KnowItAll Software Training

LC Expert

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Automatic LC-MS Processing and Analysis

How to use KnowItAll LC Expert to Perform Automatic LC-MS Component Identifications

Purpose

These exercises demonstrate how to use KnowItAll LC Expert to analyze raw LC-MS chromatograms using automatic and manual tools.

Objectives

These exercises will teach you how to:

- Use KnowItAll LC Expert to deconvolute chromatograms into peaks for further analysis
- Perform an untargeted databases search
- Perform a targeted analysis search by exact mass
- Use manual tools to allow for user-lead peak selection

Background

LC-MS chromatograms are rich in information. Analysis is challenging and curated libraries are time consuming to search through. LC Expert application allows for the automatic deconvolution of the chromatogram into peaks, which can be further analyzed and then searched for known and unknown targets. Users of LC Expert are encouraged to create user libraries with their in-house compounds to streamline their workflows using KnowItAll.

Training Files Used in This Lesson

- Folder files in C:\Users\Public\Documents\Wiley\KnowItAll\Samples\LC-MS

KnowItAll Applications Used

- KnowItAll LC Expert
- KnowItAll MineIt
- KnowItAll ReportIt

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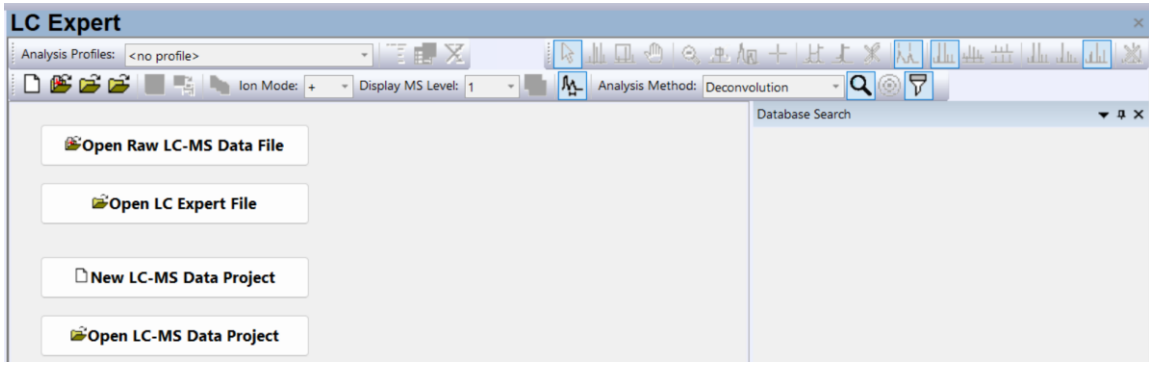
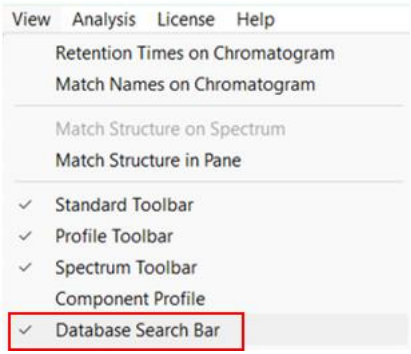
Example: Open a Chromatogram in LC Expert

	Action	Result
1	Open **LC Expert** application by clicking on the icon (), typically found in the **Spectral Processing** toolbox.	**LC Expert** application is displayed:  *Note:* **LC Expert** must be in your current license to have access to the application.
2	Go to the **View** panel and confirm that the **Database Search Bar** is selected with a checkmark. If there is no checkmark, click on the row highlighted below to select it.  	As shown in the previous step, the **Database Search Bar** is displayed in the application, typically on the righthand side of the screen. **Note:** It can be undocked and moved around by clicking on the blue title bar for the panel.

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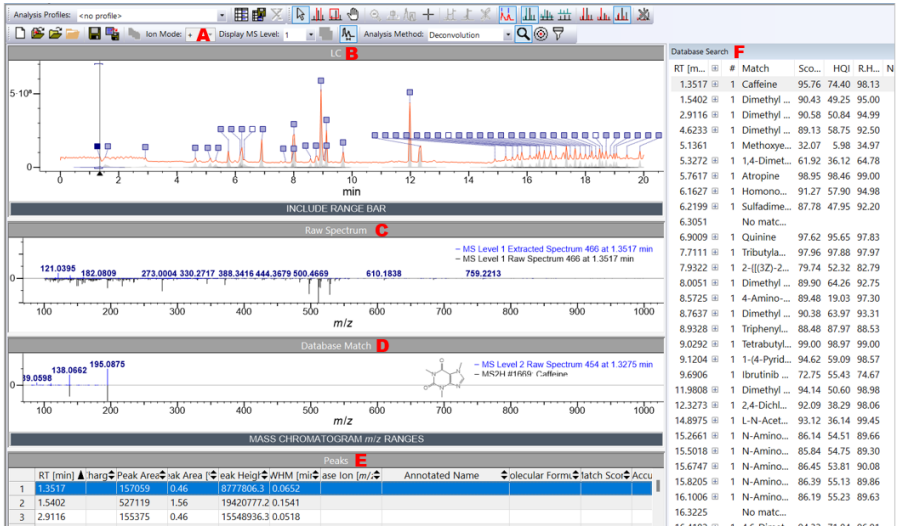
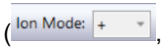
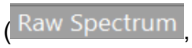
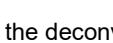
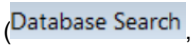
	Action	Result
3	Click **Open Raw LC-MS Data File** button. Navigate to **"C:\Users\Public\Public Documents\Wiley\KnowItAll\Samples\LC-MS\"**. Select "**TESTMIX2_180504_MAS011_06 .mzXML**" and open in the application.	The LC-MS file opens in **LC Expert** application: 
4	Investigate the different panels displayed in the **LC Expert** application.	• The detected ion polarity is displayed in the **Ion Mode** panel ( , **inset A**), located on the **Standard Toolbar**. • The top panel ( , **inset B**) displays the **Chromatogram** with **Peak Boxes** for each peak. • The second panel is the **Raw Spectrum** panel ( , **inset C**) in which the extracted spectrum and raw spectrum are displayed. • The third panel is the **Database Match** panel ( , **inset D**) which displays the search match MS/MS spectrum for the selected peak. • The bottom panel is the **Peaks Table** ( ,**inset E**) which shows the deconvoluted peaks generated from the **Chromatogram**. The rows in the table include information about each peak. • The **Database Search bar** ( , **inset F**) is located on the righthand side and displays any tandem MS search results for the peaks. **Note:** Database search matching will be discussed in greater detail in the next section.

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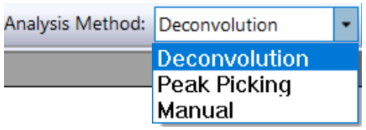
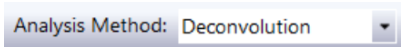
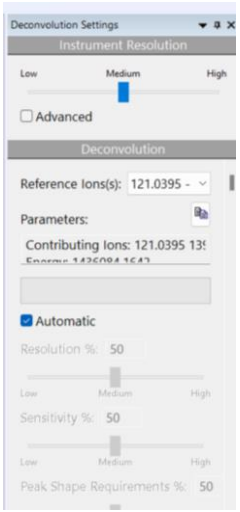
	Action	Result
5	Confirm that the **Analysis Method** dropdown menu on the **Standard Toolbar** is set to **Deconvolution** mode.  **Note**: **LC Expert** retains the last applied method.	The **Analysis Method** is set to **Deconvolution**:  Three different **Analysis Method** options are available in the dropdown menu: **Deconvolution**, **Peak Picking**, and **Manual**. • **Deconvolution**: Correlated MS scans are automatically grouped together into peaks. The averaged MS1 scan for the peak is provided in the **Raw Spectrum** pane, labelled **MS Level 1 Extracted Spectrum**. The **Peak Table** populates with peak information (*e.g.,* **Peak Area**, **Peak Area Percent [%]**). • **Peak Picking**: A single time point is automatically used to characterize the peak and the **Peak Area** and **Peak Area [%]** columns are replaced with ' – ' symbols. Using this method, the **Raw Spectrum** is equivalent to the **Extracted Spectrum**. • **Manual** method enables manual peak selection by the user and will be discussed in a following section.
6	To adjust the peak deconvolution settings, select **View > Deconvolution Settings Bar**.	The **Deconvolution Settings** bar is opened. The **Deconvolution Settings** bar can be used to tune the deconvolution and instrument resolution: 

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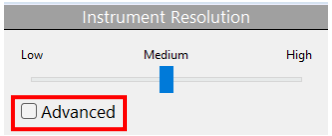
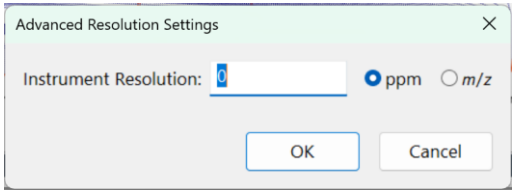
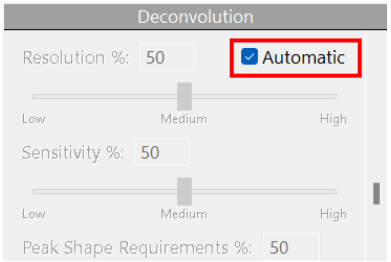
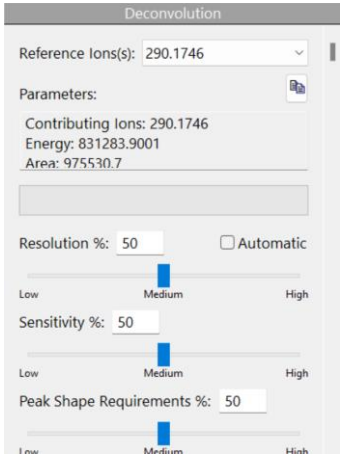
	Action	Result
7	To change the **Instrument Resolution**, go to the **Deconvolution Settings** bar and click the checkbox in the **Instrument Resolution** panel next to **Advanced**. 	The **Advanced Resolution Settings** popup appears. 
8	Click **Cancel** on the popup to close the dialog.	The popup closes.
9	Go to the **Deconvolution Settings** bar and deselect the checkbox next to **Automatic** in the **Deconvolution** panel. 	The **Resolution**, **Sensitivity** and **Peak Shape Requirements** become available for adjusting. The slider bars or numeric values can be used to control the deconvolution settings. As the deconvolution settings are changed, the number of peaks in the **Peaks Table** and **Peak Boxes** on the **Chromatogram** are changed. 
10	Click to reselect the checkbox next to **Automatic**. Select the exit icon (✕) on the **Deconvolution Settings** panel to hide the panel.	The application resumes automatic deconvolution for the peaks. The **Deconvolution Settings** panel becomes hidden.

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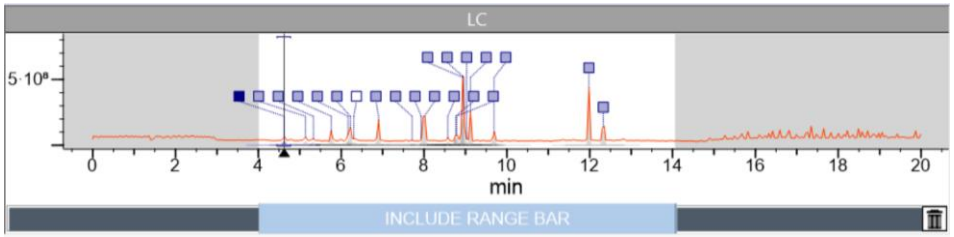
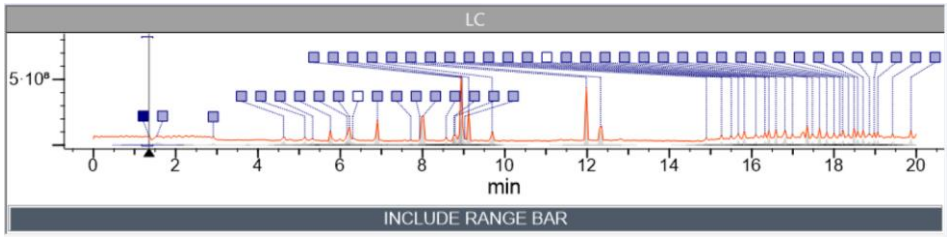
	Action	Result
11	Click the **Include Range Bar** with left mouse button and drag left/right to select a region for analysis. **Note***:* This can also be achieved by clicking on the **Include Range Bar** with the right mouse button. On the proceeding pop-up **Include Ranges** dialog, click **Add**. A space below **Low Range** will appear to manually input a value. Do the same below **High Range** to manually input a value. Click **OK**.	The **Include Range Bar** can be used to isolate analysis regions in the **Chromatogram.** Outside of these regions (shown in gray coloration on the **Chromatogram**), there is no deconvolution or additional analysis taking place: 
12	To remove the isolated regions from the **Include Range Bar**, click the trash can icon () on the right side of the **Include Range Bar**.	The isolated regions are removed from the **Chromatogram**, and the full chromatographic region is deconvoluted: 

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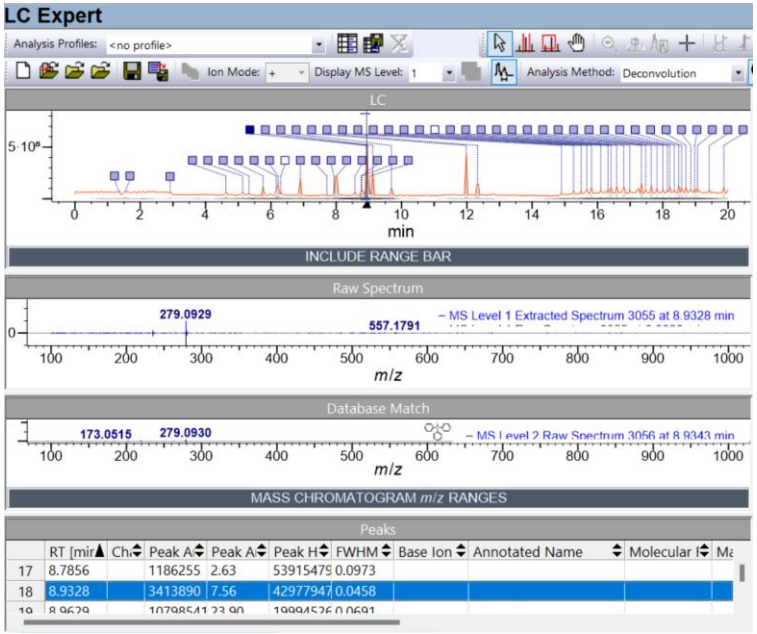
	Action	Result
13	Click on the **peak box** (☐) for the tallest peak (located at 8.93 min).	Upon peak selection for the peak at 8.93 min: • The **Peak Box** is shaded to confirm selection. • The **Peak Area** is shaded with blue coloration, displaying the deconvoluted peak area. • There is a **Bracket** above and below the peak, to visualize the retention time region for the peak. • The related row in the **Peaks Table** is highlighted. 

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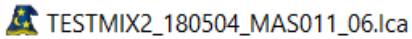
	Action	Result
14	Click on another row in the **Peaks Table** (*e.g.*, row 21).	The related peak in the **Chromatogram** becomes selected: • The **Peak Box** is shaded with a darker coloration. • The **Peak Area** is shaded. • The retention time region is indicated by the **bracket**.  **Note***:* If a peak's component can be identified by the user, its name can be manually entered into the **Peaks Table** by double clicking on the associated cell in the **Annotated Name** column.
15	To save the file, select **File > Save LC Expert File**.	An LC Expert Analysis file is saved to the location of your choosing.  This file can be reopened to re-analyze datasets, or continue processing in the future.

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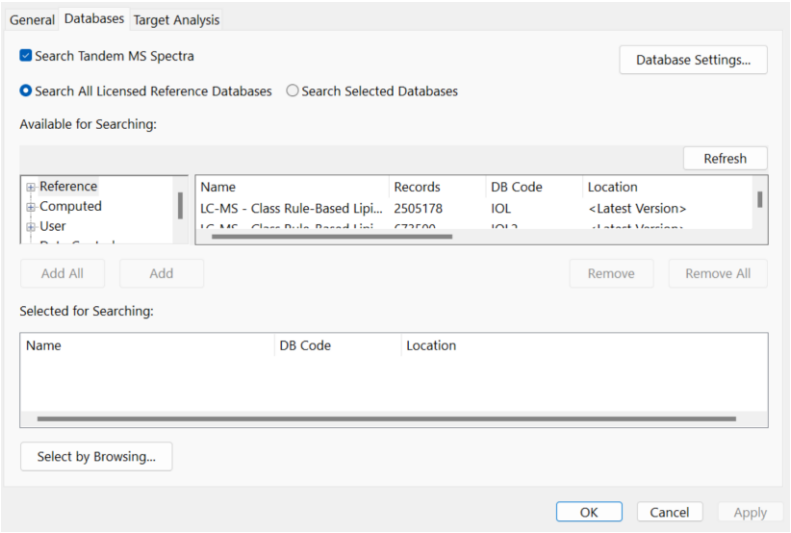
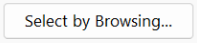
Example: Perform an Untargeted MS2 Search

This section explains how to perform an untargeted library search for MS/MS data.

	Action	Result
1	Continue using the chromatogram file from Step 2. In **LC Expert**, select **File > Settings**. In the **Settings** popup that appears, choose the **Databases** tab. Make sure the **Search Tandem MS Spectra** checkbox is selected. 	The **Settings** dialog opens.The **Databases** menu displays search settings:  The specific databases displayed in the panel below **Available for Searching** depend on the user license.
2	To choose all of the databases available for searching, select the radio button for **Search All Licensed Reference Databases**. Alternatively, select database(s) can be searched by using the radio button for **Search Selected Databases** and individually double clicking on the titles to add the desired libraries.	When the radio button for **Search All Licensed Reference Databases** is selected, then the **Selected for Searching** window is unavailable. When the radio button for **Search Selected Databases** is selected, then the **Selected for Searching** window is available. **Note***:* Specific available databases depend on the user's license. User databases can be added for searching by selecting the **Select by Browsing** button () and navigating to the desired database file (.sdbx).

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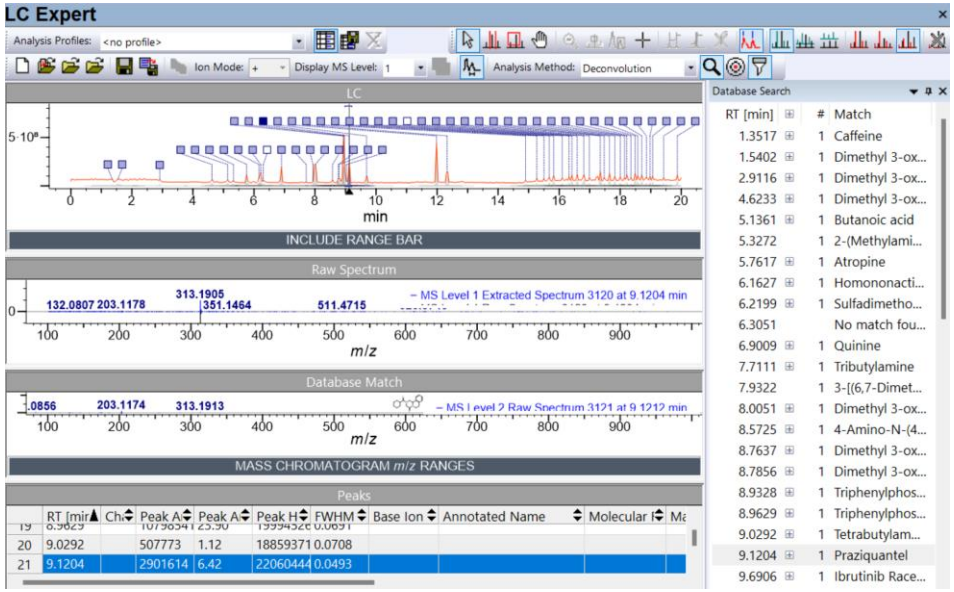
	Action	Result
3	Click **Apply** then **OK** to save any changes made in the **Settings** dialog. **Note***:* To stop the database search from taking place in the background within the software, deselect the related icon ( ) located on the **Standard Toolbar**, or turn off the database search setting from Step 1.	The **Settings** dialog is closed. In the **Database Search** panel on the righthand side: • The deconvoluted peaks were searched using the selected libraries. • The peak retention times (**RT [min]**) in the **Database Search** panel are aligned to the peaks in the **Peaks Table**. • Clicking on a row in the **Database Search** panel highlights the related row in the **Peaks Table**, and the peak in the **Chromatogram**. • The best search match for the MS2 spectrum is displayed as the top hit for each peak retention time. The software performs the Dot-Product (Cosine) search to match the MS/MS spectra against the applied databases. Using the default settings, the database search results are filtered by: • Matching ion polarity using the ion polarity applied in the **chromatogram**. • Precursor ion m/z.  **Note***:* Specific database matches in the **Database Search** panel will depend on the configured user settings and the applied licensed databases available for searching.

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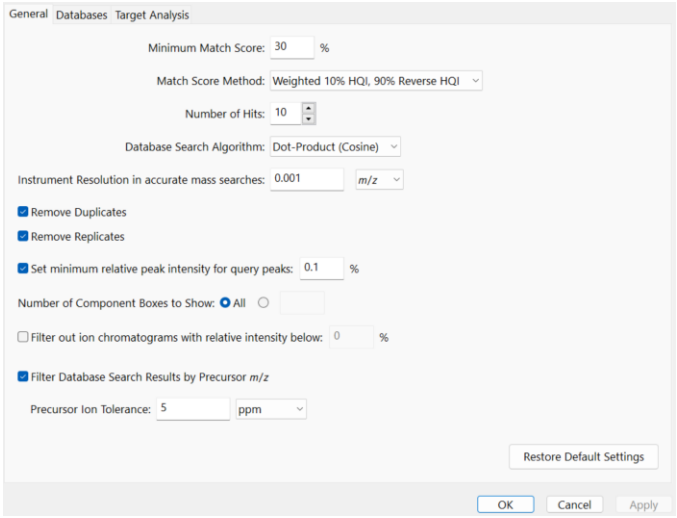
	Action	Result

4	Go to **File > Settings**. In the **Settings** dialog that launches, remain on the **General** tab. Ensure that the **Filter Database Search Results by Precursor m/z** checkbox is selected, and the value is set to “5 ppm”.	In the **Settings** dialog that is launched, there are settings for the execution of the cosine similarity search:
		• **Match Score Method** defines whether HQI or R.HQI (Reverse HQI) should be prioritized.
		  ○ By default, R.HQI is more heavily weighted in **LC Expert**.
		  ○ The scoring method can be changed using the dropdown menu.
		• **Instrument Resolution in accurate mass searches** controls the accuracy of the MS/MS search matching to a database spectrum.
		• The checkbox (when selected) for **Filter Database Search Results by Precursor m/z** forces the database search results to be filtered by the precursor m/z of the query spectrum:
		  ○ If deselected, query results will not be filtered by precursor m/z and all m/z values will be accepted.
		• The checkbox for **Set minimum relative peak intensity for query peaks** filters peaks below the defined height threshold for user data in MS/MS matching. Peaks below the threshold will not be matched to a database spectrum.
		• **Precursor Ion Tolerance** provides the match tolerance for Precursor ion m/z.
		 

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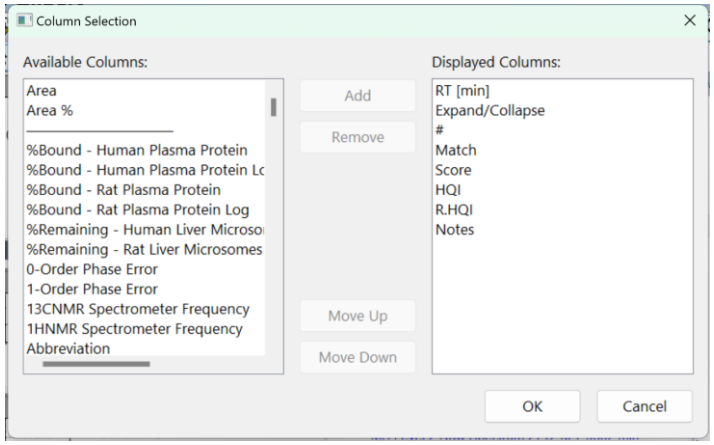
	Action	Result
5	Click **OK** in the **Settings** dialog window to close the dialog. Then in the **Database Search** panel, click on the **expand** icon (⊞) next to a search match.	The top 10 best matches are displayed (less than 10 matches will populate if <10 spectra were matched according to the search settings). Specific matches depend on the configured search settings and the applied licensed databases:  **Note***:* Double click on the cell next to the desired row in the **Notes** column to add a note.
6	Right click on the **Database Search Table** and select **Edit Component Table Columns**.	The **Column Selection** dialog window is launched. 

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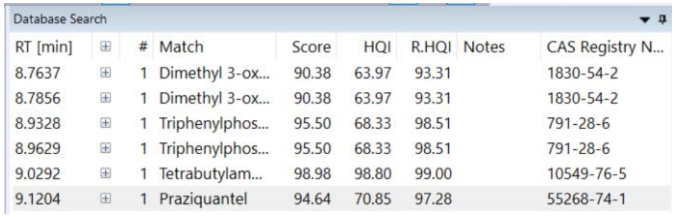
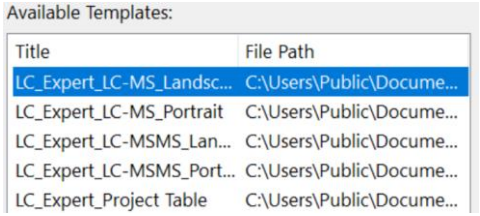
	Action	Result
7	Select a column in the **Available Columns** list, *e.g.,* "CAS Registry Number". Click on the **Available Column** and select **Add**. Click **OK** to proceed.	The column is added to the **Displayed Columns** cell at the bottom of the list. Upon clicking **OK**, the dialog window is closed. The new column is added as a column to the **Database Search Table**.  Database Search RT [min] # Match Score HQI R.HQI Notes CAS Registry N... 8.7637 1 Dimethyl 3-ox... 90.38 63.97 93.31 1830-54-2 8.7856 1 Dimethyl 3-ox... 90.38 63.97 93.31 1830-54-2 8.9328 1 Triphenylphos... 95.50 68.33 98.51 791-28-6 8.9629 1 Triphenylphos... 95.50 68.33 98.51 791-28-6 9.0292 1 Tetrabutylam... 98.98 98.80 99.00 10549-76-5 9.1204 1 Praziquantel 94.64 70.85 97.28 55268-74-1 Additional columns can be added to the **Database Search Table**, to add helpful metadata when scanning search results. **CTRL+F** can be used to quickly search the match results.
8	Go to **File > Edit Report Templates** to import report templates.	The **Report Templates** dialog window is launched. If there are templates already in the dialog, skip the next step.  Report Templates Available Templates: Title File Path No Preview Available. New... Edit... Remove Add... Set As Default Clear Default Default Template: Close
9	Click **Add** then navigate to: **"C:\Users\Public\Documents\Wiley\ KnowItAll\Report Templates\LC Expert"**. Select all 5 report templates in the folder. Click **Open**.	The report templates are added to the **Available Templates** box.  Available Templates: Title File Path LC_Expert_LC-MS_Landsc... C:\Users\Public\Docume... LC_Expert_LC-MS_Portrait C:\Users\Public\Docume... LC_Expert_LC-MSMS_Lan... C:\Users\Public\Docume... LC_Expert_LC-MSMS_Port... C:\Users\Public\Docume... LC_Expert_Project Table C:\Users\Public\Docume...

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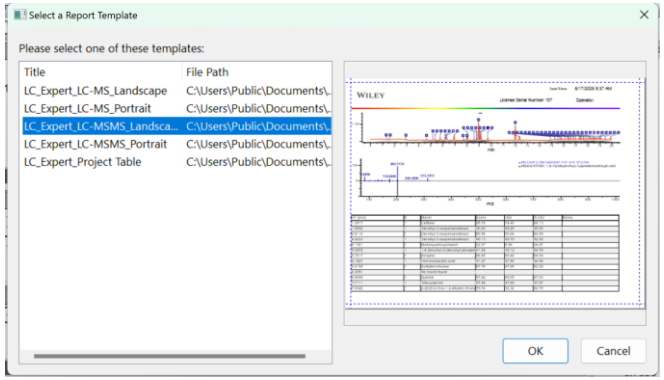
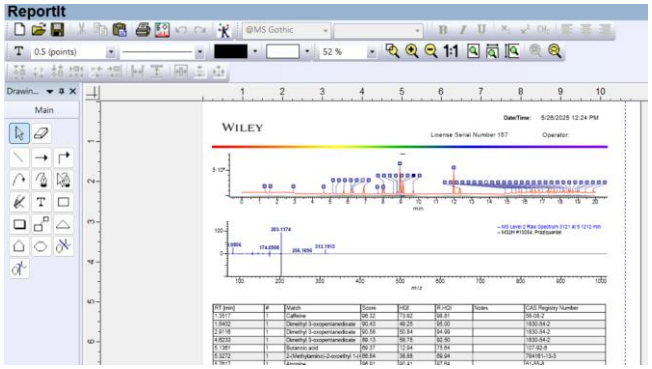
	Action	Result
10	Click **Close** on the dialog window. Using the **Transfer To** (Transfer to:) bar, click **ReportIt** (ReportIt).	The **Select a Report Template** dialog window is launched with the active chromatogram previewed in the selected report template. 
11	Click to select the **"LC_Expert_LC-MSMS_Landscape"** template. Then click **OK** button (OK) on the dialog window.	The **Report** is generated, displaying the MS/MS search information from the **Database Search Table**: 
12	Use the **Back Arrow** icon ( ) to return to **LC Expert**.	**LC Expert** is active.

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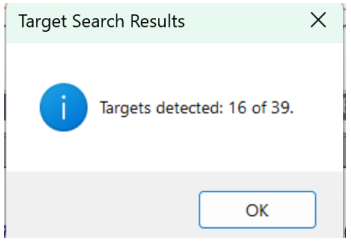
Example: Targeted Analysis Searching

This section describes how to perform a targeted analysis search within the chromatogram file. LC Expert's Targeted Analysis workflow searches the chromatogram for a list of compounds in a target list using the exact mass of the targets.

	Action	Result
1	Continue with the **Chromatogram** from the previous section. Click on the **Targeted Analysis** icon ( ) or choose **Analysis > Targeted Analysis**. Navigate to **"C:\Users\Public\ Public Documents\Wiley\KnowItAll\ Samples\LC-MS\"**. Select **"Pharmaceutical Compounds.sdbx"** and click **Open**. After reading the **Target Search Results** popup, click **OK** to close the popup window. **Note**: The sdbx file imports a list of compounds as targets to search for in the chromatogram. Individual compounds can also be searched for by transferring a structure from **ChemWindow** into **LC Expert** using the **Transfer To** bar.	Upon selecting the **Targets** button, a **File Explorer** window opens. After opening the sdbx file: • The **Target Search Results** popup provides the number of found compounds in the chromatogram.  • The **Peaks Table** updates with the detected compound information: ○ **Annotated Name** is the compound record name from the sdbx file. ○ **Base Ion [m/z]** is the base ion from the MS1 extracted spectrum. ○ **Molecular Formula** gives the chemical formula for the identified compound (*i.e.,* target). ○ **Match Score** is the match score calculation using the target's accurate mass and the calculated exact mass. ○ **Mass Accuracy** is the mass accuracy calculation using the target's accurate mass and the calculated exact mass. ○ **Detected Adducts** is the adduct which is detected in the extracted spectrum and applied in the exact mass calculation. ○ **Calculated Mass** is the exact mass for the target with the detected adduct. ○ **Database Name** is the name of the imported sdbx file used as the target list. ○ **Record ID** is the specific record ID from the sdbx file to identify the detected target.

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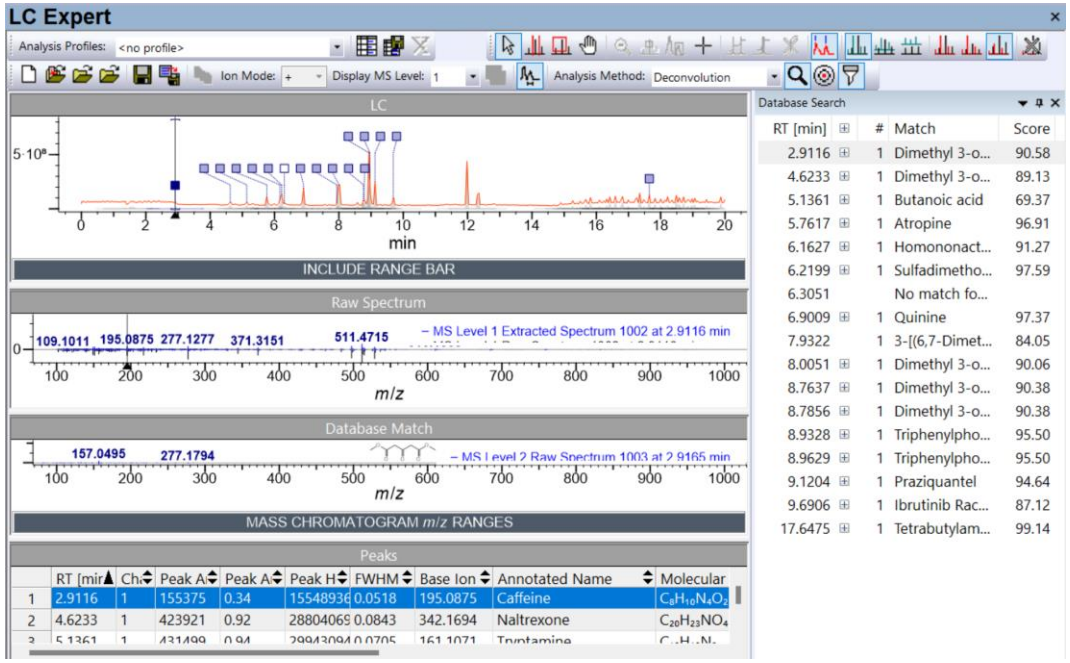
	Action	Result
2	The **Peaks Table** can be filtered/unfiltered by selecting/deselecting the **filter** icon ( ) located on the **Standard Toolbar**.	When the filter is selected: • The **Peaks Table** hides any row that does not have a detected target. • The **Peak Boxes** on the **Chromatogram** are filtered to match the displayed rows in the **Peaks Table**. • The **Database Search** table will only display matches for peaks visible in the **Peaks Table**. 

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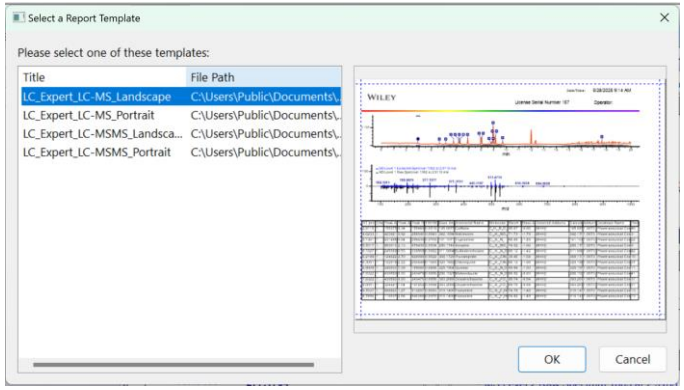
	Action	Result
3	Select the **Transfer To** (Transfer to:) bar to transfer to **ReportIt** (ReportIt).	The **Select a Report Template** dialog window is launched: 
4	Select "**LC_Expert_LC-MS_Landscape**" on the **Select a Report Template** dialog window. Click **OK**.	The **Report** is generated displaying the accurate mass information from the **Peaks Table**:  **Note***:* The active display for the **Chromatogram** will be retained in the report (*e.g.*, filtered versus unfiltered chromatogram and tables).

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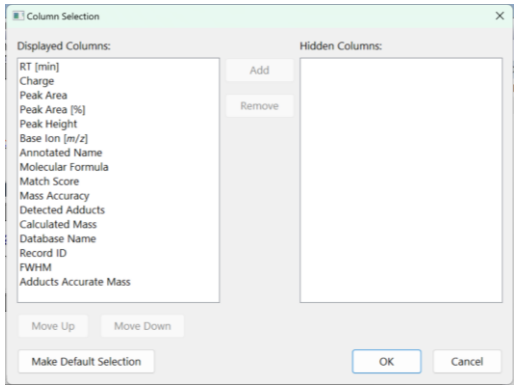
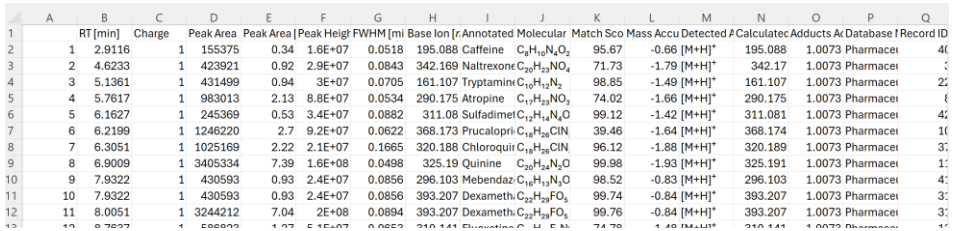
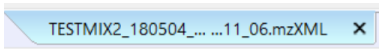
	Action	Result
5	Use the **Back Arrow** icon ( ) to return to **LC Expert**.	**LC Expert** is active.
6	The **Peaks Table** can be modified by right clicking directly on the table and selecting **Edit Column Display**. Click **Cancel** to close the dialog window.	The **Column Selection** dialog window is launched, which allows for hiding columns and rearranging their order. 
7	The **Peaks Table** can be copied into a document by right clicking on the table and selecting **Copy to Clipboard**.	The table is copied into a document:  The active display for the **Peaks Table** will be retained in the report (*e.g.*, filtered versus unfiltered table).
8	In **LC Expert**, close the **chromatogram** file by clicking the X icon ( ) on the bottom tab. 	The **chromatogram** is closed.

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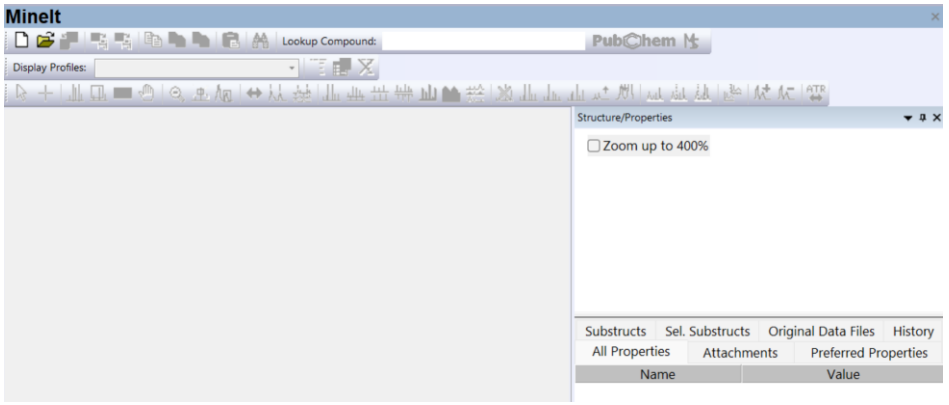
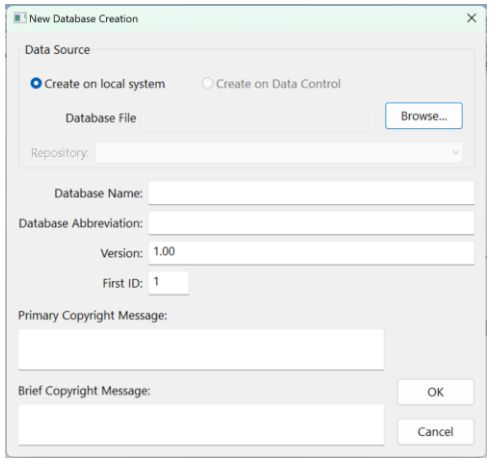
Example: Create a User Database for Targeted Analysis Search

This section describes how to prepare the user database that is used for targeted analysis searching, such as the sample file in the previous section.

	Action	Result
1	To execute targeted analysis searching within a **Chromatogram**, a user database with compounds is required for searching. Begin by opening the **MineIt** application (), typically found in the **Data** toolbox.	The **MineIt** application is displayed: 
2	Create a user database: Select **Database > New**.	The **New Database Creation** dialog window is launched. 

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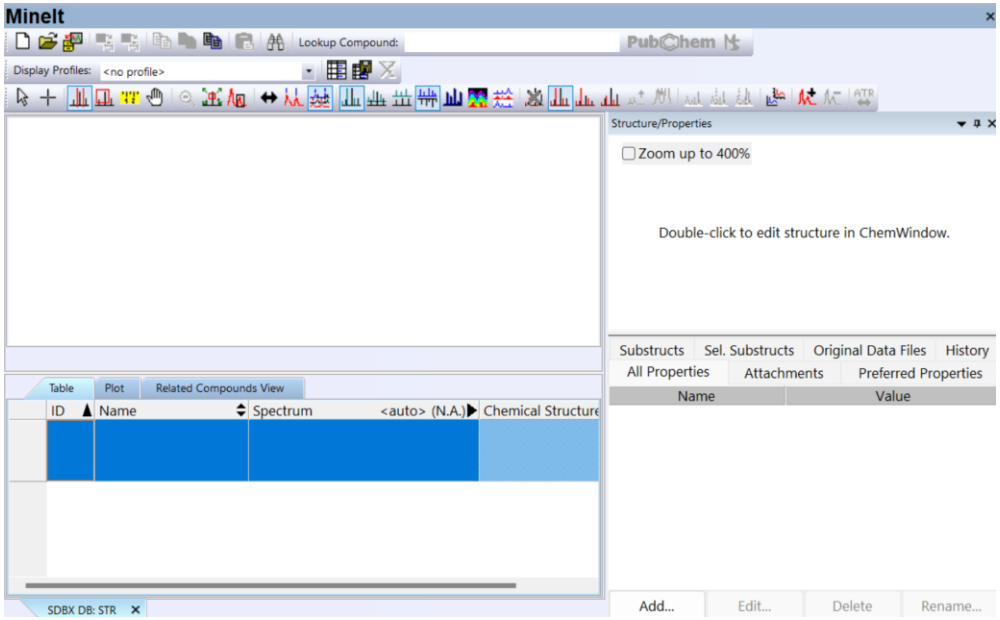
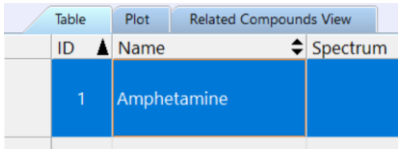
	Action	Result
3	Fill in the required database information: • **Database File** save location. Click **Browse** to select the location and give the file a name. • **Database Name**. • **Database Abbreviation** (at least 3 alphanumeric characters). Click **OK** to proceed.	A blank database is opened in **MineIt**: 
4	Double click on the **Name** cell in the first blank row of the **Table**.	The **Property** dialog window is launched.
5	Enter “Amphetamine” in the proceeding popup. Press **OK** to save.	Amphetamine is displayed as the **Name** property in the first record (ID 1). 

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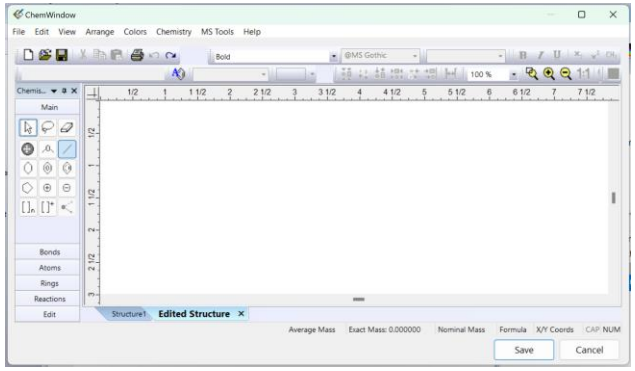
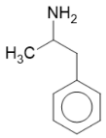
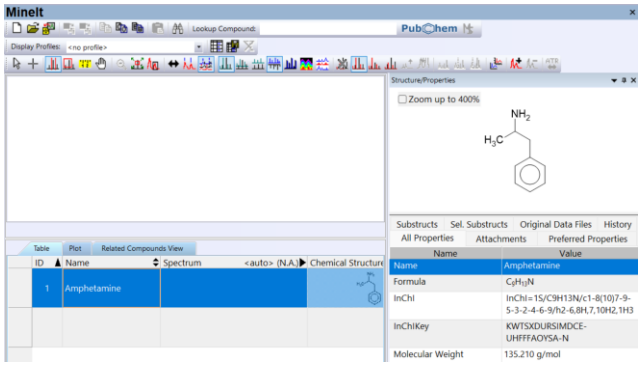
	Action	Result
6	Double click in the **Structure/Properties** window where it reads **"Double-click to edit structure in ChemWindow"**.	**ChemWindow** is launched as a popout window: 
7	Copy the given SMILES string to clipboard: c1(ccccc1)CC(N)C Then select **Edit > Paste Special** and select **SMILES.**	The struture for amphetamine appears in **ChemWindow**. 
8	Click **Save** to proceed.	The structure is displayed in the **MineIt** user database: 

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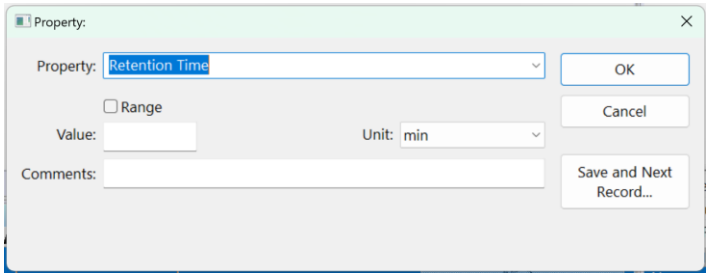
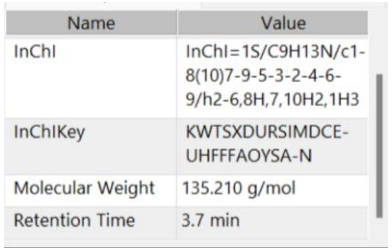
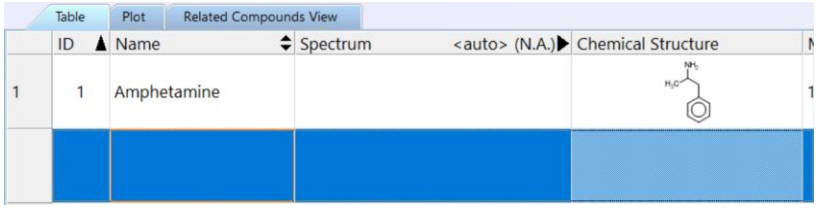
	Action	Result
9	To add the compound's expected **Retention Time**, navigate to the **Structure/Properties Table** and click **Add** button (Add...). Type **Retention Time** in the **Property** dialog window.	The **Retention Time** property value appears in the dialog window: 
10	Enter the value "3.7" in the **Property dialog window** in the cell next to **Value**. Retain the default units of "min". Select **OK** to proceed.	The **Retention Time** is added to the record.  **Note**: **Retention Time** is not required for the database file. If no value for **Retention Time** is included, then the all time points will be scanned for the compound.
11	Add more compounds to the database. Begin by clicking on the next row in the **Table**.	Upon clicking on the next row in the **Table**, it becomes highlighted to depict that it is active: 

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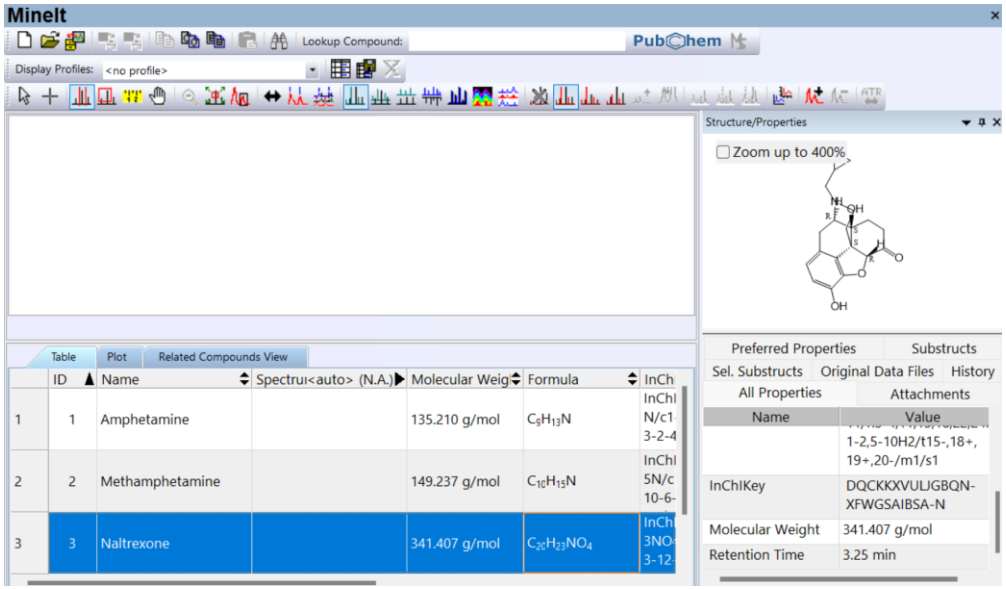
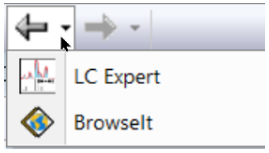
	Action	Result
12	Add the following compounds into the user database by repeating steps 4-11. **Name**: Methamphetamine **SMILES**: c1cccc(c1)C[C@H](C)NC **Retention Time**: 3.85 min **Name**: Naltrexone **SMILES:** *Provided in the Result cell* **Retention Time**: 3.25 min	**Naltrexone SMILES:** O[C@]12[C@@]3(N(CC[C@@]11C4=C(C(=CC=C4C3)O)O[C@]1(C(CC2)=O)[H])CC1CC1)[H] Methamphetamine and Naltrexon are added to the database.  This user database of in-house compounds can be used for Exact Mass searching in **LC Expert**.
13	Mouse over to the **Previous Application** icon ( ) and click on the down button ( ). Choose **LC Expert** to return to the selected application.  	**LC Expert** application is active.

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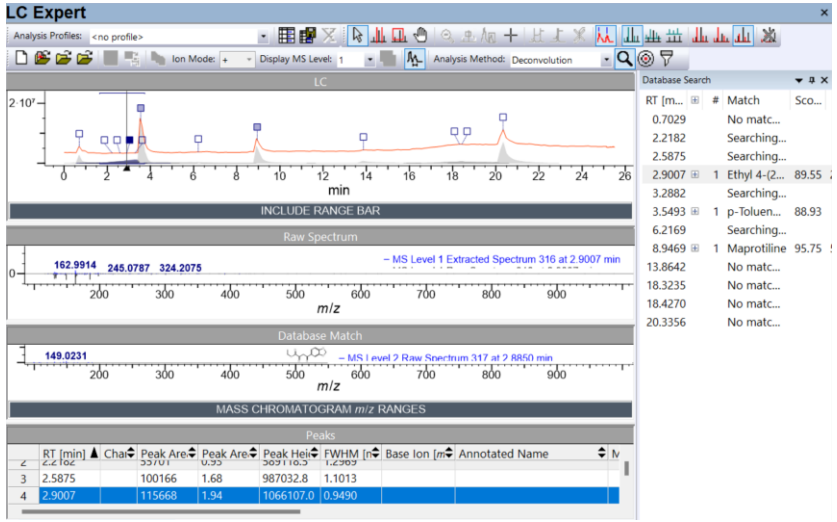
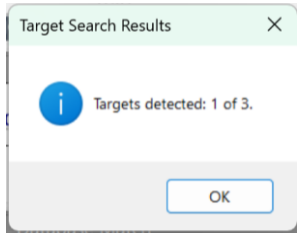
	Action	Result
14	Using the open raw file icon ( ) located in the **Standard Toolbar** of **LC Expert**, navigate to **"C:\Users\Public\Public Documents\Wiley\KnowItAll\Samples\LC-MS\"**. Open one of the raw **Chromatogram** files: • **Compound_1_POS.mzML** • **Compound_2_POS.mzML** • **Compound_3_POS.mzML**	The selected **Chromatogram** opens in **LC Expert** (*e.g.,* Compound_1_POS.mzML) and deconvolutes:  **Note:** Specific results in the **Database Search Table** depend on user settings and databases available in user license.
15	Click on the **Targets** icon ( ) or choose **Analysis > Targeted Analysis**. Navigate to and select the structure database file saved in Step 3 of this section. After reading the **Target Search Results** popup, click **OK** to close the popup window.	One target from the database is found in the chromatogram:  • Amphetamine is detected in "Compound_1_POS.mzML". • Methamphetamine is detected in "Compound_2_POS.mzML". • Naltrexone is detected in "Compound_3_POS.mzML".

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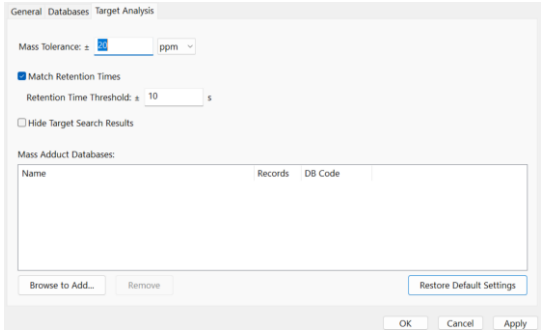
	Action	Result

16	**Note***:* User settings for **Targeted Analysis** can be updated by selecting **File > Settings** and choosing the **Target Analysis** tab.	The available **Targeted Analysis** settings are:
		• **Mass Tolerance** for accurate mass deviation tolerance.
		• **Retention Time Threshold** for the retention time tolerance in seconds.
		  ○ **Match Retention Times** checkbox turns this setting on/off.
		• **Hide Target Search Results** checkbox prevents the **Targets Search Results** popup from appearing when selected.
		• **Mass Adducts Databases** allows the user to import additional adducts in an sdbx file, to be used in the targeted analysis search.
		  ○ By default, only [M+H] or [M-H] adducts are scanned for in the **Chromatogram.**
		  ○ To add additional adducts, click the **Browse to Add** button and navigate to "Additional Adducts.sdbx" found in the LC-MS samples folder (see next step for more information).
		 

17	**Note***:* Users can create their own adduct libraries by modifying the sample sdbx file **"Additional Adducts.sdbx"** from the LC-MS samples folder (**"C:\Users\Public\Public Documents\Wiley\KnowItAll\Samples\LC-MS\"**), or by creating their own database file following the standards provided in this sample file.	To prepare an adduct library, the following information is required:
		• **Name:** used for adduct labels.
		• **Formula:** used to calculated the isotopic adduct radio.
		  ○ **KnowItAll** has been designed to recognize adduct losses by incorporation of a subtraction (-) symbol, *e.g.,* adduct [M-H] is be depicted as -H, and adduct [M+Cl-H] is depicted as Cl-H.
		• **Selected Ion Charge:** gives the ion charge and polarity where the adduct should be scanned.
		  ○ *E.g.,* The adducts [M-H] and [M+Cl-H] should be -1 and -2 correspondingly.
		  ○ Positive ions do not need a plus (+) symbol because the adduct is assumed to be positive (unless specified with a – symbol, denoting a negative adduct).

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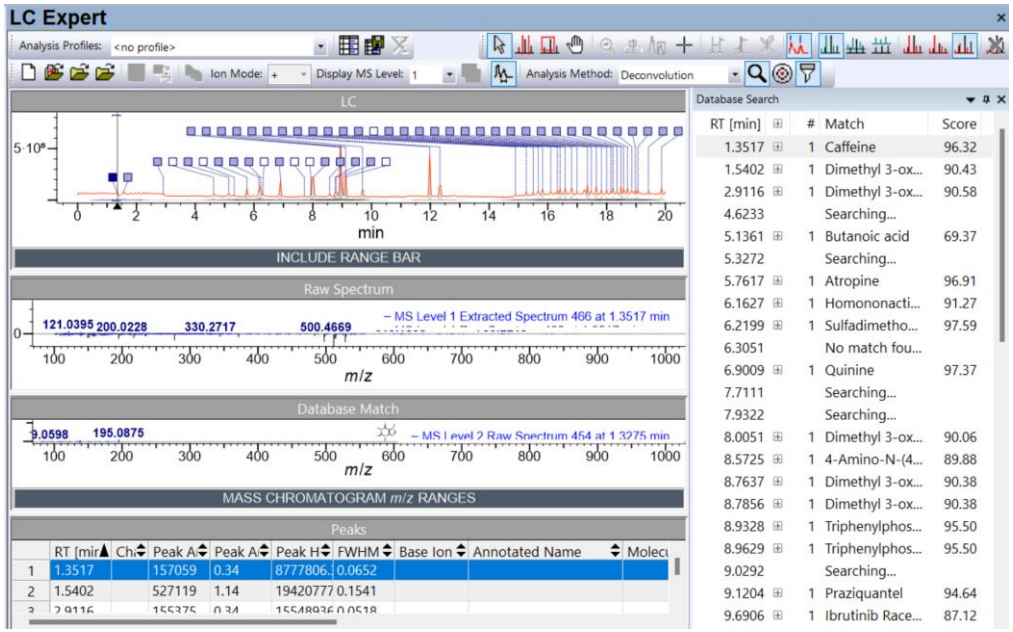
Example: Manual Deconvolution

In some cases, a user may prefer to perform their own peak selection for deconvolution, which can be performed using LC Expert's manual analysis method.

	Action	Result
1	Begin by opening an LC-MS chromatogram file in **LC Expert**. This example will use "**TESTMIX2_180504_MAS011_06 .mzXML**" located in **"C:\Users\Public\Public Documents\Wiley\KnowItAll\Samples\LC-MS\"**.	The **chromatogram** opens in **LC Expert** application. The **Analysis Method** is visible on the **Standard Toolbar**, and is set to the last applied method: 

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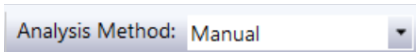
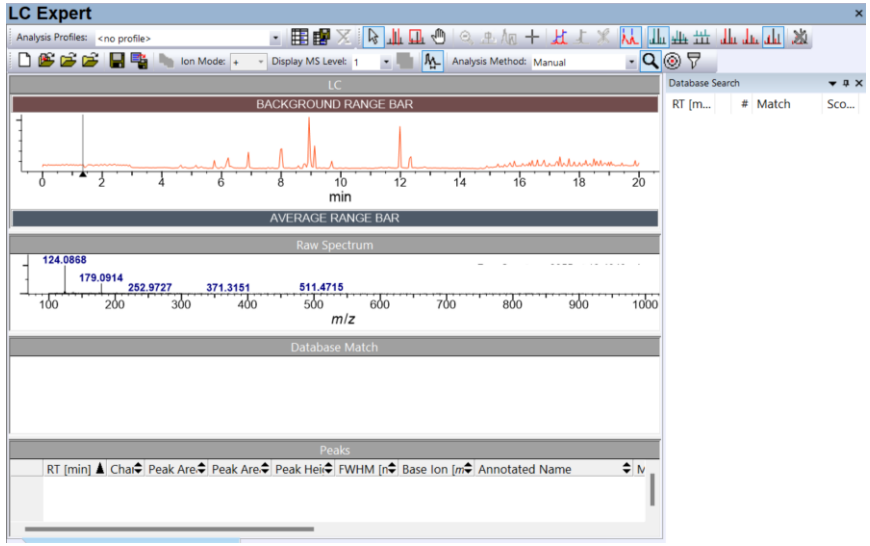
	Action	Result
2	Using the **Analysis Method** dropdown menu on the **Standard Toolbar**, select **Manual**. 	**LC Expert** enters **Manual** mode: • All previously deconvoluted peaks become cleared from the **Peaks Table** and **Database Search Table**. • The **Average Range Bar** appears below the **Chromatogram** panel. Use this to select a series of MS scans to be averaged as the analysis region. • The **Background Range Bar** appears above the **Chromatogram** panel. Use this to select a background region to subtract from the analysis region. 

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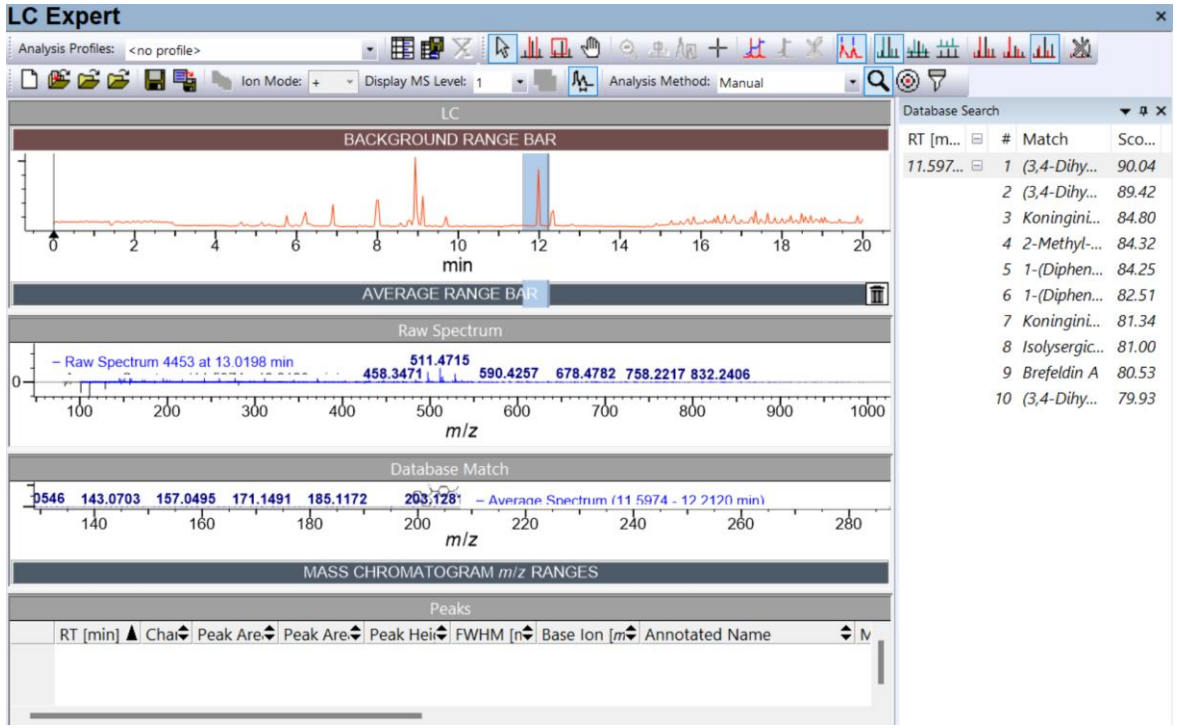
	Action	Result
3	On the **chromatogram**, navigate to a desirable peak for analysis. Using the **Average Range Bar**, click the left mouse button down and drag horizontally down the bar to select a region across a peak. Release the mouse button to accept the region.	An analysis region is highlighted using blue coloration on the **Chromatogram**. An extracted MS spectrum is created across the selected region. Additionally, if the region contains MS/MS scans, then an average of the MS/MS scans will be searched in the libraries and populate in the **Database Search Table**:  **Note:** To search a single MS/MS scan instead of an average, do not select a region using the **Average Range Bar**. Instead, click directly on the chromatogram to choose a single timepoint for searching.

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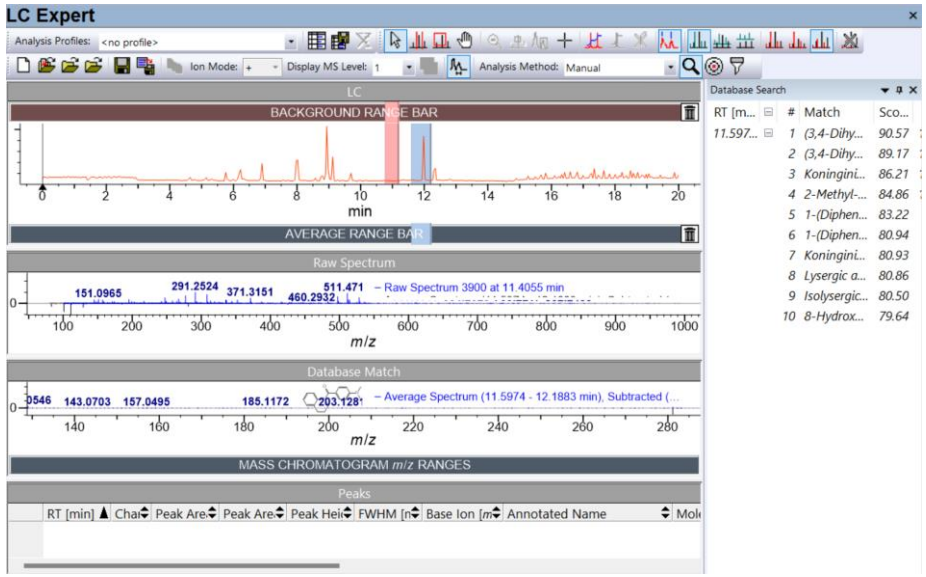
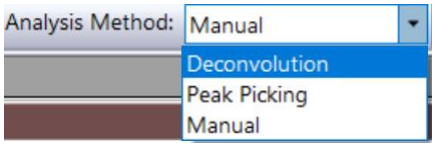
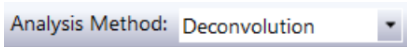
	Action	Result
4	On the **chromatogram**, navigate to a desirable baseline region to represent the background. Using the **Background Range Bar**, click the left mouse button down on the and drag horizontally down the bar to select a region. Release the mouse button to accept the region.	A background region is highlighted using red coloration on the **Chromatogram**, representing the baseline to subtract from the analysis region. The search results in the **Database Search Table** will refresh according to the updated region selection. 
5	**Note: LC Expert** will retain the last used **Analysis Method**. To prepare for future steps in this documentation, change the **Analysis Method** back to **Deconvolution.** 	The **chromatogram** becomes automatically deconvoluted, as seen in Step 1 of this section. 

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LC-MS/MS Spectrum Searching Methods

How to use KnowItAll SearchIt to Apply Advanced Algorithms for Spectrum Searching

Purpose

These exercises demonstrate how to use KnowItAll SearchIt to tune search methods for single MS/MS spectral searches.

Objectives

These exercises will teach you how to:

- Apply MSforID search algorithm
- Single spectrum search methods: Adaptive Search, Precursor Ion Filtering
- Use Peak m/z Search method when searching computed libraries

Background

KnowItAll has vast tools for LC-MS/MS spectral searches. In addition to the deconvolution tools in LC Expert, users can search single spectra in SearchIt application. Here they gain access to even more search algorithms and search controls. This includes MSforID search algorithm for high accuracy LC-MS searching, patented adaptive searching, tools for precursor ion filtering, and more.

Training Files Used in This Lesson

- Folder files in C:\Users\Public\Documents\Wiley\KnowItAll\Samples\LC-MS

KnowItAll Applications Used

- KnowItAll LC Expert
- KnowItAll SearchIt
- KnowItAll MineIt

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MSforID Searching

Introduction to MSforID Searching

The many challenges for preparing tandem MS search libraries and algorithms for unknown compound identification are well known and documented. Nonetheless, search tools and databases remain a critical part of the tandem MS workflow. The **MSforID** search method was designed to address these challenges, such as demonstrating a robustness against instrumental variability when searching quality databases and a high tolerance to variability in peak fragmentation patterns (*i.e.,* between the correct database match versus its experimental spectrum).[3] **MSforID** was positively evaluated using different instruments (*e.g.,* QqTOF, QqLIT, QqQ, LIT, LIT-FTICR and QTRAP) by different manufacturers and in different laboratories.[2-4]

The approach for **MSforID** searching is to compare the search query against a library of compounds where multiple CID spectra exist for each compound record. The compound records contain multiple spectra measured at different collision energies creating the series of spectra for the compound. The **MSforID** search algorithm then compares the query spectra to the *series* of CID spectra for the compound (Figure A). This is dissimilar to typical databases search algorithms that compare the query to a *single* spectrum per match (Figure B).

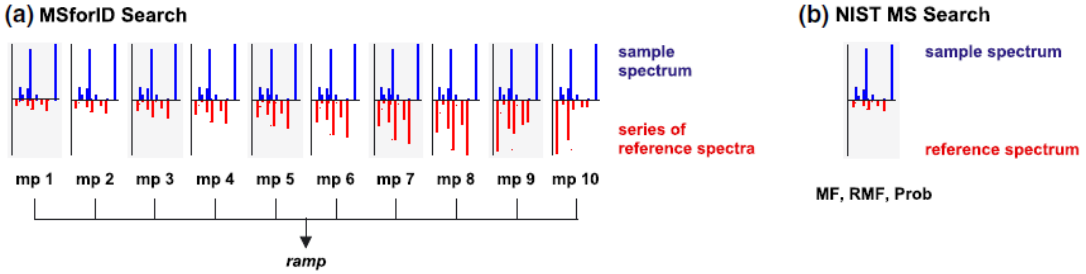

Figure A) MSforID and **B)** NIST MS Search identify search methods. (*Reprinted from Ref. 3*)

The MSforID Algorithm

The **MSforID** algorithm measures the average similarity of a query spectrum to the series of compound reference spectra. It is a probability-based matching algorithm that analyzes:

- The mass deviation for the precursor ion between the query spectrum and the database compound record.
- The number of matching fragments between the query and the database spectrum.
- The mass deviations and intensity differences for matching fragments.

For each search result, the algorithm calculates the **Average Match Probability (AMP)** for the compound's database record that contains the series of spectra. The **Relative Average Match Probability (RAMP)** is subsequently calculated, which is the normalized **AMP** value compared to the search results for the specific query (*i.e.*, from 0-100).[3] Search results are presented in **KnowItAll**'s **MineIt** by descending **RAMP** values, and the highest **RAMP** value is considered the best match. A **RAMP** value of >40.0 is considered a very good match score.[5]

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Using the MSforID Search Tool in KnowItAll

Three search methods are available for **MSforID** searching in **KnowItAll**'s **SearchIt** application: (1) **Standard Search** (default), (2) **Composite Search**, and (3) **Direct Search**. The recommended search algorithm is the **Standard Search**, which applies the main published algorithm.[3] The **Standard Search** compares all spectra in the database record to the query spectrum (as in Figure A) to compute the **RAMP**. Differently, the **Composite Search** compares the single averaged spectrum for all spectra in the database record to the query spectrum using an adapted version of the **MSforID** algorithm. The averaged spectrum is calculated in real-time during the search, and the **Composite Search** can be faster when using very large databases. The **Direct Search** is a revised edition of the **MSforID** algorithm that aims to remove false positives from the hitlist.

Preparing In-House MSforID Libraries

The "Wiley Registry of Tandem Mass Spectral Data – MS for ID" database contains highly curated spectra for use with **MSforID** searching in **SearchIt**. To prepare user libraries in-house that are highly curated for accurate **MSforID** searching, the **MSforID** database standards[1] are recommended:

1. Measure mass spectra for the standard compounds at multiple collision energies (*e.g.,* from 5 to 50 eV).
2. Filter low abundant signals in the standard spectra (*e.g.,* less than 0.01%).
3. Prepare database records in MineIt using one precursor ion (*e.g.,* M+H). If compound spectra detected from different adducts are available for your library, separate these out into different records (*e.g.*, M+H spectra in one record and M+Na spectra in a second record).

References & Additional Reading on MSforID

1. M. Pavlic, K. Libiseller, H. Oberacher. Combined use of ESI-QqTOF-MS and ESI-QqTOF-MS/MS with mass-spectral library search for qualitative analysis of drugs. *Anal. Bioanal. Chem.* **2006**, *386*, 62-82. doi: 10.1007/s00216-006-0634-8
2. H. Oberacher, M. Pavlic, K. Libiseller, B. Schubert, M. Sulyok, R. Schuhmacher, E. Csaszar, H. Köfeler. On the inter-instrument and the inter-laboratory transferability of a tandem mass spectral reference library: 1. Results of an Austrian multicenter study. *J. Mass Spectrom.* **2008**, *44*, 485-493. doi: 10.1002/jms.1545
3. H. Oberacher, M. Pavlic, K. Libiseller, B. Schubert, M. Sulyok, R. Schuhmacher, E. Csaszar, H. Köfeler. On the inter-instrument and the inter-laboratory transferability of a tandem mass spectral reference library: 2. Optimization and characterization of the search algorithm. *J. Mass Spectrom.* **2008**, *44*, 494-502. doi: 10.1002/jms.1525
4. H. Oberacher, W. Weinmann, S. Dresen. Quality evaluation of tandem mass spectral libraries. *Anal. Bioanal. Chem.* **2011**, *400,* 2641-2648. doi: 10.1007/s00216-010-4598-3
5. H. Oberacher, G. Whitley, B. Berger, W. Weinmann. Testing an alternative search algorithm for compound identification with the 'Wiley Registry of Tandem Spectral Data, MSforID'. *J. Mass Spectrom.* **2013**, *48*, 497-504. doi: 10.1002/jms.3185

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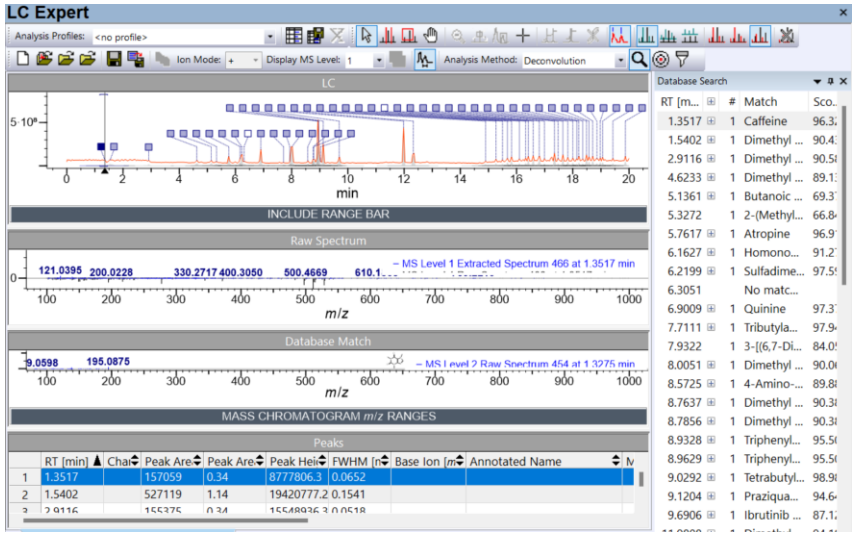
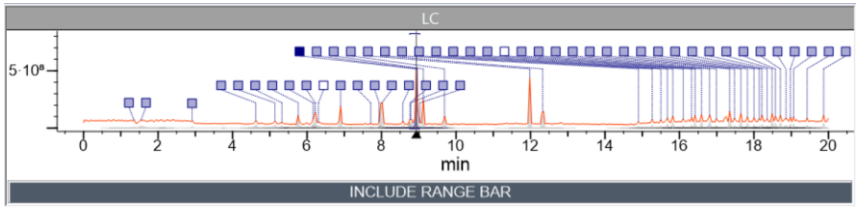
Example: MSforID Search

This section describes how to execute an MS/MS library search using the MSforID search algorithm using the SearchIt application.

	Action	Result
1	Using the **Open Raw LC-MS Data File** icon () located on the **Standard Toolbar** in **LC Expert**, navigate to **"C:\Users\Public\Public Documents\Wiley\KnowItAll\Samples\LC-MS\"**. Open **"TESTMIX2_180504_MAS011_06 .mzXML"** (**C:\Users\Public\Public Documents\Wiley\KnowItAll\Samples\LC-MS**).	The **Chromatogram** opens in **LC Expert** and deconvolutes: 
2	Click in the **Peaks Table** on the row with RT equal 8.93 min to select the peak (*i.e.*, row 18 using default deconvolution settings).	The peak at 8.93 min becomes selected in the **Chromatogram**: 

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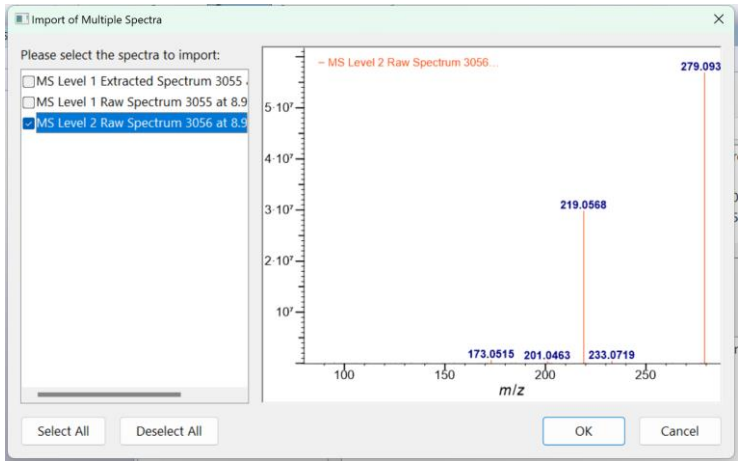
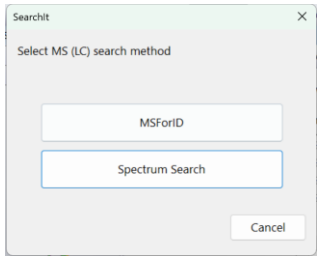
	Action	Result
3	On the **Transfer to** bar (Transfer to:), select **SearchIt** (SearchIt).	The **Import of Multiple Spectra** dialog window appears. The "MS Level 2 Raw Spectrum" option is selected by default:  **Note***:* This dialog is used to specify which MS spectral scan will be transferred using the **Transfer to** bar.
4	On the **Import of Multiple Spectra** dialog window, retain the default selection "MS Level 2 Raw Spectrum". Select **OK** to continue.	The **SearchIt** import dialog window appears with 2 different search options: • "MSForID", to open a new **MSforID** search tab in **SearchIt**. • "Spectrum Search", to use an alternative search algorithm (*e.g.*, cosine, adaptive search, partial spectrum search, *etc.*). 

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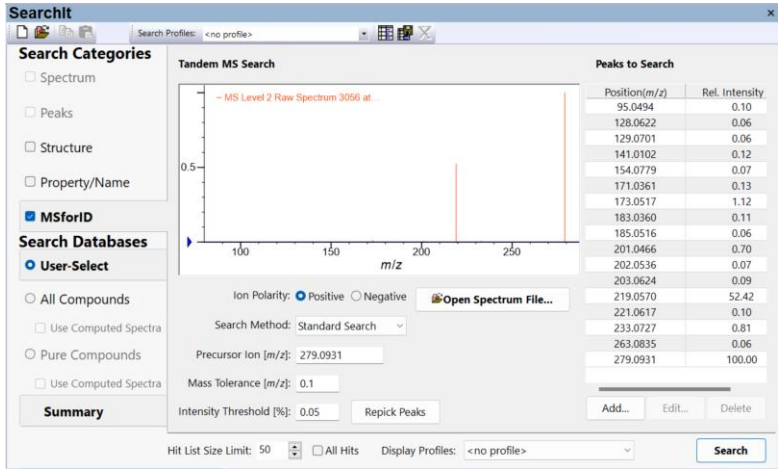
	Action	Result

5	Select **MSforID** on the **SearchIt** dialog window.
	
	Note*:* If desired, the volume of peaks included in the search can be modified using the **Intensity Threshold (%)** setting. Select **Repick Peaks** button (Repick Peaks) to update the **Peaks to Search** list using the updated threshold. The triangle symbol (▶) reveals the minimum peak height.
		The MS2 raw spectrum opens in **SearchIt**'s **MSforID Search** window. The **MSforID Search** window prepopulates the information:
		• **Ion Polarity** which is the ion polarity information in the raw chromatogram file, if included in the raw file.
		  ○ If this information is not included in the raw file, then **positive** will be selected by default, and this could be updated to **negative** by selecting the opposite radio button.
		• **Search Method** is the specific **MSforID Algorithm** that will be applied in the search. The last use search will be selected as the menu option.
		  ○ **Standard Search** (default)
		  ○ **Composite Search**
		  ○ **Direct Search**
		• **Precursor Ion (m/z)** is the MS2 scan's precursor ion information, if included in the raw file.
		• **Mass Tolerance (m/z)** parameter sets the tolerance for MS spectrum peak m/z deviations.
		• **Intensity Threshold (%)** parameter sets the minimum peak height for the MS spectrum peaks.
		 
		Note*:* A popup will display on the **Warning** dialog window if the raw spectrum is not detected to be an MS2 spectrum. This could be because the raw file does not contain MS Level information (*e.g.*, such as imported .jdx files), or it is the wrong MS Level (*e.g.,* MS Level = 1). Click **Confirm** to bypass the warning and import the MS spectrum into the window or **Cancel** to stop the process.

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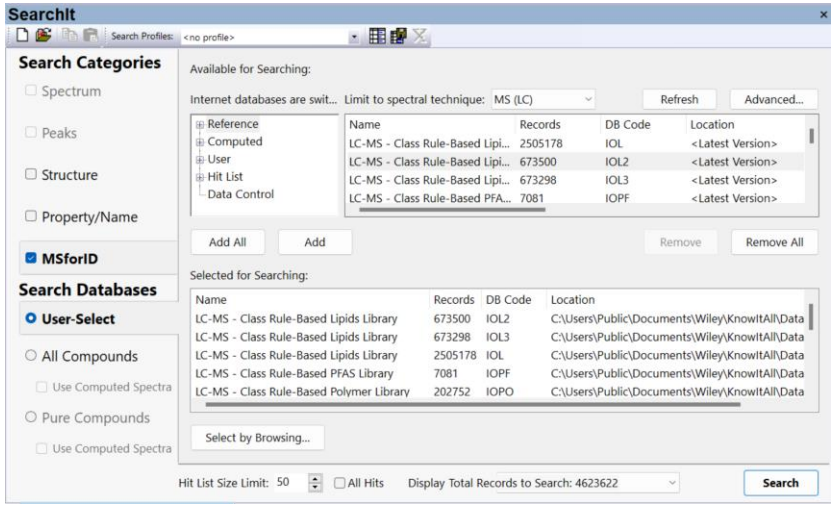
	Action	Result
6	In the **MSforID Search** window, change the **Mass Tolerance** value to “0.01" m/z.	The **Mass Tolerance** is decreased: Mass Tolerance [*m/z*]: 0.01 **Note***:* The **Mass Tolerance** value plays an impactful role in the calculation and final search results.
7	Under the **Search Databases** section, click on **User-Select** option. Search Databases User-Select Next to **Limit to spectral technique** option, choose **MS (LC)** using the dropdown menu. Limit to spectral technique: MS (LC) Then select databases for searching using by clicking on the specific database row in the database list, followed by the **Add** button (Add).	The databases selection dialog window is displayed. In the example below, all available LC-MS databases were added to **Selected for Searching** section.  **Note:** Available LC-MS databases depend on the specific user license.

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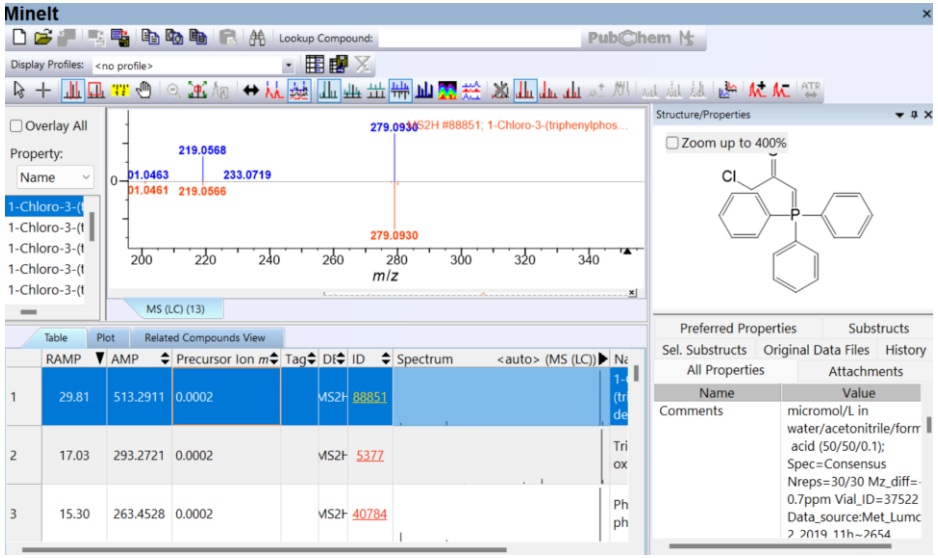
	Action	Result
8	Select **Search** button (Search) to execute the search.	The best match for the spectrum query is displayed in **MineIt**. The **Table** displays the columns: • **AMP (Average Match Probability),** which is the average probability that the reference compound record could be the query spectrum. • **RAMP (Relative Average Match Probability)**, which is the **AMP** value relative to the total search results. ○ By default, the search results will be organized by decreasing **RAMP** value. • **Precursor Ion m/z Difference** which is the m/z difference between the query's precursor ion and the database record.  **Note***:* Each row in the search results **Table** represents a match for the compound record's *series* of spectra, and each compound record can only be returned once as a match result. In the **Table**, each compound record's MS spectra will retain the order in which they exist in the database, *i.e.,* the first spectrum in the series will always be displayed first in the search results.

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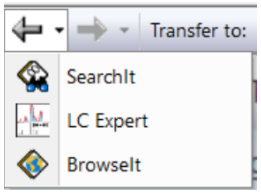
	Action	Result
9	Navigate to the **Previous Application** icon and click on the down button ( ). Choose **LC Expert** to return to the selected application. 	**LC Expert** application is active.

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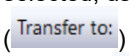
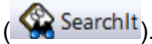
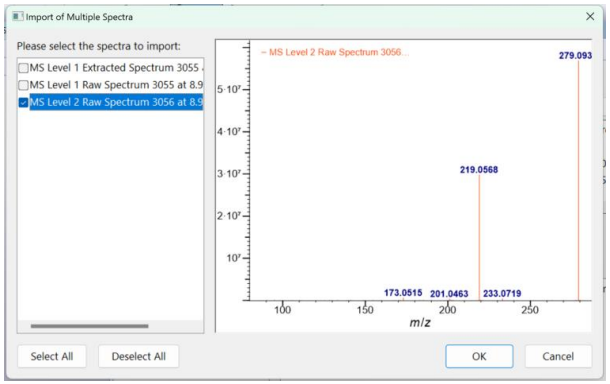
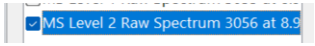
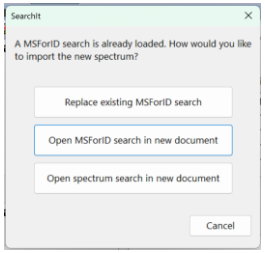
Example: Spectrum MS/MS Searches using SearchIt

This section describes how to execute an MS/MS library search using spectral search algorithms in SearchIt application: spectrum search and patented adaptive searching.

	Action	Result
1	Continue with the **Chromatogram** from the last section. In **LC Expert**, with the scan from the previous Step 2 still selected, use the **Transfer to** bar () to send the spectrum to **SearchIt** ().	The **Import of Multiple Spectra** dialog window appears. The "MS Level 2 Raw Spectrum" option is selected by default:  **Note***:* This dialog is used to denote which MS spectral scan should be transferred using the **Transfer to** bar.
2	On the **Import of Multiple Spectra** dialog window, retain the default selection "MS Level 2 Raw Spectrum". Select **OK** to continue. 	The **SearchIt** import dialog window appears with 3 different search options: • "Replace existing MSforID search", to override an existing search in **SearchIt**. • "Open MSforID in new document", to open a new search window in **SearchIt**. • "Open spectrum search in new document", to use an alternative search algorithm (*e.g.*, cosine, adaptive, etc.). 

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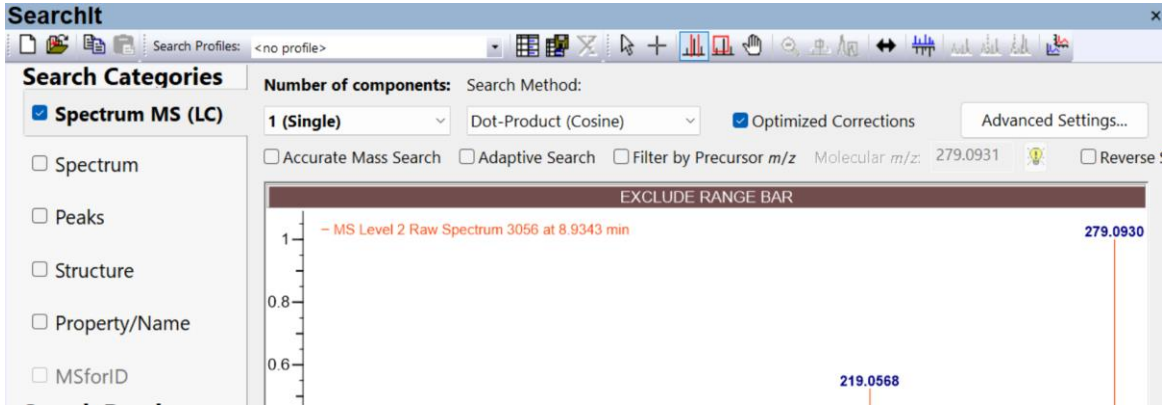
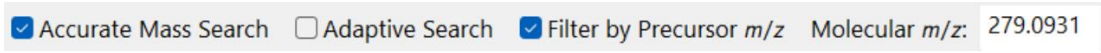
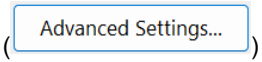
	Action	Result
3	Select “Open spectrum search in new document”.	The MS2 raw spectrum opens in **SearchIt**’s **Spectrum Search** dialog window. **SearchIt** recognizes that the spectrum search type is LC-MS and is labelled as **Spectrum MS (LC)**:  For further control of the spectrum search: • **Include Range Bar** and **Exclude Range Bar** allow for including/excluding specified areas on the spectrum. • **Search Method** allows for changing the search algorithm, such as from Dot-Product (Cosine) to Peak m/z Search (which is further described in a later section).
4	In the **Spectrum MS (LC)** window, select the checkbox next to **Accurate Mass Search** and **Filter by Precursor m/z.**	**Accurate Mass Search** is selected:  • **Accurate Mass Search** retains high resolution information included in the spectrum query and database record when performing the search. • **Filter by Precursor m/z** will filter the database search results by the precursor ion information from the raw spectrum. **Note:** Click **Advanced Settings** button () to modify the **Precursor Ion Tolerance** and **Instrument Resolution** settings for Accurate Mass searches.

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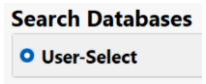
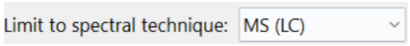
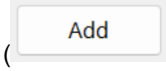
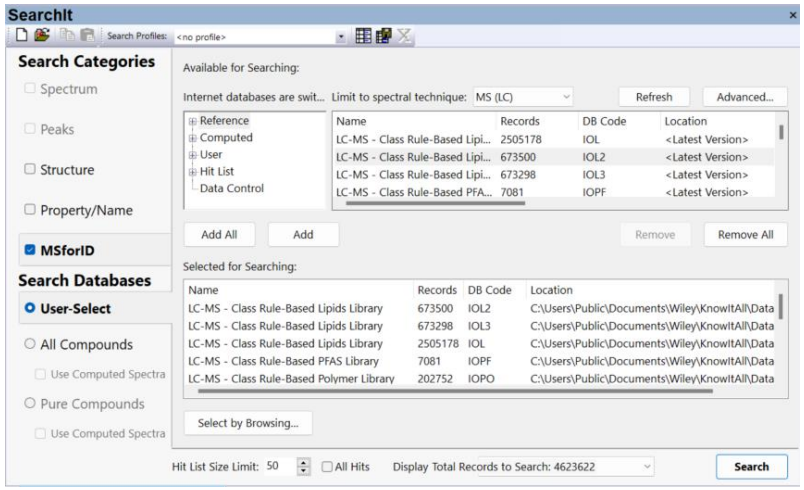
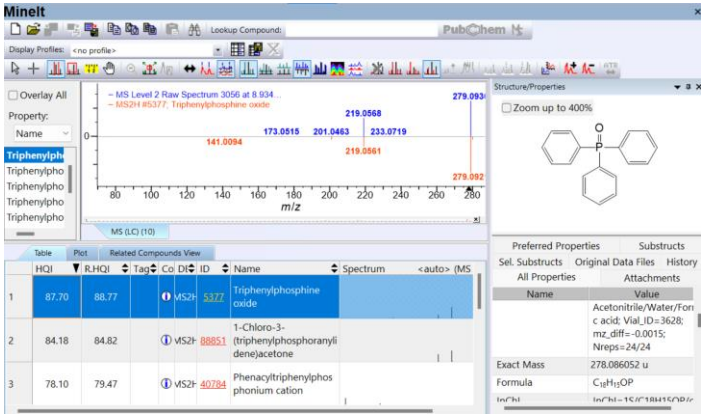
	Action	Result
5	Under the **Search Databases** section, click on **User-Select** option.  Next to **Limit to spectral technique** option, choose **MS (LC)** using the dropdown menu.  Then select databases for searching using by clicking on the specific database row in the database list, followed by the **Add** button ().	The databases selection dialog window is displayed. In the example below, all available LC-MS databases were added to **Selected for Searching** section.  **Note:** Available LC-MS databases depend on the specific user license.
6	Click **Search** to execute.	The best match for the spectrum query is displayed in **MineIt**: 

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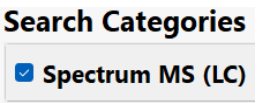
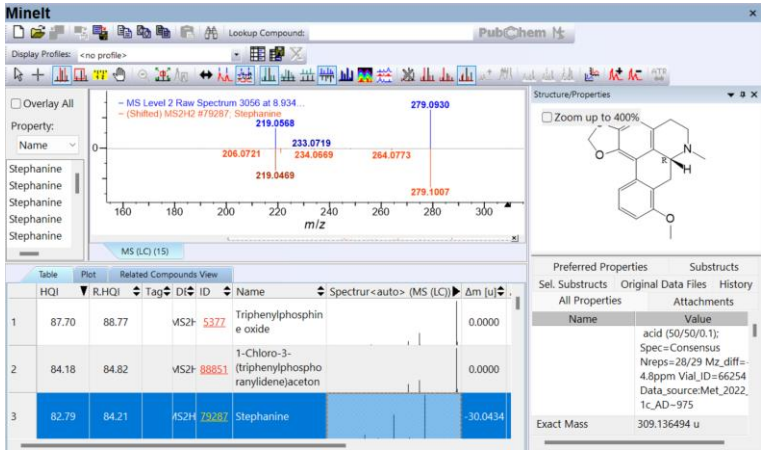
	Action	Result
7	Use the **Previous Application** arrow () to return to **SearchIt**.	**SearchIt** application is opened with the previous query loaded.
8	Click on **Spectrum MS (LC)** to modify the spectrum search settings.  Select the **Adaptive Search** checkbox.	The **Adaptive Search** is selected, and the **Filter by Precursor m/z** checkbox becomes deselected:  **Adaptive Search** method allows for scanning the spectrum peaks for available functional groups or molecular replacements between the query spectrum and the database spectrum, extending the database library to similar compounds that are not in the available spectral space. **Note***:* **Adaptive Search** method is available for low resolution data by deselecting the **Accurate Mass Search** checkbox.
9	Click **Search** to execute.	The best match for the spectral query is displayed in **MineIt**:  For the **Adaptive Search** results, regarding the spectrum query and the database record: • **Δm** column gives the difference in compound mass between the query and the database compound. • **Δm info** column contains a selectable info icon () that informs on the peak shifts that occurred to create the query result. • **Replacement** column gives the group replacement if known.

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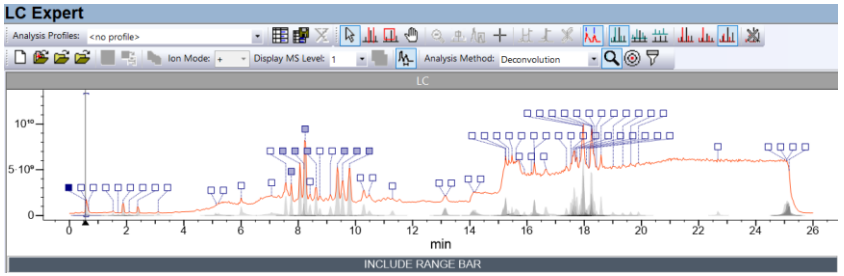
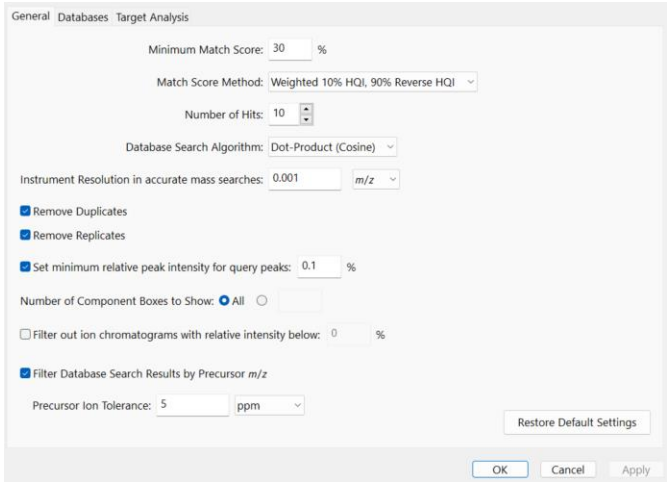
Example: Peak m/z Search Method

This section describes how to more effectively search computed libraries using Peak m/z Search algorithm. This algorithm matches the most intense peaks from query spectra to database records by Exact Mass independently of the peak intensity. This algorithm is recommended for use with Computed Libraries, where many of the database records are MS spectra with peaks at 100% intensity.

	Action	Result
1	Open a LC-MS chromatogram file in **LC Expert**. 	The **chromatogram** opens in **LC Expert**:  The example chromatogram above is a file featuring Lipids.
2	Choose **File > Settings**.	The **Settings** dialog is launched: 

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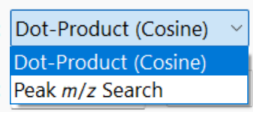
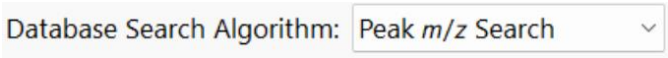
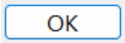
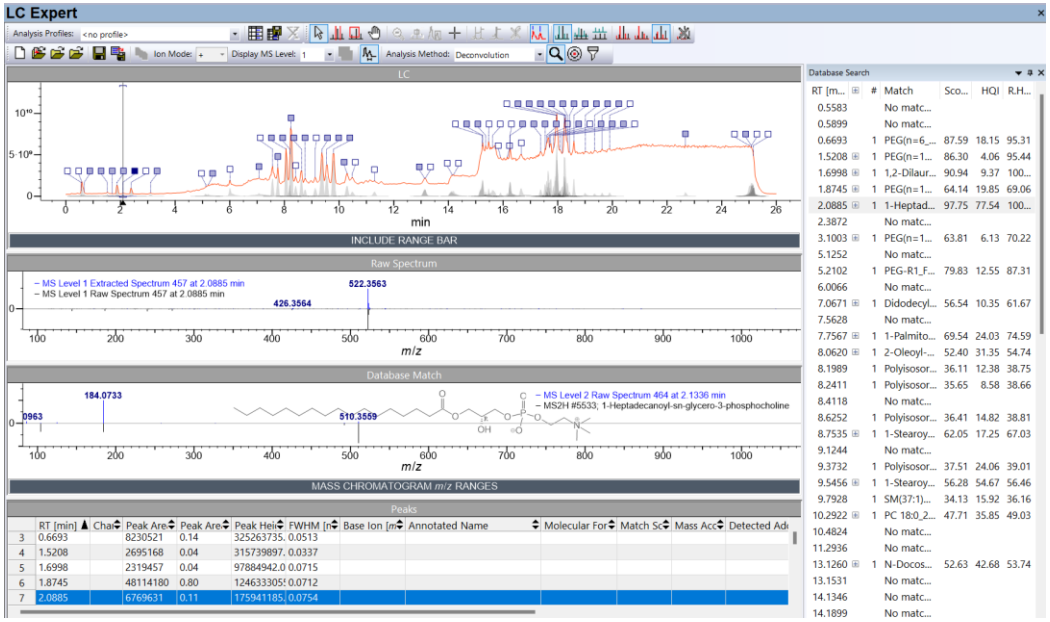
	Action	Result
3	Remain on the **General** tab. Change the **Database Search Algorithm** to **Peak m/z Search** using the dropdown menu. 	**Peak m/z Search** algorithm is selected in the dialog:  **Note:** Confirm that the checkbox for **Set minimum relative peak intensity for query peaks** is selected. When turned on, query peaks below the minimum threshold will not be matched to database spectra. 
4	On the **Settings** dialog, click **OK** button () to accept the changes.	The **Database Search Table** will refresh the search matches according to the changed algorithm. 

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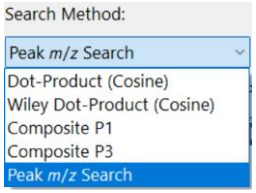
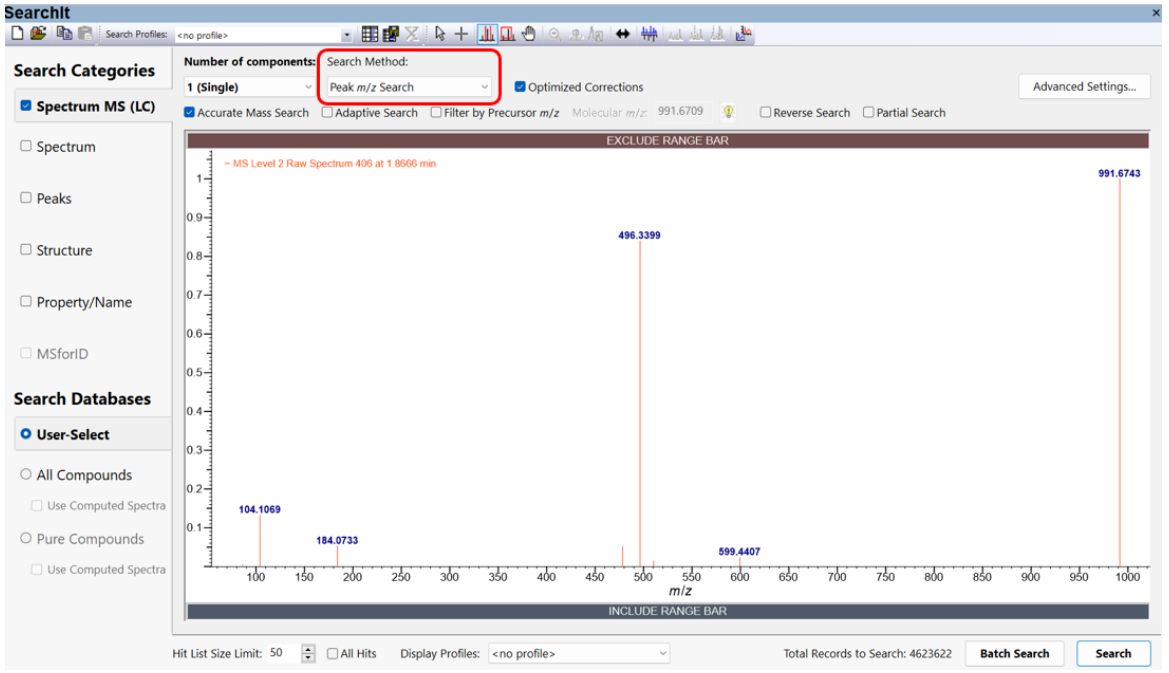
	Action	Result
5	**Note:** The **Peak m/z Search** algorithm is also available in **SearchIt**. To apply, follow instructions for a spectrum search in section **"Spectrum MS/MS Searches using SearchIt"** and change the **Search Method** to **Peak m/z Search** using the dropdown menu. 	In **SearchIt's Spectrum Search** interface, **Peak m/z Search** is selected as the search algorithm: 

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Batch LC-MS Processing

How to use KnowItAll LC Expert to Perform Automatic LC-MS Component Identifications

Purpose

This exercise demonstrates how to use KnowItAll LC Expert to process and analyze a series of raw LC-MS chromatograms.

Objectives

These exercises will teach you how to:

- Use KnowItAll LC Expert project mode to process a folder of raw chromatograms

Background

Analyzing raw chromatograms can be a time-consuming process, especially if multiple runs of similar samples are acquired. KnowItAll's LC Expert in project mode allows users to process, align, and identify folders of files in a single submission, then review result summaries as a project file.

Training Files Used in This Lesson

- N/A

KnowItAll Applications Used

- KnowItAll LC Expert

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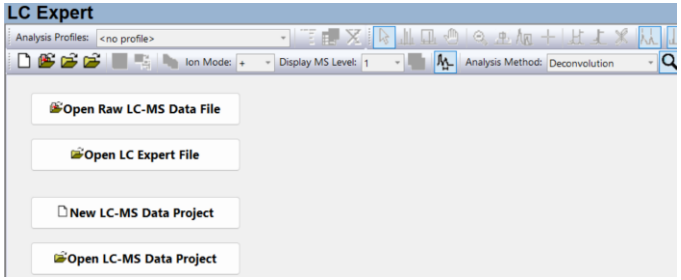
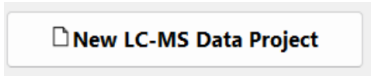
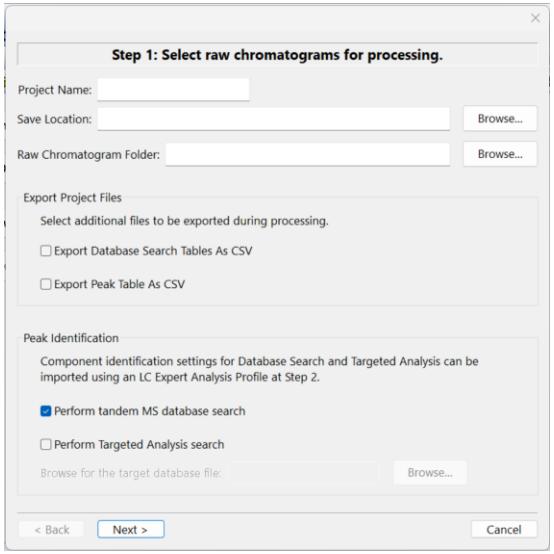
Example: Batch LC-MS Processing

Batch LC-MS processing can be used when a series of related chromatographic samples were acquired. If the samples are not related, then the singular processing workflow should be applied.

	Action	Result
1	Open **LC Expert**.	**LC Expert** application is displayed with no files previously opened: 
2	Click the **New LC-MS Data Project** button. 	The **Batch Processing** dialog window appears on **Step 1: Select raw chromatograms for processing** step:  This dialog is used to enter details for batch processing of file folder.

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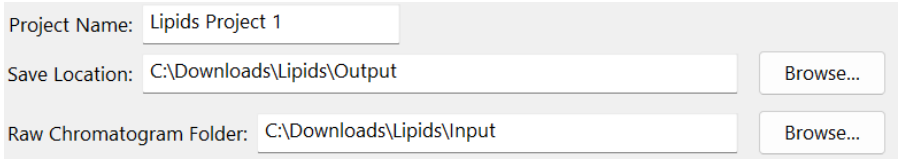
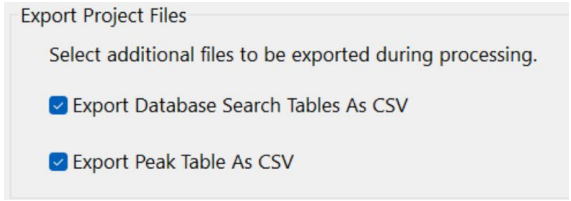
	Action	Result
3	Using the popup dialog window, enter the following details: • **Project Name**: a name for the project file • **Save Location**: click Browse button to choose the output file location • **Raw Chromatogram Folder**: click Browse button to choose the input LC-MS file folder	Project details have been filled out to give the project file a name, output location, and input file location: 
4	In the section **Export Project Files**, choose exportation settings for the individual chromatogram files. Decide if individual chromatogram tables should be exported as csv: • The checkbox for **Export Database Search Table As CSV** exports a **Database Search Table** per chromatogram file. • The checkbox for **Export Peak Table As CSV** exports a **Peaks Tables** per chromatogram file.	For each submitted project, a project file will be generated (.lcp) file that summarizes the aligned picked peaks for the series of chromatograms. Each individual chromatogram file will be saved as a file (.lca) to enable post-processing review. Additionally, the checkboxes in the **Export Project Files** section will export the corresponding tables (.csv) for the individual chromatogram files. 

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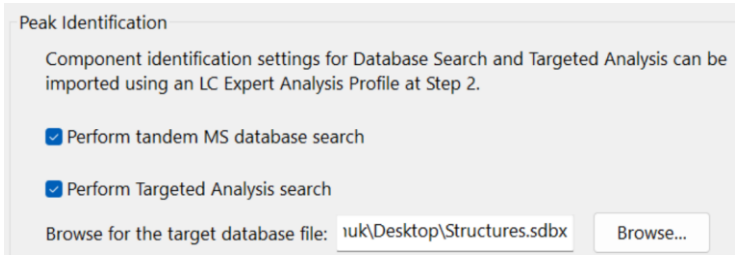
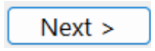
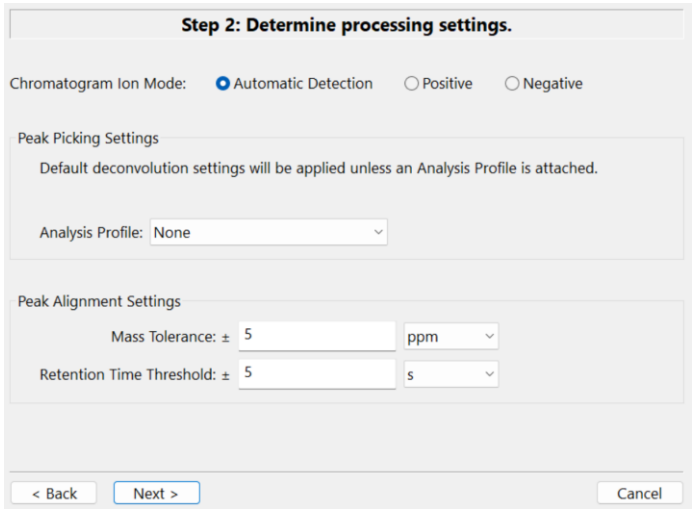
5	In the section **Peak Identification**, choose component identification requirements. • The checkbox for **Perform tandem MS database search** will require the deconvoluted peaks to be searched in the LC-MS libraries. • The checkbox for **Perform Targeted Analysis search** allows for attaching a Targeted List as .sdbx file for target identification. See training section “Create a User Database for Targeted Analysis Searching” for more information on library preparation.	The configured settings below enable component identification. **Note**: Specific database search settings are controlled at a subsequent step in the dialog. For the series of chromatogram files, when performing an MS/MS database search and/or targeted analysis search, the component with the highest match score according to the specific algorithm will be reported as the identified component in the sumary project file. 
6	Click **Next >** button to proceed in the dialog. 	**Step 2: Determine processing settings** is displayed on the dialog. Here, specific chromatogram processing and database matching controls are submitted: 

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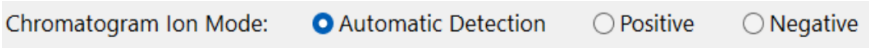
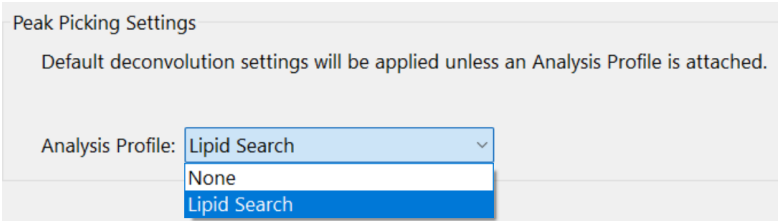
7	The dialog section **Chromatogram Ion Mode** allows for overriding the automatic detection of the ion mode.	**Automatic Detection** selection is retained:  **Note**: This control could be used for control if the chromatogram files contain both ion modes.
8	The dialog section **Peak Picking Settings** enables attaching of an **Analysis Profile**. **Note**: An **Analysis Profile** is not required. Choose "**None"** to bypass. For more information on how to create an **Analysis Profile**, follow the next step (otherwise bypass it).	An **Analysis Profile** is attached to the dialog. The **Analysis Profile** gives user control over: • Peak deconvolution settings • Applied peak picking algorithm (Dot Product (Cosine) or Peak m/z Search) • Applied LC-MS databases for searching • Precursor ion filtering If no **Analysis Profile** is attached, then the last used settings in **LC Expert** (under **File>Settings**) will be applied. 
9	**Note**: To create an **Analysis Profile**, follow these steps: • Open a single chromatogram in **LC Expert**. • Apply the desired settings (in the **Settings** dialog and the **Deconvolution Settings** panel). • On the **Standard Toobar**, select the **Save Current Profile** icon () and give the **Analysis Profile** file a name.	When an **Analysis Profile** is saved in **LC Expert**, it will automatically appear in the **Analysis Profile** section of the **Batch Processing** dialog without additional attachment requirements.

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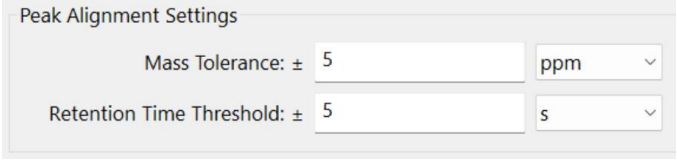
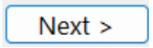
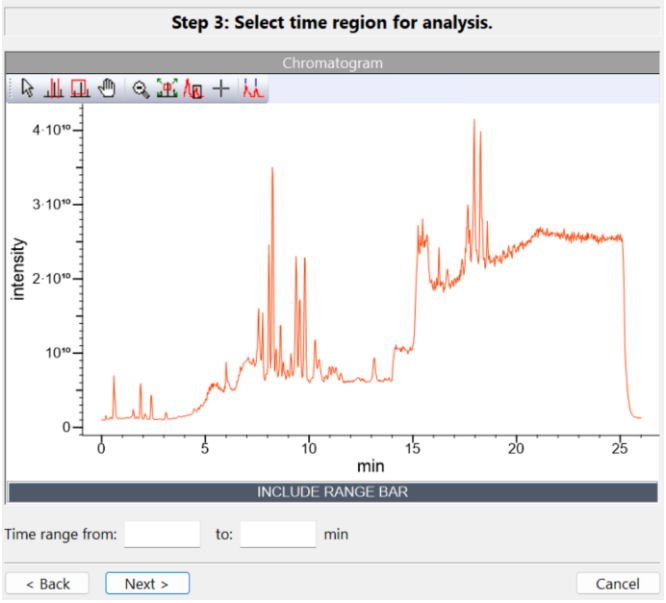
	Action	Result
10	The dialog section **Peak Alignment Settings** enables control over the tolerances for alignment: • **Mass Tolerance** controls the amount of allowed deviation for the m/z value. • **Retention Time Threshold** controls the amount of deviation for the RT value.	The **Peak Alignment** settings are defined: 
11	Click **Next >** button to proceed in the dialog. 	**Step 3: Select time region for analysis** is displayed on the dialog. The preview window displays the first chromatogram in the folder of files selected for processing.: 

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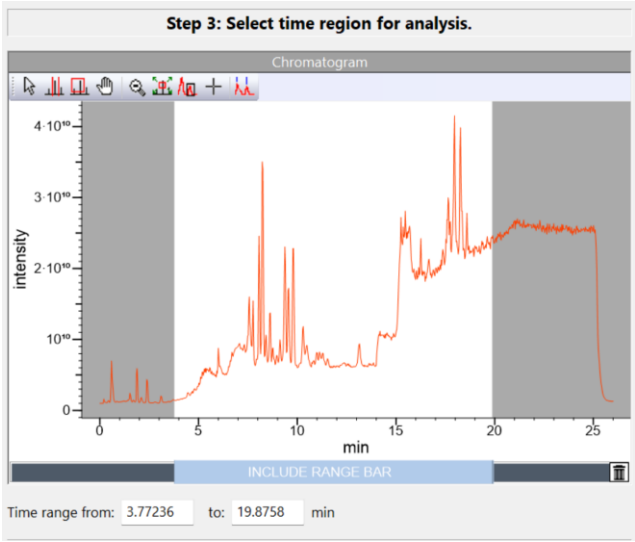
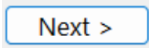
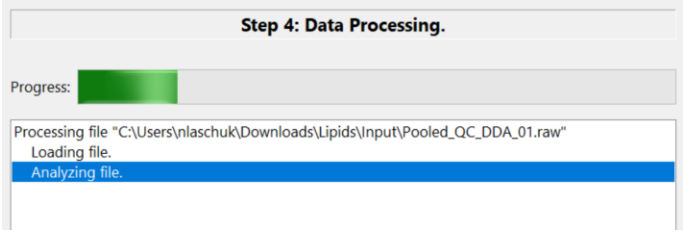
	Action	Result
12	Using the **Include Range Bar** on the dialog, click the left mouse button down and drag across the bar horizontally to select a time region. Release button to accept the region. **Note:** The end boundaries can be changed by clicking on the end of the selected region with the left mouse button and dragging to a new time point. Use the trashcan icon to remove selection entirely ().	The region for analysis is selected:  The analysis region by time is defined for the batch processing. If no region is selected, then the entire chromatogram will be processed.
13	Click **Next >** button to proceed in the dialog. 	**Step 4: Data Processing** is displayed on the dialog. Now the chromatograms are submitted for batch processing: • A **Progress Bar** applies green coloration to visualize the progress of the batch processing. • A **Log** is reported in the white box to define the active step of the batch processing. 

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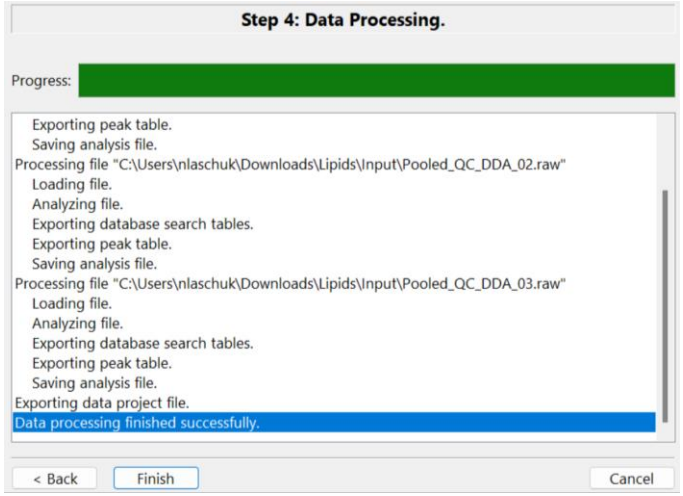
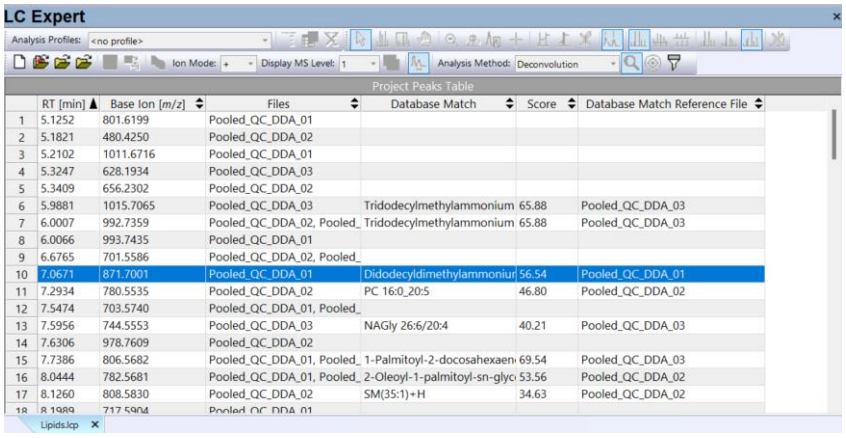
	Action	Result
14	Allow the batch processing to run to completion. **Note**: If the process needs to be aborted, click **Cancel** button (Cancel) on the dialog.	When batch processing is complete, the dialog **Log** reads “Data processing finished successfully” and the **Progress Bar** is entirely green. 
15	Click **Finish** button (Finish) on the dialog to close the dialog.	The project file (.lcp) automatically opens in **LC Expert:** 

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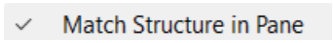
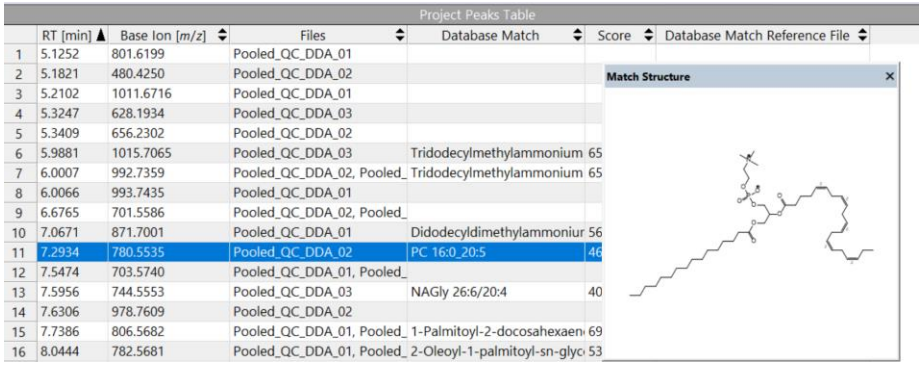
	Action	Result
16	Analyze the .lcp file results. **Note:** The **Database Match Structure** can be visualized directly in the project file. Choose **View > Match Structure in Pane.** 	The .lcp file summarizes the results of the batch processing: • **RT [min]** column gives the aligned peak retention time for the samples. • **Base Ion [m/z]** column is the detected base ion representing the aligned peak. • **Files** column gives the names of all the files for which the peak was identified in then aligned. If **Database Searching** was applied at Step 1 (otherwise, these columns will not appear): • **Database Match** gives the highest database match for the peak component. • **Score** gives the match score for the **Database Match**. • **Database Match Reference File** reports the file for which the highest **Database Match** was detected in. If **Targeted Analysis Searching** was applied at Step 1 (otherwise, these columns will not appear): • **Target Match** gives the highest match from the target list for the peak component. • **Match Score** gives the match score for the **Target Match**. • **Target Match Reference File** reports the file for which the highest **Target Match** was detected in. • **Mass Accuracy**, **Detected Adducts**, **Mass Accuracy**, **Calculated Mass**, **Adducts Accurate Mass**, **Database Name**, and **Record ID** are determined from the Target Match, and specific definitions are given in section “Targeted Analysis Searching” of this document. Below, the database **Match Structure** is visualized using the selected pane: 

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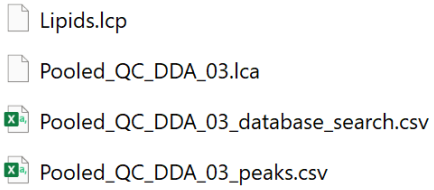
	Action	Result
17	**Note:** All project files are saved at the selected file location from Step 1.	At the described file location, there will be: • The .lcp file for the project which allows for reviewing the results. • An .lca file for each processed chromatogram, which allows for individual review of processed chromatogram files. • Any peaks selected for exporting as csv.  Lipids.lcp Pooled_QC_DDA_03.lca Pooled_QC_DDA_03_database_search.csv Pooled_QC_DDA_03_peaks.csv

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