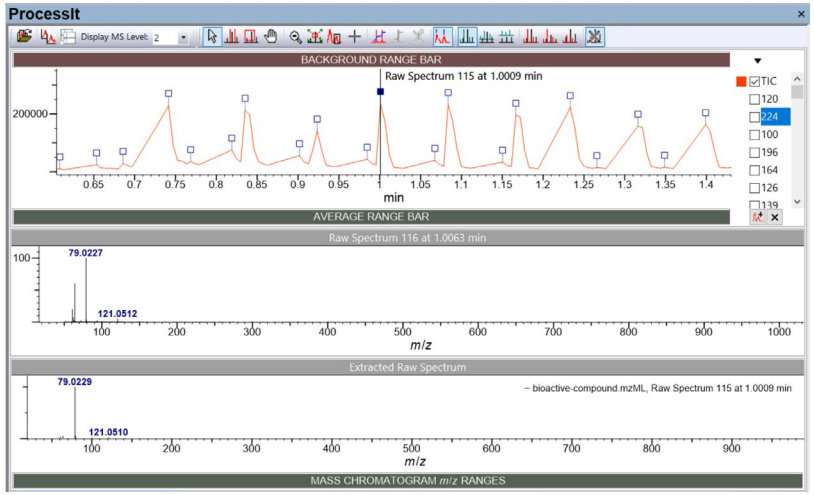
	Action	Result
13	Click on the **Background Range Bar** and left drag the mouse button across a section of the baseline. Release the mouse button.	The selected background range is displayed with a red bar on the chromatogram:  The **Background Range Bar** is used to select a background mass spectrum to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

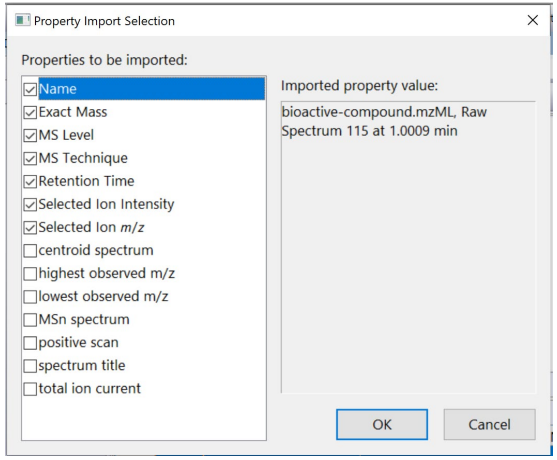
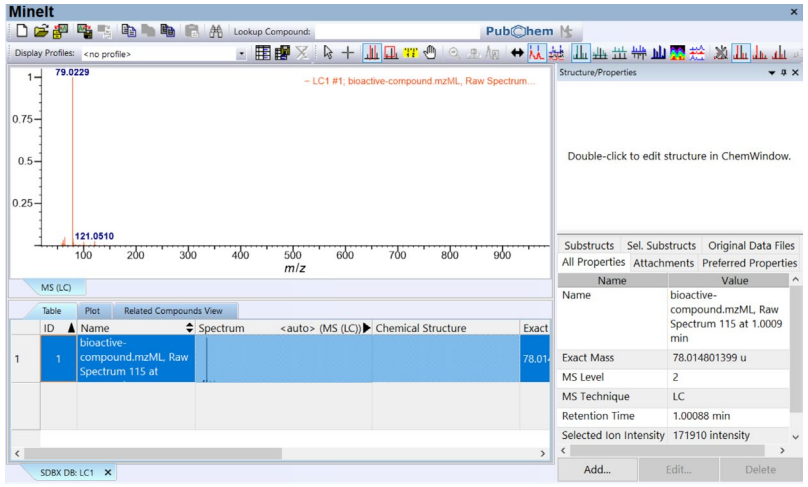
Example: Extracting MS2 data into a user database

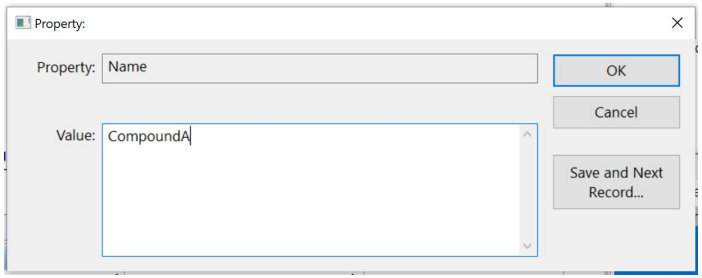
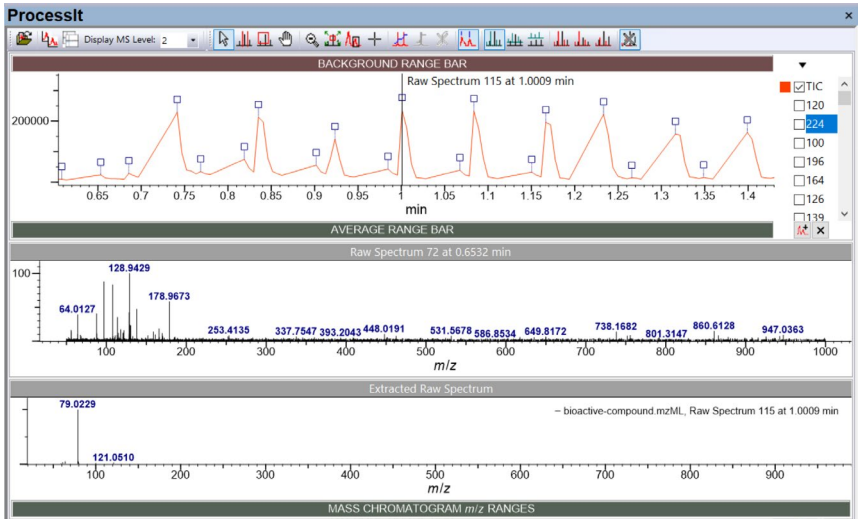
	Action	Result
15	Open the **MineIt** application by clicking its icon, typically found in the **Data** toolbox. 	**MineIt** application is displayed. 
16	Choose **Database > New**, then click **Browse** on the **New Database Creation** dialog. Give the file a name (e.g., "LCMS Samples") and choose a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

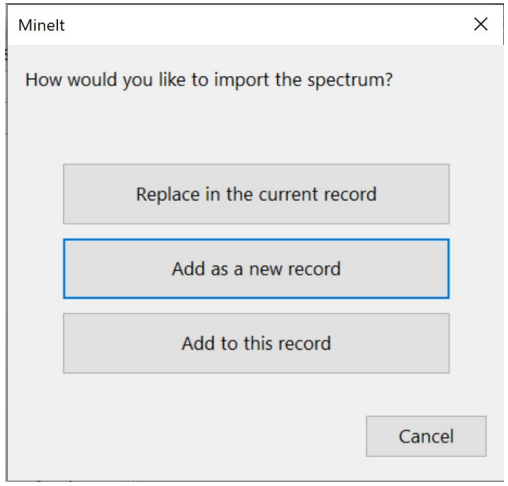
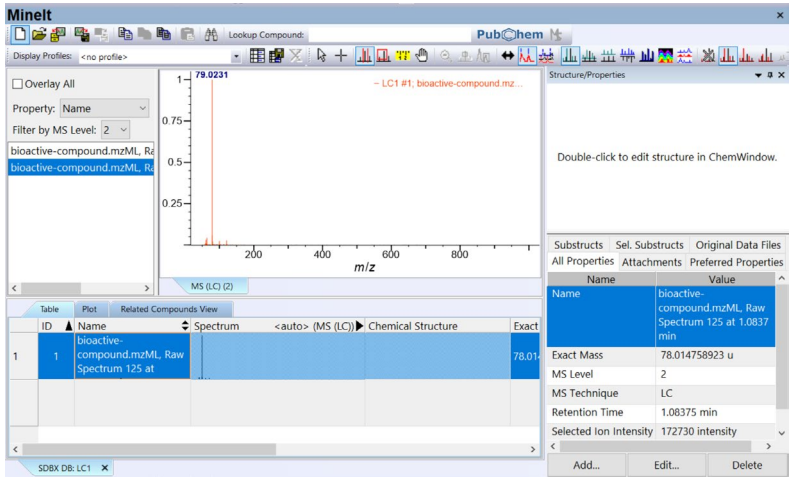
	Action	Result
17	Click **OK** to save the blank user database.	The **New Database Creation** dialog closes and a blank database in **MineIt** is displayed: 
18	Click the **Previous Application** button to return to **ProcessIt**.  **Note:** The **Previous** and **Next Application** buttons allow for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

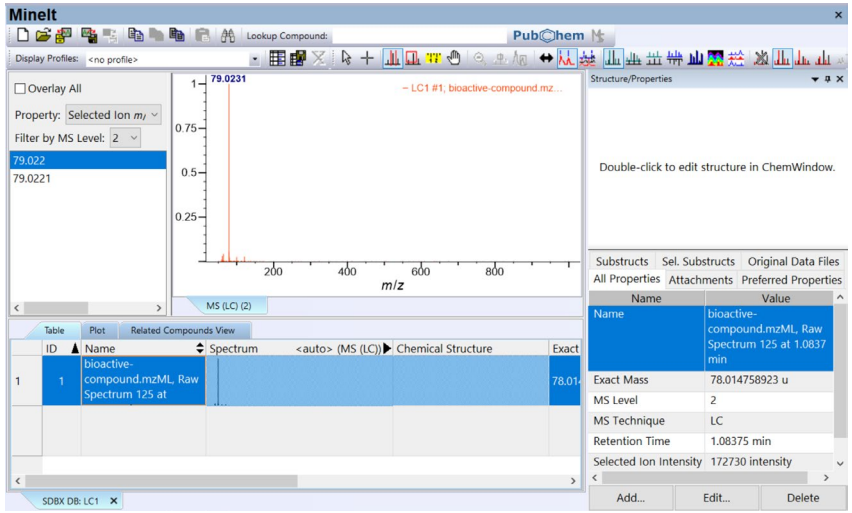
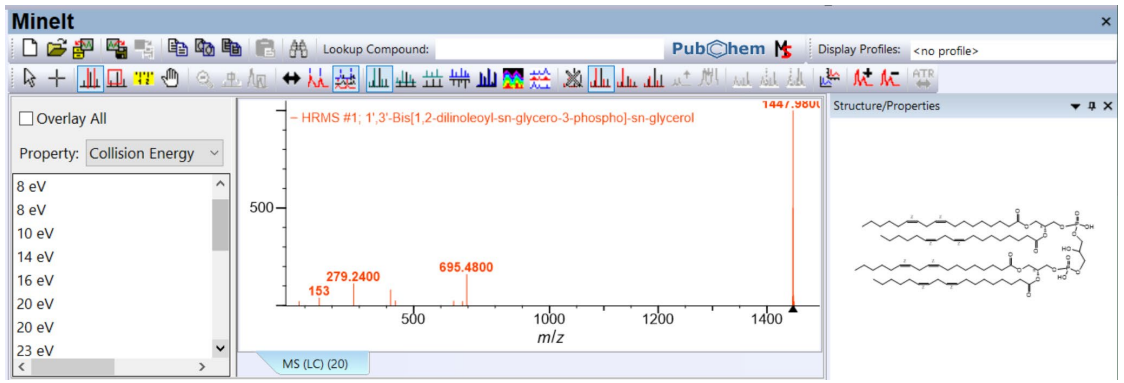
	Action	Result
19	Change the **Display MS Level** value to 2.	The mass spectra for MS Level 2 are now available for display within the chromatogram:  Using this filter, only MS Level 2 mass spectra will be displayed.
20	Choose **View > Peaks on Chromatogram** to turn the display of peak labels back on.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

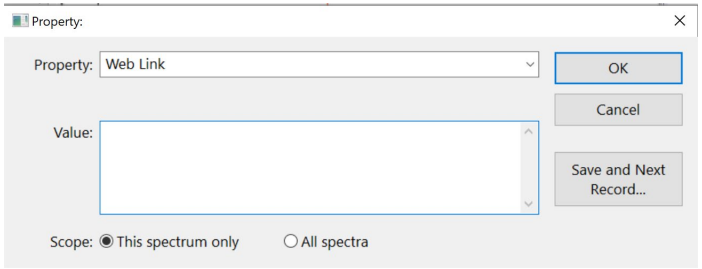
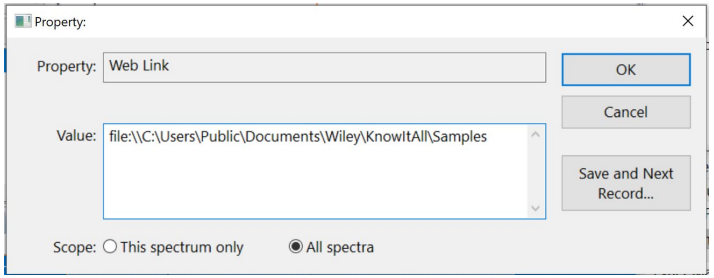
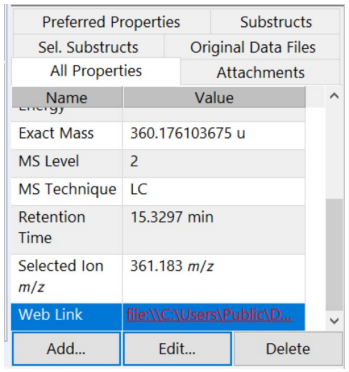
	Action	Result
21	Right click on the spectrum and select **Horizontal Zoom Mode**. Click the left mouse button and drag across the region around ~ 1 minute. Release the mouse button. Right click on the chromatogram and select **Selection Mode**.	The display is zoomed in around 1 minute: 
22	Click on any **peak box** on the chromatogram.	The selected MS spectrum is displayed in the **Extracted Raw Spectrum** panel: 

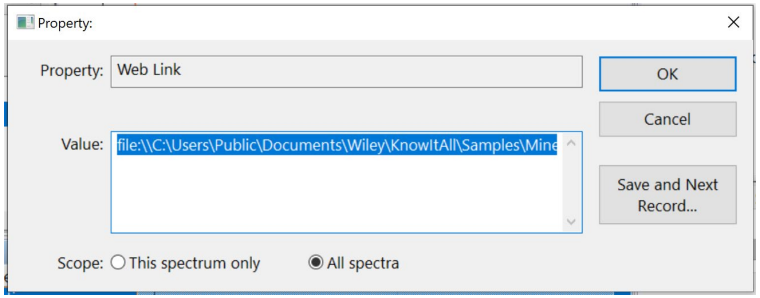
	Action	Result
23	Click **MineIt Database** using the **Transfer to bar**:	The transfer to bar is displayed:  The **Property Import Selection** dialog appears: 
24	Click **OK** on the Property Import Selection dialog.	The MS2 spectrum is added to the user database: 

	Action	Result
25	**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**. **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".	Upon selecting "Name" property and clicking **Edit**, the **Property** dialog is displayed which allows for the record name to be changed: 
26	To continue to add MS2 spectra to the user database, return to **ProcessIt** using the **Previous Application** button. 	The **chromatogram** in **ProcessIt** is displayed: 

	Action	Result
27	Click a different peak box on the chromatogram. Select **MineIt Database** on the **Transfer to bar**. **Note:** If a record was previously selected in the **MineIt** user database before transferring the MS2 spectrum, the **MineIt** import dialog will appear before the **Property Import Selection** dialog.	Upon sending another record to the database, the **MineIt** dialog will be launched: 
28	Choose “Add to this record” on the **MineIt** import dialog. Click **OK** on the **Property Import Selection** dialog.	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): 

	Action	Result
29	On the **Property Selection** menu, use the **Property Selection** dropdown menu to choose **Selected Ion m/z**. **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.	The multiple MS spectra now display in the **Property Selection** menu by Selected Ion m/z: 
30	**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.	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: 

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

	Action	Result
34	Click the Web Link for the record.	The folder described by the pathway opens in **File Explorer**.
35	**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.	Example attaching a csv file using Web Link: file:\\C:\Users\Public\Documents\Wiley\KnowItAll\Samples\MineIt\Import.csv  Example attaching a csv using **Attachments**: