

# **KnowItAll Software Training**

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## **GC-MS Analysis Using KnowItAll MS Expert, ProcessIt And SearchIt**

# Automatic GC-MS Analysis

## How to Use KnowItAll MS Expert to Perform Automatic GC-MS Analysis

### Purpose

These exercises demonstrate how to use KnowItAll MS Expert to automatically analyze GC-MS.

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### Objectives

These exercises will teach you:

- How to use KnowItAll MS Expert to auto-deconvolute GC-MS data into chemical component MS spectra which are automatically searched against millions of references.
  - How to generate reports
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### Background

GC-MS data are information rich. Analysis can be time consuming, especially when examining complex analytes. We present a computer system that combines fast, flexible automated deconvolution, automatic database search to identify knowns and unknowns. Novel compounds can be identified, and structural characteristics deduced from applying the MS Adaptive search that uses fragmentation and structural data to propose likely structural details of the unknown.

#### *Training Files Used in This Lesson*

- C:\Users\Public\Documents\Wiley\KnowItAll\Samples\MS Expert folder files

#### *KnowItAll Applications Used*

- KnowItAll MS Expert

## GC-MS Deconvolution Algorithms

Our system follows individual  $m/z$  values across multiple spectra and extracts a pure spectrum from the data for each individual component while trying to separate components with overlapping  $m/z$  value peaks. If accurate  $m/z$  value data are available and the user selects to use it instead of unit  $m/z$  values, the chosen instrument accuracy (automatic, ppm, or fixed value) is used to determine the correct accurate  $m/z$  values that exist in the entire GC-MS analysis. The  $m/z$  values in the raw data are converted into correct accurate values based on the closest value found taking the instrument resolution into account. The corrected  $m/z$  values form the basis of the following deconvolution.

During the deconvolution step, individual  $m/z$  values are pursued across multiple raw spectra, and a component spectrum is extracted from the data for each individual component while trying to separate components with overlapping  $m/z$  value peaks. The details of the algorithm are summarized to a large extent by the following papers<sup>1-4</sup>.

Additional steps are added to automatically detect components with low intensity reconstructed total Ion Current (RTIC) chromatographic peaks as long as they can well be separated from neighboring components.

The details of the algorithm are summarized to a large extent by the following papers, although we go beyond that.

1. S. E. Stein. An Integrated Method for Spectrum Extraction and Compound Identification from Gas Chromatography/Mass Spectrometry Data. *J Am Soc Mass Spectrom* 1999, **10**, 770 –781.
2. R. G. Dromey, Mark J. Stefik, Thomas C. Rindfleisch, Alan M. Duffield. Extraction of Mass Spectra Free of Background and Neighboring Component Contributions from Gas Chromatography IMass Spectrometry Data. *ANALYTICAL CHEMISTRY*, 1976, **VOL. 48, NO.9**, 1368-1375.
3. J. E. Biller, K. Biemann. Reconstructed Mass Spectra, A Novel Approach For The Utilization Of Gas Chromatography-Mass Spectrometer Data. *Analytical Letters* 1974, **7**, 515-28.
4. Bruce N. Colby. Spectral Deconvolution for Overlapping GC/MS Components. *J Am sot Mass Spectrom* 1992, **3**, 558-562.

## MS Spectral Comparison Algorithms

### Research article

Journal of  
MASS  
SPECTROMETRY

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## Evaluation of mass spectral library search algorithms implemented in commercial software

Andrey Samokhin,<sup>a\*</sup> Ksenia Sotnezova,<sup>a</sup> Vitaly Lashin<sup>b</sup> and Igor Revelsky<sup>a</sup>

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<sup>2</sup>

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

Spectrum search type – similarity (simple)

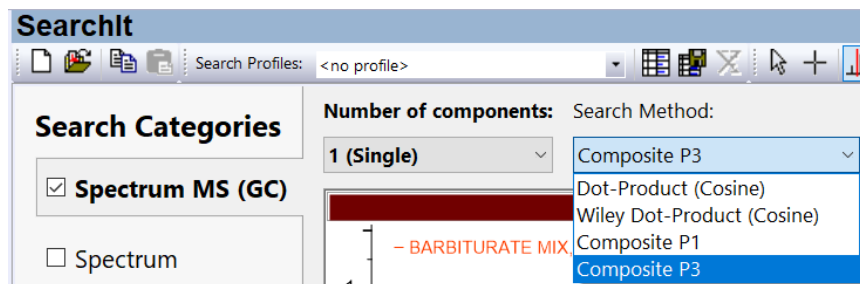
Presearch – default

Included Libs – MainLib

Apply limits – unchecked

Use constraints – unchecked

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



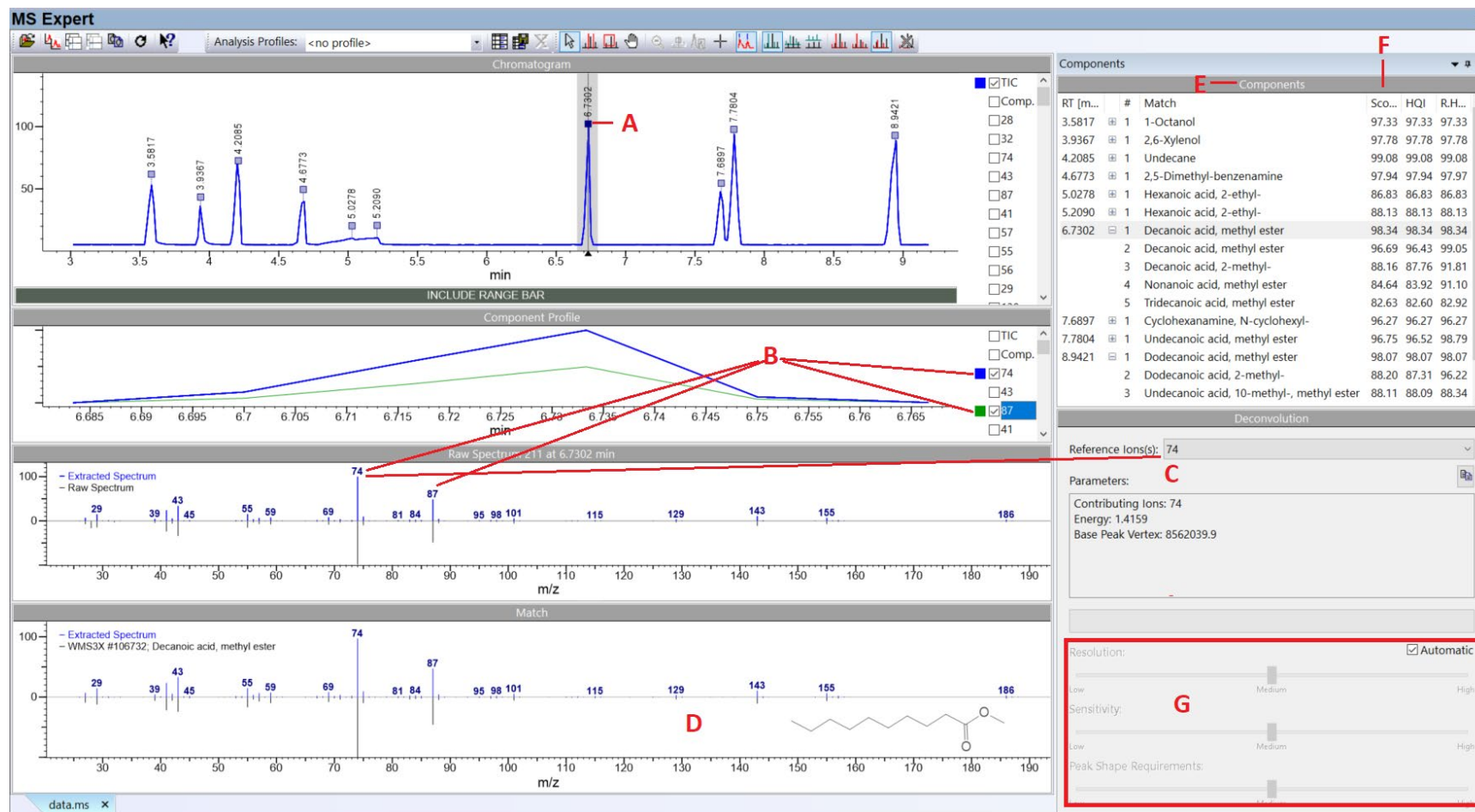
Where

- Dot-Product – second equation in above graph
- Wiley Dot-Product (old KnowItAll algorithm) - the old 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 graph
- Composite P3 – first equation in above graph  
P1 and P3 are different by the power applied to the weighted intensity of peak.

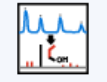
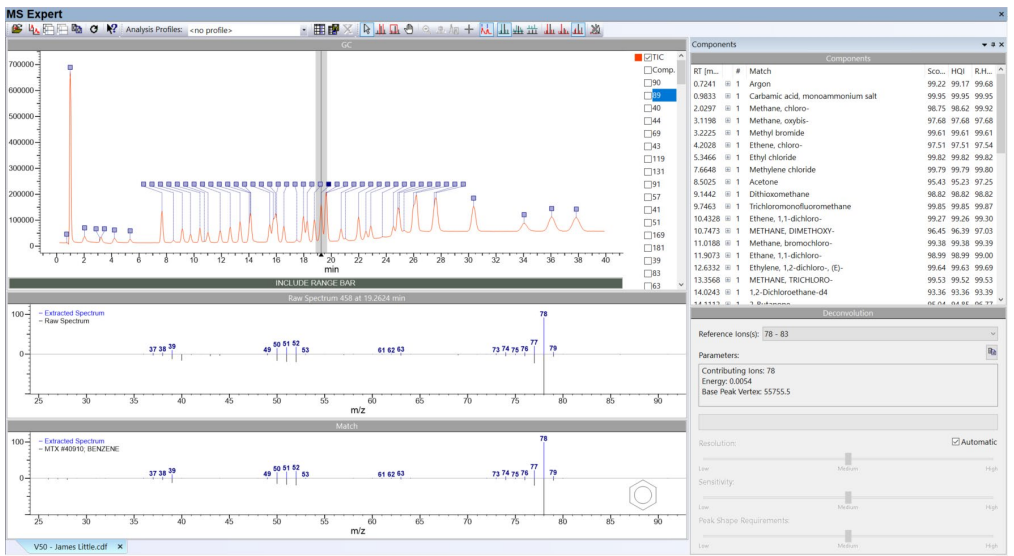
### Example 1: GC-MS of Unit m/z Values

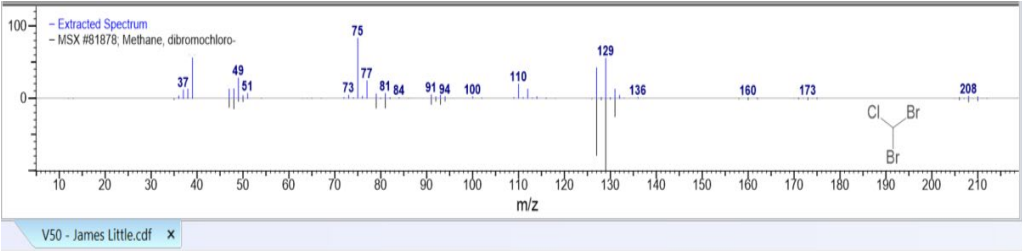
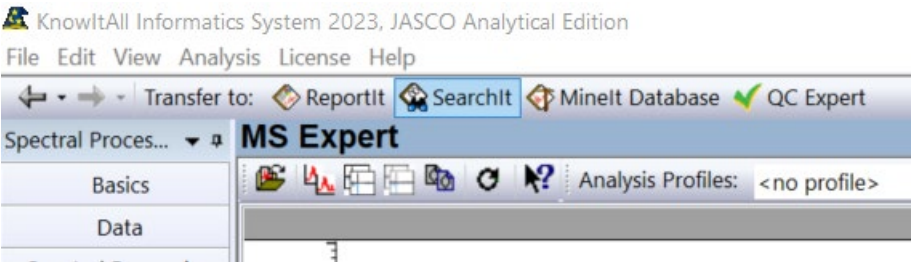
#### GUI explanation

This picture shows the deconvoluted GC-MS data of unit m/z values, and the massive Wiley MS database search results for each component.

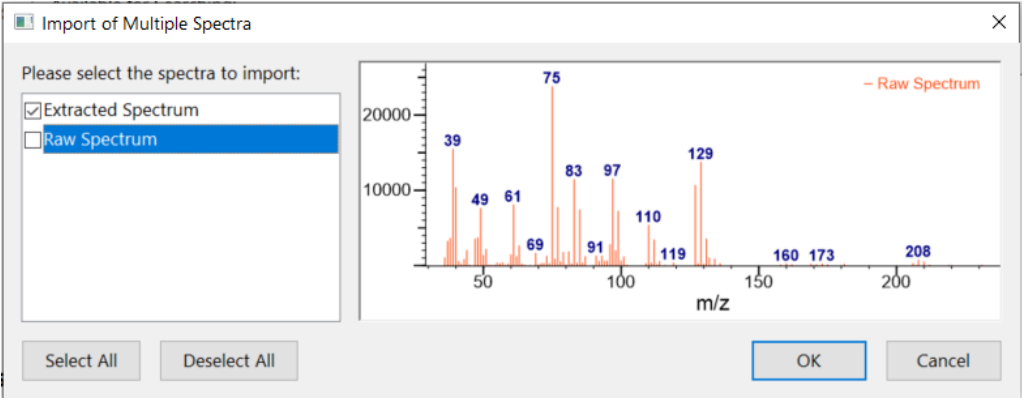
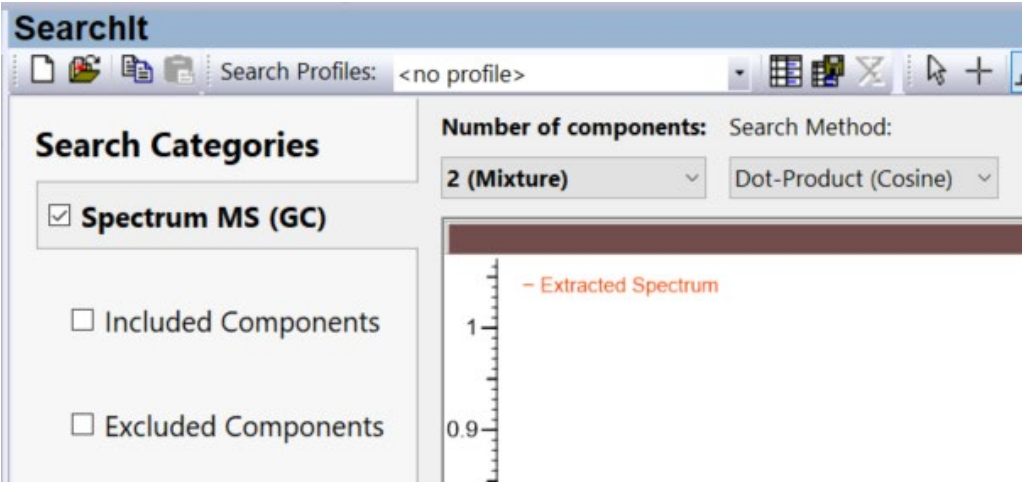


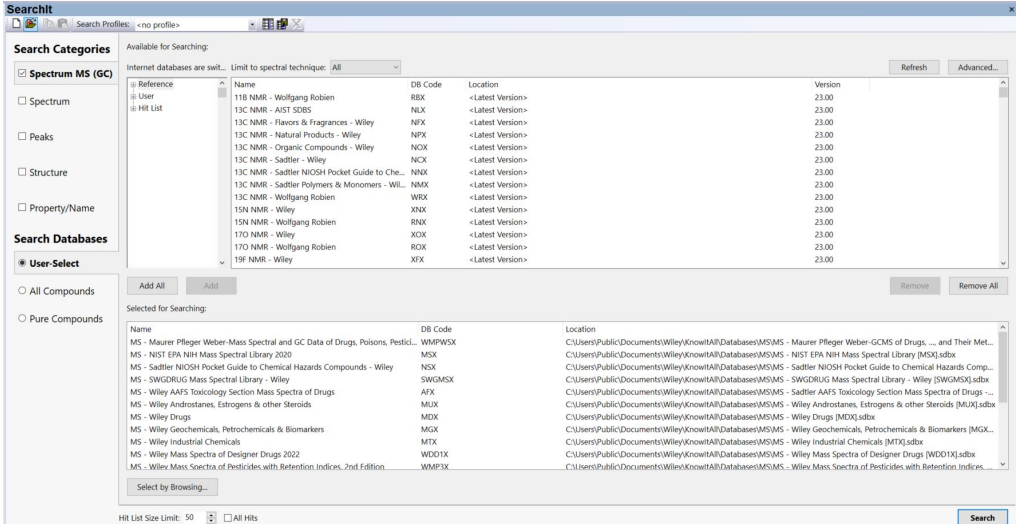
A – deconvoluted GC peak which found matches from reference MS databases; B – selected ions in a component; C – reference ion(s) used to model a component; D – extracted spectrum (top) vs reference spectrum (bottom); E – components table; F – combined spectrum search and reverse search Hit Quality Index (HQI) and, not shown in the above screenshot, each component's GC area under curve (AUC) value; G – Adjustable parameters in algorithm.

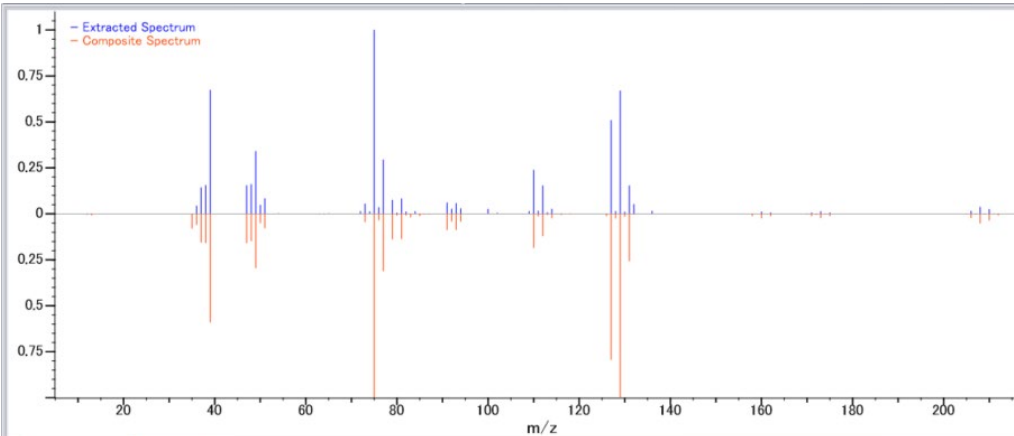
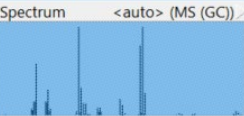
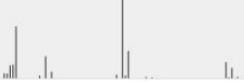
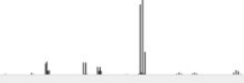
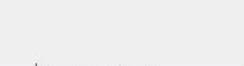
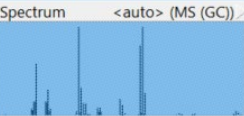
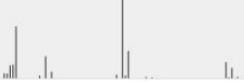
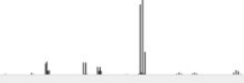
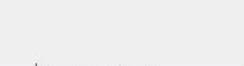
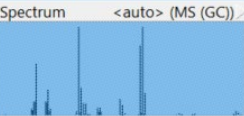
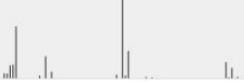
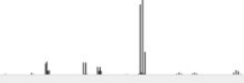
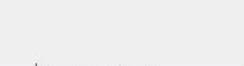
	Action	Result
1	<p>Go to the <b>MS Expert</b> application under <b>Spectral Process</b> toolbar</p>  <p>Click <b>Open Data File</b> button</p> <p>Navigate to folder C:\Users\Public\Documents\Wiley\KnowItAll\Samples\MS Expert</p> <p>V50 - James Little.CDF file</p> <p><b>Note:</b> in 2024 release, one can use button <i>Open Analysis</i> to open an externally saved analysis file.</p>	<p><b>MS Expert</b> automatically performs</p> <ol style="list-style-type: none"> <li>GC deconvolution to component extracted MS</li> <li>Search extracted MS against reference databases</li> <li>Report top hits</li> </ol>  <p><b>Note:</b> in 2024 release, we add a column "Area%" to the Components table to give user a rough idea for the component percentage of chromatogram peak intensity.</p>

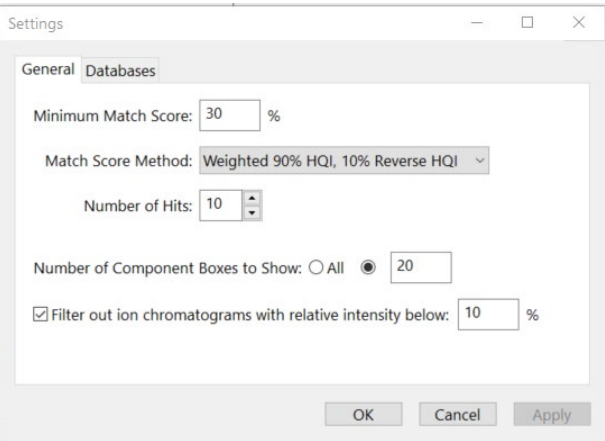
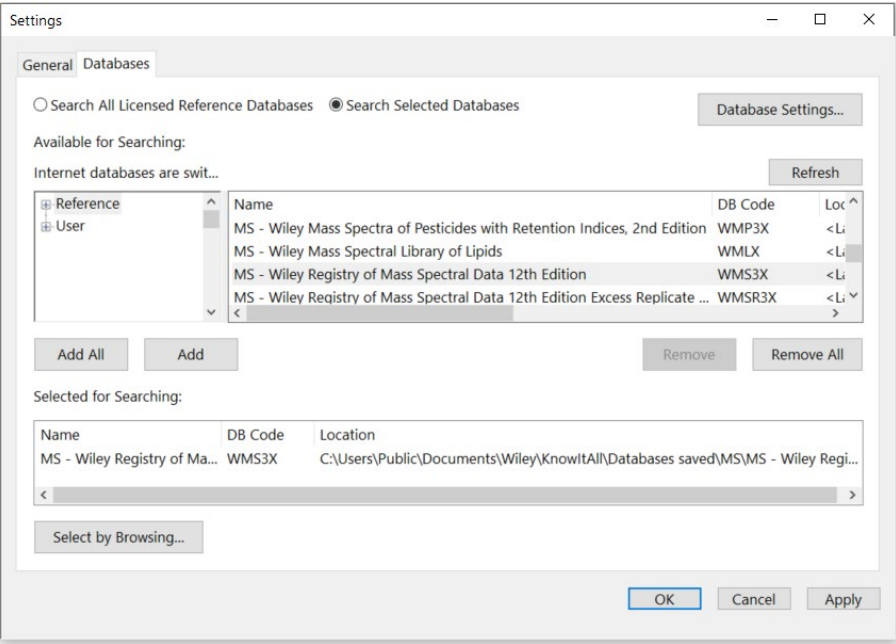
	Action	Result
2	<p>Examine the hit lists for each deconvoluted components in the <b>Components</b> table</p> <p>Go to <b>RT (MIN)</b> 19.6127, notice the <b>Score</b> for this hit is very low, 56.74.</p>	 <p>From the comparison of <b>Extracted Spectrum</b> (top) and reference spectrum of methane, dibromochloro- in the bottom pane, we can see that the <b>Extract Spectrum</b> contains methane, dibromochloro- as well as other components.</p>
3	Transfer the <b>Extracted Spectrum</b> to <b>SearchIt</b>	

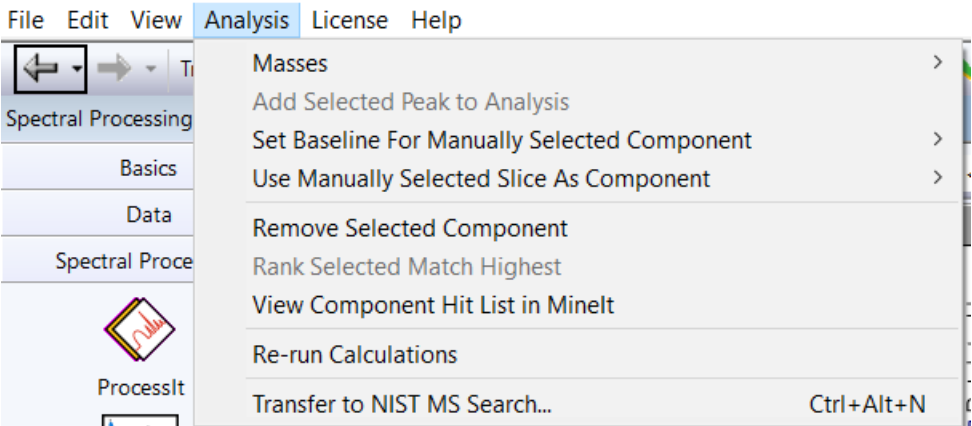


	Action	Result
4	Only check the <b>Extract Spectrum</b> at prompt  OK	
5	In <b>SearchIt</b> , set to search for <b>2 (Mixture)</b>	

	Action	Result																																																																																																
6	<p>Click the <b>User-Select</b> tab to add all licensed MS databases</p> <p><b>Search</b></p>	 <p><b>Search Categories</b></p> <ul style="list-style-type: none"> <li><input checked="" type="checkbox"/> Spectrum MS (GC)</li> <li><input type="checkbox"/> Spectrum</li> <li><input type="checkbox"/> Peaks</li> <li><input type="checkbox"/> Structure</li> <li><input type="checkbox"/> Property/Name</li> </ul> <p><b>Search Databases</b></p> <ul style="list-style-type: none"> <li><input checked="" type="radio"/> User-Select</li> <li><input type="radio"/> All Compounds</li> <li><input type="radio"/> Pure Compounds</li> </ul> <p><b>Available for Searching:</b></p> <table border="1"> <thead> <tr> <th>Name</th> <th>DB Code</th> <th>Location</th> <th>Version</th> </tr> </thead> <tbody> <tr><td>11B NMR - Wollgang Robien</td><td>RBX</td><td>&lt;Latest Version&gt;</td><td>23.00</td></tr> <tr><td>13C NMR - ADST SDBS</td><td>NLX</td><td>&lt;Latest Version&gt;</td><td>23.00</td></tr> <tr><td>13C NMR - Flavors &amp; Fragrances - Wiley</td><td>NFX</td><td>&lt;Latest Version&gt;</td><td>23.00</td></tr> <tr><td>13C NMR - Natural Products - Wiley</td><td>NPX</td><td>&lt;Latest Version&gt;</td><td>23.00</td></tr> <tr><td>13C NMR - Organic Compounds - Wiley</td><td>NOX</td><td>&lt;Latest Version&gt;</td><td>23.00</td></tr> <tr><td>13C NMR - Sadtler - Wiley</td><td>NCK</td><td>&lt;Latest Version&gt;</td><td>23.00</td></tr> <tr><td>13C NMR - Sadtler NIOSH Pocket Guide to Chem...</td><td>NNX</td><td>&lt;Latest Version&gt;</td><td>23.00</td></tr> <tr><td>13C NMR - Sadtler Polymers &amp; Monomers - WIL...</td><td>NMX</td><td>&lt;Latest Version&gt;</td><td>23.00</td></tr> <tr><td>13C NMR - Wollgang Robien</td><td>WRX</td><td>&lt;Latest Version&gt;</td><td>23.00</td></tr> <tr><td>15N NMR - Wiley</td><td>XNX</td><td>&lt;Latest Version&gt;</td><td>23.00</td></tr> <tr><td>15N NMR - Wollgang Robien</td><td>RNX</td><td>&lt;Latest Version&gt;</td><td>23.00</td></tr> <tr><td>17O NMR - Wiley</td><td>XOX</td><td>&lt;Latest Version&gt;</td><td>23.00</td></tr> <tr><td>17O NMR - Wollgang Robien</td><td>ROX</td><td>&lt;Latest Version&gt;</td><td>23.00</td></tr> <tr><td>19F NMR - Wiley</td><td>XFX</td><td>&lt;Latest Version&gt;</td><td>23.00</td></tr> </tbody> </table> <p><b>Selected for Searching:</b></p> <table border="1"> <thead> <tr> <th>Name</th> <th>DB Code</th> <th>Location</th> </tr> </thead> <tbody> <tr><td>MS - Maurer Pflieger Weber-Mass Spectral and GC Data of Drugs, Poisons, Pestic...</td><td>WMPWSX</td><td>C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - Maurer Pflieger Weber-GCMS of Drugs, ... and Their Met...</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 Spectral Library [MSX].sdbx</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 Guide to Chemical Hazards Comp...</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 Library - Wiley [SWGMSX].sdbx</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 Section Mass Spectra of Drugs ...</td></tr> <tr><td>MS - Wiley Androstanes, Estrogens &amp; 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MS - Wiley Mass Spectra of Pesticides with Retention Indices. 2nd Edition	WMP1X	C:\Users\Public\Documents\Wiley\KnowItAll\Databases\MS\MS - Wiley Mass Spectra of Pesticides with Retention Indices...																																																																																																

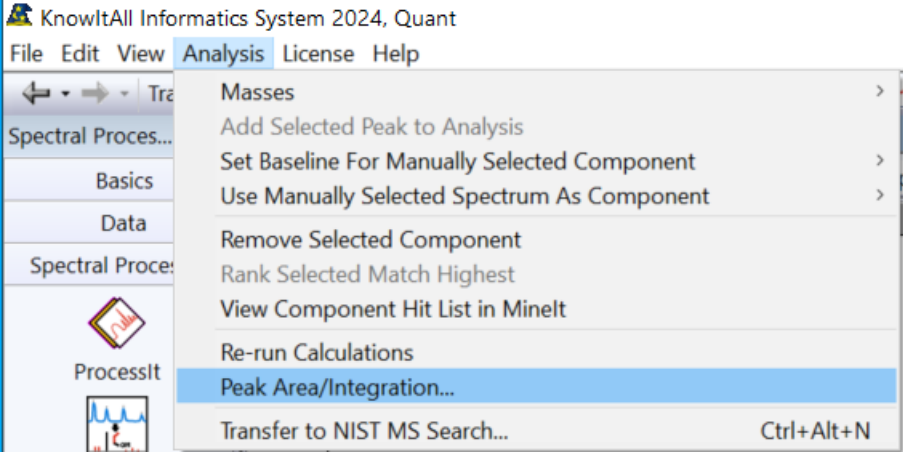
	Action	Result																																																										
7		<p>Mixture analysis returns a very good <b>Composite Spectrum</b> match top of the hit list:</p>  <p>MS (GC) NMR</p> <table border="1"> <thead> <tr> <th colspan="2">Table</th> <th colspan="2">Plot</th> <th colspan="2">Related Compounds View</th> <th colspan="2"></th> </tr> <tr> <th>HQI</th> <th>Ratio</th> <th>Exclude</th> <th>Co</th> <th>Df</th> <th>ID</th> <th>Name</th> <th>Chemical Structure</th> <th>Spectrum</th> <th>&lt;auto&gt; (MS (GC))</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>94.09</td> <td>N.A.</td> <td></td> <td></td> <td></td> <td>Composite Spectrum</td> <td><chem>ClC=CClC(Br)C(Br)C</chem></td> <td></td> <td></td> </tr> <tr> <td></td> <td></td> <td>0.59</td> <td><input type="radio"/></td> <td></td> <td></td> <td>MSR: 3579 1-Propene, 1,3-dichloro-, (Z)-</td> <td><chem>ClC=CCl</chem></td> <td></td> <td></td> </tr> <tr> <td></td> <td></td> <td>0.41</td> <td><input type="radio"/></td> <td></td> <td></td> <td>MSX: 81878 Methane, dibromochloro-</td> <td><chem>ClC(Br)C(Br)C</chem></td> <td></td> <td></td> </tr> <tr> <td></td> <td></td> <td>N.A.</td> <td></td> <td></td> <td></td> <td>Residual Spectrum</td> <td></td> <td></td> <td></td> </tr> </tbody> </table> <p>The <b>Composite Spectrum</b> in the first row is a mix of two components: 1-propene, 1,3-dichloro- (new component) and methane, dibromochloro-. The <b>Residual Spectrum</b> in the last row, the difference between <b>Extracted Spectrum</b> and <b>Composite Spectrum</b> is negligible, indicating no more components remain undetected.</p>	Table		Plot		Related Compounds View				HQI	Ratio	Exclude	Co	Df	ID	Name	Chemical Structure	Spectrum	<auto> (MS (GC))	1	94.09	N.A.				Composite Spectrum	<chem>ClC=CClC(Br)C(Br)C</chem>					0.59	<input type="radio"/>			MSR: 3579 1-Propene, 1,3-dichloro-, (Z)-	<chem>ClC=CCl</chem>					0.41	<input type="radio"/>			MSX: 81878 Methane, dibromochloro-	<chem>ClC(Br)C(Br)C</chem>					N.A.				Residual Spectrum			
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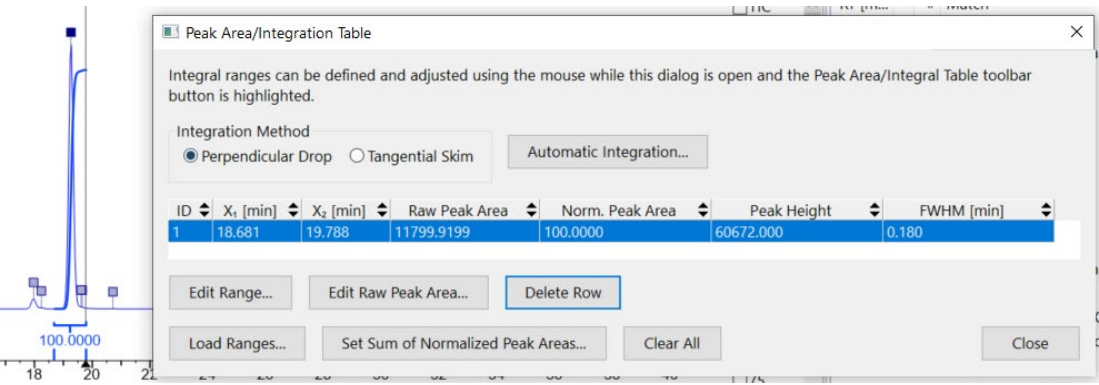
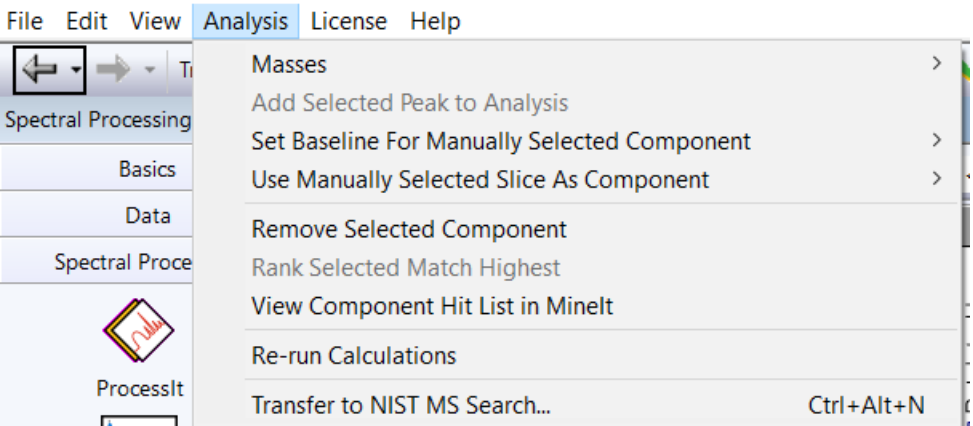
	Action	Result
8	<p>Go back to <b>MS Expert</b>, One can go to <b>File &gt; Settings</b> menu to adjust component hit list parameters</p>	<p>Hits evaluation parameters:</p>  <p>Reference database choices</p> 

	Action	Result
9	<p>One can click the TIC pane <b>INCLUDE RANGE BAR</b> to add peaks manually, then select <b>Re-run Calculations</b> to perform a new analysis</p> <p>One can select <b>View Component Hit List in Minelt</b> to see Component table contents in <b>Minelt</b></p>	

## Example 2: Evaluate Chromatogram Peak Area Under Curve Values

This is a new feature in KnowItAll 2024 release. With this capability, one can get the chromatogram AUC value per component.

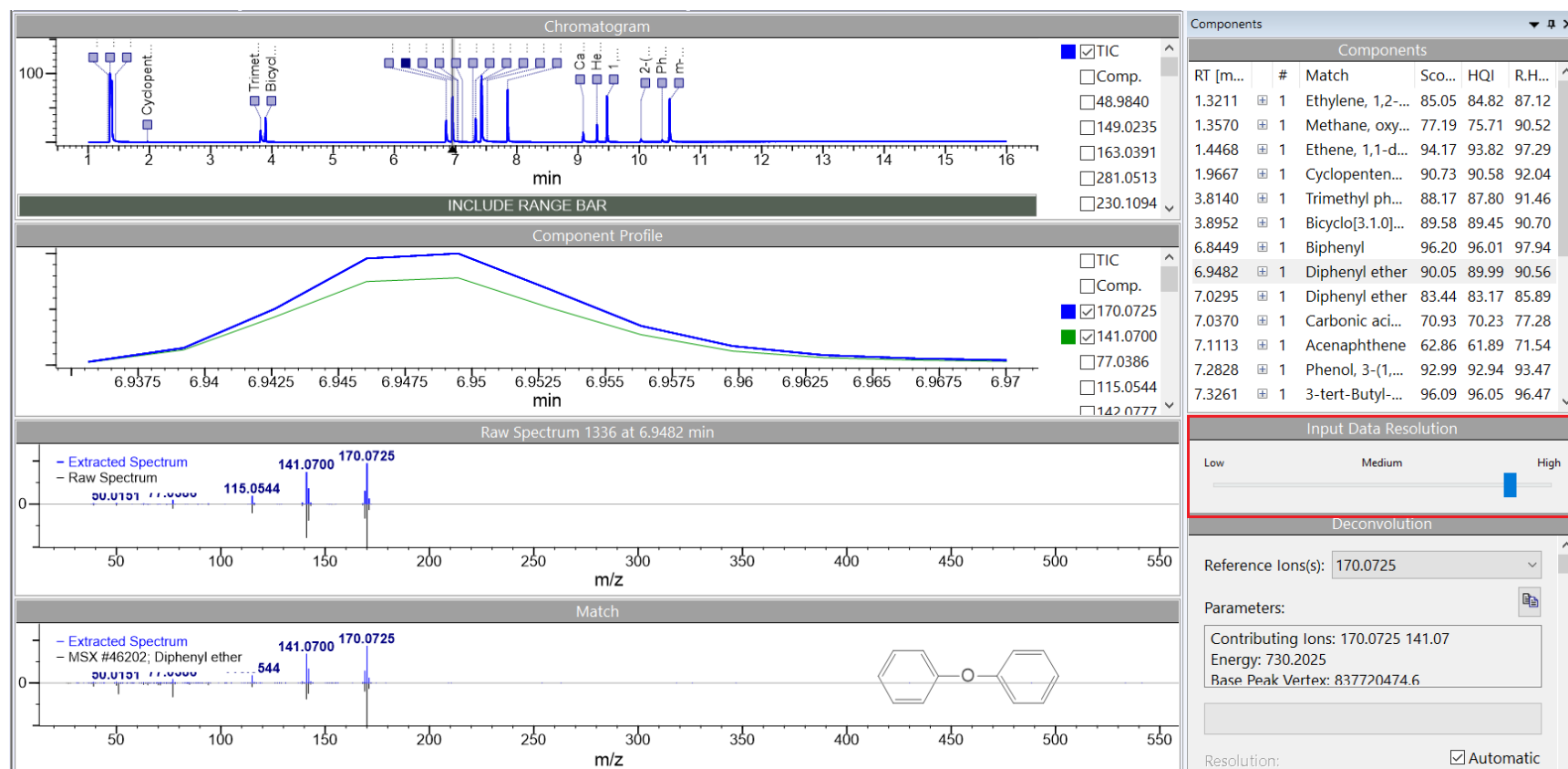
	Action	Result
1	<p>Continue with the above example. Turn off TIC check and turn on the ion m/z value of 78</p> <p><input type="checkbox"/> TIC <input type="checkbox"/> Comp. <input type="checkbox"/> 44 <input checked="" type="checkbox"/> 78</p> <p>Select menu-item <b>Analysis &gt; Peak Area/Integration</b></p>	

	Action	Result														
2	<p>Mouse over the chromatogram pane, an integration sign  occurs, one can drag and drop the mouse to define a region of AUC calculation.</p>	 <p>Or one can use the pop-up window to auto populate the AUC table. Tangential skim is a method to calculate AUC and peak height for a sloping baseline.</p> <table border="1" data-bbox="913 511 1837 560"> <thead> <tr> <th>ID</th> <th>X<sub>1</sub> [min]</th> <th>X<sub>2</sub> [min]</th> <th>Raw Peak Area</th> <th>Norm. Peak Area</th> <th>Peak Height</th> <th>FWHM [min]</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>18.681</td> <td>19.788</td> <td>11799.9199</td> <td>100.0000</td> <td>60672.000</td> <td>0.180</td> </tr> </tbody> </table>	ID	X <sub>1</sub> [min]	X <sub>2</sub> [min]	Raw Peak Area	Norm. Peak Area	Peak Height	FWHM [min]	1	18.681	19.788	11799.9199	100.0000	60672.000	0.180
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### Example 3: High-resolution GC-MS

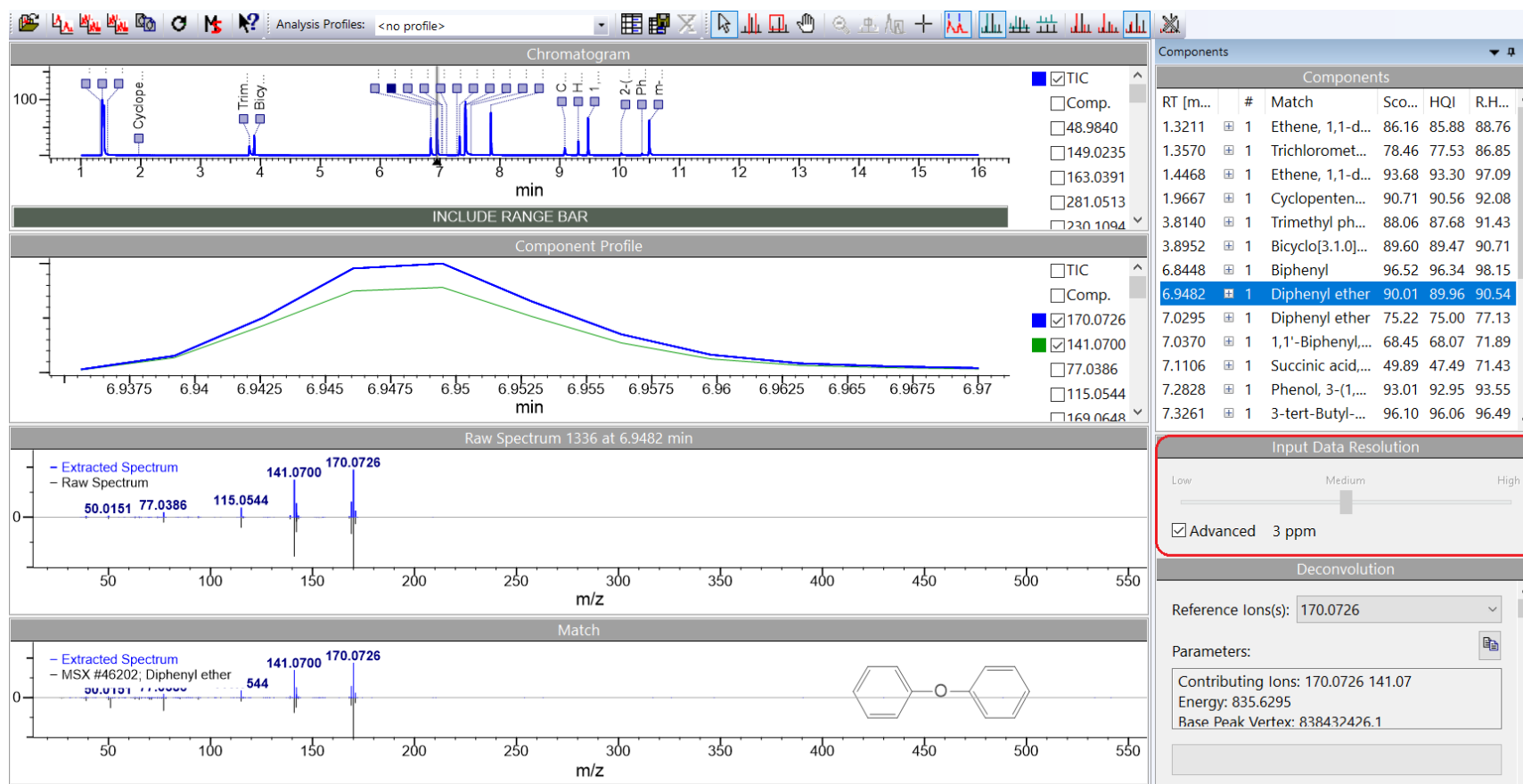
#### GUI explanation

For accurate  $m/z$  value data, without knowing the instrument resolving power, there is no safe algorithm to automatically calculate this value. Therefore, we use what our research considers a reasonable value by default that has a constant component and a variable component depending on mass (ppm). Empirically, this works in most cases. Increasing the  $m/z$  value accuracy too much incurs the danger of splitting an individual  $m/z$  value into individual mass spectral peaks that should be considered as just one. Decreasing the  $m/z$  value accuracy too much may cause individual mass spectral peaks to be merged resulting incorrect accurate  $m/z$  values to be reported.



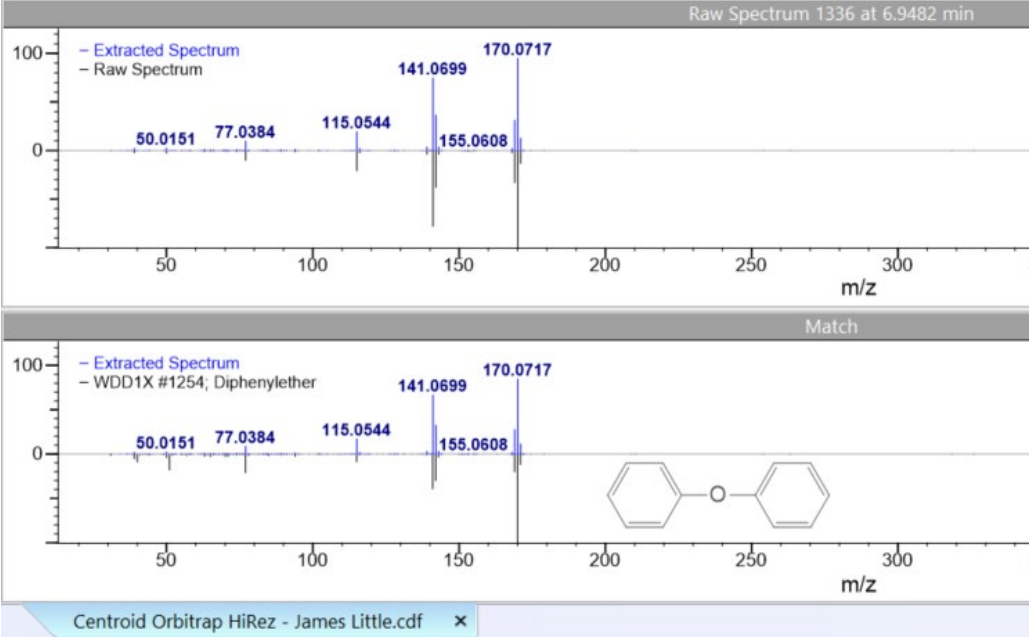


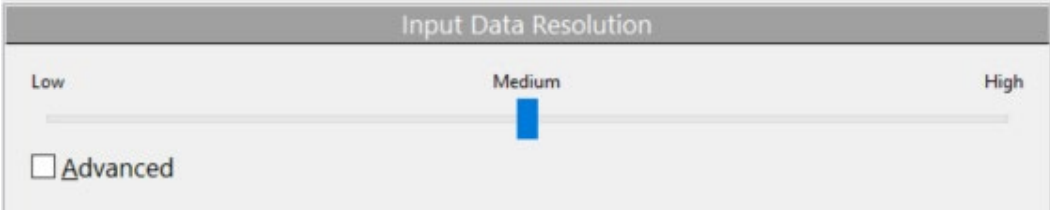
If a user knows the instrument's resolving power, that value should be entered in the highlighted dialog in above figure:

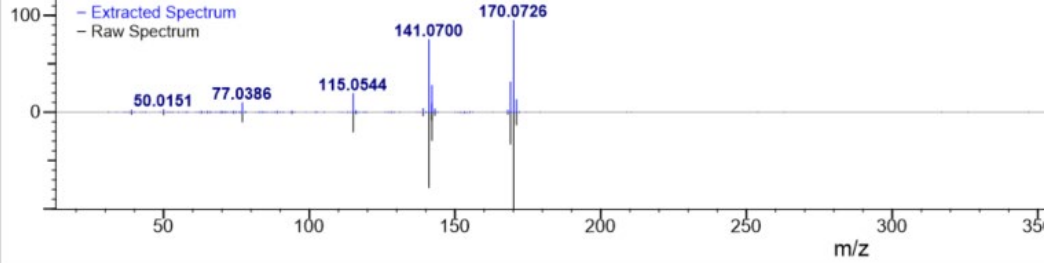
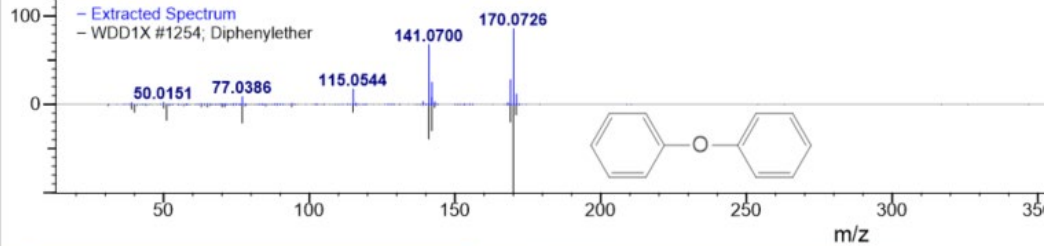


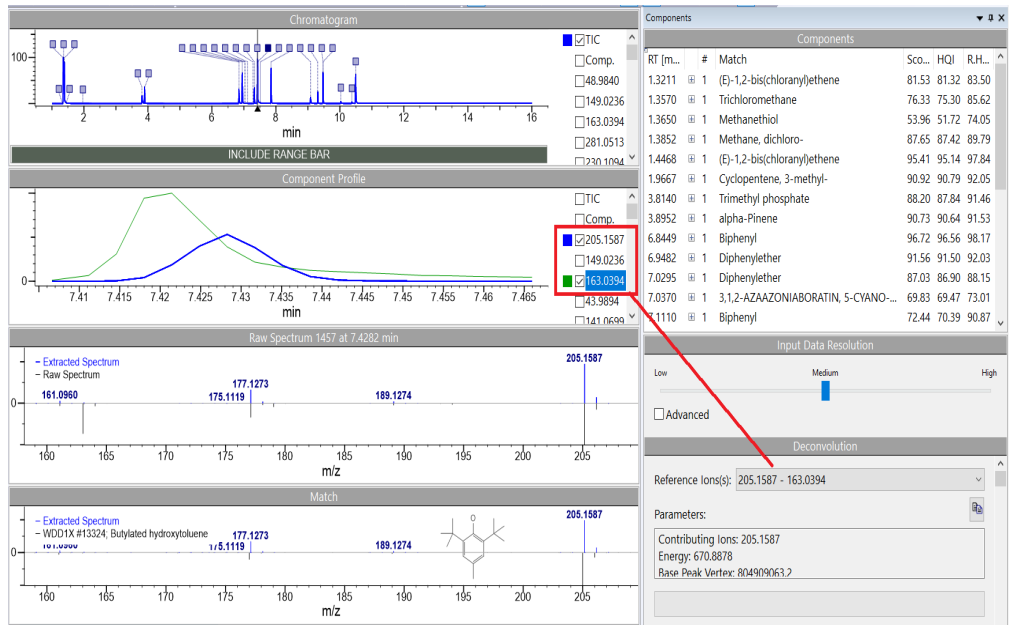
It is possible to save it as part of a profile, which can then be selected depending on the type of instrument. A user can create several profiles with different resolution settings for different types of data (and instruments).

## Excise 1

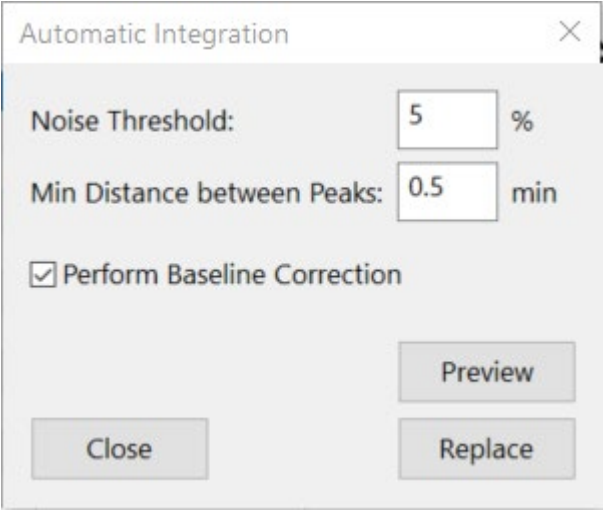
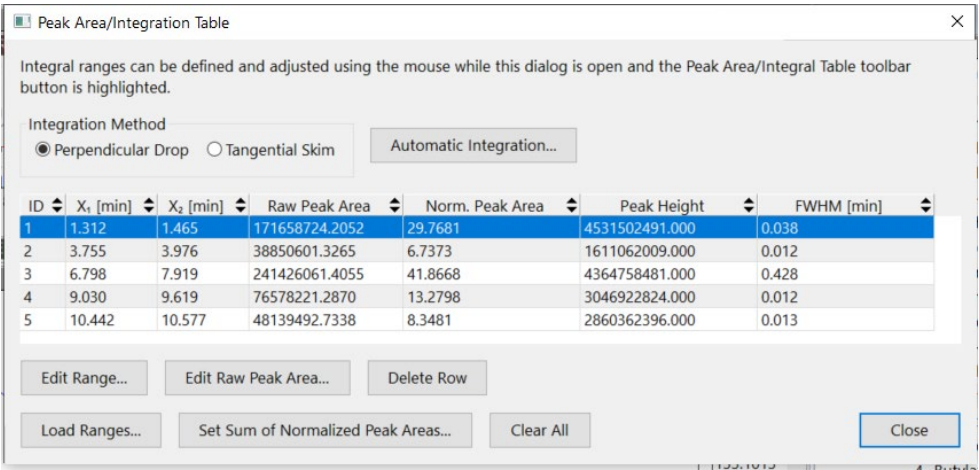
	Action	Result
1	Open a high-resolution GC-MS file in the same folder Centroid Orbitrap HiRez - James Little Navigate to hit list RT (MIN) 6.9482	 <p>The m/z value of 170.0717 is slightly off for this compound.</p>

	Action	Result
2	Check <b>Advanced</b> Set the instrument resolution at 3 ppm, <b>OK</b>	<p><b>MS Expert</b> recognizes this is a high-resolution data file and uniquely allows user to fill in resolution information or adjust resolution:</p> 

	Action	Result
3		<p data-bbox="1539 332 1827 354">Raw Spectrum 1336 at 6.9482 min</p>  <p data-bbox="1654 646 1711 667">Match</p>  <p data-bbox="930 933 1333 954">Centroid Orbitrap HiRez - James Little.cdf x</p> <p data-bbox="865 982 1753 1003">The m/z value of 170.0726 is correct. This instrument resolution set-up is persistent.</p>

	Action	Result
4	Navigate to component TIC 7.4282	<p>One can see <b>MS Expert</b> separates two components characterized by peaking at slightly different TIC values. The m/z values are 205.1587 and 163.0394, respectively. Component 205.1587 is in the extracted spectrum in this case.</p>  <p>The screenshot displays the following panels:</p> <ul style="list-style-type: none"> <li><b>Chromatogram:</b> Shows a Total Ion Chromatogram (TIC) with peaks at various retention times. A range bar is visible at the bottom.</li> <li><b>Component Profile:</b> Shows two overlapping peaks. The peak at 7.4282 min is highlighted in green, and the peak at 7.435 min is highlighted in blue. A red box highlights the m/z values 205.1587 and 163.0394 in the legend.</li> <li><b>Raw Spectrum 1457 at 7.4282 min:</b> Shows the mass spectrum of the selected peak with major peaks at m/z 161.0960, 177.1273, 175.1119, 189.1274, and 205.1587.</li> <li><b>Match:</b> Shows the reference mass spectrum for WDD1X #13324, Butylated hydroxytoluene, with a chemical structure. Major peaks are at m/z 177.1273, 175.1119, 189.1274, and 205.1587.</li> <li><b>Components Table:</b> A table listing identified components with their retention times, match scores, and other parameters. The entry for 7.0295 min (Diphenylether) is highlighted in red.</li> <li><b>Input Data Resolution:</b> A slider set to Medium.</li> <li><b>Deconvolution:</b> Reference ions are listed as 205.1587 - 163.0394.</li> <li><b>Parameters:</b> Contributing ions: 205.1587; Energy: 670.8878; Base Peak Vertex: 804909063.7.</li> </ul>

	Action	Result																																																																																																																														
5	Expand a few component hits, for example, RT 7.4	<div data-bbox="865 331 1885 1198" style="border: 1px solid gray; padding: 5px;"> <p>Components <span style="float: right;">▼ 🔍 ✕</span></p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th colspan="6" style="background-color: #cccccc;">Components</th> </tr> <tr> <th style="width: 15%;">RT [m...</th> <th style="width: 5%;">#</th> <th style="width: 55%;">Match</th> <th style="width: 10%;">Sco...</th> <th style="width: 10%;">HQI</th> <th style="width: 5%;">R.H...</th> </tr> </thead> <tbody> <tr> <td>7.3261</td> <td>1</td> <td>3-tert-Butyl-4-hydroxyanisole</td> <td>96.10</td> <td>96.06</td> <td>96.49</td> </tr> <tr> <td>7.4200</td> <td>1</td> <td>Dimethylisophthalate</td> <td>90.14</td> <td>90.08</td> <td>90.75</td> </tr> <tr style="background-color: #007bff; color: white;"> <td>7.4282</td> <td>1</td> <td>Butylated hydroxytoluene</td> <td>93.49</td> <td>93.40</td> <td>94.27</td> </tr> <tr> <td></td> <td>2</td> <td>Phenol, 2,4,6-tris(1-methylethyl)-</td> <td>88.83</td> <td>88.72</td> <td>89.82</td> </tr> <tr> <td></td> <td>3</td> <td>Terbutol</td> <td>88.80</td> <td>88.75</td> <td>89.27</td> </tr> <tr> <td></td> <td>4</td> <td>Butylated hydroxy toluene</td> <td>87.90</td> <td>87.39</td> <td>92.47</td> </tr> <tr> <td></td> <td>5</td> <td>Phenol, 2,4-bis(1,1-dimethylethyl)-6-methyl-</td> <td>86.80</td> <td>86.73</td> <td>87.44</td> </tr> <tr> <td></td> <td>6</td> <td>Ethanone, 1-(5,6,7,8-tetrahydro-2,8,8-trimethyl-...</td> <td>78.26</td> <td>78.22</td> <td>78.65</td> </tr> <tr> <td></td> <td>7</td> <td>4,6-di-tert-Butyl-m-cresol</td> <td>76.87</td> <td>76.83</td> <td>77.26</td> </tr> <tr> <td></td> <td>8</td> <td>Propanoic acid, 2-methyl-3-[4-t-butyl]phenyl-</td> <td>76.40</td> <td>76.35</td> <td>76.85</td> </tr> <tr> <td></td> <td>9</td> <td>Ethyl 4-oxo-2-phenylpentanoate</td> <td>75.38</td> <td>73.99</td> <td>87.91</td> </tr> <tr> <td></td> <td>10</td> <td>2,6-Di-t-butyl-4-methylphenol acetate(ester)</td> <td>75.18</td> <td>75.11</td> <td>75.83</td> </tr> <tr> <td>7.5162</td> <td>1</td> <td>Dimethylisophthalate</td> <td>76.39</td> <td>75.65</td> <td>83.08</td> </tr> <tr> <td>7.8488</td> <td>1</td> <td>Diethylphthalate</td> <td>94.54</td> <td>94.40</td> <td>95.76</td> </tr> <tr> <td>9.0830</td> <td>1</td> <td>Caffeine</td> <td>95.71</td> <td>95.63</td> <td>96.49</td> </tr> <tr> <td>9.3082</td> <td>1</td> <td>Hexadecanoic acid, methyl ester</td> <td>93.22</td> <td>93.01</td> <td>95.14</td> </tr> <tr> <td>9.4719</td> <td>1</td> <td>1,4-Dibutyl benzene-1,4-dicarboxylate</td> <td>95.51</td> <td>95.41</td> <td>96.46</td> </tr> <tr> <td>9.4730</td> <td>1</td> <td>Dibutyl phthalate</td> <td>93.64</td> <td>93.59</td> <td>94.09</td> </tr> <tr> <td>10.0282</td> <td>1</td> <td>Drometrizole</td> <td>95.72</td> <td>95.71</td> <td>95.95</td> </tr> </tbody> </table> </div>	Components						RT [m...	#	Match	Sco...	HQI	R.H...	7.3261	1	3-tert-Butyl-4-hydroxyanisole	96.10	96.06	96.49	7.4200	1	Dimethylisophthalate	90.14	90.08	90.75	7.4282	1	Butylated hydroxytoluene	93.49	93.40	94.27		2	Phenol, 2,4,6-tris(1-methylethyl)-	88.83	88.72	89.82		3	Terbutol	88.80	88.75	89.27		4	Butylated hydroxy toluene	87.90	87.39	92.47		5	Phenol, 2,4-bis(1,1-dimethylethyl)-6-methyl-	86.80	86.73	87.44		6	Ethanone, 1-(5,6,7,8-tetrahydro-2,8,8-trimethyl-...	78.26	78.22	78.65		7	4,6-di-tert-Butyl-m-cresol	76.87	76.83	77.26		8	Propanoic acid, 2-methyl-3-[4-t-butyl]phenyl-	76.40	76.35	76.85		9	Ethyl 4-oxo-2-phenylpentanoate	75.38	73.99	87.91		10	2,6-Di-t-butyl-4-methylphenol acetate(ester)	75.18	75.11	75.83	7.5162	1	Dimethylisophthalate	76.39	75.65	83.08	7.8488	1	Diethylphthalate	94.54	94.40	95.76	9.0830	1	Caffeine	95.71	95.63	96.49	9.3082	1	Hexadecanoic acid, methyl ester	93.22	93.01	95.14	9.4719	1	1,4-Dibutyl benzene-1,4-dicarboxylate	95.51	95.41	96.46	9.4730	1	Dibutyl phthalate	93.64	93.59	94.09	10.0282	1	Drometrizole	95.72	95.71	95.95
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6	Select <b>Analysis &gt; Peak Area/Integration</b> Select <b>Automatic Integration</b> Set the parameters in the <b>Automatic Integration</b> pop-up window																																											
7	Click <b>Replace</b> Click <b>Close</b> button	<p>We only use a few peaks to demonstrate this feature.</p>  <table border="1" data-bbox="863 894 1835 1360"> <thead> <tr> <th>ID</th> <th>X<sub>1</sub> [min]</th> <th>X<sub>2</sub> [min]</th> <th>Raw Peak Area</th> <th>Norm. Peak Area</th> <th>Peak Height</th> <th>FWHM [min]</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>1.312</td> <td>1.465</td> <td>171658724.2052</td> <td>29.7681</td> <td>4531502491.000</td> <td>0.038</td> </tr> <tr> <td>2</td> <td>3.755</td> <td>3.976</td> <td>38850601.3265</td> <td>6.7373</td> <td>1611062009.000</td> <td>0.012</td> </tr> <tr> <td>3</td> <td>6.798</td> <td>7.919</td> <td>241426061.4055</td> <td>41.8668</td> <td>4364758481.000</td> <td>0.428</td> </tr> <tr> <td>4</td> <td>9.030</td> <td>9.619</td> <td>76578221.2870</td> <td>13.2798</td> <td>3046922824.000</td> <td>0.012</td> </tr> <tr> <td>5</td> <td>10.442</td> <td>10.577</td> <td>48139492.7338</td> <td>8.3481</td> <td>2860362396.000</td> <td>0.013</td> </tr> </tbody> </table>	ID	X <sub>1</sub> [min]	X <sub>2</sub> [min]	Raw Peak Area	Norm. Peak Area	Peak Height	FWHM [min]	1	1.312	1.465	171658724.2052	29.7681	4531502491.000	0.038	2	3.755	3.976	38850601.3265	6.7373	1611062009.000	0.012	3	6.798	7.919	241426061.4055	41.8668	4364758481.000	0.428	4	9.030	9.619	76578221.2870	13.2798	3046922824.000	0.012	5	10.442	10.577	48139492.7338	8.3481	2860362396.000	0.013
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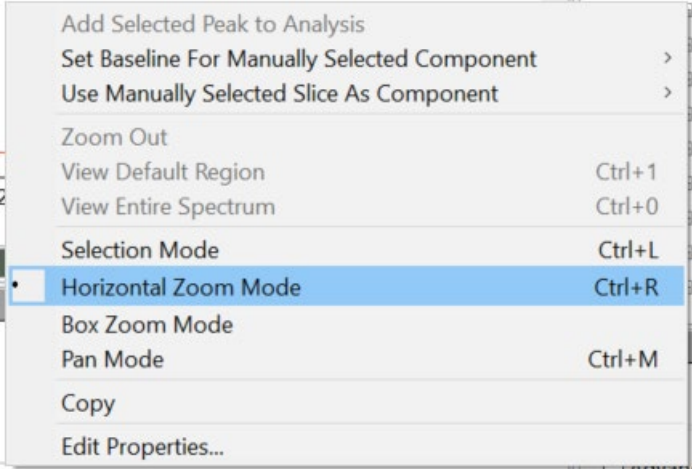
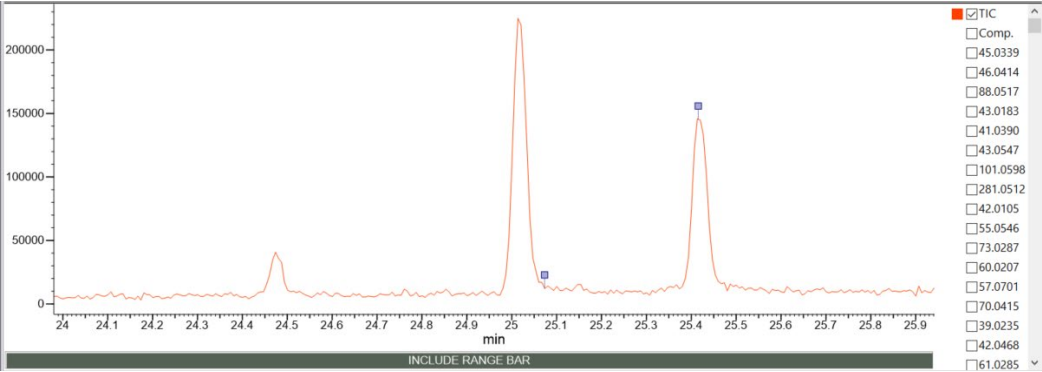
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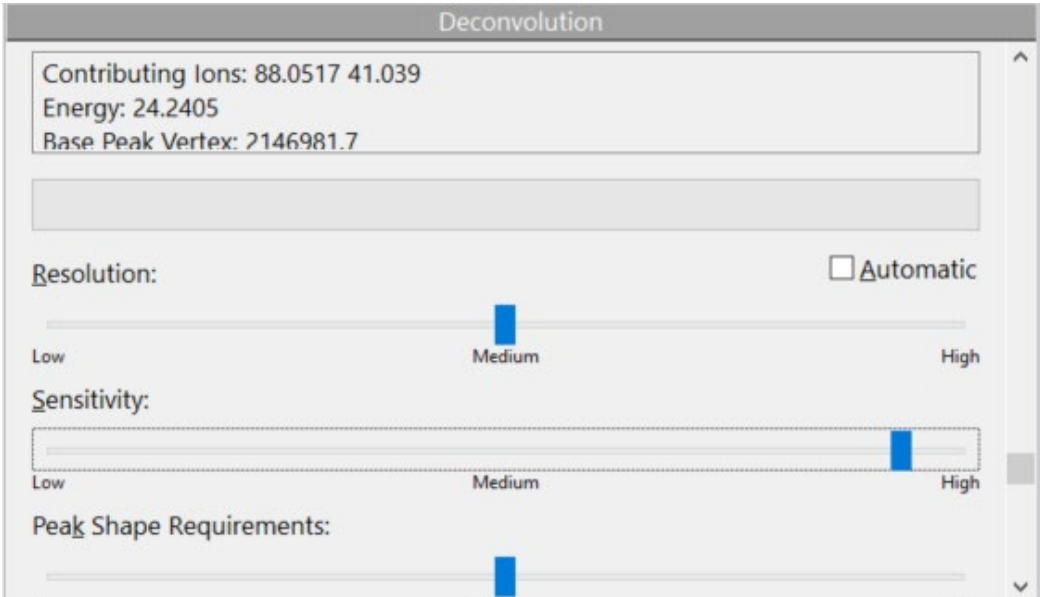


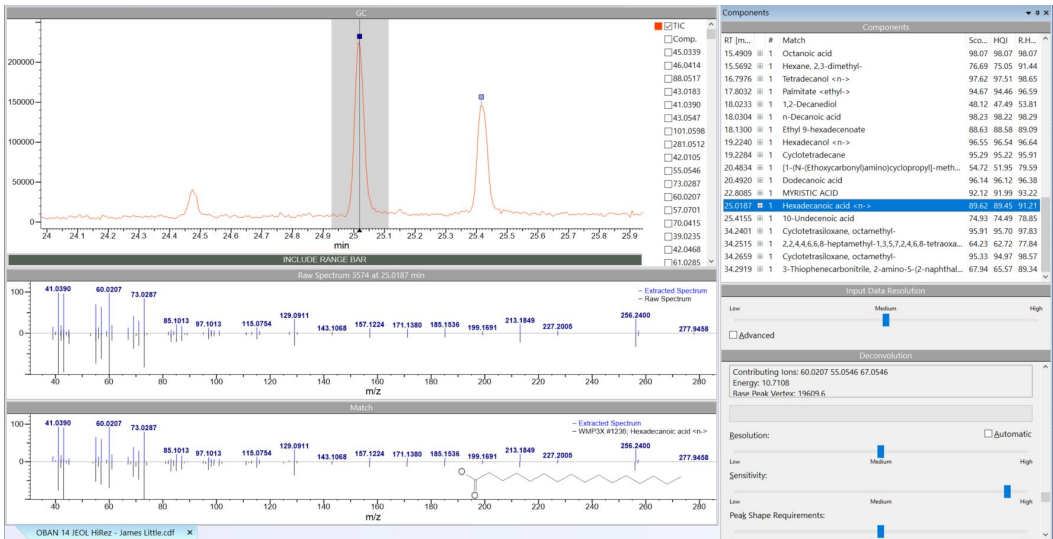
Action		Result							
10	Go back to <b>MS Expert</b>  Right-mouse click the <b>Components</b> table and select <b>Copy All Component Information</b> . One can then paste this information to Excel or Word, etc..	RT [min]	#	Match	Score	HQI	R.HQI	DB	ID
		1.3211	1	(E)-1,2-bis(chloranyl)ethene	88.34	88.04	90.99	WMS3X	4144
			2	Ethene, 1,1-dichloro-	86.55	86.26	89.15	MSX	3048
			3	Ethene, 1,1-dichloro-	84.14	82.84	95.9	WMSR3X	1983
			4	2,2,3-Trichloropropionaldehyde	78.8	78.56	80.92	WMS3X	56863
			5	2,2,3-Trichloropropane-1,1-diol	75.53	75.3	77.6	WMS3X	88275
			6	Dihydro-4,5-dichloro-2(3H)furanone	72.69	71.59	82.61	MSX	30922
			7	Propanoic acid, 2,2,3-trichloro-	68.81	68.59	70.83	WMS3X	84684
			8	Propanoic acid, 2,2,3-trichloro-	59.81	57.45	80.97	WMS3X	84685
			9	Chloromethylmethyl sulfide	57.41	57.23	59.07	MSX	3054
			10	Methyl 2,3,3-trichloropropanoate	53.98	53.77	55.85	MSX	64271
		1.357	1	Trichloromethane	78.5	77.57	86.84	MSX	9775
			2	Methane, oxybis[dichloro-	78.48	77	91.75	MSX	56380
			3	Methane, trichloro-	78.05	76.69	90.33	WMS3X	13436
			4	Ethane, 2-bromo-1,1-dichloro-	69.34	68	81.41	WMSD3X	330
			5	Ethane, 1,1,2,2-tetrachloro-	68.51	68.31	70.3	WMSR3X	13033
			6	1,1',3-trimethyl-3,3'-biindolin-2-one	67.98	65.35	91.65	WMSD3X	1193
			7	Methane, dichloronitro-	67.11	65.71	79.7	MSX	14617
			8	N,N-Dimethyl-2H-pyran-2-iminium chloride	64.16	62.24	81.49	WMSD3X	219
			9	Ethane, 1,2,2-trichloro-1,1-difluoro-	63.71	62.79	71.95	MSX	42934
			10	N-(Phenyl MIDA boronate-4-yl)-S-methyl-S-phe	63.11	59.87	92.28	WMS3X	576845
		1.4468	1	(E)-1,2-bis(chloranyl)ethene	93.9	93.52	97.26	WMS3X	4143
			2	Ethene, 1,1-dichloro-	93.88	93.5	97.28	MSX	3048
			3	(E)-1,2-bis(chloranyl)ethene	93.86	93.48	97.32	WMS3X	4144
			4	Ethene, 1,1-dichloro-	92.61	91.89	99.11	WMSR3X	1983
			5	2,2,3-Trichloropropionaldehyde	81.63	81.34	84.21	WMS3X	56863
			6	Dihydro-4,5-dichloro-2(3H)furanone	81.56	81.04	86.23	MSX	30922
			7	2,2,3-Trichloropropane-1,1-diol	77.78	77.5	80.3	WMS3X	88275
			8	Propanoic acid, 2,2,3-trichloro-	76.16	75.88	78.67	WMS3X	84684
			9	Propanoic acid, 2,2,3-trichloro-	68.17	66.78	80.63	WMS3X	84685
			10	Chloromethylmethyl sulfide	65.61	65.46	66.99	MSX	3054
		1.9667	1	Cyclopentene, 3-methyl-	90.7	90.55	92.08	MSX	1382
			2	Cyclopentene, 1-methyl-	90.19	90.07	91.25	MSX	1367
			3	Cyclopentene, 4-methyl-	89.11	88.84	91.55	WMS3X	1919
			4	Cyclopentene, 4-methyl-	89.05	88.94	90.1	MSX	1389
			5	(Z),(Z)-2,4-Hexadiene	87.84	87.69	89.17	MSX	1356

**Note: component Area% column is exported but removed from the above Excel display**

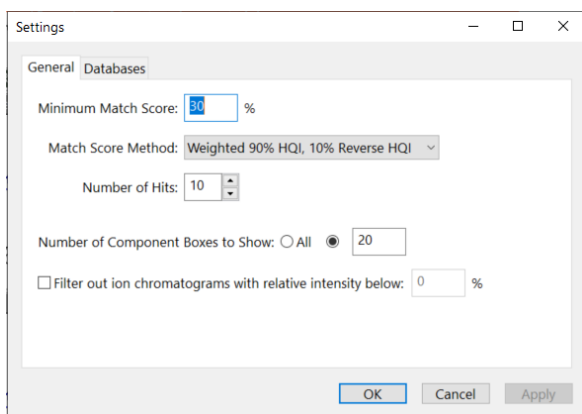
**Excise 2**

	Action	Result
1	<p>Open another high-resolution GC-MS file in the same folder</p> <p>OBAN 14 JEOL HiRez - James Little</p> <p>Right-mouse-click the <b>TIC</b> pane</p> <p>Choose Horizontal Zoom or Box Zoom mode</p>	
2	<p>Zoom to region 24 – 26 min (3 tiny peaks):</p>	 <p>We have two issues here: first, tiny peak was not picked up for analysis; and second, component was assigned to baseline.</p>

	Action	Result
3	<p>We can increase the algorithm Sensitivity to resolve the second issue.</p> <p>Check off <b>Automatic</b> under <b>Deconvolution</b> and move the <b>Sensitivity</b> bar to <b>High</b></p>	 <p>The screenshot shows the 'Deconvolution' settings panel. At the top, it lists 'Contributing Ions: 88.0517 41.039', 'Energy: 24.2405', and 'Base Peak Vertex: 2146981.7'. Below this, there are three sliders: 'Resolution' (set to Medium), 'Sensitivity' (set to High), and 'Peak Shape Requirements' (set to Medium). The 'Automatic' checkbox is unchecked.</p>

Action	Result																																																																																																																		
4	<p>Now the second component is correctly identified as the missing hexadecanoic acid</p>  <p>The screenshot displays a GC-MS analysis interface. At the top, a chromatogram shows two peaks. The first peak is at 25.0187 minutes, and the second is at 25.4 minutes. Below the chromatogram are mass spectra plots comparing the extracted spectrum to a library match for Hexadecanoic acid. On the right, a 'Components' table lists various compounds with their retention times, scores, and HQI values. The 'Input Data Resolution' and 'Deconvolution' settings are also visible.</p> <table border="1"> <caption>Components Table</caption> <thead> <tr> <th>RT [m.]</th> <th>#</th> <th>Match</th> <th>Score</th> <th>HQI</th> <th>R.H.</th> </tr> </thead> <tbody> <tr><td>15.4909</td><td>1</td><td>Octanoic acid</td><td>98.07</td><td>98.07</td><td>98.07</td></tr> <tr><td>15.5692</td><td>1</td><td>Hexane, 2,3-dimethyl-</td><td>76.69</td><td>75.05</td><td>91.44</td></tr> <tr><td>16.7976</td><td>1</td><td>Tetradecanol &lt;n-&gt;</td><td>97.62</td><td>97.51</td><td>96.65</td></tr> <tr><td>17.9032</td><td>1</td><td>Palmitate &lt;ethyl-&gt;</td><td>94.67</td><td>94.46</td><td>96.59</td></tr> <tr><td>18.0233</td><td>1</td><td>1,2-Decanediol</td><td>48.12</td><td>47.49</td><td>53.81</td></tr> <tr><td>18.0304</td><td>1</td><td>n-Decanoic acid</td><td>98.23</td><td>98.22</td><td>98.29</td></tr> <tr><td>18.1300</td><td>1</td><td>Ethyl 9-hexadecanoate</td><td>86.63</td><td>88.58</td><td>89.09</td></tr> <tr><td>18.2240</td><td>1</td><td>Hexadecanol &lt;n-&gt;</td><td>96.55</td><td>96.54</td><td>96.64</td></tr> <tr><td>19.2284</td><td>1</td><td>Cyclohexadecane</td><td>95.29</td><td>95.22</td><td>95.91</td></tr> <tr><td>20.4834</td><td>1</td><td>[1-N-(Ethoxycarbonylamino)cyclopropyl]-meth...</td><td>54.72</td><td>51.95</td><td>79.59</td></tr> <tr><td>20.4920</td><td>1</td><td>Dodecanoic acid</td><td>96.14</td><td>96.12</td><td>96.38</td></tr> <tr><td>22.8005</td><td>1</td><td>MYRISTIC ACID</td><td>92.12</td><td>91.99</td><td>91.22</td></tr> <tr><td>25.0187</td><td>1</td><td>Hexadecanoic acid &lt;n-&gt;</td><td>97.74</td><td>97.56</td><td>97.71</td></tr> <tr><td>25.4155</td><td>1</td><td>10-Lindolenic acid</td><td>74.93</td><td>74.49</td><td>78.85</td></tr> <tr><td>34.2401</td><td>1</td><td>Cycloletrasiloxane, octamethyl-</td><td>95.91</td><td>95.70</td><td>97.83</td></tr> <tr><td>34.2515</td><td>1</td><td>2,2,4,4,6,6,8-heptamethyl-1,3,5,7,2,4,6,8-tetraoxa...</td><td>64.23</td><td>62.72</td><td>77.84</td></tr> <tr><td>34.2659</td><td>1</td><td>Cycloletrasiloxane, octamethyl-</td><td>95.33</td><td>94.97</td><td>98.57</td></tr> <tr><td>34.2919</td><td>1</td><td>3-Thiopheneacetonitrile, 2-amino-5-(2-naphthal...</td><td>67.94</td><td>65.57</td><td>89.34</td></tr> </tbody> </table>	RT [m.]	#	Match	Score	HQI	R.H.	15.4909	1	Octanoic acid	98.07	98.07	98.07	15.5692	1	Hexane, 2,3-dimethyl-	76.69	75.05	91.44	16.7976	1	Tetradecanol <n->	97.62	97.51	96.65	17.9032	1	Palmitate <ethyl->	94.67	94.46	96.59	18.0233	1	1,2-Decanediol	48.12	47.49	53.81	18.0304	1	n-Decanoic acid	98.23	98.22	98.29	18.1300	1	Ethyl 9-hexadecanoate	86.63	88.58	89.09	18.2240	1	Hexadecanol <n->	96.55	96.54	96.64	19.2284	1	Cyclohexadecane	95.29	95.22	95.91	20.4834	1	[1-N-(Ethoxycarbonylamino)cyclopropyl]-meth...	54.72	51.95	79.59	20.4920	1	Dodecanoic acid	96.14	96.12	96.38	22.8005	1	MYRISTIC ACID	92.12	91.99	91.22	25.0187	1	Hexadecanoic acid <n->	97.74	97.56	97.71	25.4155	1	10-Lindolenic acid	74.93	74.49	78.85	34.2401	1	Cycloletrasiloxane, octamethyl-	95.91	95.70	97.83	34.2515	1	2,2,4,4,6,6,8-heptamethyl-1,3,5,7,2,4,6,8-tetraoxa...	64.23	62.72	77.84	34.2659	1	Cycloletrasiloxane, octamethyl-	95.33	94.97	98.57	34.2919	1	3-Thiopheneacetonitrile, 2-amino-5-(2-naphthal...	67.94	65.57	89.34
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**Tip:** Sometimes, there are too many GC peaks crowding the GC pane. One can control how to display by using **File > Settings** to set preferences:



# Manual GC-MS Analysis

## How to Use KnowItAll ProcessIt Perform Manual GC-MS Analysis

### Purpose

These exercises demonstrate how to use KnowItAll ProcessIt manually analyze GC-MS.

---

### Objectives

These exercises will teach you:

- How to use KnowItAll ProcessIt.
- 

### Background

The KnowItAll ProcessIt software reviews GC-MS data and, allow a user to control spectral subtraction. Subtracted spectrum is searched for matches in reference data.

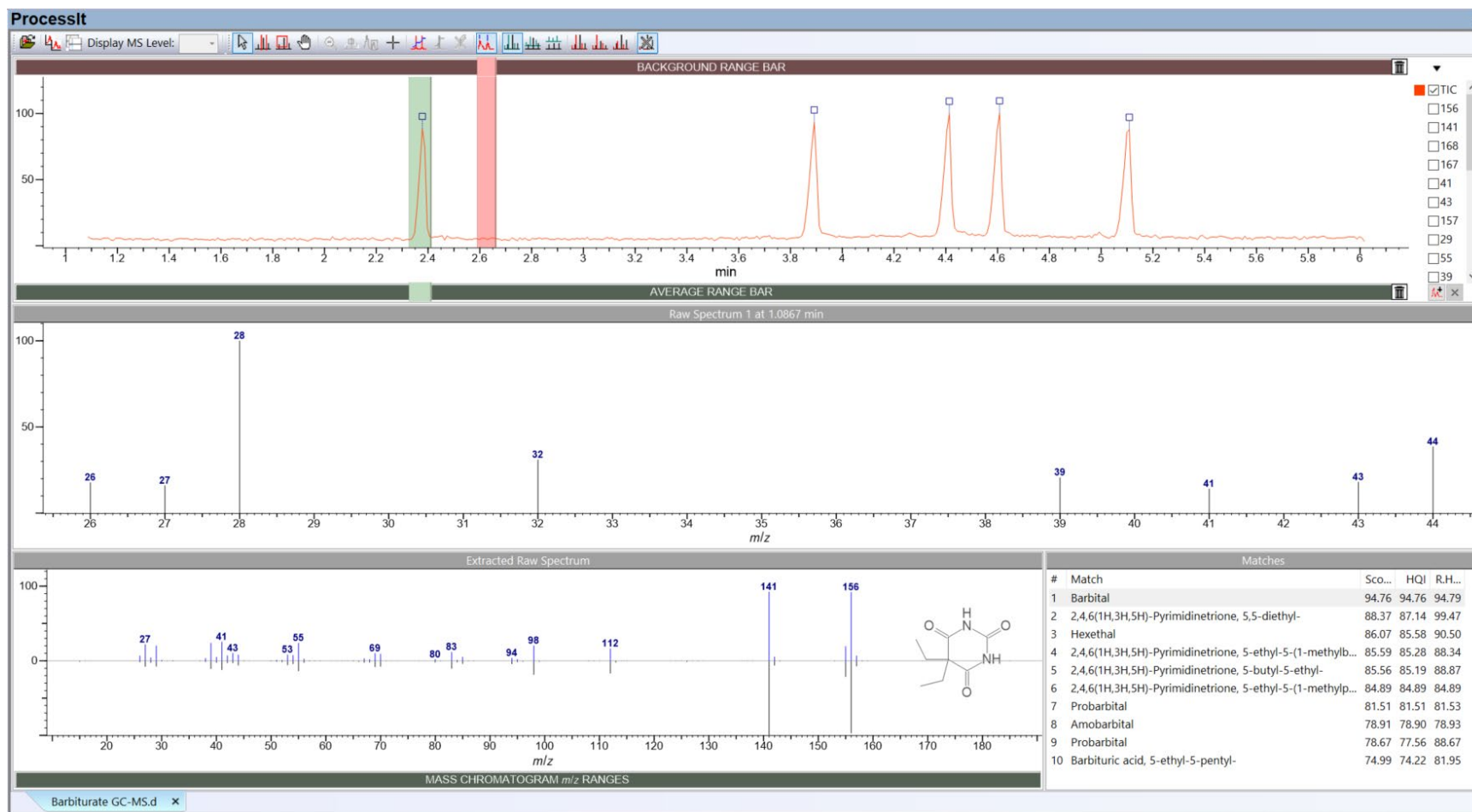
#### *Training Files Used in This Lesson*

- C:\Users\Public\Documents\Wiley\KnowItAll\Samples\GC-MS\Barbiturate GC-MS.d

#### *KnowItAll Applications Used*

- KnowItAll ProcessIt

## GUI explanation



The bottom spectrumPane holds

- Selected MS, defined by left mouse clicking or
- Selected MS – background (red bar in GC pane), defined by clicking the mouse on the bar, drag and drop

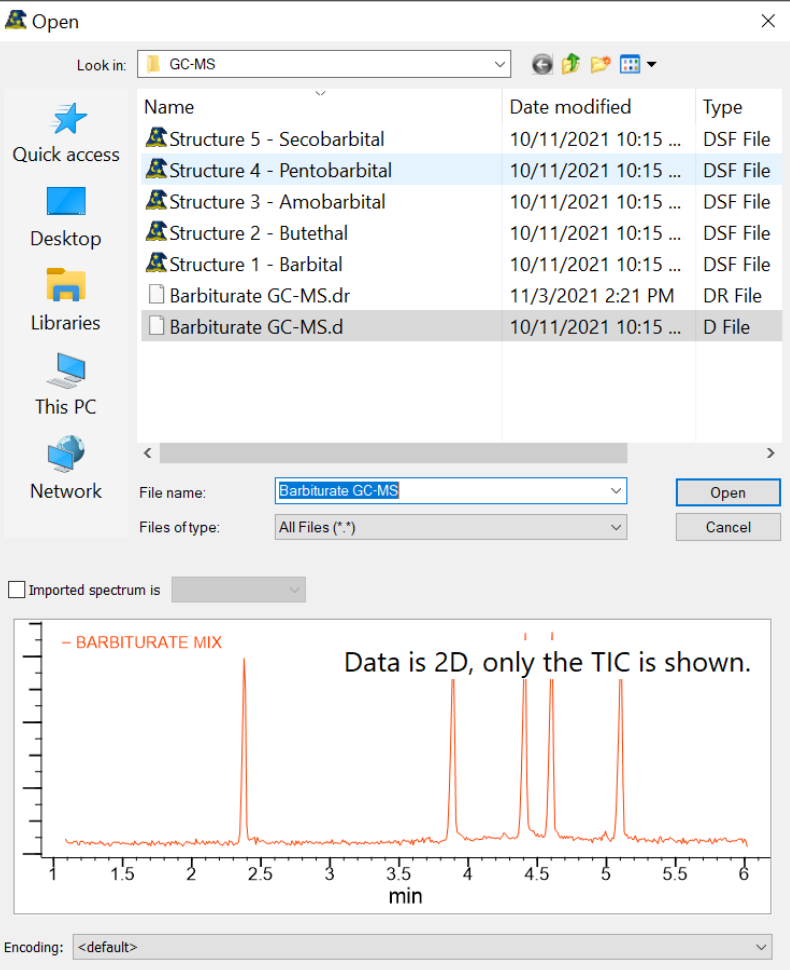
- Average MS (green bar in GC pane), defined by clicking the mouse on the bar, drag and drop
- Average MS (green bar) - background (red bar)

The **Matches** table are database search hit list for the selected MS.

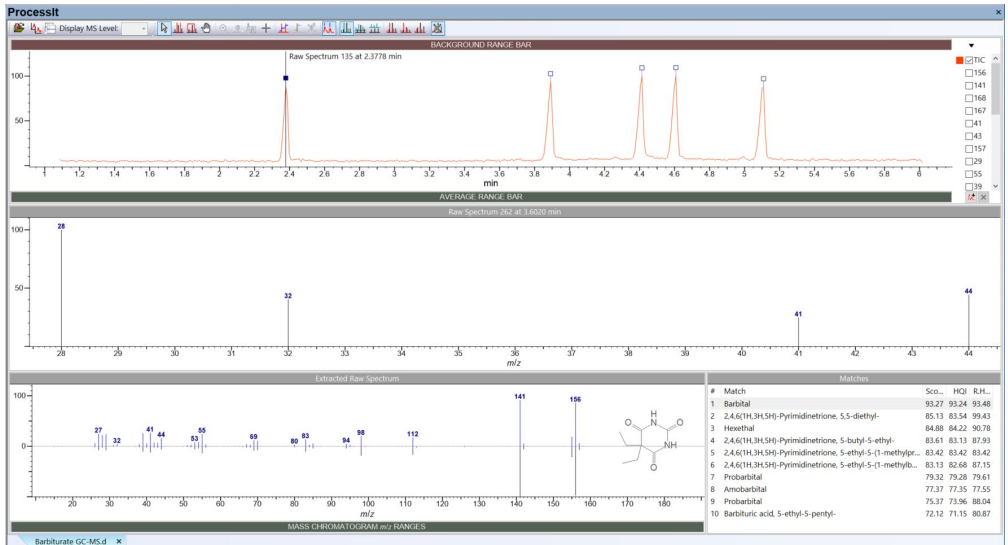
The selected MS can be transferred to **SearchIt** application for spectrum search.

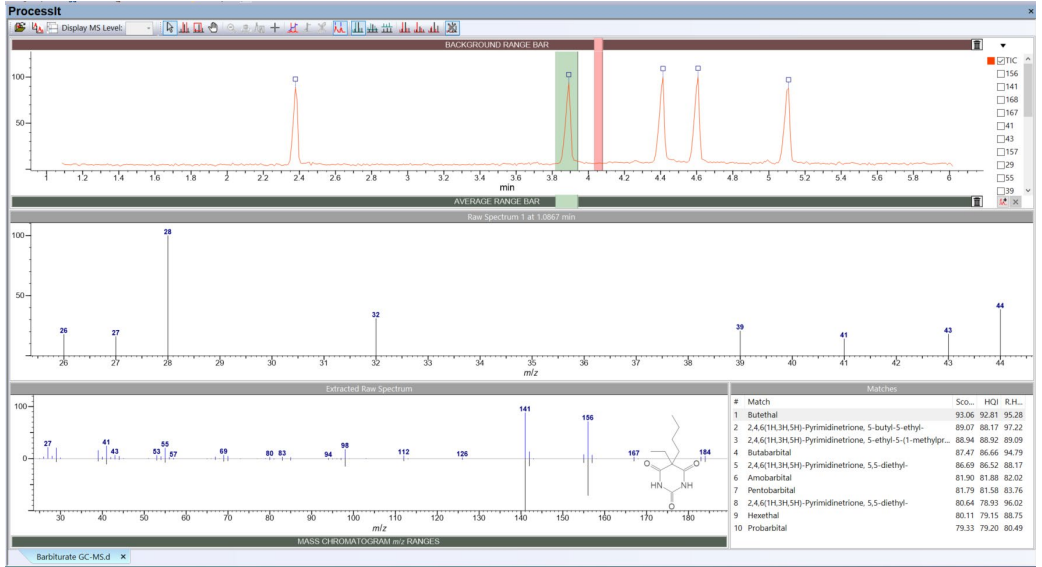
The area under curve (AUC) and peak height values for the ion chromatogram can be calculated by using the **Analysis > Peak Area/Integration** menu-item.

### Example: ProcessIt MS

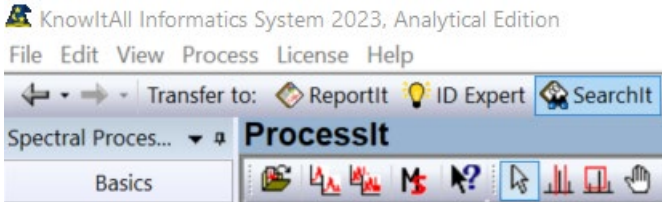
	Action	Result
<p>1 In <b>ProcessIt</b>, click <b>Open Data File</b> button</p> <p>Navigate to “C:\Users\Public\Documents\Wiley\KnowItAll\Samples\GC-MS”</p> <p>Select Barbiturate GC-MS.d</p> <p><b>Open</b></p>	 <p>The screenshot shows a Windows-style 'Open' dialog box. The 'Look in:' field is set to 'GC-MS'. A list of files is displayed with columns for Name, Date modified, and Type. The file 'Barbiturate GC-MS.d' is selected. Below the list, the 'File name:' field contains 'Barbiturate GC-MS' and the 'Files of type:' field is set to 'All Files (*.*)'. At the bottom, there is a section for 'Imported spectrum is' and a chromatogram plot. The plot shows a Total Ion Chromatogram (TIC) with a peak at approximately 2.4 minutes and several peaks between 4 and 5 minutes. The x-axis is labeled 'min' and ranges from 1 to 6. The y-axis represents intensity. The plot is titled 'Data is 2D, only the TIC is shown.'</p>	

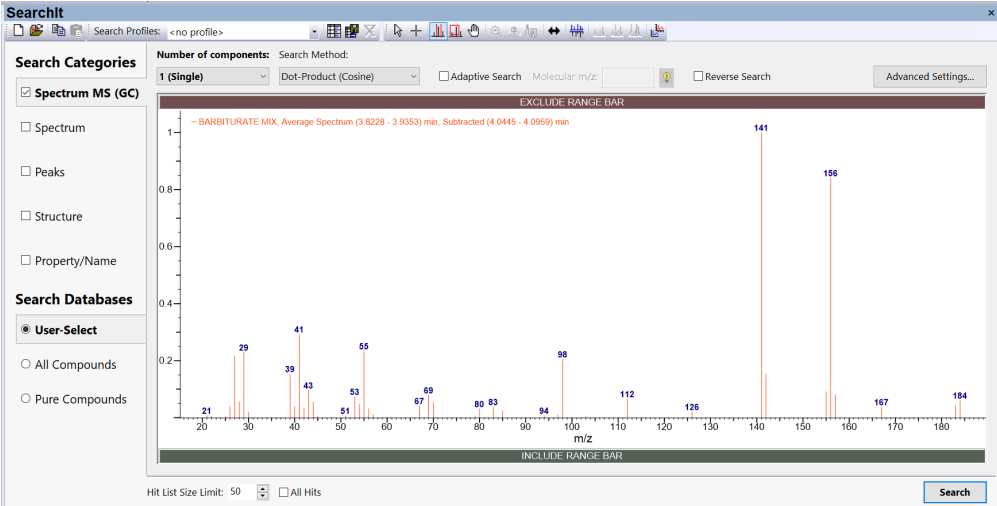
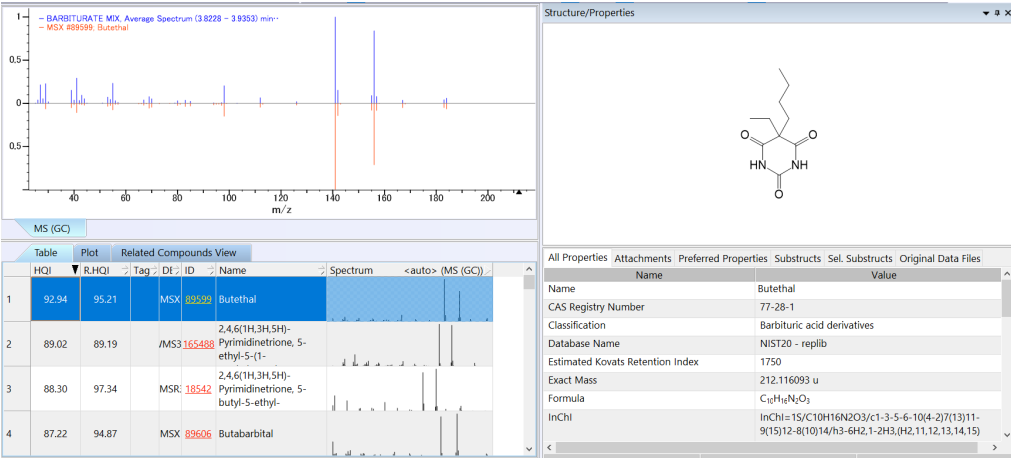


	Action	Result																																																							
2	Click a peak in chromatogram pane (top pane)	<p>You would see that the corresponding MS and hit database search results are reflected in the bottom pane:</p>  <p>The screenshot displays the ProcessIt software interface. The top panel shows a chromatogram with a peak at 2.3778 min. The middle panel shows the raw mass spectrum for that peak. The bottom panel shows the extracted mass spectrum and a list of matches from a database.</p> <table border="1"> <thead> <tr> <th>#</th> <th>Match</th> <th>Score</th> <th>HQ</th> <th>R.H.</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>Barbital</td> <td>93.27</td> <td>93.24</td> <td>93.48</td> </tr> <tr> <td>2</td> <td>2,4,6-(1H,3H,5H)-Pyrimidinetrione, 5,5-diethyl-</td> <td>85.13</td> <td>83.54</td> <td>90.43</td> </tr> <tr> <td>3</td> <td>Hexethyl</td> <td>84.88</td> <td>84.22</td> <td>90.78</td> </tr> <tr> <td>4</td> <td>2,4,6-(1H,3H,5H)-Pyrimidinetrione, 5-butyl-5-ethyl-</td> <td>83.61</td> <td>83.13</td> <td>87.93</td> </tr> <tr> <td>5</td> <td>2,4,6-(1H,3H,5H)-Pyrimidinetrione, 5-ethyl-5-(1-methylp...</td> <td>83.42</td> <td>83.42</td> <td>83.42</td> </tr> <tr> <td>6</td> <td>2,4,6-(1H,3H,5H)-Pyrimidinetrione, 5-ethyl-5-(1-methyl...</td> <td>83.13</td> <td>82.68</td> <td>87.15</td> </tr> <tr> <td>7</td> <td>Probarbital</td> <td>79.32</td> <td>79.28</td> <td>79.61</td> </tr> <tr> <td>8</td> <td>Amobarbital</td> <td>77.37</td> <td>77.35</td> <td>77.55</td> </tr> <tr> <td>9</td> <td>Probarbital</td> <td>75.37</td> <td>73.96</td> <td>88.04</td> </tr> <tr> <td>10</td> <td>Barbituric acid, 5-ethyl-5-pentyl-</td> <td>72.12</td> <td>71.15</td> <td>80.87</td> </tr> </tbody> </table>	#	Match	Score	HQ	R.H.	1	Barbital	93.27	93.24	93.48	2	2,4,6-(1H,3H,5H)-Pyrimidinetrione, 5,5-diethyl-	85.13	83.54	90.43	3	Hexethyl	84.88	84.22	90.78	4	2,4,6-(1H,3H,5H)-Pyrimidinetrione, 5-butyl-5-ethyl-	83.61	83.13	87.93	5	2,4,6-(1H,3H,5H)-Pyrimidinetrione, 5-ethyl-5-(1-methylp...	83.42	83.42	83.42	6	2,4,6-(1H,3H,5H)-Pyrimidinetrione, 5-ethyl-5-(1-methyl...	83.13	82.68	87.15	7	Probarbital	79.32	79.28	79.61	8	Amobarbital	77.37	77.35	77.55	9	Probarbital	75.37	73.96	88.04	10	Barbituric acid, 5-ethyl-5-pentyl-	72.12	71.15	80.87
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3	<p>Define average <b>spectrum range</b></p> <ul style="list-style-type: none"> <li>Click the top (green) bar</li> <li>Drag and</li> <li>Drop</li> </ul> <p>Define background spectrum <b>range</b></p> <ul style="list-style-type: none"> <li>Click the top (red) bar</li> <li>Drag and</li> </ul> <p>Drop</p>	 <p>The screenshot displays the ProcessIt software interface. The top plot is a chromatogram showing several peaks, with a green bar highlighting a peak at approximately 3.8 minutes and a red bar highlighting a peak at approximately 4.1 minutes. The middle plot is a mass spectrum showing relative intensity versus m/z, with a base peak at m/z 28. The bottom plot is an extracted raw spectrum showing relative intensity versus m/z, with a chemical structure of a barbiturate derivative and a table of matches.</p> <table border="1" data-bbox="1606 706 1906 860"> <thead> <tr> <th>#</th> <th>Match</th> <th>Score</th> <th>HQI</th> <th>R.H.</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>Hexethal</td> <td>93.06</td> <td>92.81</td> <td>95.28</td> </tr> <tr> <td>2</td> <td>2,4,6(1H,3H,5H)-Pyrimidinetrione, 5-butyl-5-ethyl-</td> <td>89.07</td> <td>88.17</td> <td>87.22</td> </tr> <tr> <td>3</td> <td>2,4,6(1H,3H,5H)-Pyrimidinetrione, 5-ethyl-5-(1-methylpr...</td> <td>88.94</td> <td>88.92</td> <td>89.09</td> </tr> <tr> <td>4</td> <td>Butabarbital</td> <td>87.47</td> <td>86.66</td> <td>94.79</td> </tr> <tr> <td>5</td> <td>2,4,6(1H,3H,5H)-Pyrimidinetrione, 5,5-diethyl-</td> <td>86.69</td> <td>86.52</td> <td>88.17</td> </tr> <tr> <td>6</td> <td>Amobarbital</td> <td>81.90</td> <td>81.88</td> <td>82.02</td> </tr> <tr> <td>7</td> <td>Pentobarbital</td> <td>81.79</td> <td>81.58</td> <td>83.76</td> </tr> <tr> <td>8</td> <td>2,4,6(1H,3H,5H)-Pyrimidinetrione, 5,5-diethyl-</td> <td>80.64</td> <td>78.93</td> <td>96.02</td> </tr> <tr> <td>9</td> <td>Hexethal</td> <td>80.11</td> <td>79.15</td> <td>88.75</td> </tr> <tr> <td>10</td> <td>Probarbital</td> <td>79.33</td> <td>79.20</td> <td>80.49</td> </tr> </tbody> </table> <p>The database search result is default to display, however, one can turn off this by <b>Process &gt; Search Extracted MS Spectrum for Database Matches</b> menu-item.</p>	#	Match	Score	HQI	R.H.	1	Hexethal	93.06	92.81	95.28	2	2,4,6(1H,3H,5H)-Pyrimidinetrione, 5-butyl-5-ethyl-	89.07	88.17	87.22	3	2,4,6(1H,3H,5H)-Pyrimidinetrione, 5-ethyl-5-(1-methylpr...	88.94	88.92	89.09	4	Butabarbital	87.47	86.66	94.79	5	2,4,6(1H,3H,5H)-Pyrimidinetrione, 5,5-diethyl-	86.69	86.52	88.17	6	Amobarbital	81.90	81.88	82.02	7	Pentobarbital	81.79	81.58	83.76	8	2,4,6(1H,3H,5H)-Pyrimidinetrione, 5,5-diethyl-	80.64	78.93	96.02	9	Hexethal	80.11	79.15	88.75	10	Probarbital	79.33	79.20	80.49
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### Example: Transfer to SearchIt

	Action	Result
1	Click <b>Transfer to: SearchIt</b>	 <p>KnowItAll Informatics System 2023, Analytical Edition          File Edit View Process License Help          Transfer to: ReportIt ID Expert SearchIt          Spectral Proces... # <b>ProcessIt</b>          Basics</p>

	Action	Result
2	<p>In <b>SearchIt</b>, one can perform various MS spectral searches.</p> <p>Check off <b>Adaptive Search</b></p> <p>Make sure all licensed databases are selected in <b>User-Select Search</b></p>	
3		 <p>The above is an expected hit.</p>

