

KnowItAll Software Training

Mass Spectrometry Search

Mass Spectrometry Spectral Searching

How to Perform a Mass Spectrometry Spectral Search

Purpose

This exercise demonstrates how to perform a Mass Spectrometry spectral search.

Objectives

This exercise will teach you:

- How to perform a straightforward search
- How to perform a reverse search
- How to complete a mixture analysis
- How to perform an adaptive search (similar compound search)
- How to perform simultaneous multiple MS spectra search

Background

Spectral searching against reference databases is frequently used in the analysis of unknown compounds. KnowItAll has full-featured MS spectrum comparison tools for this purpose.

Training Files Used in This Lesson

In C:\Users\Public\Documents\Wiley\KnowItAll\Samples folder

- \MS\1,1,1-Trichlorobutane - Adaptive Search demo
- \MS\2-Hydroxybenzoic acid
- \Mixture Analysis\MS Examples\MS Mixture of Two 1
- \Mixture Analysis\MS Examples\MS Mixture of Two 2
- \Mixture Analysis\MS Examples\MS Mixture of Three
- \GC-MS\Barbiturate GC-MS.d
- \Mixture Analysis\MS Examples\Components.SDBX

KnowItAll Applications Used

- SearchIt
- Minelt

Algorithms

Research article

Journal of
MASS
SPECTROMETRY

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Evaluation of mass spectral library search algorithms implemented in commercial software

Andrey Samokhin,^{a*} Ksenia Sotnezova,^a Vitaly Lashin^b and Igor Revelsky^a

MS SEARCH

Composite algorithm

$$SI = \frac{N_U \cdot \left[\frac{\left(\sum W_L \cdot W_U \right)^2}{\sum W_L^2 \cdot \sum W_U^2} \right] + \left[\sum \left(\frac{R_U}{R_L} \right)^n \right]}{N_U + N_{U\&L}}$$

Spectrum search type – identity (normal)

Presearch – default

Included Libs – MainLib

Apply limits – unchecked

Use constraints – unchecked

Dot-product algorithm

$$SI = \frac{\left(\sum W_L \cdot W_U \right)^2}{\sum W_L^2 \cdot \sum W_U^2}$$

Spectrum search type – similarity (simple)

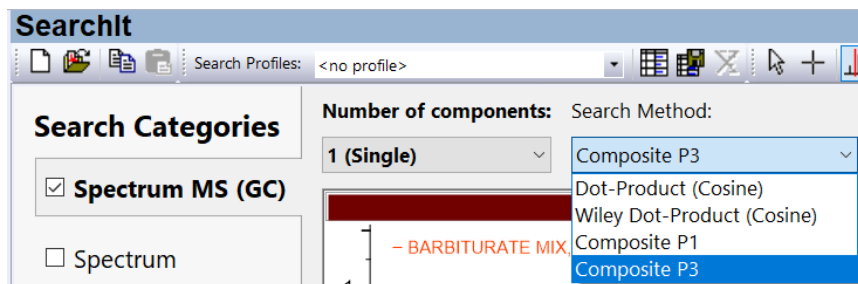
Presearch – default

Included Libs – MainLib

Apply limits – unchecked

Use constraints – unchecked

A. Samokhin, K. Sotnezova, V. Lashin, I. Revelsky. Evaluation of mass spectral library search algorithms implemented in commercial software. *Journal of Mass Spectrometry*. 2015, 50, 820-825.



Search Methods:

- Dot-Product (Cosine): second equation in above figure
- Wiley Dot-Product (Cosine): the Finnigan algorithm that verified at least 12 of the largest 16 peaks and the base peak match before continuing with the dot-product calculation
- Composite P1: first equation in above figure
- Composite P3: first equation in above figure
P1 and P3 are different by the power applied to the weighted intensity of peak.

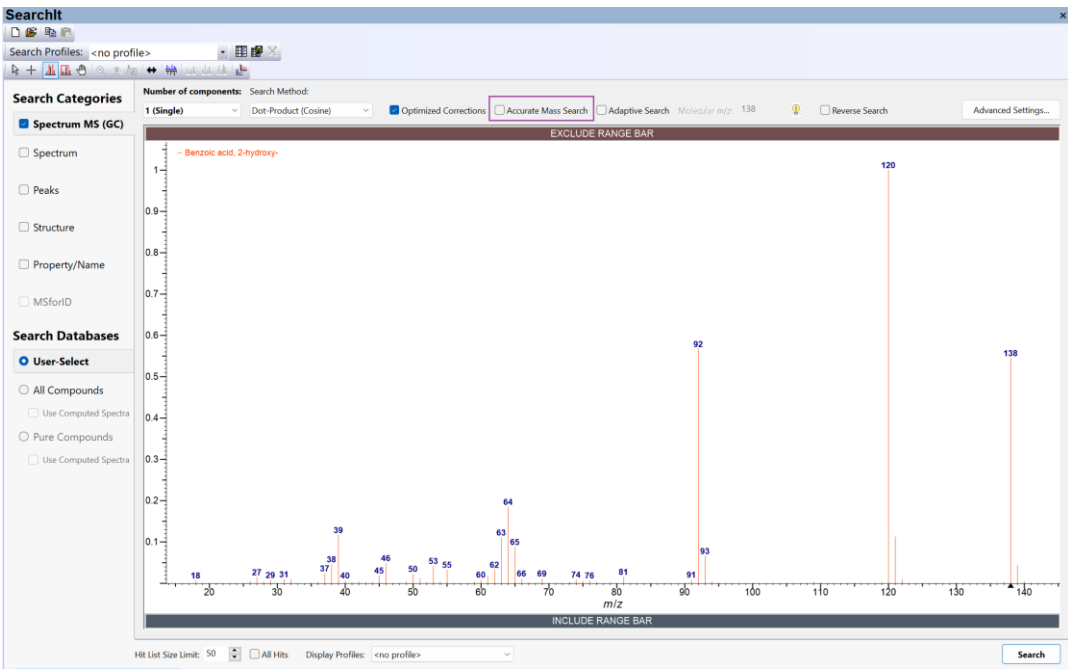
Optimized Corrections:

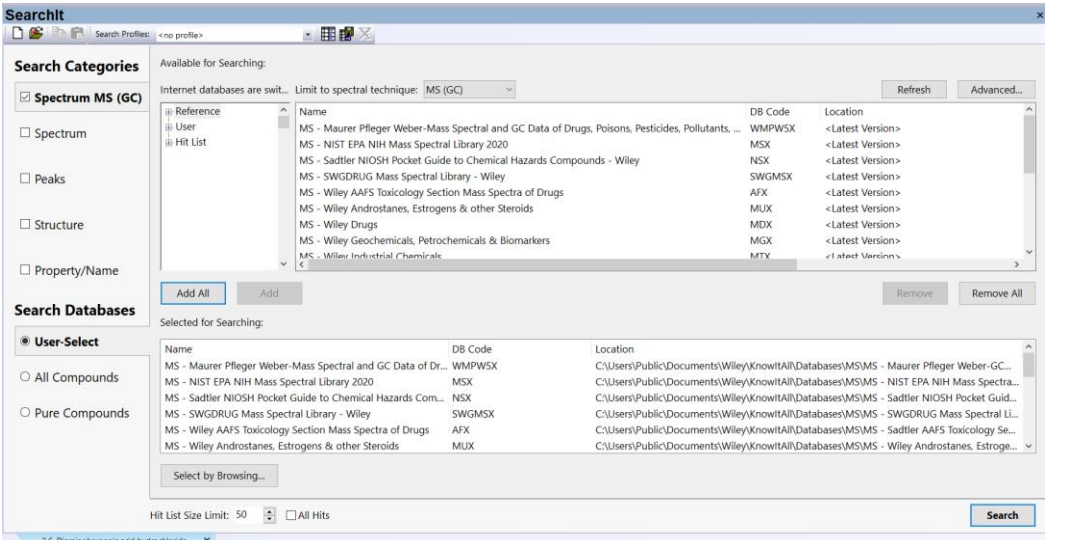
- Mass Defection is the difference between a compound's exact mass and its nominal mass. It is automatically applied for a MS search. Examples:
 - For hydrocarbon compounds, when m/z value is over 500, use 0.99888.
 - For polybrominated compounds, when there are more than 5 Br atoms and m/z value is over 800, use 1.00087.
- Spectral Skewing is caused by analyte's concentration changing during scan. A linear compensation factor (positive for ascending or negative for descending slope) is calculated for each search match. This factor corrects the intensities as follows:


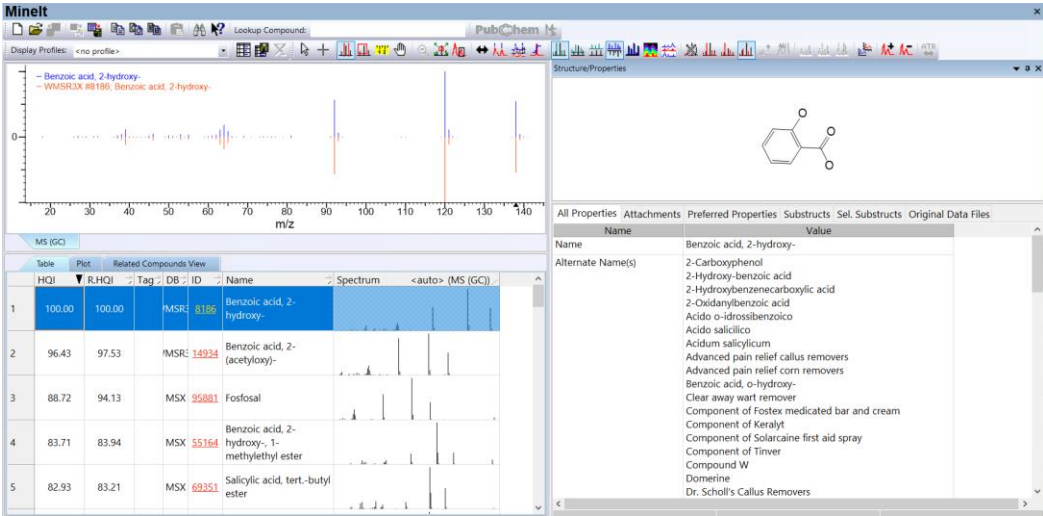
$$I(\text{corrected})=I * f * m,$$

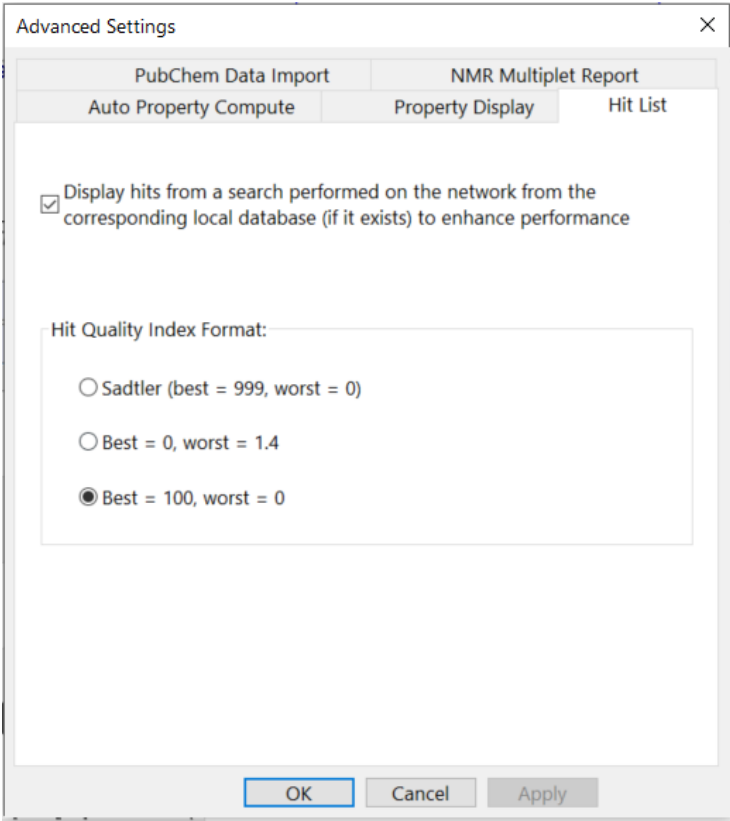
where I=intensity, f=OC factor, and $m=m/z$ value

Straightforward Search

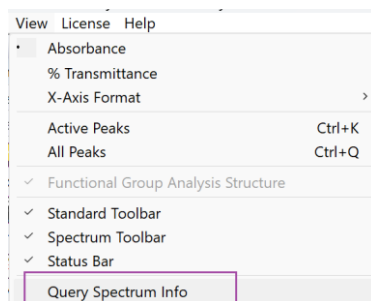
	Action	Result
1	<p>Open the KnowItAll SearchIt application by clicking its icon in the KnowItAll application.</p> <p>Check Spectrum and navigate to C:\Users\Public\Documents\Wiley\KnowItAll\Samples\MS folder</p> <p>Select the file 2-Hydroxybenzoic acid.</p> <p>Click Open.</p> <p>Ensure Adaptive Search or Reverse Search is unchecked.</p>	 <p>The screenshot shows the SearchIt application window. The search profile is set to '<no profile>'. The search categories are 'Spectrum MS (GC)', 'Spectrum', 'Peaks', 'Structure', 'Property/Name', and 'MSforID'. The search databases are 'User-Select', 'All Compounds', 'Use Computed Spectra', 'Pure Compounds', and 'Use Computed Spectra'. The search method is 'Dot-Product (Cosine)' and the molecular m/z is 138. The mass spectrum plot shows relative intensity on the y-axis (0 to 1) and m/z on the x-axis (20 to 140). The base peak is at m/z 120. Other significant peaks are at m/z 92 and 138. The plot is titled 'Benzoic acid, 2-hydroxy-' and includes 'EXCLUDE RANGE BAR' and 'INCLUDE RANGE BAR' labels.</p> <p>Note: in KnowItAll 2025 release, one can perform accurate mass search in addition to the default unit mass search</p>

	Action	Result																														
2	<p>Click User-Select.</p> <p>Use Remove All to clean selected databases.</p> <p>Use Limit to spectral technique to select MS (GC).</p> <p>Use Add All to add all MS (GC) databases.</p> <p>Click Search.</p>	 <p>The screenshot shows the SearchIt application window. On the left, under 'Search Categories', 'Spectrum MS (GC)' is selected. Under 'Search Databases', 'User-Select' is selected. The 'Limit to spectral technique' dropdown is set to 'MS (GC)'. The 'Selected for Searching' table lists several databases with their names, DB codes, and file locations.</p> <table border="1"> <thead> <tr> <th>Name</th> <th>DB Code</th> <th>Location</th> </tr> </thead> <tbody> <tr> <td>MS - Maurer Pfleger Weber-Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants, ...</td> <td>WMPWSX</td> <td>C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - Maurer Pfleger Weber-GC...</td> </tr> <tr> <td>MS - NIST EPA NIH Mass Spectral Library 2020</td> <td>MSX</td> <td>C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - NIST EPA NIH Mass Spectra...</td> </tr> <tr> <td>MS - Sadtler NIOSH Pocket Guide to Chemical Hazards Compounds - Wiley</td> <td>NSX</td> <td>C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - Sadtler NIOSH Pocket Guid...</td> </tr> <tr> <td>MS - SWGDRUG Mass Spectral Library - Wiley</td> <td>SWGMSX</td> <td>C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - SWGDRUG Mass Spectral Li...</td> </tr> <tr> <td>MS - Wiley AAFS Toxicology Section Mass Spectra of Drugs</td> <td>AFX</td> <td>C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - Sadtler AAFS Toxicology Se...</td> </tr> <tr> <td>MS - Wiley Androstanes, Estrogens & other Steroids</td> <td>MUX</td> <td>C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - Wiley Androstanes, Estroge...</td> </tr> <tr> <td>MS - Wiley Drugs</td> <td>MDX</td> <td></td> </tr> <tr> <td>MS - Wiley Geochemicals, Petrochemicals & Biomarkers</td> <td>MGX</td> <td></td> </tr> <tr> <td>MS - Wiley Industrial Chemicals</td> <td>MTX</td> <td></td> </tr> </tbody> </table>	Name	DB Code	Location	MS - Maurer Pfleger Weber-Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants, ...	WMPWSX	C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - Maurer Pfleger Weber-GC...	MS - NIST EPA NIH Mass Spectral Library 2020	MSX	C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - NIST EPA NIH Mass Spectra...	MS - Sadtler NIOSH Pocket Guide to Chemical Hazards Compounds - Wiley	NSX	C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - Sadtler NIOSH Pocket Guid...	MS - SWGDRUG Mass Spectral Library - Wiley	SWGMSX	C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - SWGDRUG Mass Spectral Li...	MS - Wiley AAFS Toxicology Section Mass Spectra of Drugs	AFX	C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - Sadtler AAFS Toxicology Se...	MS - Wiley Androstanes, Estrogens & other Steroids	MUX	C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - Wiley Androstanes, Estroge...	MS - Wiley Drugs	MDX		MS - Wiley Geochemicals, Petrochemicals & Biomarkers	MGX		MS - Wiley Industrial Chemicals	MTX	
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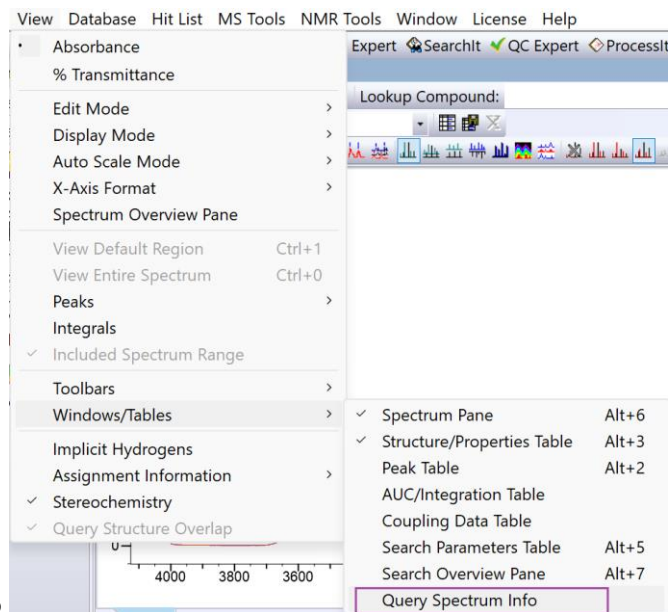
	Action	Result
3	Click the Butterfly view icon  to place the unknown and reference spectrum in the opposite Y-direction.	<p>A target from a database is found.</p>  <p>Hits are initially sorted by the Hit Quality Index (HQI). Reverse Hit Quality Index (R.HQI) is also calculated for each reference spectrum.</p>

	Action	Result
4	HQI (Hit Quality Index)	<p>The HQI value measures how close the reference spectrum is to that of the query. The default scale of HQI is 0-100. You can change the scale from Minelt > File > Preferences > Hit List.</p>  <p>Note: Reverse Search ignores peaks that are in unknown but not in reference. One scenario is that the unknown spectrum might be a mixture and the reference spectrum might be a component.</p>

Note: one can view the metadata of query spectrum by


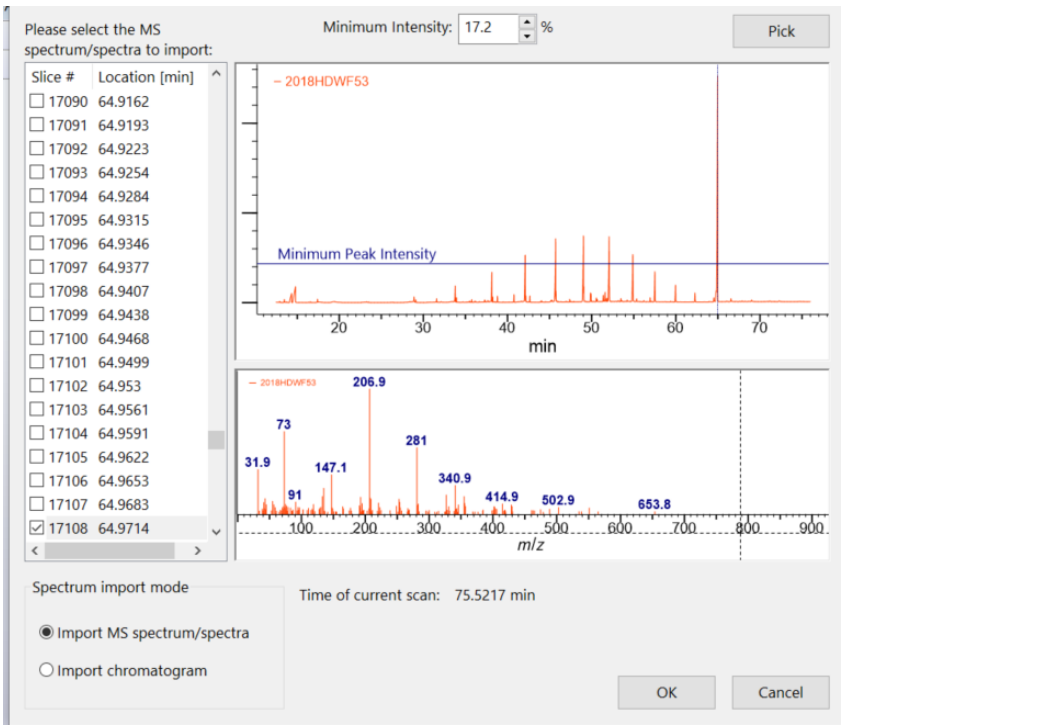


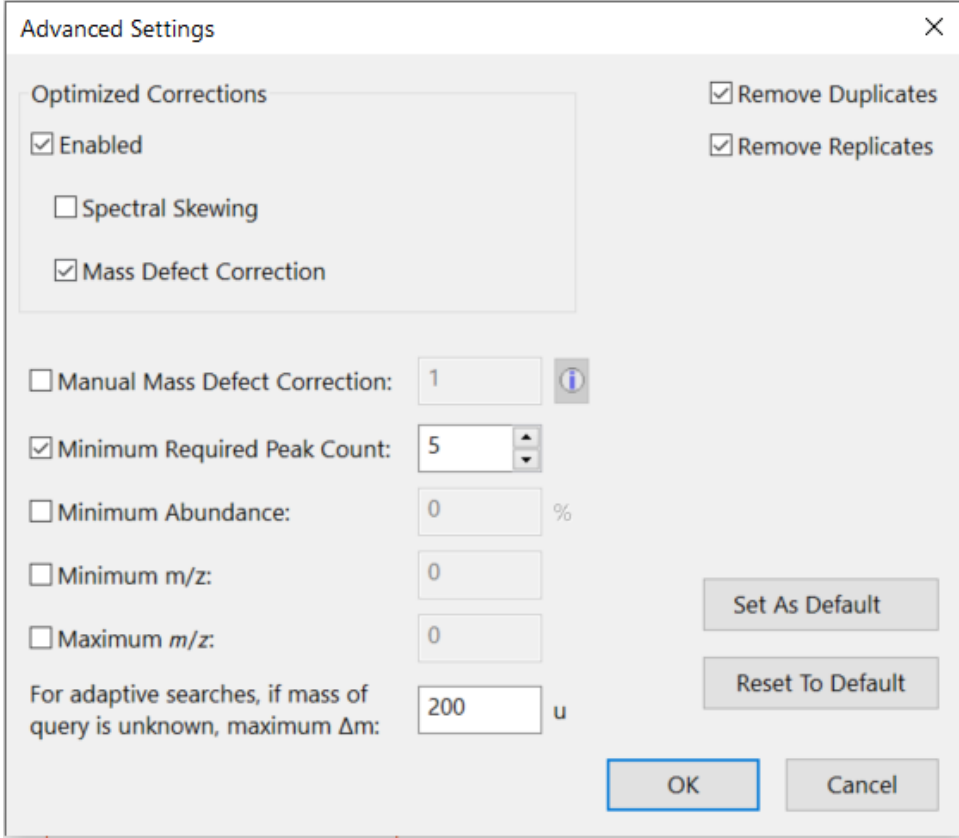
- **View > Query Spectrum Info** in **SearchIt** and

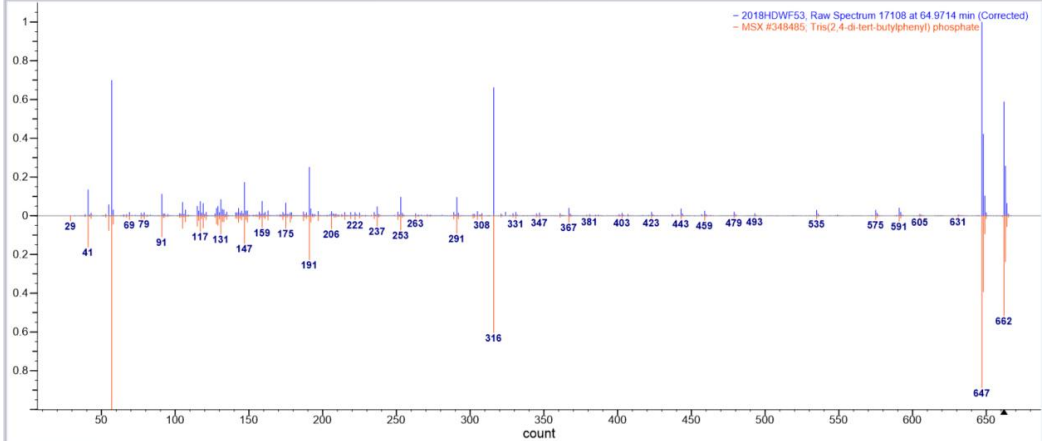
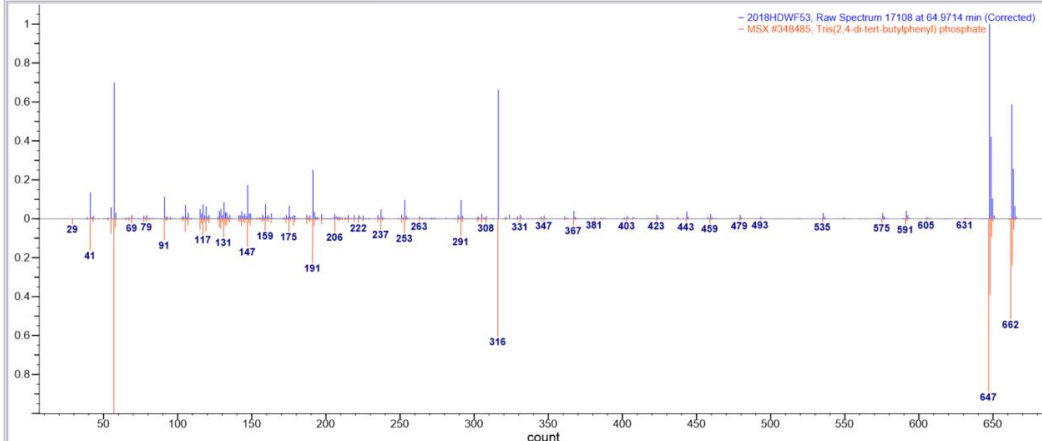


- **View > Windows/Tables > Query Spectrum Info** in a **Minelt** hit list.

Optimized Corrections


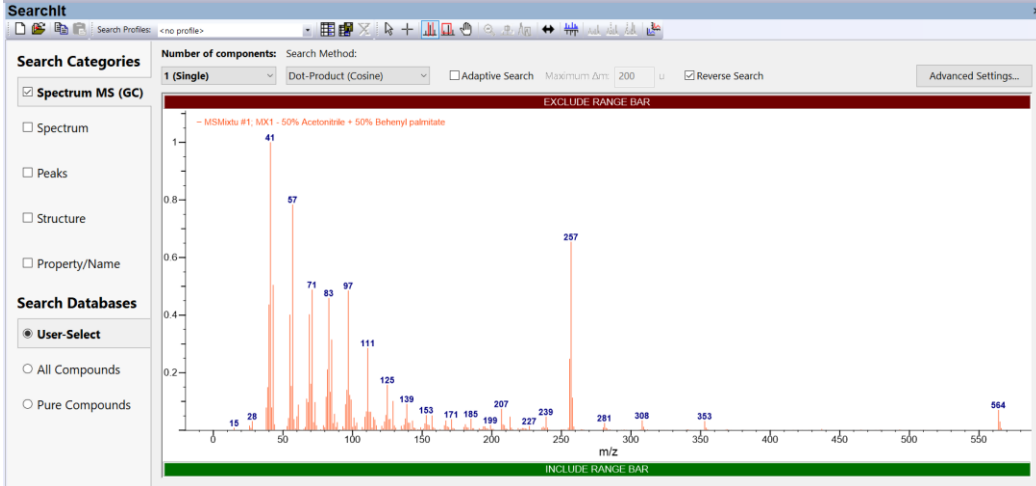
	Action	Result
1	<p>Go back to SearchIt.</p> <p>Click  to start a new search.</p> <p>Click Spectrum and navigate to C:\Users\Public\Documents\Wiley\KnowItAll\Samples\MS</p> <p>Select the file Mass defect correction example Click Open.</p> <p>Click OK (use the default MS 17108)</p>	 <p>The screenshot displays the 'Please select the MS spectrum/spectra to import:' dialog box. On the left, a list of spectra is shown with columns for 'Slice #' and 'Location [min]'. Spectrum 17108 at 64.9714 min is selected. On the right, two mass spectra are shown. The top plot is a chromatogram showing intensity over time (0-70 min) with a 'Minimum Peak Intensity' line. The bottom plot is a mass spectrum (0-900 m/z) with labeled peaks at 31.9, 73, 91, 147.1, 206.9, 281, 340.9, 414.9, 502.9, and 653.8. The 'Spectrum import mode' section has 'Import MS spectrum/spectra' selected. The 'Time of current scan' is 75.5217 min. 'OK' and 'Cancel' buttons are at the bottom right.</p>

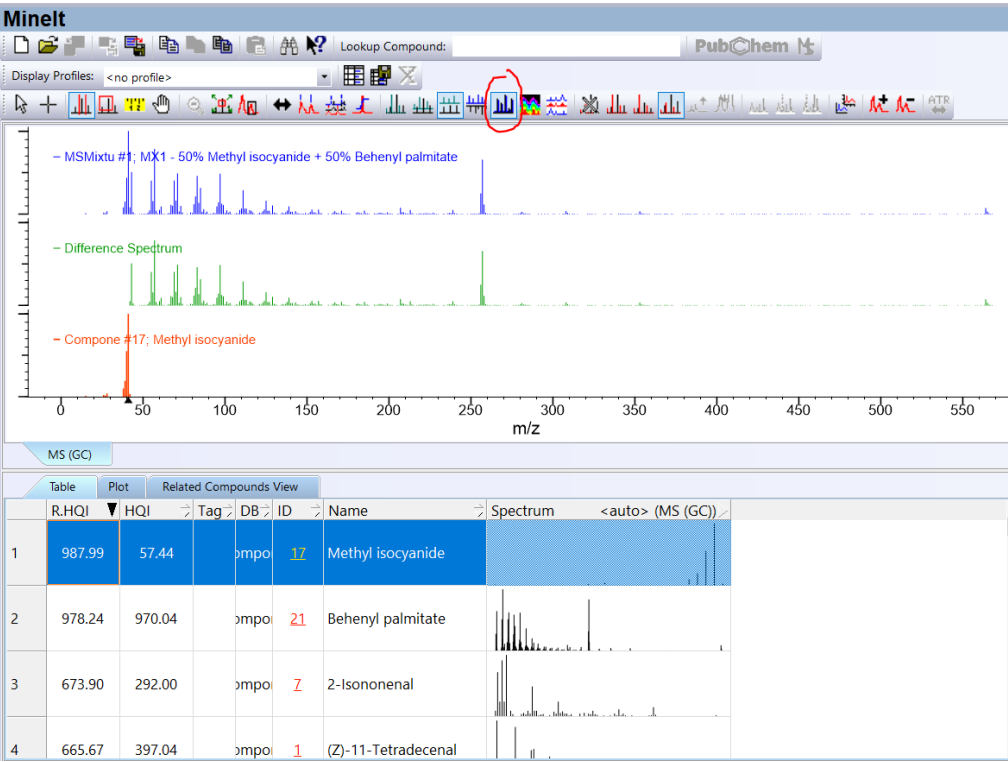

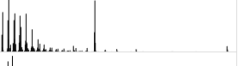



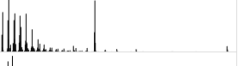



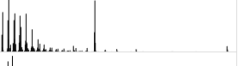


	Action	Result
2	<p>Optimized Corrections for MS spectrum is default to be checked.</p> <p><input checked="" type="checkbox"/> Optimized Corrections</p> <p>Click Advanced Setting button</p> <p><input type="button" value="Advanced Settings..."/></p> <p>Click OK to close this dialog</p> <p>Click the Search button</p>	<p>One can see that Mass Defection (the difference between a compound's exact mass and its nominal mass) is automatically applied. Many other criterion, such as Spectral Skewing (analyte's concentration changing during scan), can be applied by users choice to improve a search.</p> 

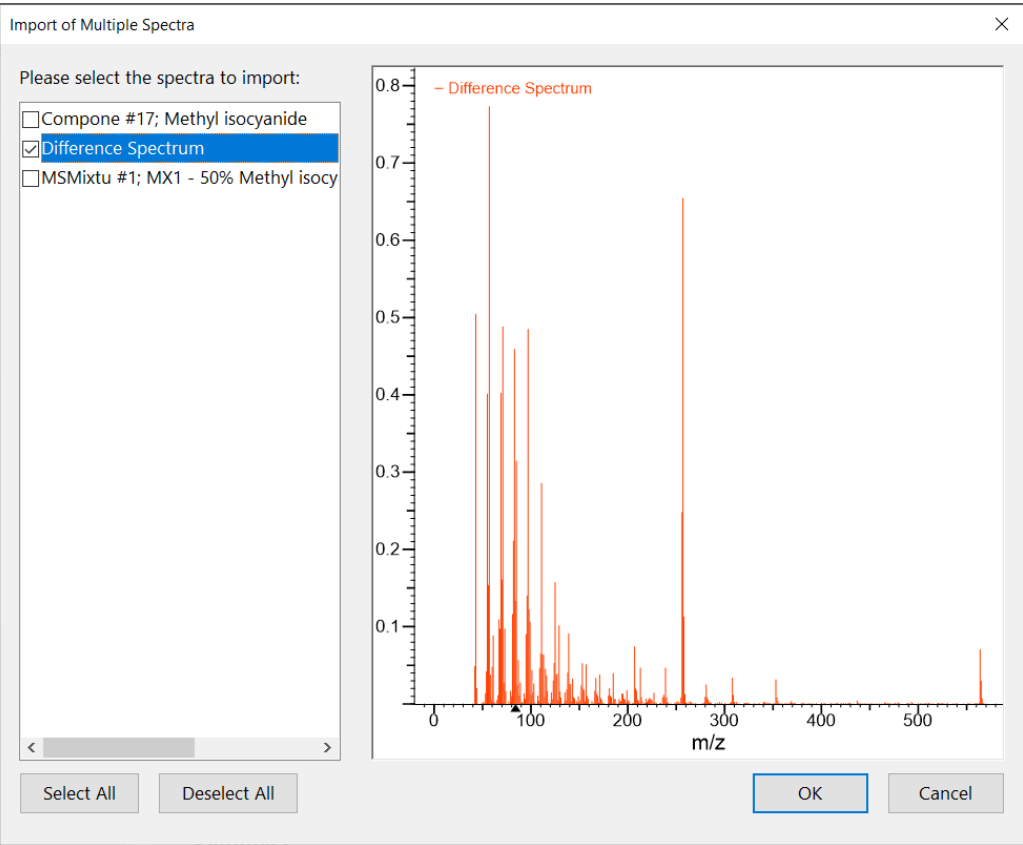
	Action	Result
3		 <p data-bbox="856 805 1650 829">We can see analyte MS and reference MS are perfectly aligned at m/z 662.</p>
4	<p data-bbox="233 854 449 878">Go back to SearchIt</p> <p data-bbox="233 919 594 943">Check off Optimized Corrections</p> <p data-bbox="233 984 380 1008">Search again</p>	 <p data-bbox="856 1333 1667 1357">At high m/z range, analyte peaks and reference peaks are not aligned nicely.</p>

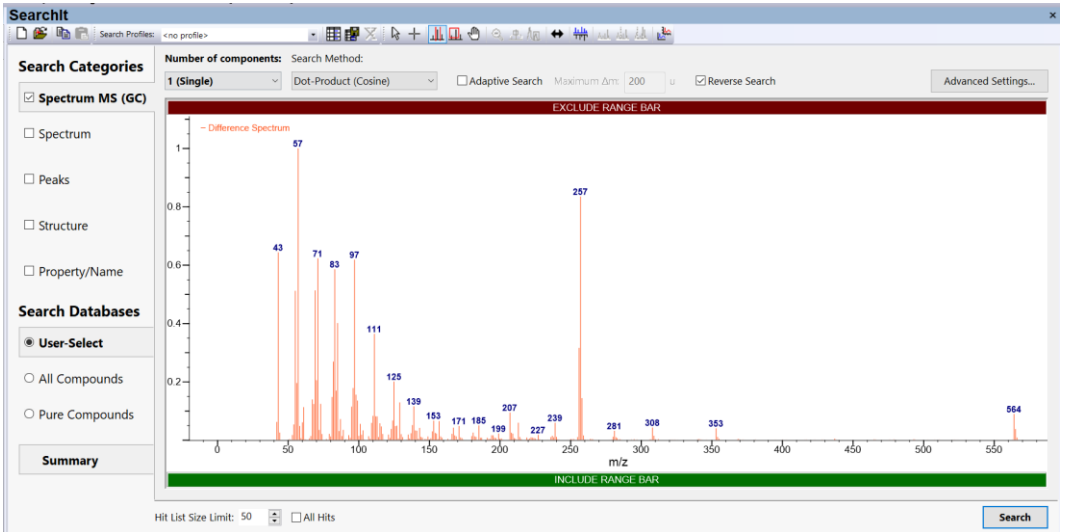
Reverse Search

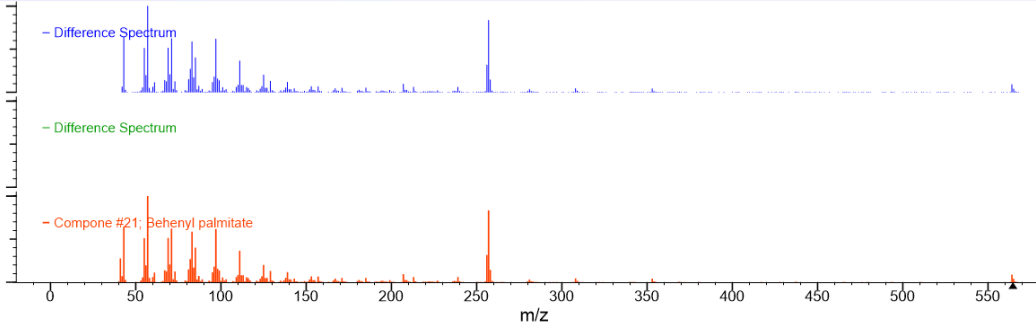






This search ignores peaks that are in unknown but not in reference

	Action	Result
1	<p>Go back to SearchIt</p> <p>Click  to start a new search.</p> <p>Click Spectrum and navigate to C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Mixture Analysis\MS Examples folder</p> <p>Select MS Mixture of Two 1 to open</p> <p>Check Reverse Search.</p>	
2	<p>Click User-Select button</p> <p>Use button Remove All to clean current database selection</p> <p>Then, use Select by Browsing button to add the example database: C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Mixture Analysis\MS Examples\Components.SDBX</p> <p>Search</p>	

Action	Result																																								
<p>3 In Minelt, make sure that Subtract View (circled) is selected.</p> <p>In the spectrum pane, the first row is the unknown, the last row is the reference spectrum, and the middle row is the difference between the two.</p>	 <p>The hits are initially sorted by the Reverse Search HQI (R.HQI).</p> <table border="1" data-bbox="863 792 1591 1079"> <thead> <tr> <th></th> <th>R.HQI</th> <th>HQI</th> <th>Tag</th> <th>DB</th> <th>ID</th> <th>Name</th> <th>Spectrum</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>987.99</td> <td>57.44</td> <td>ompo</td> <td></td> <td>17</td> <td>Methyl isocyanide</td> <td></td> </tr> <tr> <td>2</td> <td>978.24</td> <td>970.04</td> <td>ompo</td> <td></td> <td>21</td> <td>Behenyl palmitate</td> <td></td> </tr> <tr> <td>3</td> <td>673.90</td> <td>292.00</td> <td>ompo</td> <td></td> <td>7</td> <td>2-Isononenal</td> <td></td> </tr> <tr> <td>4</td> <td>665.67</td> <td>397.04</td> <td>ompo</td> <td></td> <td>1</td> <td>(Z)-11-Tetradecenal</td> <td></td> </tr> </tbody> </table>		R.HQI	HQI	Tag	DB	ID	Name	Spectrum	1	987.99	57.44	ompo		17	Methyl isocyanide		2	978.24	970.04	ompo		21	Behenyl palmitate		3	673.90	292.00	ompo		7	2-Isononenal		4	665.67	397.04	ompo		1	(Z)-11-Tetradecenal	
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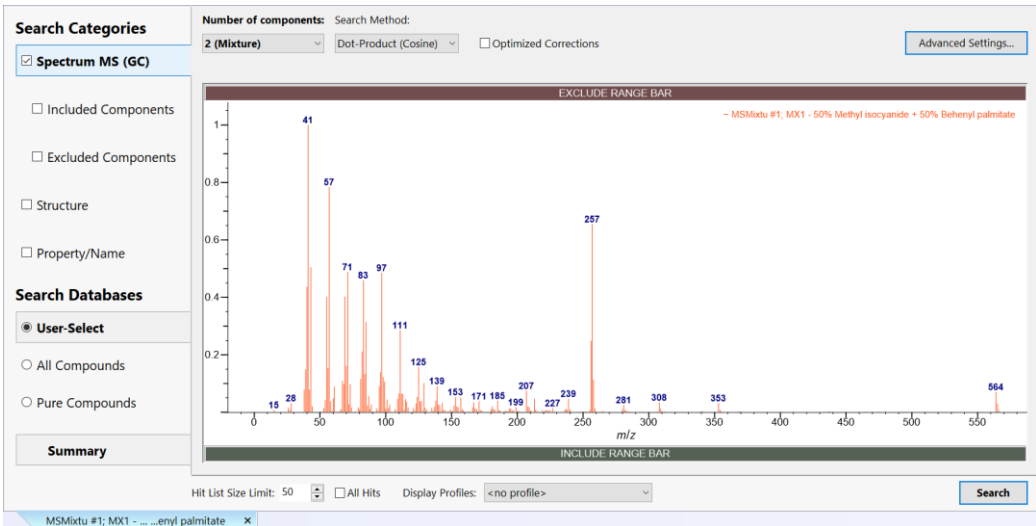
	Action	Result
4	<p>Transfer spectra in spectrum pane to SearchIt using the Transfer to: menu at the top of the application.</p> <p>Select to transfer only the Difference Spectrum. Click OK.</p> <p>When prompted by SearchIt, choose Start a new search.</p>	

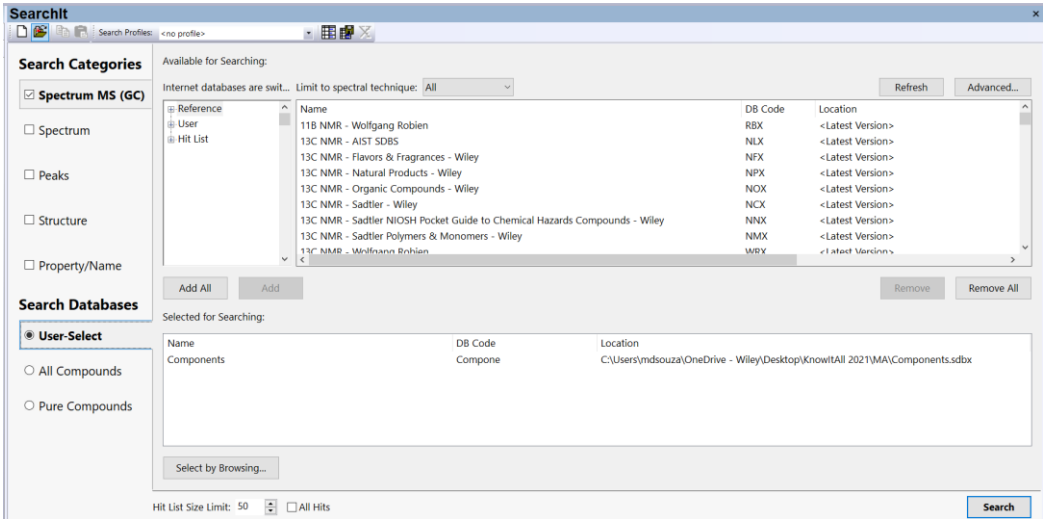
	Action	Result
5	Click Search .	 <p>The screenshot displays the SearchIt software interface. On the left, there are search categories and databases. The main area shows a mass spectrum plot with the x-axis labeled 'm/z' ranging from 0 to 550 and the y-axis representing relative intensity from 0 to 1.0. The plot is titled 'Difference Spectrum' and features a red 'EXCLUDE RANGE BAR' at the top and a green 'INCLUDE RANGE BAR' at the bottom. Several peaks are labeled with their m/z values: 43, 71, 83, 97, 111, 125, 139, 153, 171, 185, 199, 207, 227, 239, 257, 281, 308, 353, and 564. The peak at m/z 257 is the most prominent. At the bottom left, there is a 'Hit List Size Limit: 50' and an 'All Hits' checkbox. A 'Search' button is located at the bottom right.</p>

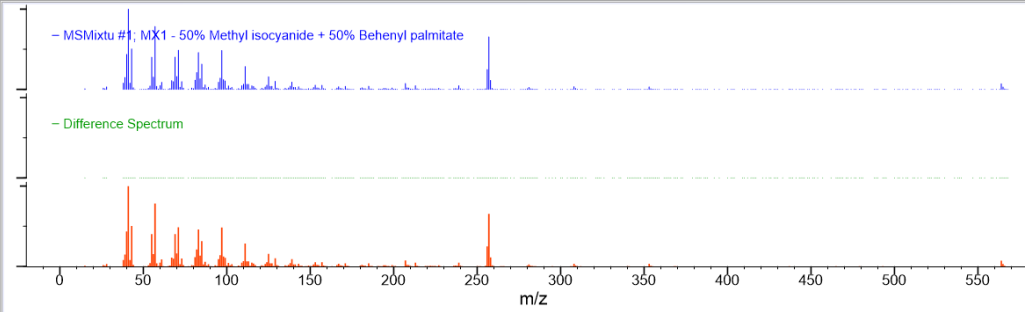
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6	 <p>MS (GC)</p> <table border="1" data-bbox="869 688 1898 867"> <thead> <tr> <th></th> <th>R.HQI</th> <th>HQI</th> <th>Tag</th> <th>DB</th> <th>ID</th> <th>Name</th> <th>Spectrum</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>991.32</td> <td>991.32</td> <td>mpo</td> <td></td> <td>21</td> <td>Behenyl palmitate</td> <td></td> </tr> <tr> <td>2</td> <td>680.09</td> <td>449.83</td> <td>mpo</td> <td></td> <td>38</td> <td>Octatriacontane</td> <td></td> </tr> </tbody> </table> <p>A second component is identified.</p> <p>Notice that the Difference Spectrum is empty, which means there are no more components.</p>		R.HQI	HQI	Tag	DB	ID	Name	Spectrum	1	991.32	991.32	mpo		21	Behenyl palmitate		2	680.09	449.83	mpo		38	Octatriacontane	
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Mixture Analysis


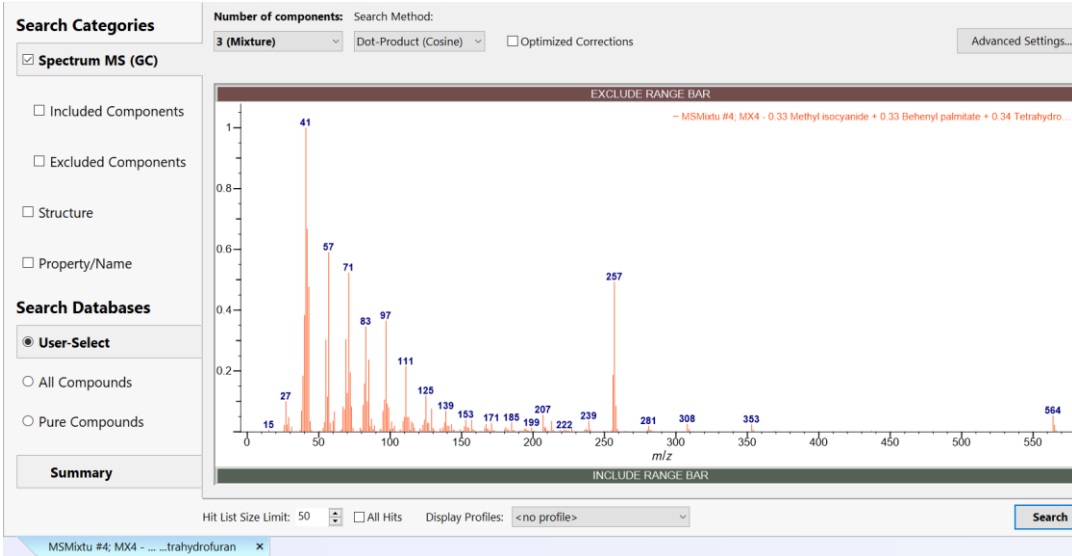
A mixture wherein two-component MS spectra have limited overlap, and one of them has a large MS range

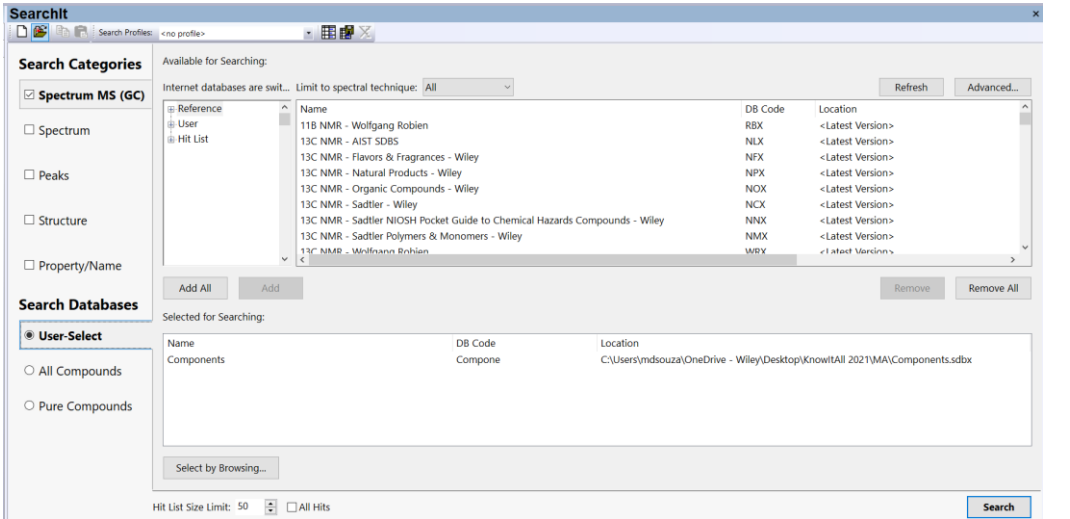
	Action	Result
1	<p>Go back to SearchIt.</p> <p>Start a new search by clicking .</p> <p>Select MS Mixture of Two 1.</p> <p>Ensure Reverse Search or Adaptive Search is unchecked.</p> <p>Use the drop-down menu to set the Number of components to 2.</p>	 <p>The screenshot displays the SearchIt interface. On the left, the 'Search Categories' section has 'Spectrum MS (GC)' checked. Below it, 'Included Components', 'Excluded Components', 'Structure', and 'Property/Name' are all unchecked. The 'Search Databases' section has 'User-Select' selected, with 'All Compounds' and 'Pure Compounds' unselected. A 'Summary' section is also visible. On the right, the search parameters are set to '2 (Mixture)' components and 'Dot-Product (Cosine)' search method. The 'Optimized Corrections' checkbox is unchecked. The main area shows a mass spectrum plot with the x-axis labeled 'm/z' ranging from 0 to 550. The y-axis represents relative intensity from 0 to 1.0. The plot is titled 'MSMixture #1, MX1 - 50% Methyl isocyanide + 50% Behenyl palmitate'. The spectrum shows several peaks, with the most prominent ones at m/z 41, 57, 71, 83, 97, 111, 125, 139, 153, 171, 185, 199, 207, 227, 239, 257, 281, 308, 353, and 564. The peak at m/z 257 is the base peak. The plot is framed by 'EXCLUDE RANGE BAR' at the top and 'INCLUDE RANGE BAR' at the bottom. At the bottom of the interface, there is a 'Hit List Size Limit' set to 50, an 'All Hits' checkbox, a 'Display Profiles' dropdown set to '<no profile>', and a 'Search' button.</p>

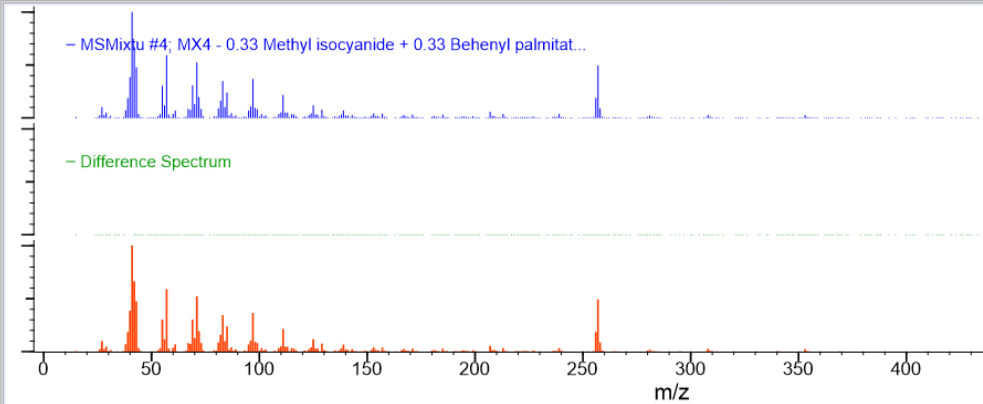
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2	<p>Click on User-Select.</p> <p>Make sure Components database is selected to use.</p> <p>Click Search.</p>	 <p>The screenshot shows the SearchIt application window. On the left, under 'Search Categories', 'Spectrum MS (GC)' is checked. Under 'Search Databases', 'User-Select' is selected. The main area shows a list of available internet databases with columns for Name, DB Code, and Location. The 'Components' database is highlighted in the 'Selected for Searching' section.</p> <table border="1"> <thead> <tr> <th>Name</th> <th>DB Code</th> <th>Location</th> </tr> </thead> <tbody> <tr> <td>11B NMR - Wolfgang Robien</td> <td>RBX</td> <td><Latest Version></td> </tr> <tr> <td>13C NMR - AIST SDBS</td> <td>NLX</td> <td><Latest Version></td> </tr> <tr> <td>13C NMR - Flavors & Fragrances - Wiley</td> <td>NFX</td> <td><Latest Version></td> </tr> <tr> <td>13C NMR - Natural Products - Wiley</td> <td>NPX</td> <td><Latest Version></td> </tr> <tr> <td>13C NMR - Organic Compounds - Wiley</td> <td>NOX</td> <td><Latest Version></td> </tr> <tr> <td>13C NMR - Sadtler - Wiley</td> <td>NCX</td> <td><Latest Version></td> </tr> <tr> <td>13C NMR - Sadtler NIOSH Pocket Guide to Chemical Hazards Compounds - Wiley</td> <td>NNX</td> <td><Latest Version></td> </tr> <tr> <td>13C NMR - Sadtler Polymers & Monomers - Wiley</td> <td>NMX</td> <td><Latest Version></td> </tr> <tr> <td>13C NMR - Wolthann Bohlen</td> <td>WBX</td> <td><Latest Version></td> </tr> </tbody> </table> <table border="1"> <thead> <tr> <th>Name</th> <th>DB Code</th> <th>Location</th> </tr> </thead> <tbody> <tr> <td>Components</td> <td>Compone</td> <td>C:\Users\ymdsouza\OneDrive - Wiley\Desktop\KnowItAll 2021\MA\Components.sdbx</td> </tr> </tbody> </table>	Name	DB Code	Location	11B NMR - Wolfgang Robien	RBX	<Latest Version>	13C NMR - AIST SDBS	NLX	<Latest Version>	13C NMR - Flavors & Fragrances - Wiley	NFX	<Latest Version>	13C NMR - Natural Products - Wiley	NPX	<Latest Version>	13C NMR - Organic Compounds - Wiley	NOX	<Latest Version>	13C NMR - Sadtler - Wiley	NCX	<Latest Version>	13C NMR - Sadtler NIOSH Pocket Guide to Chemical Hazards Compounds - Wiley	NNX	<Latest Version>	13C NMR - Sadtler Polymers & Monomers - Wiley	NMX	<Latest Version>	13C NMR - Wolthann Bohlen	WBX	<Latest Version>	Name	DB Code	Location	Components	Compone	C:\Users\ymdsouza\OneDrive - Wiley\Desktop\KnowItAll 2021\MA\Components.sdbx
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A more complex example

	Action	Result
1	<p>Go back to SearchIt.</p> <p>Start a new search by clicking .</p> <p>Select the file MS Mixture of Three.</p> <p>Use the drop-down menu to set the Number of components to 3 (Mixture).</p>	 <p>The screenshot displays the SearchIt interface. On the left, the 'Search Categories' panel is expanded to 'Spectrum MS (GC)'. Under 'Search Databases', 'User-Select' is selected. The 'Number of components' is set to '3 (Mixture)'. The search method is 'Dot-Product (Cosine)'. The main area shows a mass spectrum plot with the x-axis labeled 'm/z' ranging from 0 to 550. The y-axis represents relative intensity from 0 to 1.0. The base peak is at m/z 41. Other significant peaks are labeled at m/z 27, 57, 71, 83, 97, 111, 125, 139, 153, 171, 185, 199, 207, 222, 239, 257, 281, 308, 353, and 564. A legend above the plot identifies the mixture as 'MSMixture #4, MX4 - 0.33 Methyl isocyanide + 0.33 Behenyl palmitate + 0.34 Tetrahydro...'. The interface also includes a 'Summary' section at the bottom with a 'Hit List Size Limit' of 50 and a 'Search' button.</p>


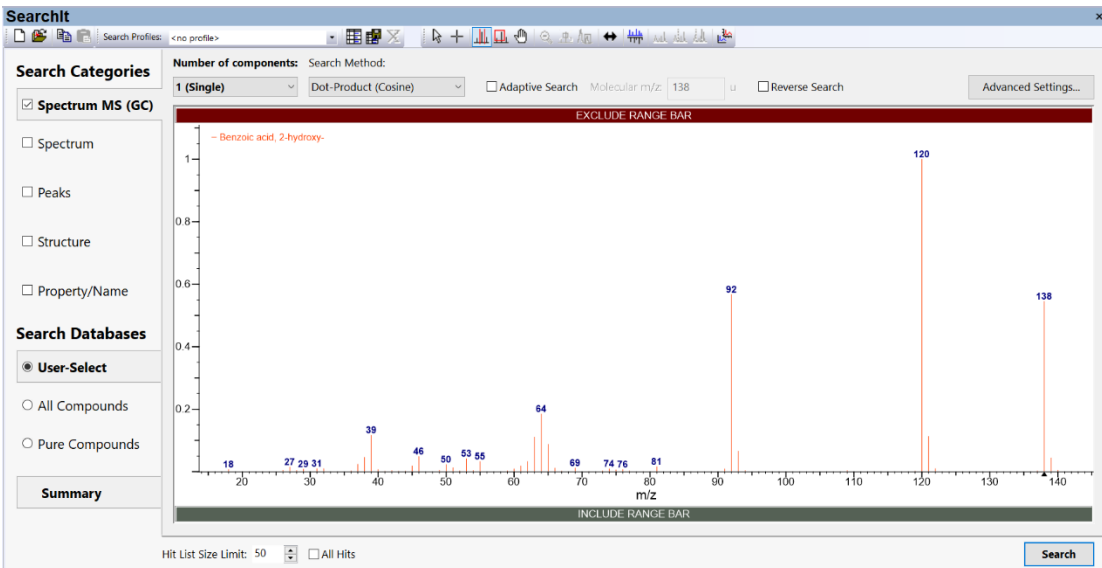
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2	<p>Click on User-Select.</p> <p>Make sure the Components database is selected for use.</p> <p>Click Search.</p>	 <p>The screenshot shows the SearchIt application window. On the left, under 'Search Categories', 'Spectrum MS (GC)' is checked. Under 'Search Databases', 'User-Select' is selected. The main area shows a list of available internet databases with columns for Name, DB Code, and Location. The 'Components' database is highlighted in the 'Selected for Searching' section below.</p> <table border="1"><thead><tr><th>Name</th><th>DB Code</th><th>Location</th></tr></thead><tbody><tr><td>11B NMR - Wolfgang Robien</td><td>RBX</td><td><Latest Version></td></tr><tr><td>13C NMR - AIST SDBS</td><td>NLX</td><td><Latest Version></td></tr><tr><td>13C NMR - Flavors & Fragrances - Wiley</td><td>NFX</td><td><Latest Version></td></tr><tr><td>13C NMR - Natural Products - Wiley</td><td>NPX</td><td><Latest Version></td></tr><tr><td>13C NMR - Organic Compounds - Wiley</td><td>NOX</td><td><Latest Version></td></tr><tr><td>13C NMR - Sadtler - Wiley</td><td>NCX</td><td><Latest Version></td></tr><tr><td>13C NMR - Sadtler NIOSH Pocket Guide to Chemical Hazards Compounds - Wiley</td><td>NNX</td><td><Latest Version></td></tr><tr><td>13C NMR - Sadtler Polymers & Monomers - Wiley</td><td>NMX</td><td><Latest Version></td></tr><tr><td>13C NMR - Wolthann Bohlen</td><td>WBX</td><td><Latest Version></td></tr></tbody></table> <table border="1"><thead><tr><th>Name</th><th>DB Code</th><th>Location</th></tr></thead><tbody><tr><td>Components</td><td>Compone</td><td>C:\Users\ymdsouza\OneDrive - Wiley\Desktop\KnowItAll 2021\MA\Components.sdbx</td></tr></tbody></table>	Name	DB Code	Location	11B NMR - Wolfgang Robien	RBX	<Latest Version>	13C NMR - AIST SDBS	NLX	<Latest Version>	13C NMR - Flavors & Fragrances - Wiley	NFX	<Latest Version>	13C NMR - Natural Products - Wiley	NPX	<Latest Version>	13C NMR - Organic Compounds - Wiley	NOX	<Latest Version>	13C NMR - Sadtler - Wiley	NCX	<Latest Version>	13C NMR - Sadtler NIOSH Pocket Guide to Chemical Hazards Compounds - Wiley	NNX	<Latest Version>	13C NMR - Sadtler Polymers & Monomers - Wiley	NMX	<Latest Version>	13C NMR - Wolthann Bohlen	WBX	<Latest Version>	Name	DB Code	Location	Components	Compone	C:\Users\ymdsouza\OneDrive - Wiley\Desktop\KnowItAll 2021\MA\Components.sdbx
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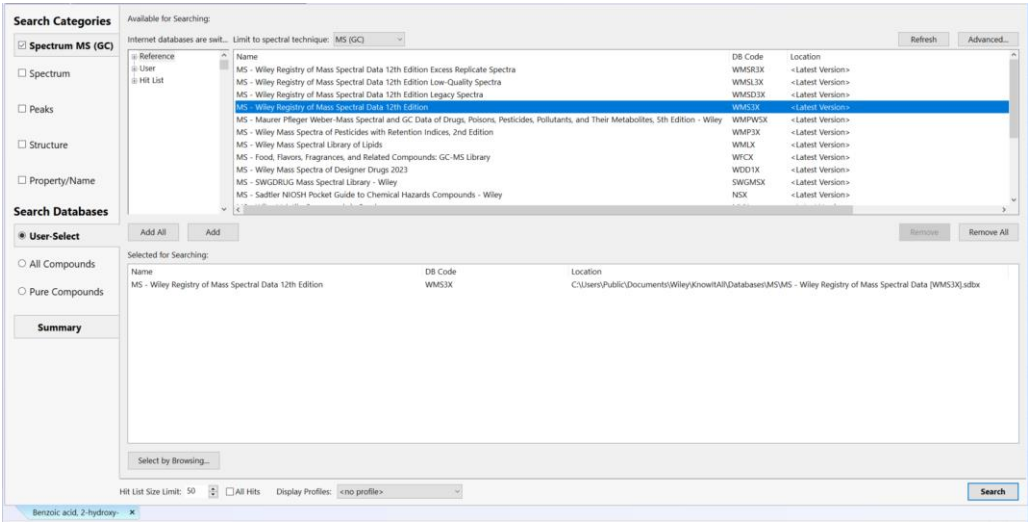

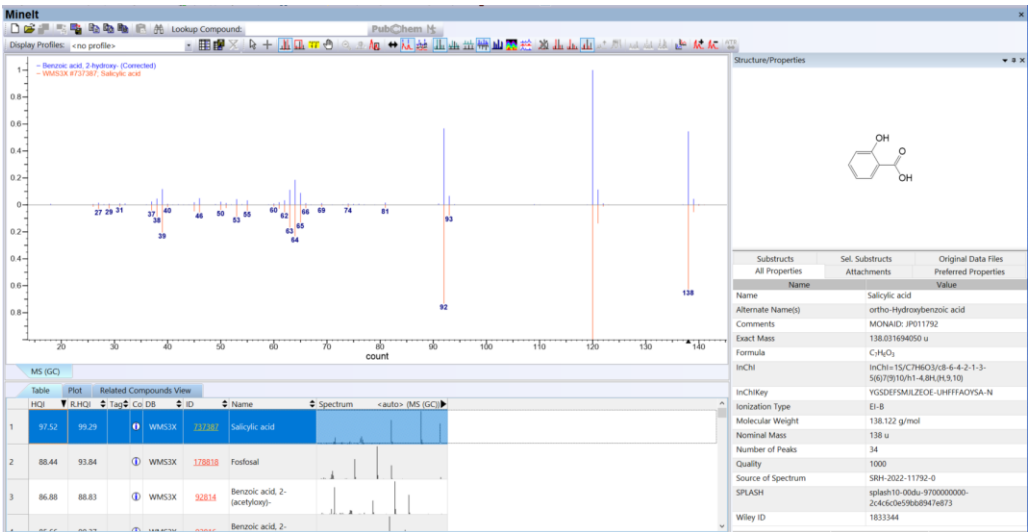
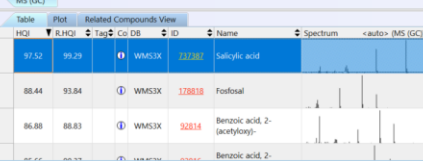
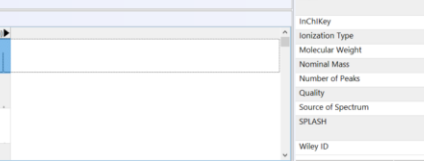
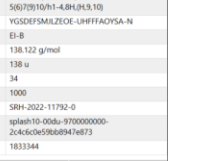
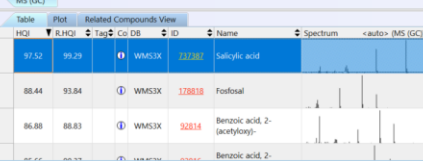
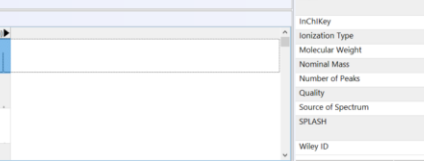
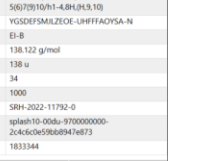
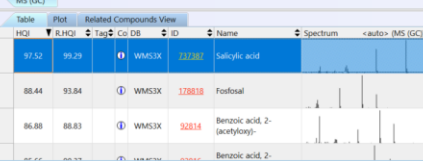
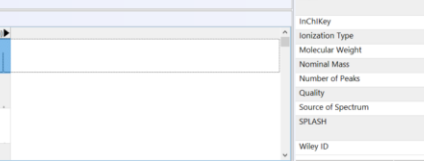
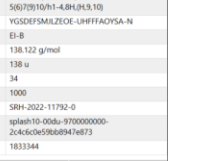
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	 <p data-bbox="863 735 1839 764">NMR MS (GC)</p> <table border="1" data-bbox="863 773 1839 1135"> <thead> <tr> <th>Table</th> <th>Plot</th> <th colspan="6">Related Compounds View</th> </tr> <tr> <th>HQI</th> <th>Weight</th> <th>Exclude</th> <th>DB</th> <th>ID</th> <th>Name</th> <th>Spectrum</th> <th><auto> (NMR)</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>999.00</td> <td>N.A.</td> <td></td> <td></td> <td>Composite Spectrum</td> <td></td> <td></td> </tr> <tr> <td></td> <td>0.34</td> <td><input type="radio"/></td> <td>mpo</td> <td>44</td> <td>Tetrahydrofuran</td> <td></td> <td></td> </tr> <tr> <td></td> <td>0.33</td> <td><input type="radio"/></td> <td>mpo</td> <td>17</td> <td>Methyl isocyanide</td> <td></td> <td></td> </tr> <tr> <td></td> <td>0.33</td> <td><input type="radio"/></td> <td>mpo</td> <td>21</td> <td>Behenyl palmitate</td> <td></td> <td></td> </tr> <tr> <td></td> <td>N.A.</td> <td></td> <td></td> <td></td> <td>Residual Spectrum</td> <td></td> <td></td> </tr> </tbody> </table> <p data-bbox="856 1177 1919 1258">KnowItAll presents a 3-component search result. The top spectrum is unknown; the bottom spectrum is the composite spectrum of 3 components. The middle spectrum is the difference between the two. In this case, it is next to nothing, indicating that there are no more residual peaks.</p> <p data-bbox="856 1295 1808 1349">This process accomplishes many steps in one. It also avoids negative peaks from spectral subtraction.</p>	Table	Plot	Related Compounds View						HQI	Weight	Exclude	DB	ID	Name	Spectrum	<auto> (NMR)	1	999.00	N.A.			Composite Spectrum				0.34	<input type="radio"/>	mpo	44	Tetrahydrofuran				0.33	<input type="radio"/>	mpo	17	Methyl isocyanide				0.33	<input type="radio"/>	mpo	21	Behenyl palmitate				N.A.				Residual Spectrum		
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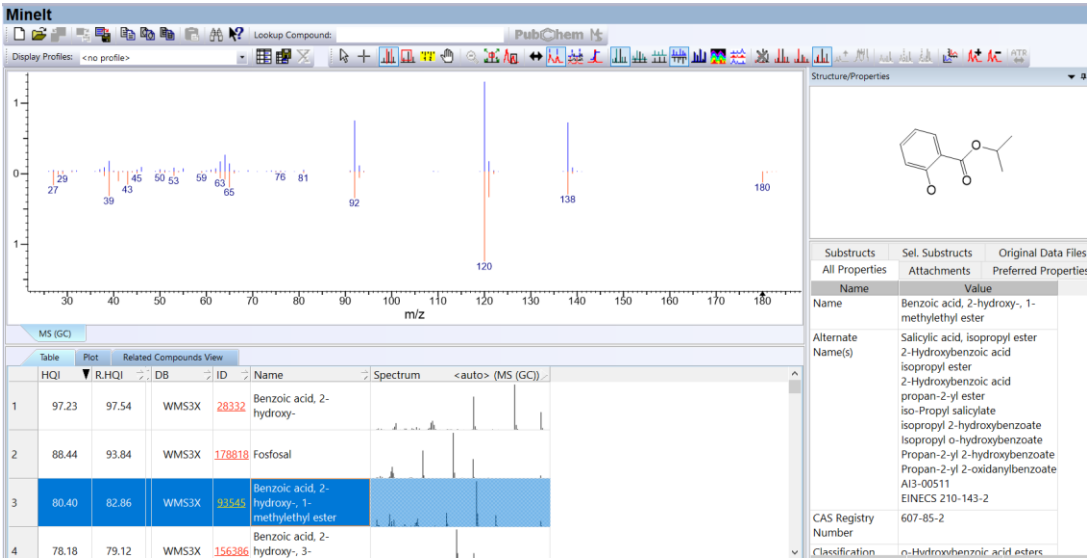

Adaptive Search

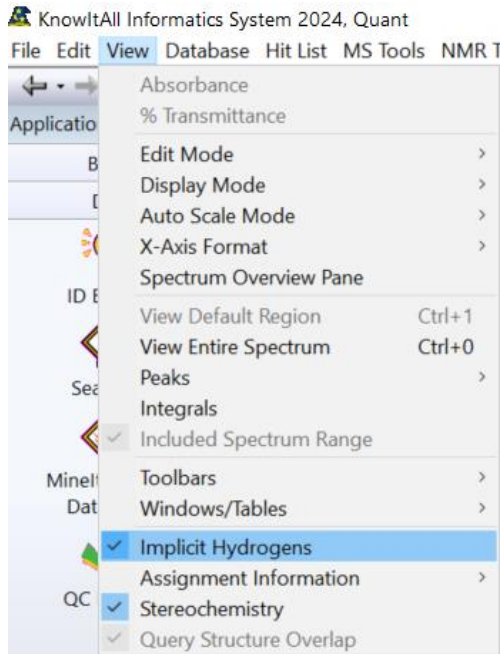
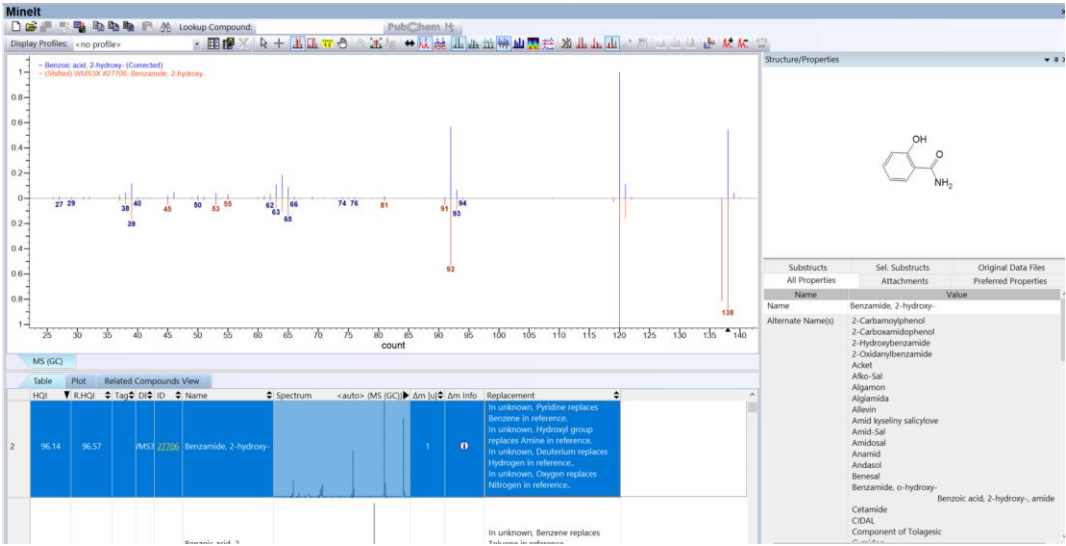
This search finds similar compounds where a fragment group can be present or missing compared to the unknown. The presence/absence of a fragment causes some peak positions in reference MS differing to that of unknown by a delta mass (Δm). KnowItAll shifts some peaks by the Δm to achieve a better matching score. Because of the better matching score, similar compounds come atop of the hit list. To clearly mark the shifts done by Adaptive Search, dotted lines are used to show reference spectrum before and after shifting in the pop-up window when you click on the (i) button in a hit.

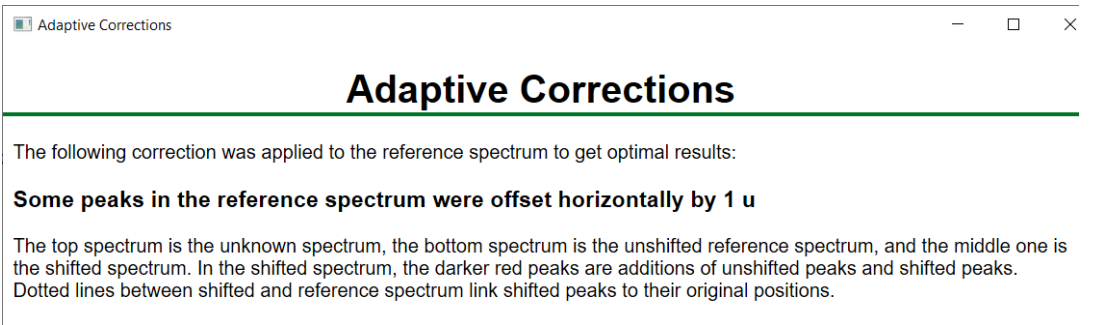
Example wherein exact mass is in a spectrum

	Action	Result
1	<p>Start a new search by clicking .</p> <p>Click Spectrum to navigate to C:\Users\Public\Documents\Wiley\KnowItAll\Samples\MS folder</p> <p>Select the file 2-Hydroxybenzoic acid.</p> <p>Use the drop-down menu to set the Number of components to 1 (Single).</p> <p>Ensure Adaptive Search or Reverse Search is unchecked.</p>	 <p>This spectrum file contains a molecular ion mass of 138. KnowItAll will use this value for the Adaptive Search.</p>

Action	Result																																
<p>2 Click User-Select button</p> <p>Use Remove All to clean selected databases.</p> <p>Use Limit to spectral technique: and select MS (GC).</p> <p>Scroll down the list and highlight the WMS3X database.</p> <p>Use Add to add MS (GC) databases by codes: WMS3X.</p> <p>Click Search.</p>	 <p>The screenshot shows the 'Search Categories' panel on the left with 'Spectrum MS (GC)' selected. The 'Search Databases' panel below it shows a list of databases, with 'MS - Wiley Registry of Mass Spectral Data 12th Edition' (WMS3X) highlighted. The 'User-Select' section is active, and the 'Add' button is visible. The 'Search' button is at the bottom right.</p>																																
<p>3 Click the Butterfly view icon .</p>	 <p>The screenshot shows the 'Mineit' window with a mass spectrum plot and a table of search results. The 'Butterfly view icon' is highlighted in the top toolbar. The mass spectrum plot shows relative intensity versus count, with peaks at 97.52, 99.29, 88.44, 93.84, 86.88, 88.83, 71.78, 178.18, 92.14, and 138. The table below the plot shows search results for 'Salicylic acid' and 'Benzoic acid, 2-(acetyloxy)-'.</p> <table border="1"> <thead> <tr> <th>ICSI</th> <th>RHCS</th> <th>Pub</th> <th>CU DB</th> <th>ID</th> <th>Name</th> <th>Spectrum</th> <th>«auto» (MS (GC))</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>97.52</td> <td>99.29</td> <td>WMS3X</td> <td>71738</td> <td>Salicylic acid</td> <td></td> <td></td> </tr> <tr> <td>2</td> <td>88.44</td> <td>93.84</td> <td>WMS3X</td> <td>17818</td> <td>Fosfosal</td> <td></td> <td></td> </tr> <tr> <td>3</td> <td>86.88</td> <td>88.83</td> <td>WMS3X</td> <td>9214</td> <td>Benzoic acid, 2-(acetyloxy)-</td> <td></td> <td></td> </tr> </tbody> </table>	ICSI	RHCS	Pub	CU DB	ID	Name	Spectrum	«auto» (MS (GC))	1	97.52	99.29	WMS3X	71738	Salicylic acid			2	88.44	93.84	WMS3X	17818	Fosfosal			3	86.88	88.83	WMS3X	9214	Benzoic acid, 2-(acetyloxy)-		
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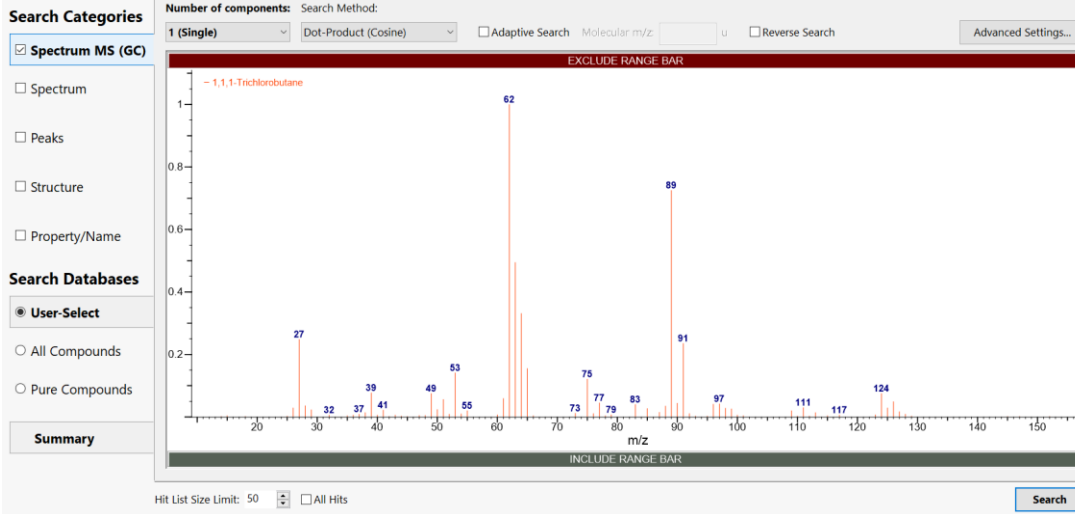
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<p>4 Highlight the 2nd then the 3rd hit.</p>	 <p>The screenshot shows the Minelt software interface. At the top, there is a search bar and a toolbar. Below that is a mass spectrum plot with the x-axis labeled 'm/z' ranging from 30 to 180. The y-axis represents relative intensity. A table below the plot lists search results with columns for HQI, R.HQI, DB, ID, Name, and Spectrum. The 3rd hit is highlighted in blue.</p> <table border="1"> <thead> <tr> <th>HQI</th> <th>R.HQI</th> <th>DB</th> <th>ID</th> <th>Name</th> <th>Spectrum</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>97.23</td> <td>97.54</td> <td>WMS3X</td> <td>28332 Benzoic acid, 2-hydroxy-</td> <td></td> </tr> <tr> <td>2</td> <td>88.44</td> <td>93.84</td> <td>WMS3X</td> <td>178810 Fosfosal</td> <td></td> </tr> <tr> <td>3</td> <td>80.40</td> <td>82.86</td> <td>WMS3X</td> <td>93545 Benzoic acid, 2-hydroxy-, 1-methylethyl ester</td> <td></td> </tr> <tr> <td>4</td> <td>78.18</td> <td>79.12</td> <td>WMS3X</td> <td>156386 Benzoic acid, 2-hydroxy-, 3-</td> <td></td> </tr> </tbody> </table> <p>Assume that we do not have the first hit in our database. The HQI values for the 2nd and 3rd hits are not totally convincing that they are good matches.</p>	HQI	R.HQI	DB	ID	Name	Spectrum	1	97.23	97.54	WMS3X	28332 Benzoic acid, 2-hydroxy-		2	88.44	93.84	WMS3X	178810 Fosfosal		3	80.40	82.86	WMS3X	93545 Benzoic acid, 2-hydroxy-, 1-methylethyl ester		4	78.18	79.12	WMS3X	156386 Benzoic acid, 2-hydroxy-, 3-	
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<p>5 Go back to SearchIt.</p> <p>Click Spectrum MS(GC)</p> <p>Check Adaptive Search.</p> <p>Click Search.</p>	 <p>The screenshot shows the search interface. The 'Adaptive Search' checkbox is checked. The 'Molecular m/z:' box contains the value '138'. To the right of this box is a lightbulb icon and an unchecked 'Reverse Search' checkbox. Below the search bar is a dark red bar labeled 'EXCLUDE RANGE BAR' and a button that says 'Estimates the molecular m/z value'.</p> <ul style="list-style-type: none"> • If a spectrum file contains the molecular ion mass, it will be displayed in the “Molecular <i>m/z</i>” box, and a solid triangle marks this position in the spectrumPane. • You can type in an appropriate value as well. This value is used to assist Adaptive Search. • Or, you can ask KnowItAll to estimate a molecular mass for you by clicking the bulb icon. • However, if molecular ion mass is unknown, this box would be empty. KnowItAll Adaptive Search will estimate this value from the input unknown MS spectrum. 																														


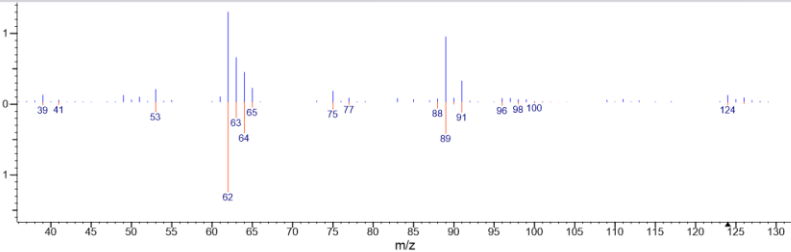
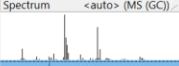

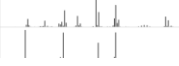
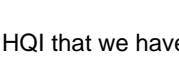

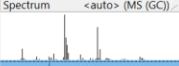

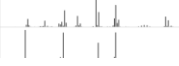
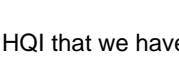
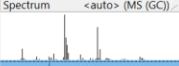

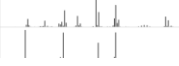
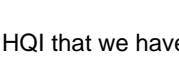
Action	Result
<p>6 Ignore the 1st hit and move to the 2nd hit.</p> <p>Make sure KnowItAll displays implicit Hydrogens.</p> <p>Go to the View menu and check Implicit Hydrogens.</p> 	 <p>The result looks good. Δm of 1 suggests a few possibilities wherein the unknown's molecular ion mass (m/z) is 1 unit more than that of the reference. One of them is "In unknown, Oxygen replaces Nitrogen in reference."</p> <p>Columns of interest:</p> <ul style="list-style-type: none"> • Δm: the molecular ion mass of unknown minus that of the reference • Δm Info: details on reference peak shifts to make the match • Replacement: suggestions of possible group exchange which would cause the difference between unknown and reference molecular ion masses


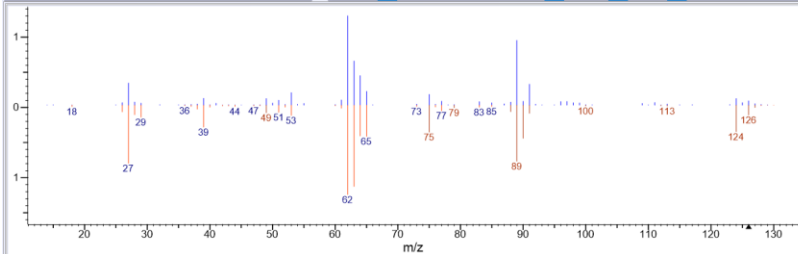

	Action	Result
7	Click (i) in the Δm Info column. This brings up the Adaptive Corrections html page which explains how selective peaks have been shifted to obtain good Hit Quality Index (HQI) .	 <p>The following correction was applied to the reference spectrum to get optimal results:</p> <p>Some peaks in the reference spectrum were offset horizontally by 1 u</p> <p>The top spectrum is the unknown spectrum, the bottom spectrum is the unshifted reference spectrum, and the middle one is the shifted spectrum. In the shifted spectrum, the darker red peaks are additions of unshifted peaks and shifted peaks. Dotted lines between shifted and reference spectrum link shifted peaks to their original positions.</p>


8	Action	Result
		<p>— Query</p> <p>— Corrected Reference</p> <p>— Uncorrected Reference</p> <p>m/z</p> <ul style="list-style-type: none">• The top spectrum is the unknown• The bottom spectrum is the unshifted reference• The middle “spectrum” contains shifted peaks• Dotted lines indicate what peaks have been moved by an Δm• In the middle “spectrum,” darker red peaks are the additions of existing peaks and moved ones


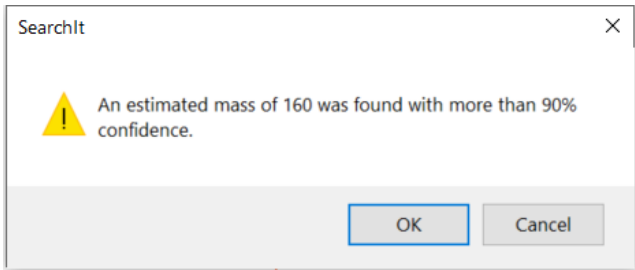
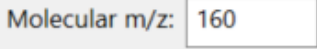

Example wherein exact mass is NOT in a spectrum

	Action	Result
1	<p>Start a new search by clicking .</p> <p>Click Spectrum and navigate to C:\Users\Public\Documents\Wiley\KnowItAll\Samples\MS folder.</p> <p>Select file 1,1,1-Trichlorobutane.</p> <p>Use the drop-down menu to set the Number of components to 1 (Single).</p> <p>Ensure Adaptive Search or Reverse Search is unchecked.</p> <p>Click User-Select.</p> <p>Use Remove All to clean selected databases.</p> <p>Use Add to add MS (GC) databases by codes: WMS3X.</p> <p>Click Search.</p>	 <p>As we can see, this spectrum does not contain molecular ion mass. KnowItAll will estimate this value and use it for Adaptive Search.</p>

Action	Result																																																														
<p>2</p> <p>Click the Butterfly view icon .</p> <p>Go to the 2nd hit since the 1st hit is the exact match (pretend that we do not have the exact match in our databases).</p>	 <div style="display: flex; justify-content: space-between;"> <div data-bbox="863 576 1648 820"> <p>MS (GC)</p> <table border="1"> <thead> <tr> <th>Table</th> <th>Plot</th> <th>Related Compounds View</th> </tr> <tr> <th>HQI</th> <th>R.HQI</th> <th>Tag</th> <th>ID</th> <th>Name</th> <th>Spectrum</th> <th><auto> (MS (GC))</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>100.00</td> <td>100.00</td> <td>VMS3_56884</td> <td>1,1,1-Trichlorobutane</td> <td></td> <td></td> </tr> <tr style="background-color: #e0f0ff;"> <td>2</td> <td>78.93</td> <td>92.51</td> <td>VMS3_16200</td> <td>Cyclobutane, 1,2-dichloro-</td> <td></td> <td></td> </tr> <tr> <td>3</td> <td>64.80</td> <td>65.18</td> <td>VMS3_16286</td> <td>2-Butene, 1,4-dichloro-</td> <td></td> <td></td> </tr> <tr> <td>4</td> <td>64.23</td> <td>64.59</td> <td>VMS3_16299</td> <td>1-Butene, 3,4-dichloro-</td> <td></td> <td></td> </tr> </tbody> </table> </div> <div data-bbox="1648 326 1948 820"> <p>Structure/Properties</p>  <table border="1"> <thead> <tr> <th>Name</th> <th>Value</th> </tr> </thead> <tbody> <tr> <td>Name</td> <td>Cyclobutane, 1,2-dichloro-</td> </tr> <tr> <td>Alternate Name(s)</td> <td>1,2-Dichlorocyclobutane 1,2-Bis(chloranyl)cyclobutane</td> </tr> <tr> <td>CAS Registry Number</td> <td>17437-39-7</td> </tr> <tr> <td>Classification</td> <td>Organochlorides</td> </tr> <tr> <td>Estimated Kovats Retention Index</td> <td>813</td> </tr> <tr> <td>Exact Mass</td> <td>123.984656 u</td> </tr> <tr> <td>Formula</td> <td>C4H6Cl2</td> </tr> <tr> <td>InChI</td> <td>InChI=1S/C4H6Cl2/c5-3-1-2-4...</td> </tr> <tr> <td>InChIKey</td> <td>MPWHMPULOPKVDQ-UHFFFA...</td> </tr> <tr> <td>Molecular Weight</td> <td>124.996 g/mol</td> </tr> <tr> <td>Nominal Mass</td> <td>124 u</td> </tr> </tbody> </table> </div> </div> <p>We are not sure by looking at the HQI that we have a hit that is structurally similar to our unknown.</p>	Table	Plot	Related Compounds View	HQI	R.HQI	Tag	ID	Name	Spectrum	<auto> (MS (GC))	1	100.00	100.00	VMS3_56884	1,1,1-Trichlorobutane			2	78.93	92.51	VMS3_16200	Cyclobutane, 1,2-dichloro-			3	64.80	65.18	VMS3_16286	2-Butene, 1,4-dichloro-			4	64.23	64.59	VMS3_16299	1-Butene, 3,4-dichloro-			Name	Value	Name	Cyclobutane, 1,2-dichloro-	Alternate Name(s)	1,2-Dichlorocyclobutane 1,2-Bis(chloranyl)cyclobutane	CAS Registry Number	17437-39-7	Classification	Organochlorides	Estimated Kovats Retention Index	813	Exact Mass	123.984656 u	Formula	C4H6Cl2	InChI	InChI=1S/C4H6Cl2/c5-3-1-2-4...	InChIKey	MPWHMPULOPKVDQ-UHFFFA...	Molecular Weight	124.996 g/mol	Nominal Mass	124 u
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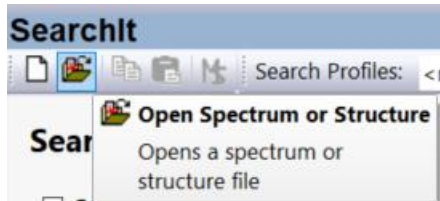
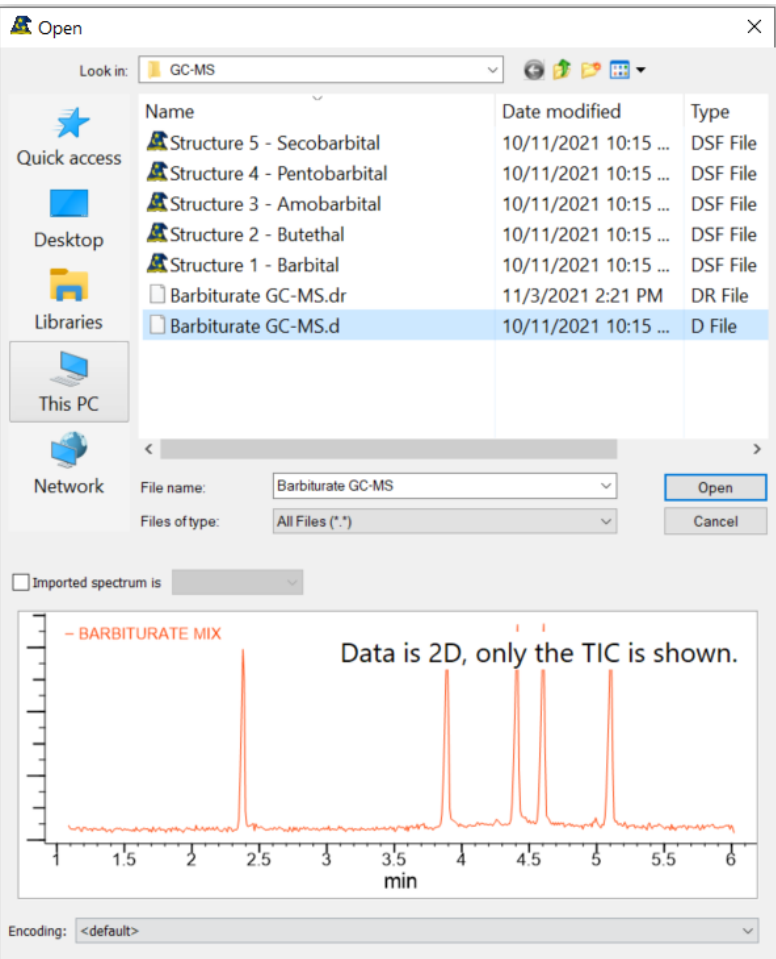
Action	Result																																																			
<p>3 Go back to SearchIt</p> <p>Check Adaptive Search</p> <p><input checked="" type="checkbox"/> Adaptive Search Molecular <i>m/z</i>: <input type="text"/></p> <p>Search</p> <p>In Minelt, Click the Butterfly view icon .</p> <p>Go to the 2nd hit (1st being the unknown which is already in a database).</p>	 <table border="1" data-bbox="856 576 1648 812"> <thead> <tr> <th>Table</th> <th>Plot</th> <th>Related Compounds View</th> </tr> <tr> <th>HQI</th> <th>R.H.C</th> <th>DB</th> <th>ID</th> <th>Name</th> <th>Spectrum</th> <th><auto> (MS (GC))</th> <th>Δm [u]</th> <th>Δm Info</th> <th>Replacement</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>100.00</td> <td>100.00</td> <td>VMS3</td> <td>56884</td> <td>1,1,1-Trichlorobutane</td> <td></td> <td>0</td> <td></td> <td></td> </tr> <tr> <td>2</td> <td>82.79</td> <td>67.26</td> <td>VMS3</td> <td>17731</td> <td>Butane, 2,3-dichloro-</td> <td></td> <td>34</td> <td></td> <td>In unknown, Chlorine replaces Hydrogen in reference In unknown, -CF3 group replaces Chlorine in reference</td> </tr> </tbody> </table> <div data-bbox="1648 324 1942 812"> <p>Structure/Properties</p>  <table border="1"> <thead> <tr> <th>Substructs</th> <th>Sel. Substructs</th> <th>Original Data Files</th> </tr> <tr> <th>All Properties</th> <th>Attachments</th> <th>Preferred Properties</th> </tr> <tr> <th>Name</th> <th colspan="2">Value</th> </tr> </thead> <tbody> <tr> <td>Name</td> <td colspan="2">Butane, 2,3-dichloro-</td> </tr> <tr> <td>Alternate Name(s)</td> <td colspan="2">2,3-Dichlorobutane (-+/-)-2,3-Dichlorobutane 2,3-Bis(chloranyl)butane Butane, 2,3-dichloro-, (-+/-)- Butane, 2,3-dichloro-, (R*,S*)- Butane, 2,3-dichloro-, (R*,R*)-(+)- Butane, 2,3-dichloro-, (R*,R*)-(+/-)- Butane, 2,3-dichloro-, meso- di-2,3-Dichlorobutane meso-2,3-Dichlorobutane racemic-2,3-Dichlorobutane EINECS 231-486-4</td> </tr> <tr> <td>CAS Registry Number</td> <td colspan="2">7581-97-7</td> </tr> </tbody> </table> </div> <p>One of the suggestions in the Replacement cell makes sense. The unknown should have 1 more Chlorine for a delta mass of 34.</p>	Table	Plot	Related Compounds View	HQI	R.H.C	DB	ID	Name	Spectrum	<auto> (MS (GC))	Δm [u]	Δm Info	Replacement	1	100.00	100.00	VMS3	56884	1,1,1-Trichlorobutane		0			2	82.79	67.26	VMS3	17731	Butane, 2,3-dichloro-		34		In unknown, Chlorine replaces Hydrogen in reference In unknown, -CF3 group replaces Chlorine in reference	Substructs	Sel. Substructs	Original Data Files	All Properties	Attachments	Preferred Properties	Name	Value		Name	Butane, 2,3-dichloro-		Alternate Name(s)	2,3-Dichlorobutane (-+/-)-2,3-Dichlorobutane 2,3-Bis(chloranyl)butane Butane, 2,3-dichloro-, (-+/-)- Butane, 2,3-dichloro-, (R*,S*)- Butane, 2,3-dichloro-, (R*,R*)-(+)- Butane, 2,3-dichloro-, (R*,R*)-(+/-)- Butane, 2,3-dichloro-, meso- di-2,3-Dichlorobutane meso-2,3-Dichlorobutane racemic-2,3-Dichlorobutane EINECS 231-486-4		CAS Registry Number	7581-97-7	
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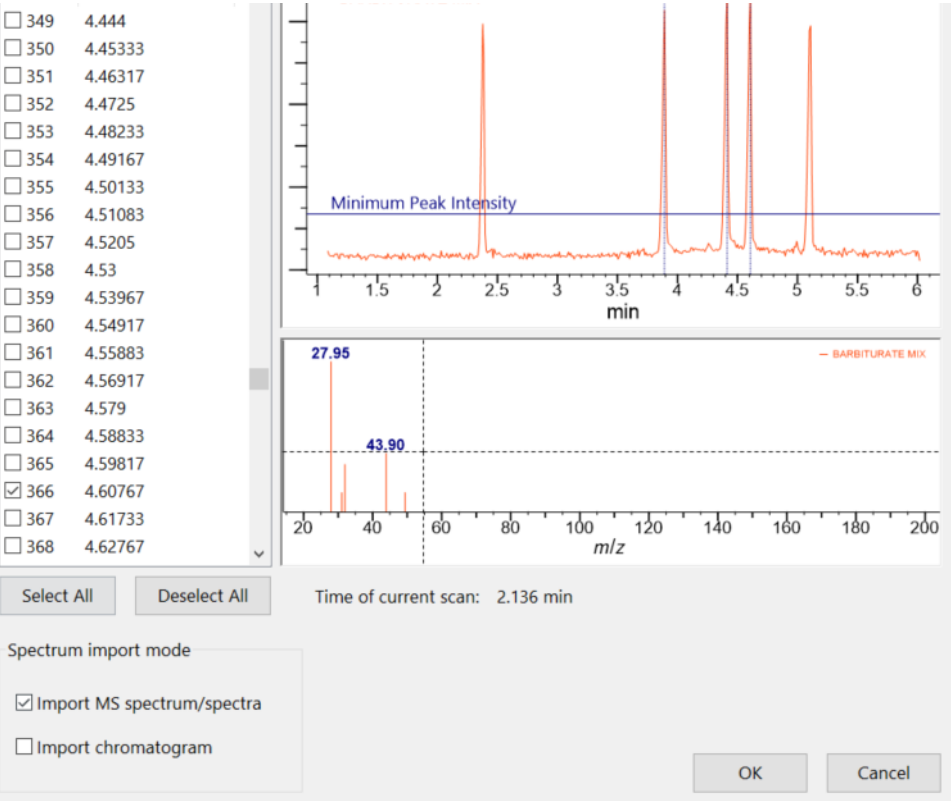
Alternatively, one can use the molecular ion mass estimation icon  to estimate the molecular ion mass:

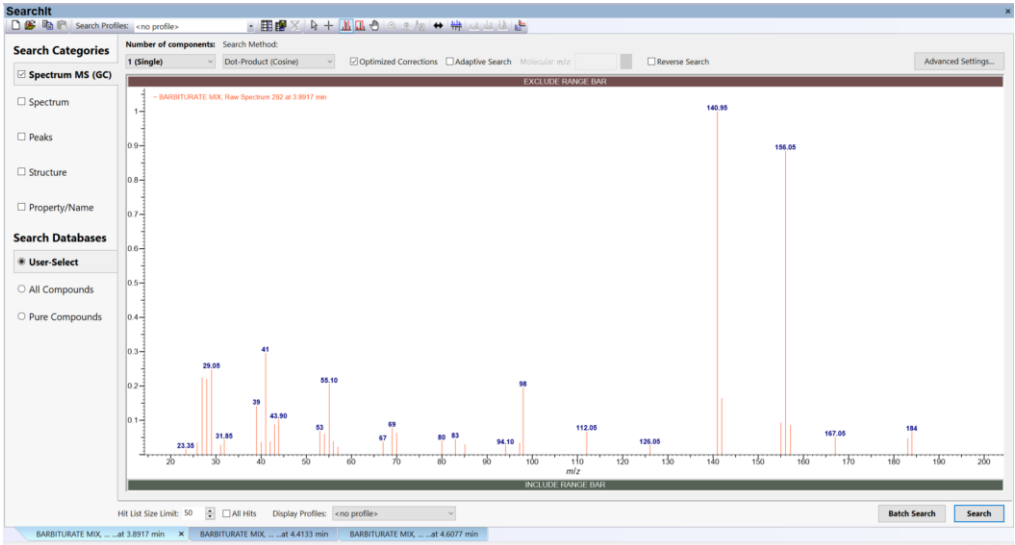
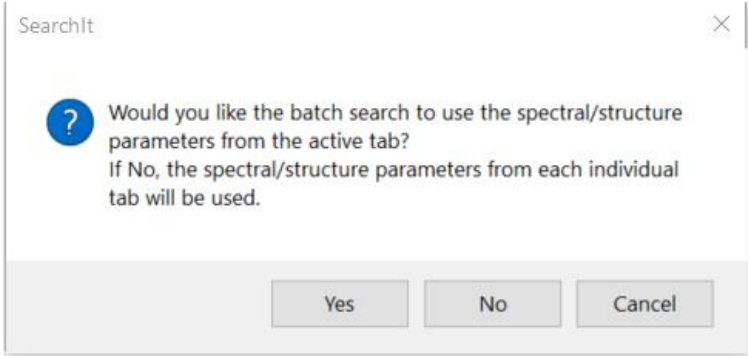
	Action	Result
1	Go Back to SearchIt . Click the  icon. Let the calculation complete.	
3	Click OK button	The estimated molecular ion mass value fills the box  

NOTE: KnowItAll runs an adaptive search of the unknown against all of our MS databases. The resulting hit list is then analyzed in steps. The mass of every hit list entry is calculated as the nominal mass of the compound in the database record plus the Δm found for the match. Matches of equal mass are then grouped together into clusters. The higher the found HQI, the higher the score for an individual match. Scores for clusters are then calculated as a combination of individual match scores with additional information such as the number of entries in the cluster and the separation from the next best cluster. The cluster with the best score determines the found mass. As side information of this procedure, information on confidence that the found mass will be correct is reported. The confidence values found by the algorithm were determined by running statistics with thousands of very diverse compound spectra run against our MS data.

Simultaneous Multiple MS Spectra Search

	Action	Result
<p>1 In SearchIt, select Open Spectrum or Structure</p>  <p>Navigate to C:\Users\Public\Documents\Wiley\KnowItAll\Samples\GC-MS\Barbiturate GC-MS.d</p> <p>Click Open in previous dialog.</p>		

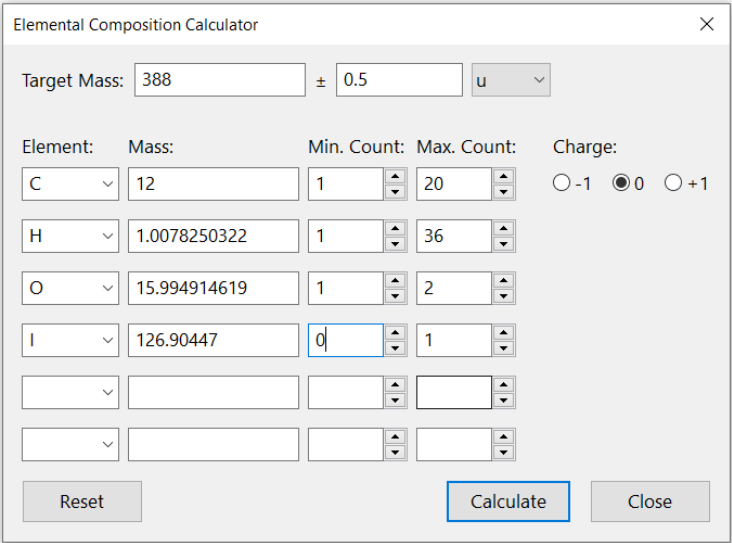
	Action	Result
2	<p>Pick multiple MS spectrum in the MS Spectra Scan Selection dialog</p> <p>For example, 292, 346, 366</p> <p>Click OK button</p>	 <p> <input type="checkbox"/> 349 4.444 <input type="checkbox"/> 350 4.45333 <input type="checkbox"/> 351 4.46317 <input type="checkbox"/> 352 4.4725 <input type="checkbox"/> 353 4.48233 <input type="checkbox"/> 354 4.49167 <input type="checkbox"/> 355 4.50133 <input type="checkbox"/> 356 4.51083 <input type="checkbox"/> 357 4.5205 <input type="checkbox"/> 358 4.53 <input type="checkbox"/> 359 4.53967 <input type="checkbox"/> 360 4.54917 <input type="checkbox"/> 361 4.55883 <input type="checkbox"/> 362 4.56917 <input type="checkbox"/> 363 4.579 <input type="checkbox"/> 364 4.58833 <input type="checkbox"/> 365 4.59817 <input checked="" type="checkbox"/> 366 4.60767 <input type="checkbox"/> 367 4.61733 <input type="checkbox"/> 368 4.62767 </p> <p> <input type="button" value="Select All"/> <input type="button" value="Deselect All"/> </p> <p> Spectrum import mode <input checked="" type="checkbox"/> Import MS spectrum/spectra <input type="checkbox"/> Import chromatogram </p> <p>Time of current scan: 2.136 min</p> <p> <input type="button" value="OK"/> <input type="button" value="Cancel"/> </p>

	Action	Result
3	Multiple search tabs are created	
4	<p>One can either search these MS spectra one by one by clicking the Search button in each tab.</p> <p>Or use Do Batch Search button and reply Yes when prompted.</p>	
5		Multiple hits are transferred to Minelt if Do Batch Search was selected

Action	Result																																																																					
	<div data-bbox="856 315 1837 1078"> <p>Minett</p> <p>Display Profiles: <no profile> Lookup Compound: PubChem</p> <p>MS (GC)</p> <table border="1"> <thead> <tr> <th>Table</th> <th>Plot</th> <th>Related Compounds View</th> </tr> </thead> <tbody> <tr> <th>HQI</th> <th>R.HQI</th> <th>Tag</th> <th>DI</th> <th>ID</th> <th>Name</th> <th>Spectrum</th> <th><auto> (MS (GC))</th> <th>Δm (u)</th> <th>Δm Info</th> <th>Replacement</th> </tr> <tr> <td>1</td> <td>91.90</td> <td>95.75</td> <td></td> <td>/MS3_165479</td> <td>2,4,6(1H,3H,5H)- Pyrimidinetrione, 5- butyl-5-ethyl-</td> <td></td> <td><auto> (MS (GC))</td> <td>0</td> <td>ⓘ</td> <td></td> </tr> <tr> <td>2</td> <td>88.47</td> <td>89.62</td> <td></td> <td>/MS3_165488</td> <td>2,4,6(1H,3H,5H)- Pyrimidinetrione, 5- ethyl-5-(1-</td> <td></td> <td><auto> (MS (GC))</td> <td>0</td> <td>ⓘ</td> <td></td> </tr> <tr> <td>3</td> <td>88.42</td> <td>91.28</td> <td></td> <td>/MS3_101117</td> <td>2,4,6(1H,3H,5H)- Pyrimidinetrione, 5,5- diethyl-</td> <td></td> <td><auto> (MS (GC))</td> <td>89</td> <td>ⓘ</td> <td></td> </tr> <tr> <td>4</td> <td>87.26</td> <td>84.84</td> <td></td> <td>/MS3_201919</td> <td>Pentobarbital</td> <td></td> <td><auto> (MS (GC))</td> <td>-14</td> <td>ⓘ</td> <td>In unknown, CH2 group replaces C=O group in reference. In unknown, Methyl group</td> </tr> <tr> <td>5</td> <td>87.08</td> <td>17.86</td> <td></td> <td>/MS3_238804</td> <td>2,4,6(1H,3H,5H)- Pyrimidinetrione, 5- butyl-3-ethyl-1,3- 2,4,6(1H,3H,5H)-</td> <td></td> <td><auto> (MS (GC))</td> <td>-28</td> <td>ⓘ</td> <td>In unknown, CH2 group replaces</td> </tr> </tbody> </table> <p>Hit List: BARBITURATE MIX, Raw Spectrum 345 at 4.4038 min Hit List: BARBITURATE MIX, Raw Spectrum 366 at 4.6077 min Hit List: BARBITURATE MIX, Raw Spectrum 292 at 3.8917 m</p> <p>14 < 1 200 Records</p> </div>	Table	Plot	Related Compounds View	HQI	R.HQI	Tag	DI	ID	Name	Spectrum	<auto> (MS (GC))	Δm (u)	Δm Info	Replacement	1	91.90	95.75		/MS3_165479	2,4,6(1H,3H,5H)- Pyrimidinetrione, 5- butyl-5-ethyl-		<auto> (MS (GC))	0	ⓘ		2	88.47	89.62		/MS3_165488	2,4,6(1H,3H,5H)- Pyrimidinetrione, 5- ethyl-5-(1-		<auto> (MS (GC))	0	ⓘ		3	88.42	91.28		/MS3_101117	2,4,6(1H,3H,5H)- Pyrimidinetrione, 5,5- diethyl-		<auto> (MS (GC))	89	ⓘ		4	87.26	84.84		/MS3_201919	Pentobarbital		<auto> (MS (GC))	-14	ⓘ	In unknown, CH2 group replaces C=O group in reference. In unknown, Methyl group	5	87.08	17.86		/MS3_238804	2,4,6(1H,3H,5H)- Pyrimidinetrione, 5- butyl-3-ethyl-1,3- 2,4,6(1H,3H,5H)-		<auto> (MS (GC))	-28	ⓘ	In unknown, CH2 group replaces
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Other Tools for Mass Spectrometry

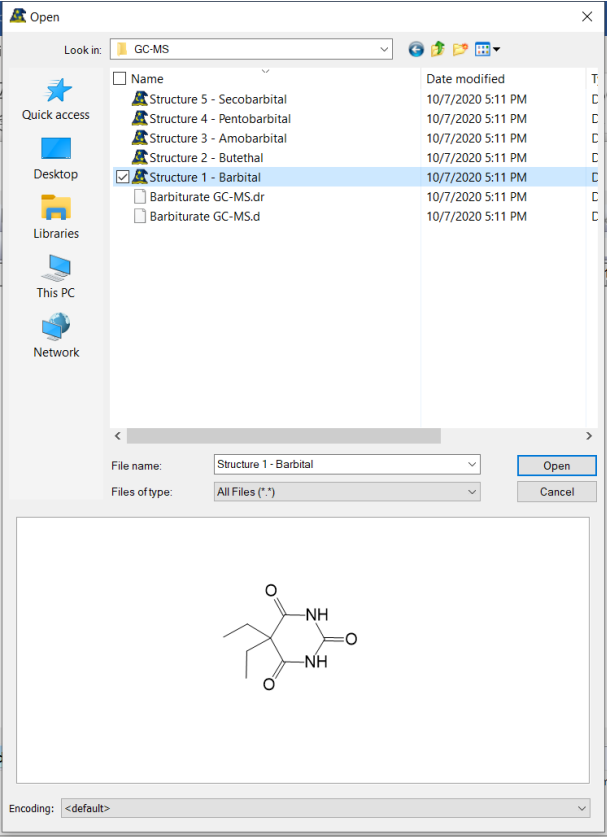
Elemental Composition

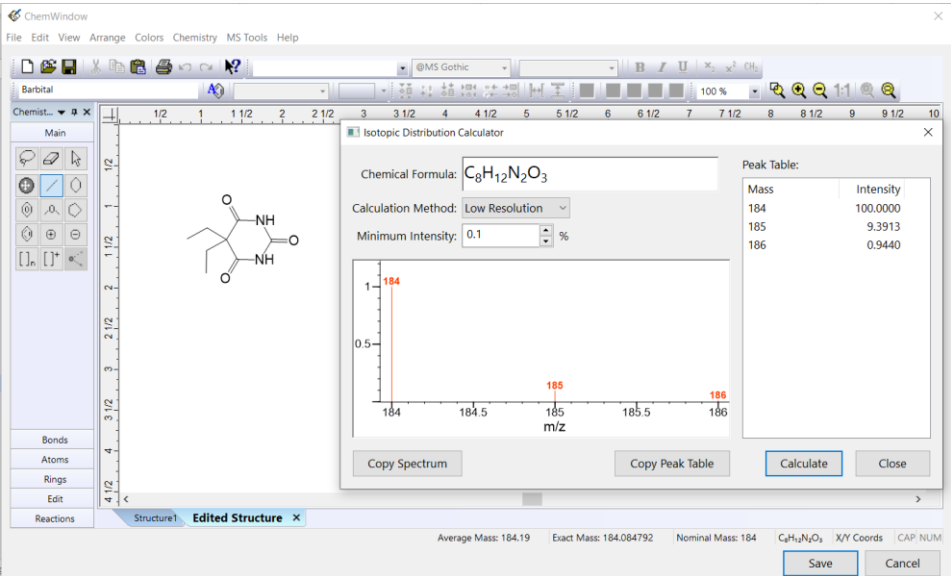
	Action	Result																																			
1	From the Menu , navigate to MS Tools > Calculate Elemental Composition .	 <p>The screenshot shows a dialog box titled "Elemental Composition Calculator". At the top, there is a "Target Mass" field set to 388, followed by a tolerance field set to ± 0.5 and a unit dropdown menu set to "u". Below this, there is a table of elements with columns for "Element", "Mass", "Min. Count", "Max. Count", and "Charge". The elements listed are C, H, O, and I. The "Calculate" button is highlighted with a blue border.</p> <table border="1"> <thead> <tr> <th>Element</th> <th>Mass</th> <th>Min. Count</th> <th>Max. Count</th> <th>Charge</th> </tr> </thead> <tbody> <tr> <td>C</td> <td>12</td> <td>1</td> <td>20</td> <td><input type="radio"/> -1 <input checked="" type="radio"/> 0 <input type="radio"/> +1</td> </tr> <tr> <td>H</td> <td>1.0078250322</td> <td>1</td> <td>36</td> <td></td> </tr> <tr> <td>O</td> <td>15.994914619</td> <td>1</td> <td>2</td> <td></td> </tr> <tr> <td>I</td> <td>126.90447</td> <td>0</td> <td>1</td> <td></td> </tr> <tr> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> </tbody> </table> <p>Buttons: Reset, Calculate, Close</p>	Element	Mass	Min. Count	Max. Count	Charge	C	12	1	20	<input type="radio"/> -1 <input checked="" type="radio"/> 0 <input type="radio"/> +1	H	1.0078250322	1	36		O	15.994914619	1	2		I	126.90447	0	1											
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You can fill in this dialog with target mass and elemental information.

	Action	Result																																																	
2	Click Calculate .	<div data-bbox="877 321 1633 1209"> <p>Elemental Composition Results ×</p> <p>Target Mass: 388 ± 0.5 u</p> <p>Charge: 0 Result Count: 6</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>C</th> <th>H</th> <th>O</th> <th>I</th> <th>m</th> <th>Δm [u]</th> <th>Δm [ppm]</th> </tr> </thead> <tbody> <tr> <td>18</td> <td>13</td> <td>2</td> <td>1</td> <td>387.9960</td> <td>-0.0040</td> <td>-10.2457</td> </tr> <tr> <td>19</td> <td>17</td> <td>1</td> <td>1</td> <td>388.0324</td> <td>0.0324</td> <td>83.5314</td> </tr> <tr> <td>20</td> <td>5</td> <td>1</td> <td>1</td> <td>387.9385</td> <td>-0.0615</td> <td>-158.4799</td> </tr> <tr> <td>17</td> <td>25</td> <td>2</td> <td>1</td> <td>388.0899</td> <td>0.0899</td> <td>231.7656</td> </tr> <tr> <td>19</td> <td>1</td> <td>2</td> <td>1</td> <td>387.9021</td> <td>-0.0979</td> <td>-252.2570</td> </tr> <tr> <td>18</td> <td>29</td> <td>1</td> <td>1</td> <td>388.1263</td> <td>0.1263</td> <td>325.5427</td> </tr> </tbody> </table> <p style="text-align: right;"> <input type="button" value="Copy To Clipboard"/> <input type="button" value="Close"/> </p> </div> <p>KnowItAll provides combinations of these elements.</p>	C	H	O	I	m	Δm [u]	Δm [ppm]	18	13	2	1	387.9960	-0.0040	-10.2457	19	17	1	1	388.0324	0.0324	83.5314	20	5	1	1	387.9385	-0.0615	-158.4799	17	25	2	1	388.0899	0.0899	231.7656	19	1	2	1	387.9021	-0.0979	-252.2570	18	29	1	1	388.1263	0.1263	325.5427
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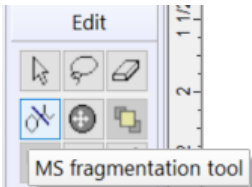
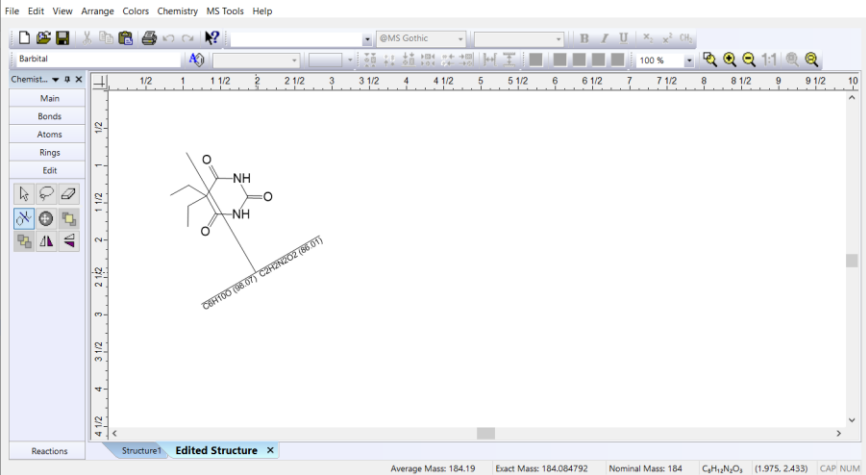
Isotopic Distribution

	Action	Result																
1	<p>Double-click the structure pane to bring up ChemWindow or use the Transfer to: bar and select ChemWindow at the top of the application.</p> <p>Click File > Open.</p> <p>Select a structure from the Samples > GC-MS folder.</p>	 <p>The screenshot shows an 'Open' dialog box with the following details:</p> <ul style="list-style-type: none">Look in: GC-MSFiles list:<table border="1"><thead><tr><th>Name</th><th>Date modified</th></tr></thead><tbody><tr><td>Structure 5 - Secobarbital</td><td>10/7/2020 5:11 PM</td></tr><tr><td>Structure 4 - Pentobarbital</td><td>10/7/2020 5:11 PM</td></tr><tr><td>Structure 3 - Amobarbital</td><td>10/7/2020 5:11 PM</td></tr><tr><td>Structure 2 - Butethal</td><td>10/7/2020 5:11 PM</td></tr><tr><td><input checked="" type="checkbox"/> Structure 1 - Barbital</td><td>10/7/2020 5:11 PM</td></tr><tr><td><input type="checkbox"/> Barbiturate GC-MS.dr</td><td>10/7/2020 5:11 PM</td></tr><tr><td><input type="checkbox"/> Barbiturate GC-MS.d</td><td>10/7/2020 5:11 PM</td></tr></tbody></table>File name: Structure 1 - BarbitalFiles of type: All Files (*.*)Chemical structure of Barbital is displayed below the file list.	Name	Date modified	Structure 5 - Secobarbital	10/7/2020 5:11 PM	Structure 4 - Pentobarbital	10/7/2020 5:11 PM	Structure 3 - Amobarbital	10/7/2020 5:11 PM	Structure 2 - Butethal	10/7/2020 5:11 PM	<input checked="" type="checkbox"/> Structure 1 - Barbital	10/7/2020 5:11 PM	<input type="checkbox"/> Barbiturate GC-MS.dr	10/7/2020 5:11 PM	<input type="checkbox"/> Barbiturate GC-MS.d	10/7/2020 5:11 PM
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	Action	Result								
2	<p>Navigate to MS Tools > Calculate Isotopic Distribution.</p> <p>Click Calculate.</p>	 <p>The screenshot shows the ChemWindow interface with the 'Isotopic Distribution Calculator' dialog box open. The dialog box contains the following information:</p> <ul style="list-style-type: none">Chemical Formula: $C_8H_{12}N_2O_3$Calculation Method: Low ResolutionMinimum Intensity: 0.1 %Mass Spectrum Plot: Shows peaks at m/z 184 (base peak), 185, and 186.Peak Table: <table border="1"><thead><tr><th>Mass</th><th>Intensity</th></tr></thead><tbody><tr><td>184</td><td>100.0000</td></tr><tr><td>185</td><td>9.3913</td></tr><tr><td>186</td><td>0.9440</td></tr></tbody></table> <p>Buttons: Copy Spectrum, Copy Peak Table, Calculate, Close, Save, Cancel.</p>	Mass	Intensity	184	100.0000	185	9.3913	186	0.9440
Mass	Intensity									
184	100.0000									
185	9.3913									
186	0.9440									

Molecular Fragmentation

In ChemWindow, you can use the MS fragmentation tools to view possible fragments and corresponding masses.

	Action	Result
1	<p>In the Edit toolbar on the left, click on the MS fragmentation tool.</p>  <p>Point to a position, then drag a line—this is the fragmentation line.</p>	

Solid Triangle Marks Nominal Mass

- In the spectrum display, KnowItAll marks the nominal mass of the structure that corresponds to the spectrum. This mass is shown as a black triangle.
- When importing a spectrum from a data file, a number of import formats define fields for molecular m/z (also called precursor m/z or base peak m/z in some cases) and the charge of the molecular ion. To convert from molecular m/z to exact mass, the following formulae are used:
 - For positive charges:
 - $M_{\text{exact}} = (Mz - M(\text{H}) + M(\text{e})) * \text{charge}$, where $M(\text{H})$ is the mass of a hydrogen atom, and $M(\text{e})$ is the mass of an electron.
 - For negative charges:
 - $M_{\text{exact}} = (Mz - M(\text{e})) * (-\text{charge})$.
 - If no charge is defined, a default charge of +1 is assumed.
- If a data file does not have the molecular m/z field defined, the exact mass is calculated from the formula field, if available.