

Qualitative Analysis Workflows

MassHunter Qualitative Analysis Webinar Series

Howard Sanford

Stephen Harnos

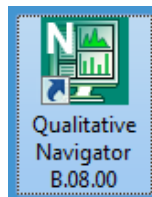
With Tom Barrett & Matt Leyden & Kevin Costalunga

MassHunter Qualitative Analysis Software B.08.00 SP1

Navigator and Workflows

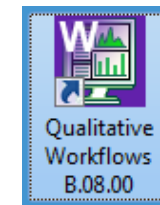
Navigator

- User centric
- Interactive Browsing
 - Walk the Chromatogram
 - Spectrum Preview
- Spectrum ID
 - Library/Database Search
 - Molecular Formula Generator
- No concept of compounds
- No Feature Finding – ‘Find by...’
- What does the chromatography look like? System suitability, S/N, plates...
- What does the spectrum look of each peak look like?
- Can the spectrum be identified by searching a library?



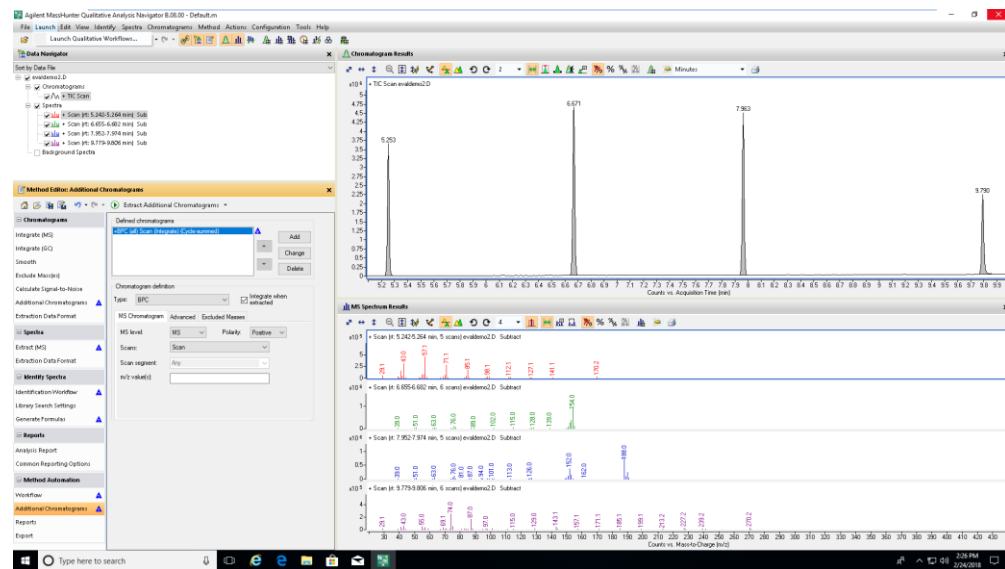
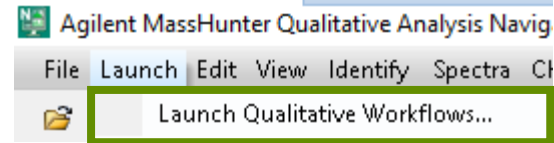
Workflows

- Compound centric
- Automated Routine Workflows
 - Sample Purity
 - Compound Discovery
 - Compound Identification
- Feature Finding – ‘Find by...’
- No Spectrum Preview
- No ‘User Spectra’
- All Spectra are compound based.
- What compounds can be found and identified?



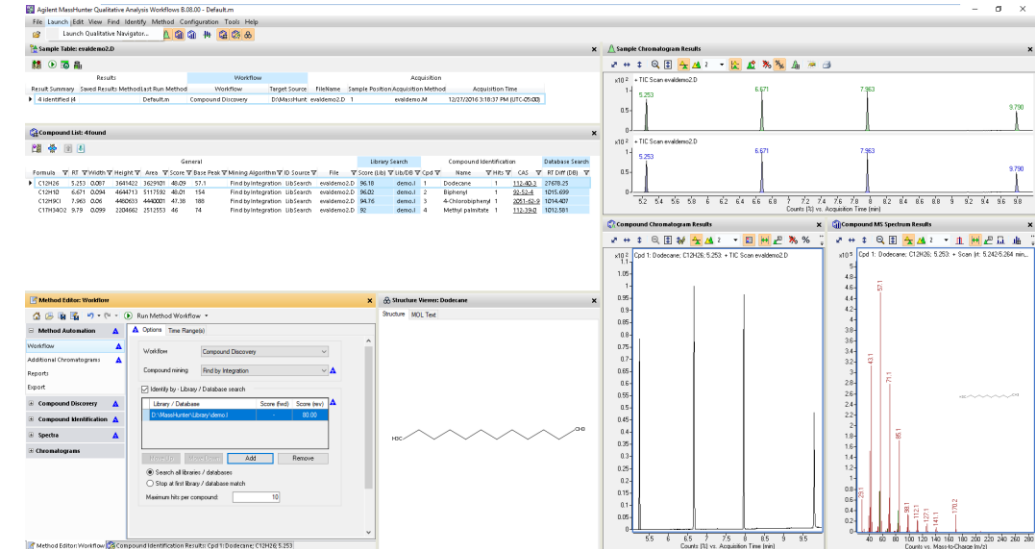
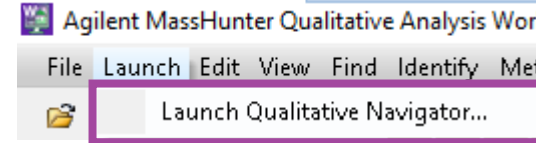
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Navigator and Workflows



File opens in Workflows.
The sample chromatogram(s) extracted.
Spectra not extracted.
Method is NOT passed to other context.

Workflows



File opens in Navigator.
The sample chromatogram(s) are extracted.
No compound results.
Method is NOT passed to other context.

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Topics

Qualitative Analysis Workflows

Workflow Definitions

- Target/Suspect Screening
- Compound Discovery
- Sample Purity
- Custom

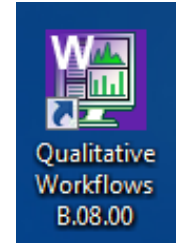
Compound Mining Algorithms

- Find by Integration
- Find by Chromatogram Deconvolution
- Molecular Feature Extraction
- Find by Formula

Compound Identification

- Library and Databases

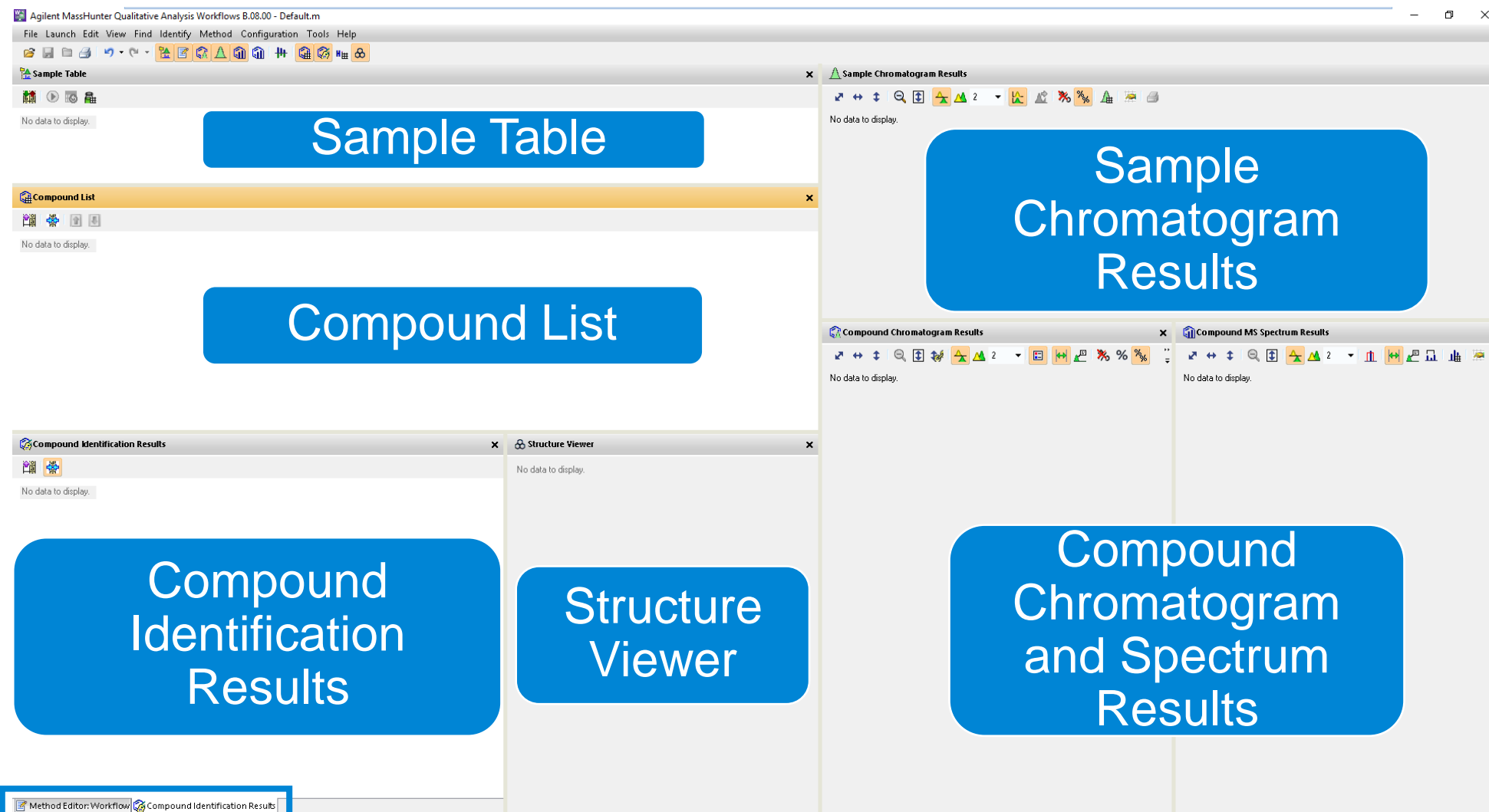
Generating Reports



Basic Qualitative Analysis features were covered in the previous webinar available at www.agilent.com/en-us/training-events/eseminars/236e

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Workflows Default Layout



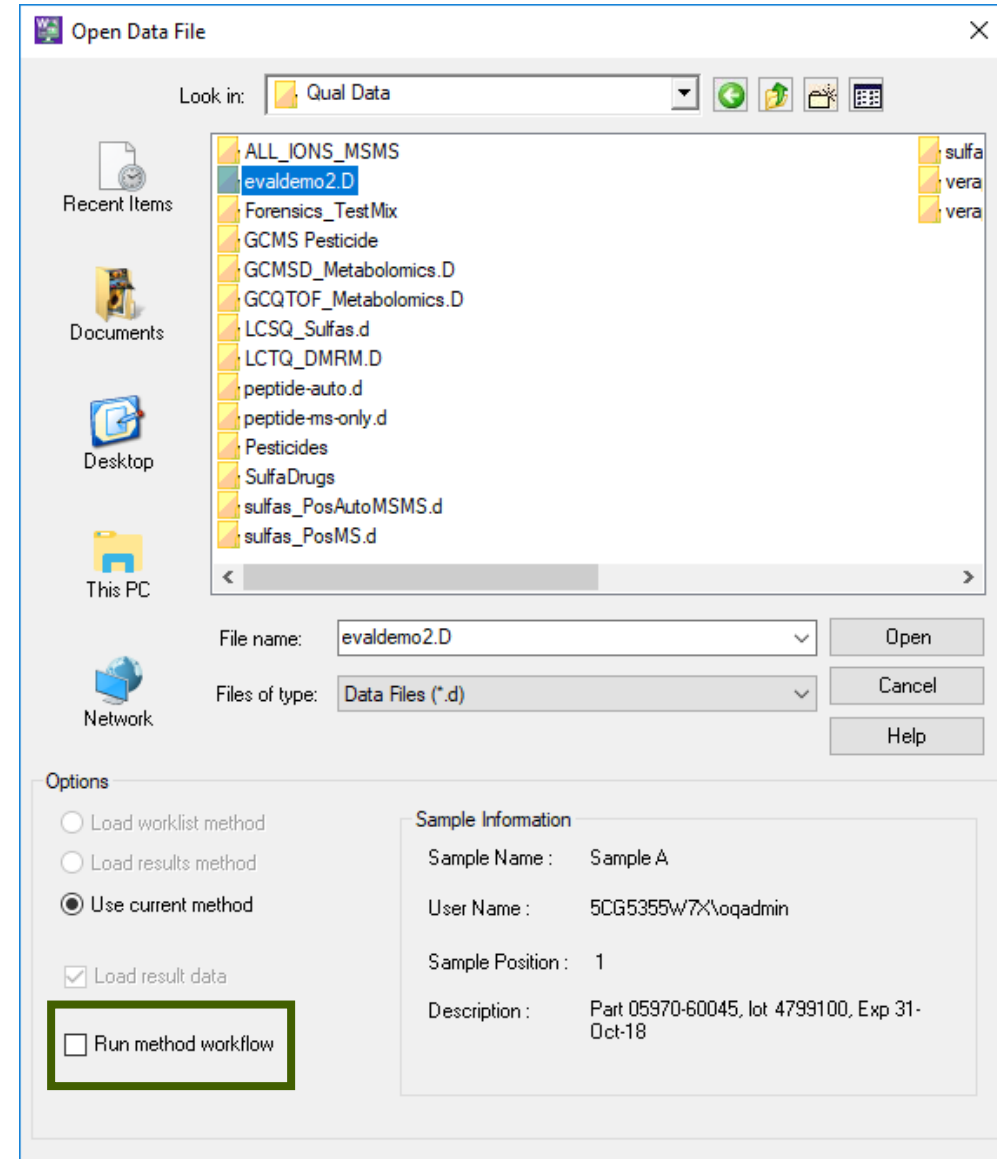
Method Editor is a tab.

Workflows

Open Data File

Open Data File Dialog Box

- Options
 - Load Worklist Method
 - Load Results Method
 - Use Current Method
- Sample Information
- Load result data
- Run method workflow
 - **NEW FEATURE!**



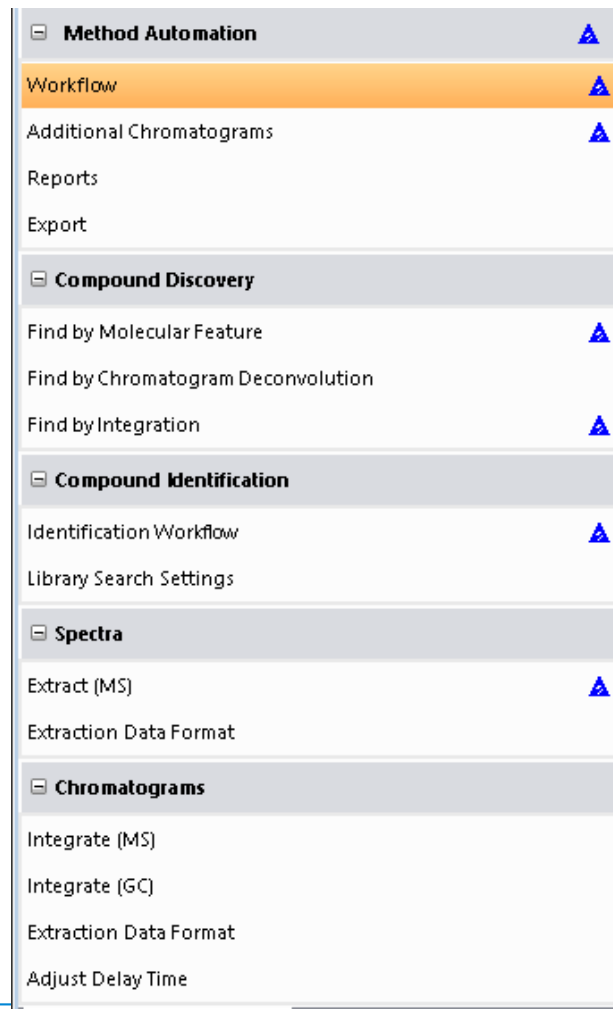
Adaptive User Interface – simplifies Method Editor



The screenshot shows the Method Editor interface with no data file loaded. The left sidebar is fully expanded, showing all available options under three main categories: Method Automation, Target/Suspect Screening, Compound Discovery, and Compound Identification. The 'Workflow' option under Method Automation is highlighted in light blue.

- Method Automation**
 - Workflow
 - Additional Chromatograms
 - Reports
 - Export
- Target/Suspect Screening**
 - Find by Formula
 - Find by Fragments
 - Find by MRM
- Compound Discovery**
 - Find by Molecular Feature
 - Find by Auto MS/MS
 - Find by Targeted MS/MS
 - Find by Chromatogram Deconvolution
 - Find by Integration
- Compound Identification**
 - Identification Workflow
 - Database Search Settings
 - Library Search Settings
 - Generate Formulas

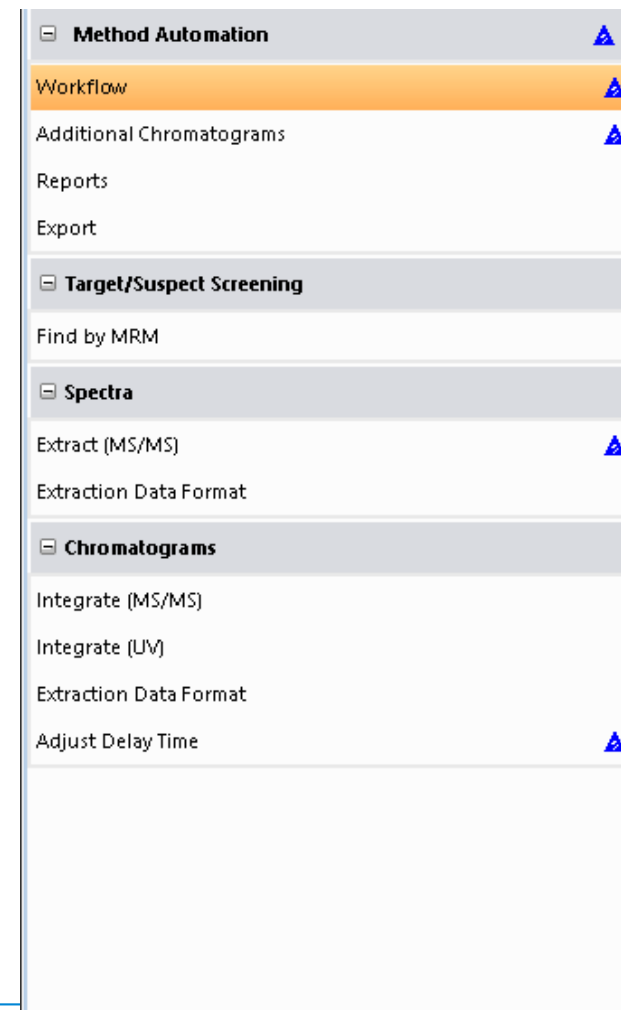
No Data File Loaded



The screenshot shows the Method Editor interface with GCMS data loaded. The left sidebar is partially collapsed, showing only the 'Workflow' option under Method Automation, which is highlighted in orange. The other categories are hidden.

- Method Automation**
 - Workflow
 - Additional Chromatograms
 - Reports
 - Export
- Compound Discovery**
 - Find by Molecular Feature
 - Find by Chromatogram Deconvolution
 - Find by Integration
- Compound Identification**
 - Identification Workflow
 - Library Search Settings
- Spectra**
 - Extract (MS)
 - Extraction Data Format
- Chromatograms**
 - Integrate (MS)
 - Integrate (GC)
 - Extraction Data Format
 - Adjust Delay Time

GCMS Data Loaded

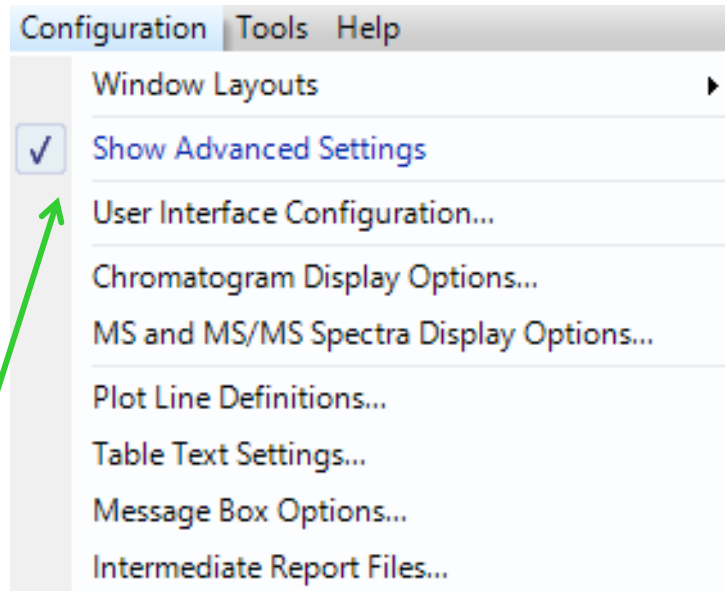


The screenshot shows the Method Editor interface with LC QQQ dMRM data loaded. The left sidebar is further simplified, showing only the 'Workflow' option under Method Automation (highlighted in orange) and the 'Adjust Delay Time' option under Chromatograms (highlighted in light blue). All other options are hidden.

- Method Automation**
 - Workflow
 - Additional Chromatograms
 - Reports
 - Export
- Target/Suspect Screening**
 - Find by MRM
- Spectra**
 - Extract (MS/MS)
 - Extraction Data Format
- Chromatograms**
 - Integrate (MS/MS)
 - Integrate (UV)
 - Extraction Data Format
 - Adjust Delay Time

LC QQQ dMRM Data Loaded

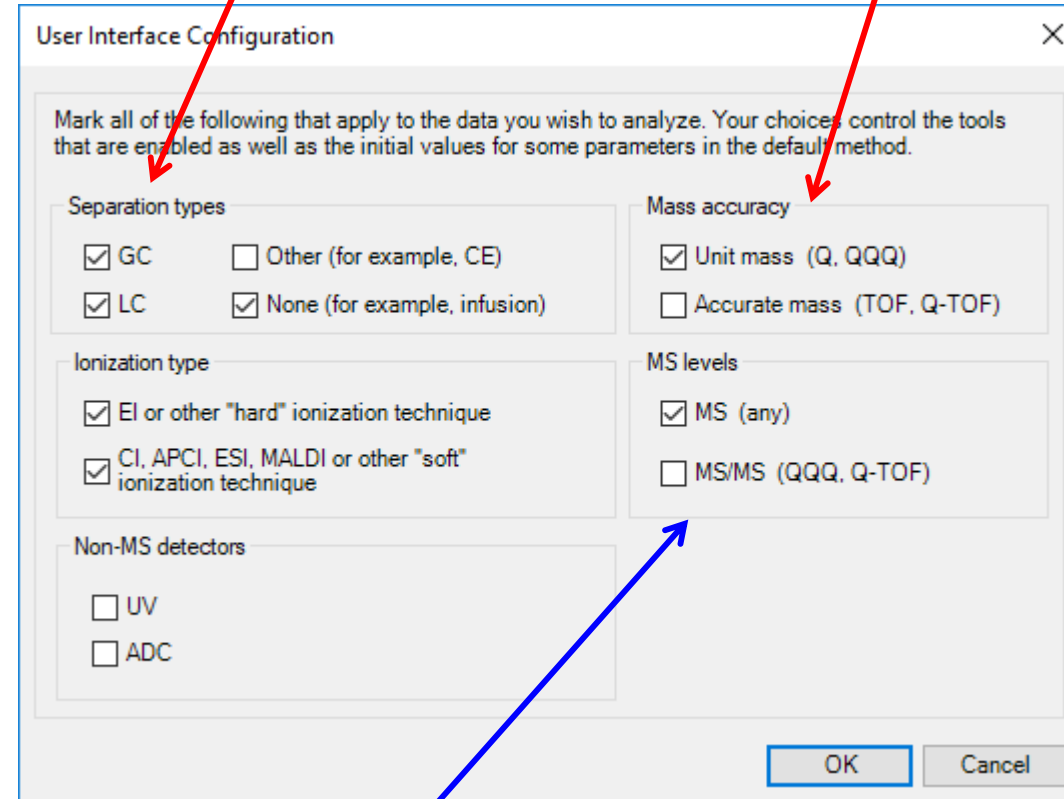
User Interface Configuration



- Check Show Advanced Settings from the Configuration menu to access the User Interface Configuration.
- Also shows advanced Method Editor features.

Separation types
(Check GC or LC)

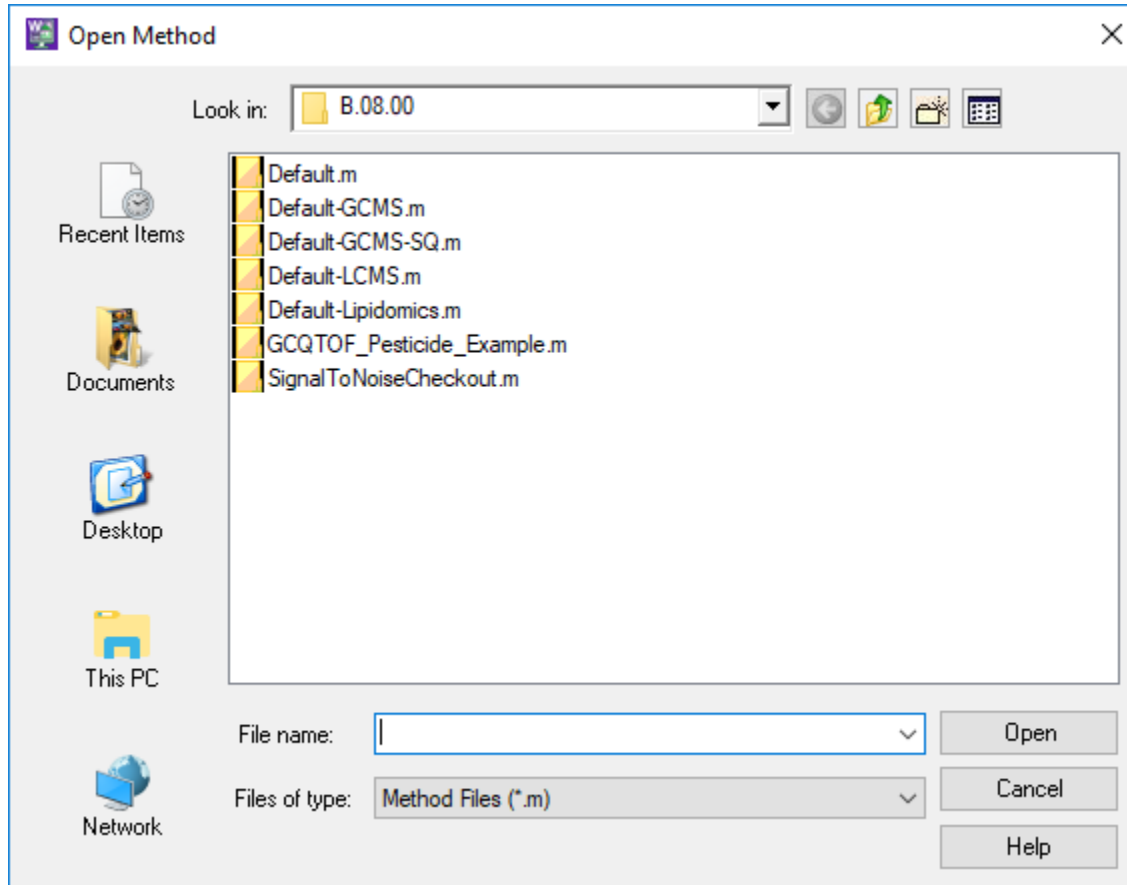
Unit Mass (SQ, TQ)
Accurate Mass (TOF, QTOF)



MS Levels MS or MS/MS

Qualitative Analysis Methods

Open Method



Open Method

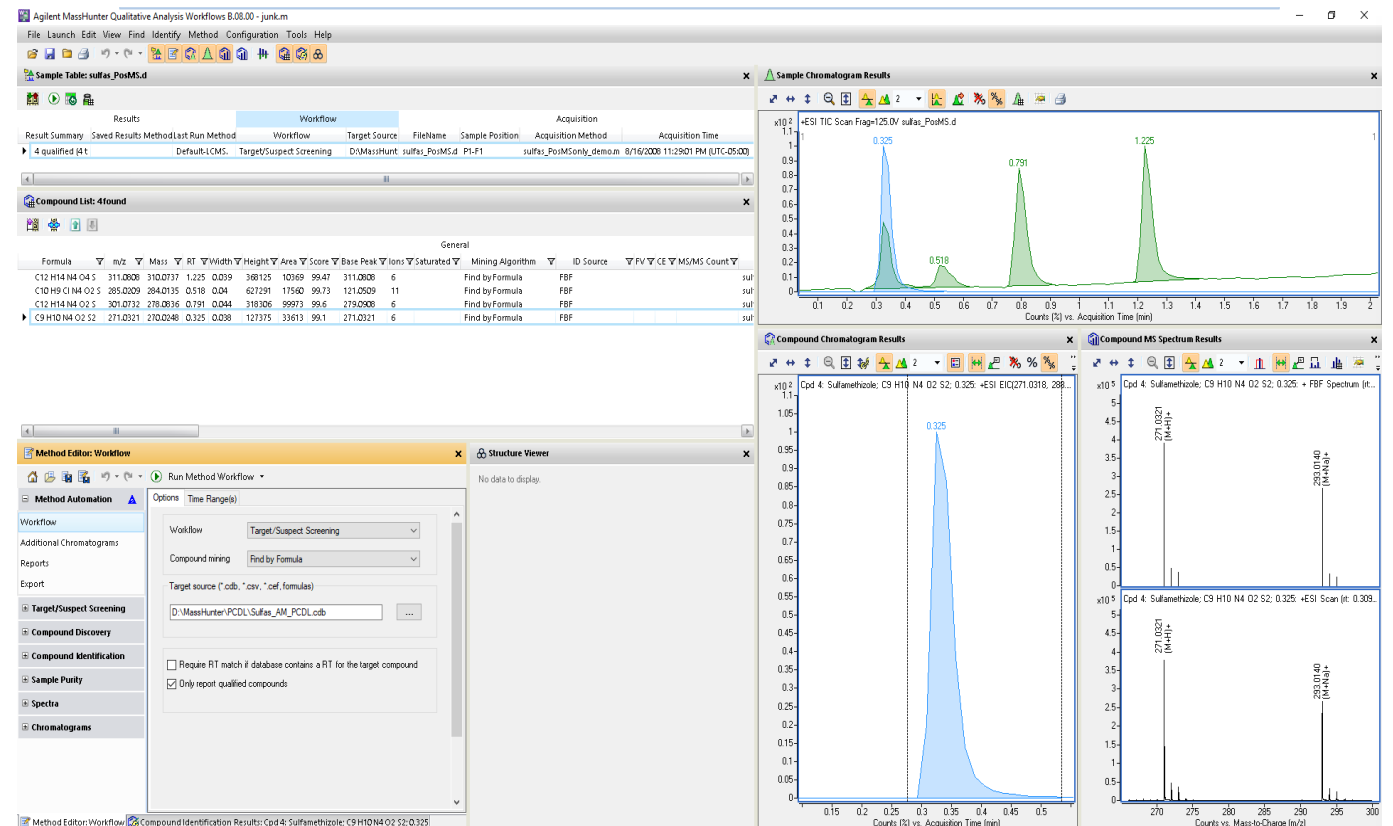
- Load the method relevant to the data set.
- Method contains recommended/default parameters.
- Method Editor values can be modified.
- Saves time developing specific values.
- Good starting point.

• **Tip: Load the applicable method.**

- Default-GCMS-SQ.M → GCMS and GC QQQ
- Default-GCMS.M → GC QTOF
- Default-LCMS.M → LCMS

Definition

- Intended for routine and automated analysis.
 - Select a workflow.
 - Select the mining algorithm.
 - Develop method parameters.
- Limited tools for manual spectral extraction.
 - No spectral preview.
 - Manual extraction always creates a compound.
- Double-click on sample in Sample Table to process data files with loaded method.
- Compound report only (no analysis reports).

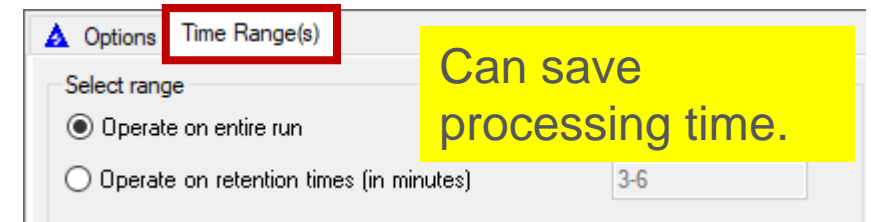
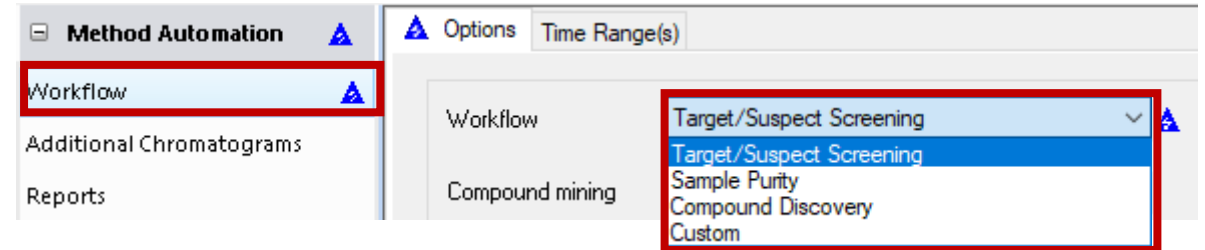


Workflows

Definition

A workflow is an automated sequence of steps to complete a task and generate results.

- Workflow is the table of contents.
- All the steps are defined via the Method Editor.
- Default workflows depend upon data set.
- Workflows
 - Target/Suspect Screening
 - Sample Purity
 - Compound Discovery
 - Custom
- The workflow parameters can be saved as part of the Qualitative Analysis Workflows method.

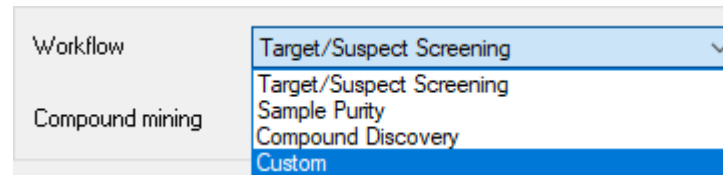


Workflows

Method Groupings

The Method Editor groups method sections to match workflows.

- Method Automation via workflows.
- **Target/Suspect Screening**
- **Compound Discovery**
- **Sample Purity**
- **Custom**
- **Compound Identification**
- Spectra
- Chromatograms



Agilent
CrossLab
From Insight to Outcome

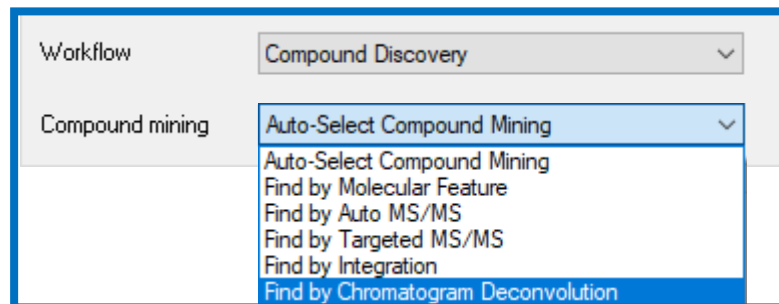
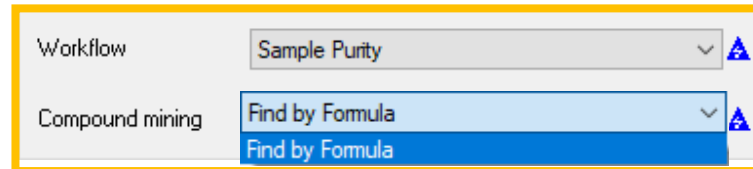
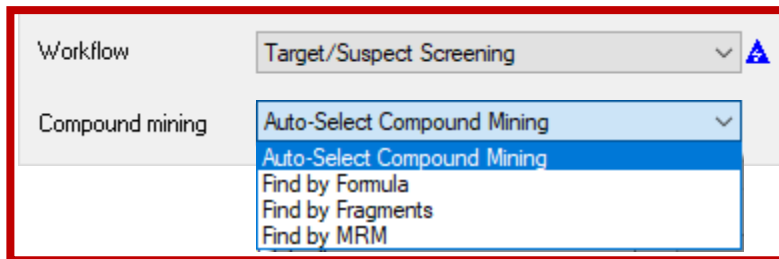
A screenshot of the Agilent CrossLab Method Editor interface. The interface is organized into several sections, each with a header and a list of sub-items. The sections are: 'Method Automation' (with a sub-section 'Workflow'), 'Additional Chromatograms', 'Reports', 'Export', 'Target/Suspect Screening' (highlighted with a red border), 'Compound Discovery' (highlighted with a blue border), 'Compound Identification' (with a sub-section 'Sample Purity' highlighted with a yellow border), 'Spectra', and 'Chromatograms'. The 'Target/Suspect Screening' section includes 'Find by Formula', 'Find by Fragments', and 'Find by MRM'. The 'Compound Discovery' section includes 'Find by Molecular Feature', 'Find by Auto MS/MS', 'Find by Targeted MS/MS', 'Find by Chromatogram Deconvolution', and 'Find by Integration'. The 'Sample Purity' section includes 'Sample Purity' and 'Find by Formula'. The 'Spectra' section includes 'Extract (MS)', 'Extract (MS/MS)', and 'Extraction Data Format'. The 'Chromatograms' section includes 'Integrate (MS)', 'Integrate (MS/MS)', 'Integrate (UV)', 'Integrate (GC)', 'Integrate (ADC)', 'Extraction Data Format', and 'Adjust Delay Time'.

Compound Mining

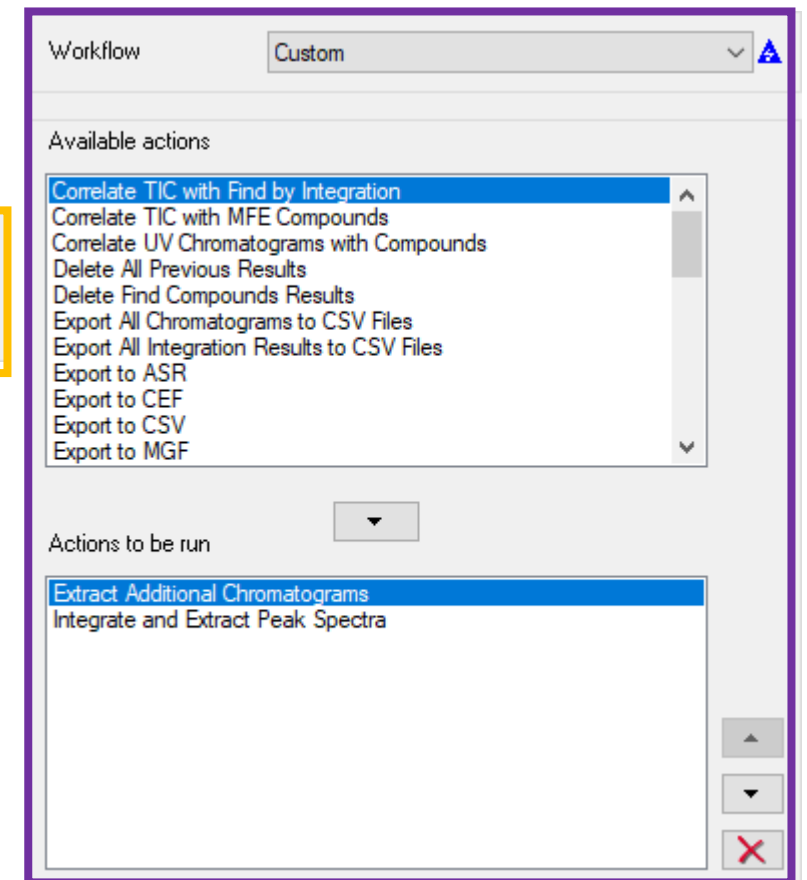
Definition

Compound Mining defines the algorithm to be run to find compounds.

- All the steps are defined via the Method Editor.
- Mining Algorithms depend upon data set selected.
- Mining Algorithms depend upon workflow selected.



All features may not be visible with the Adaptive User Interface.

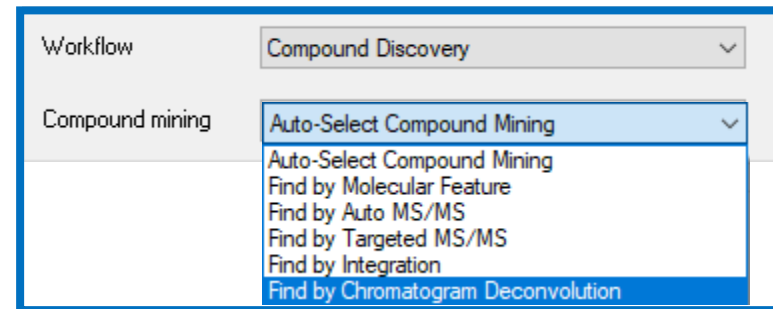
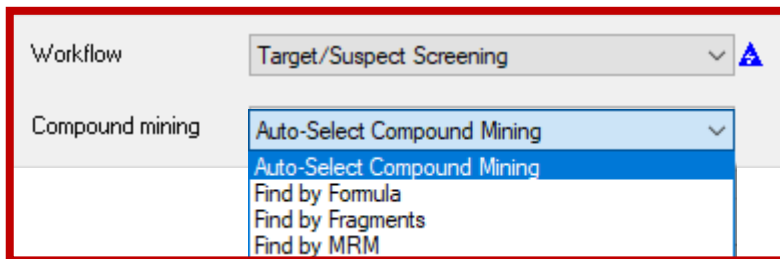


Compound Mining

Auto-Select Compound Mining

Auto-Select Compound Mining defines the algorithm to be run to find compounds.

- Based on data set loaded.
- Not always present, for example MRM data.
- Available with only Target/Suspect Screening and Compound Discovery.
- Good starting point.



Workflows

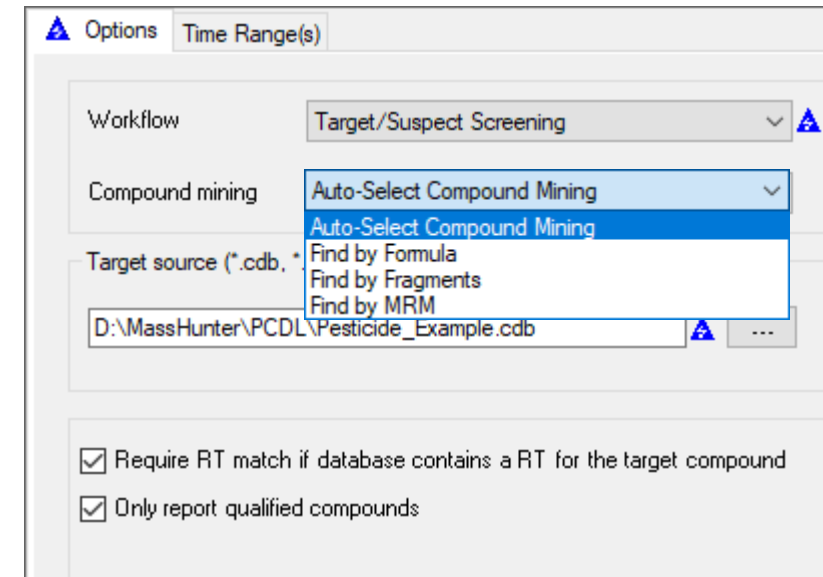
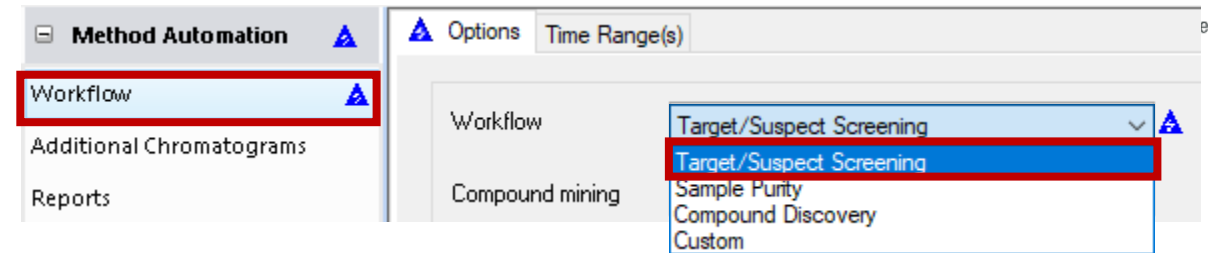
Target/Suspect Screening

“I know what I want, is it there?”

- Useful with accurate mass data (TOF, QTOF).
- Requires a library or a database
 - List of compound formulas (formulae)
 - In the form of cdb, csv, cef files
 - Can enter the neutral mass
- Mining Algorithms (data dependent)
 - Find by Formula
 - Find by Fragment (accurate mass data)
 - Are the fragments logical losses?
 - Find by MRM
 - Used with MRM data (QQQ)

Require RT match

Only report qualified compounds



Workflows

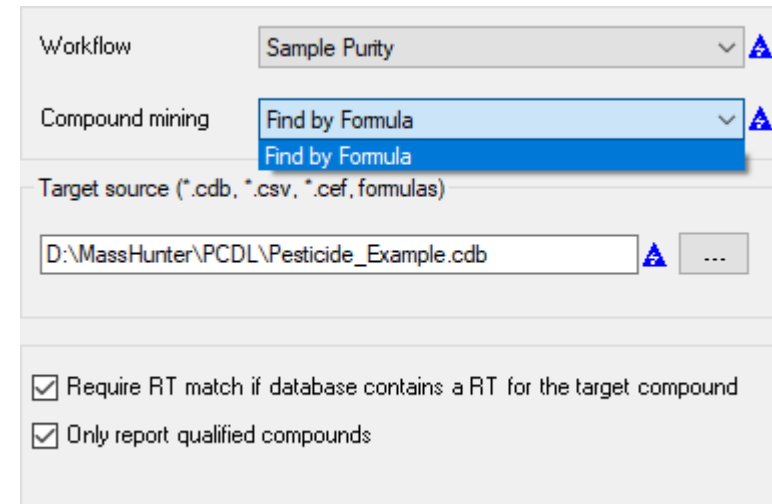
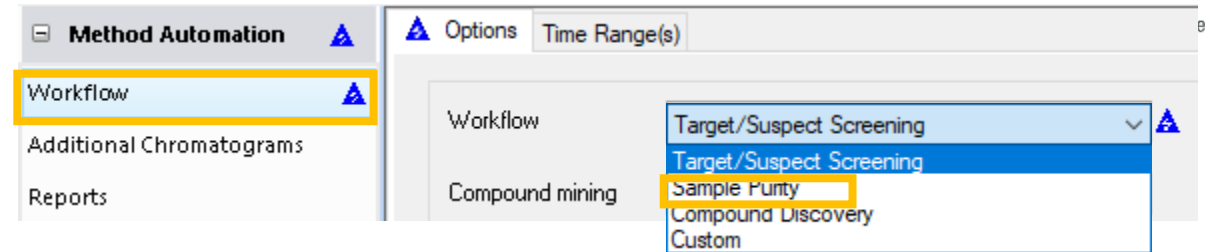
Sample Purity

“I know what I want, how pure is it?”

- Uses high resolution data.
- Requires a library or a database
 - List of compound formulas (formulae)
 - In the form of cdb, csv, cef files
- Mining Algorithms (data dependent)
 - Find by Formula
 - Adds sample purity calculations
 - Adds the area percent calculations and report.

Require RT match

Only report qualified compounds

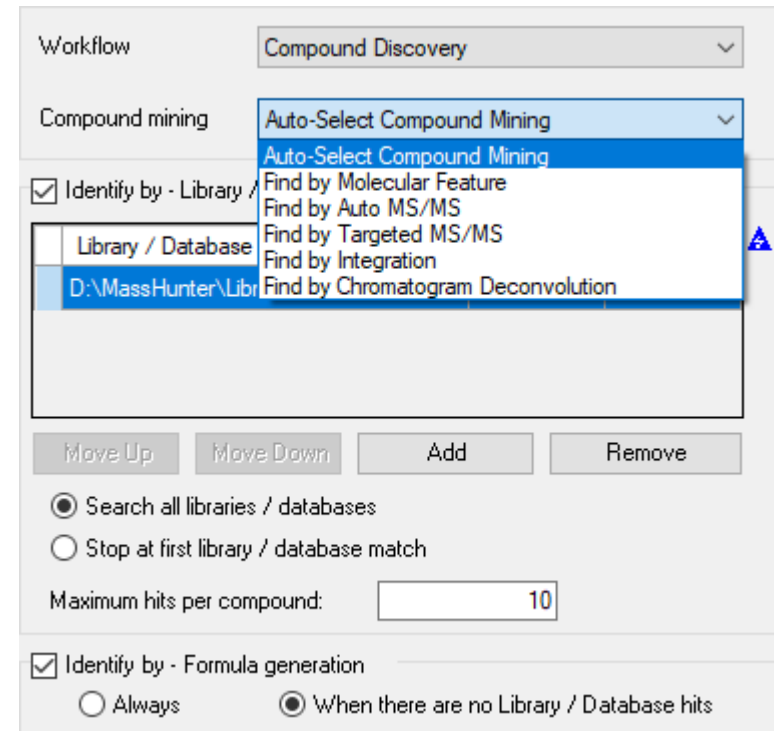
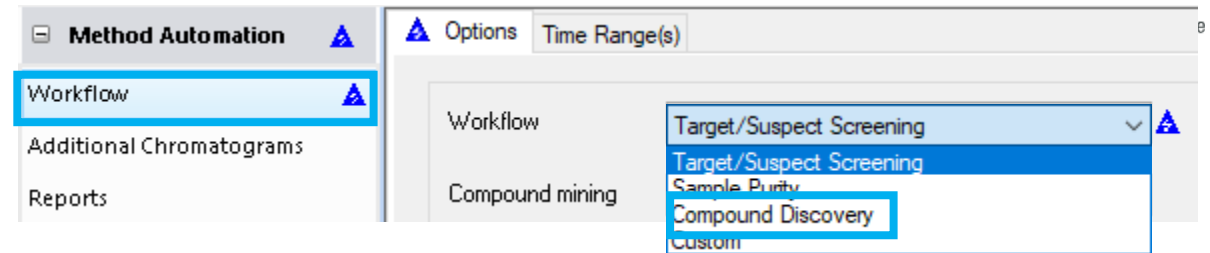


Workflows

Compound Discovery

“I don't know what I have, find the compounds and identify them”

- Library or database is optional.
 - Usually one database or library is specified.
 - List of compound formulas (formulae)
 - In the form of cdb, csv, cef files
- Compound Mining
 - Auto Select Compound Mining
 - Find by Molecular Feature
 - Find by Auto MS/MS
 - Find by Targeted MS/MS
 - Find by Integration
 - Find by Chromatogram Deconvolution

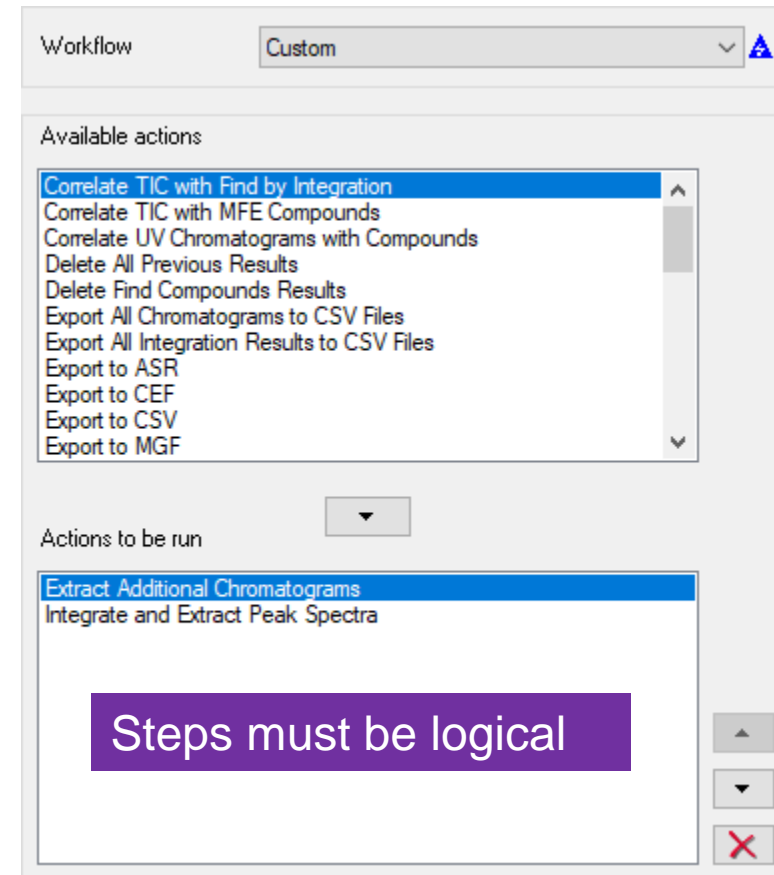
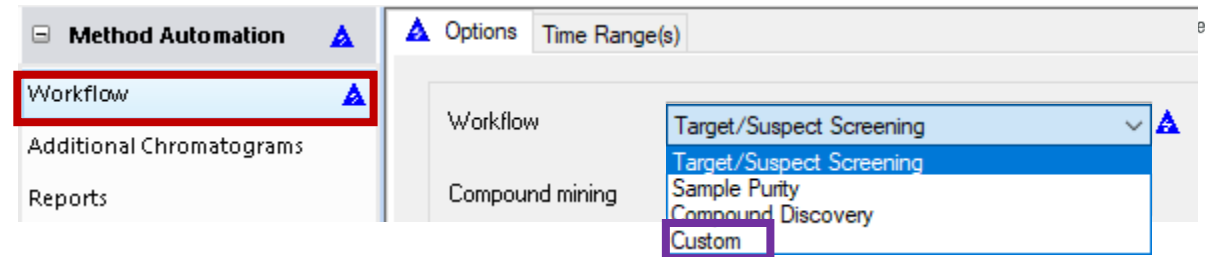


Workflows

Custom

“I know what I need to do, generate the compounds!”

- Selecting Custom provides Available Actions.
- Define and refine the actions in Method Editor.
- Available for all datasets.



Compound Mining

Target/Suspect Screening Method Editor

The image displays the Target/Suspect Screening Method Editor interface. On the left, a sidebar shows the workflow selection menu with 'Target/Suspect Screening' expanded and 'Find by Formula' selected. A red arrow points from this menu to the main configuration window. The main window has tabs for 'EIC Peak Filters', 'Charge State', 'Fragment Annotation', and 'Fragment Peak Filter'. The 'Target Source' tab is active, showing a text field with the path 'D:\MassHunter\PCDL\default.csv'. Below this, there are 'Values to match' options, including 'Require RT match if database compound' which is checked. A second red arrow points from the 'Find by Fragments' option in the sidebar to the 'Fragment Options' tab. This tab shows similar settings for 'Target Source' and 'Values to match'. A third red arrow points from the 'Find by MRM' option in the sidebar to the 'Peak Spectrum (MS)' tab. This tab includes 'Signal to Noise Options', 'Integrator', and 'Chromatogram Peak Filters'. Under 'Find compound settings', 'Group transitions by compound name' is selected. Under 'Compound results', 'Detect most abundant peak by' is set to 'Peak area'.

- Method Editor reveals 3 algorithms
 - Find by Formula
 - Find by Fragments
 - Find by MRM
- Selecting the ‘Find by’ in the Method Editor opens additional parameters.

Compound Mining

Sample Purity Method Editor

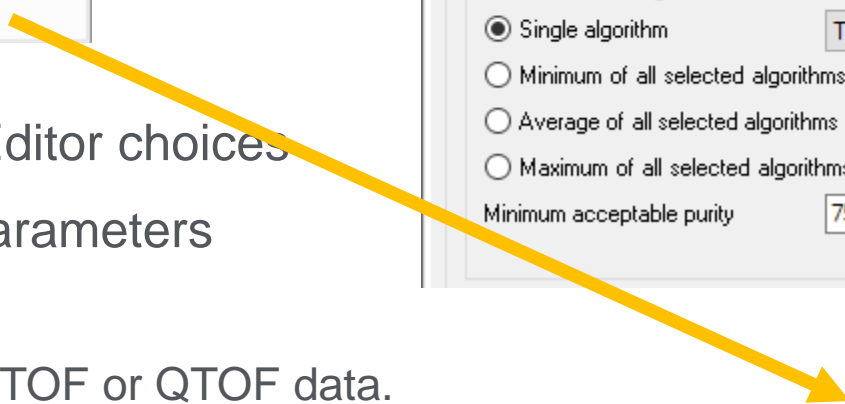
Workflow:

Compound mining:



Sample Purity

- Sample Purity
- Find by Formula



Integrate (UV) Suitability (UV) Peaks (UV) Integrate (ADC) Peaks (ADC)

Options Delay Time Exclude Masses Integrate (MS) Peaks (MS)

Sample purity

Algorithms to use

TIC % UV A % UV C %

EIC/TIC % UV B % TWC %

ADC %

Calculation settings

Use peak areas Use peak heights

RT acceptance window: +/- min

Qualification settings

Single algorithm

Minimum of all selected algorithms

Average of all selected algorithms

Maximum of all selected algorithms

Minimum acceptable purity:

Target Source Ion Species EIC Integration EIC Peak Filters Charge State

Target source (*.cdb, *.csv, *.cef, formulas, neutral masses)

Values to match

Require RT match if database contains a RT for the target compound.

- Only 2 Method Editor choices
- Sample Purity parameters
- Find by Formula
 - Useful for LC/TOF or QTOF data.
 - Requires a formula database.

Compound Mining

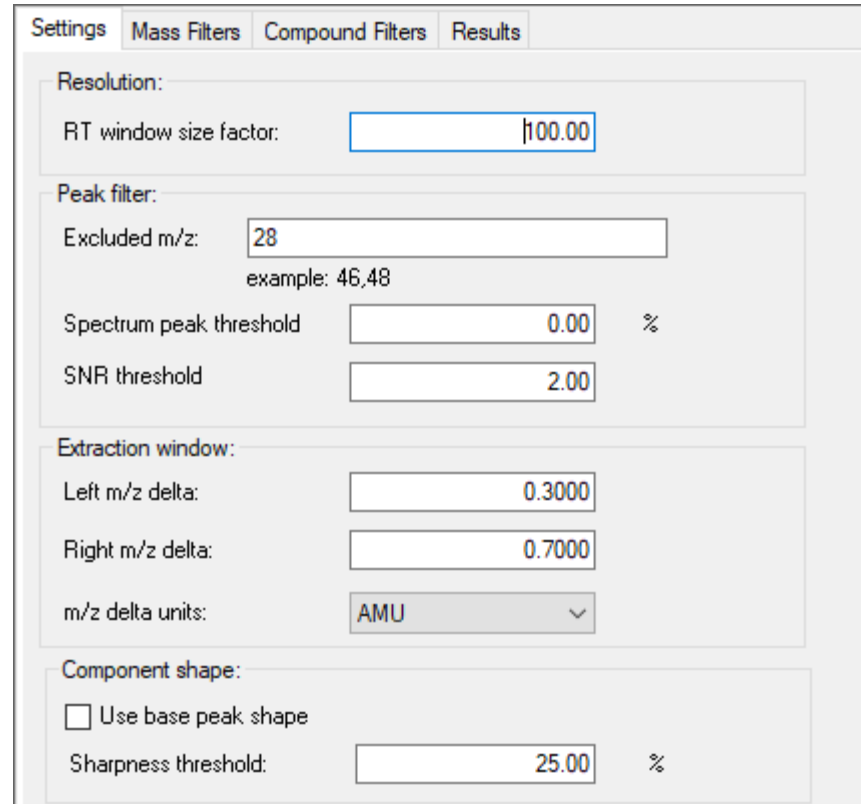
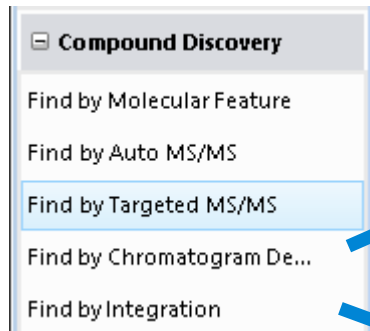
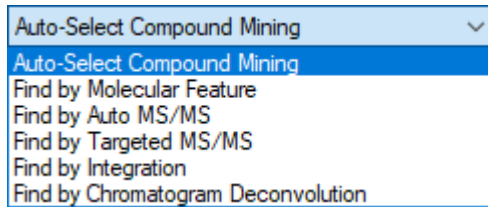
Compound Discovery Method Editor (1)

The image displays the Compound Discovery Method Editor interface. On the left, a dropdown menu is open, showing options: 'Auto-Select Compound Mining', 'Find by Molecular Feature', 'Find by Auto MS/MS', 'Find by Targeted MS/MS', 'Find by Integration', and 'Find by Chromatogram Deconvolution'. Below this is a 'Compound Discovery' section with sub-options: 'Find by Molecular Feature', 'Find by Auto MS/MS', 'Find by Targeted MS/MS', 'Find by Chromatogram De...', and 'Find by Integration'. The main interface is divided into several panels: 'Mass Filters' (with sub-sections 'Extraction', 'Ion Species', 'Charge State', 'Compound Filters'), 'Mass Defect', 'Fragment Peak Filter', and 'Results'. The 'Extraction' panel includes 'Extraction algorithm', 'Target data type', 'Input data range' (with checkboxes for 'Restrict retention time to' and 'Restrict m/z to'), 'Peak filters' (with 'Use peaks with height'), and 'Compound ion count threshold'. The 'Processing' panel includes 'Compound identification' (with fields for 'Retention time window', 'Positive MS/MS TIC threshold', 'Negative MS/MS TIC threshold', 'Mass match tolerance', and 'Limit to the largest') and 'Compound fragment filters' (with options for 'Filter results by fragments', 'Fragment mass filter', 'Neutral loss filter', 'Match specified fragments', and 'Mass match tolerance'). The 'Results' panel includes 'Persistent background compounds' and 'Integrator selection' (set to 'Agile 2'). Blue arrows indicate the flow from the dropdown menu to the 'Compound Discovery' section, and from the 'Find by Auto MS/MS' and 'Find by Targeted MS/MS' options to their respective configuration panels.

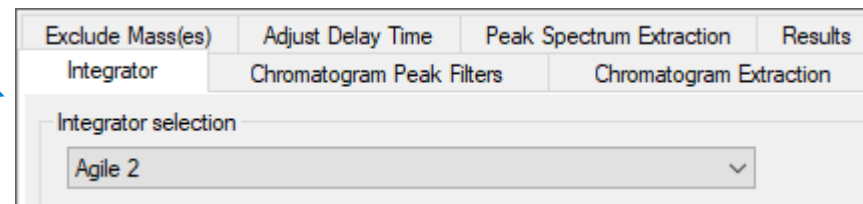
- Multiple Compound Mining algorithms available.
- Find by Auto MS/MS and Find by Targeted MS/MS specific to QTOF data.

Compound Mining

Compound Discovery Method Editor (2)



- Find by Chromatogram Deconvolution
 - Similar to Quantitative Analysis and Unknowns Analysis.



Compound Identification Definition

Identify All Compounds ▾

Identify by - Library / Database search

Library / Database	Score (fwd)	Score (rev)
D:\MassHunter\PCDL\default.csv	-	-

Move Up Move Down Add Remove

Search all libraries / databases
 Stop at first library / database match

Maximum hits per compound:

Identify by - Formula generation

Always When there are no Library / Database hits

Search Library / DB for All Compounds ▾

Search Criteria Peak Limits Positive Ions Negative Ions Search Results

Values to match

Molecular formula
 Mass
 Mass and retention time (retention time optional)
 Mass and retention time (retention time required)

Match tolerance

Mass ppm
Retention time minutes

Search Library / DB for All Compounds ▾

Search Criteria RT Scoring Peak Filters Tolerances

Search criteria (.cdb)

Restrict spectral comparison based on

Ionization mode
 Instrument type
 Collision energy +/- eV

Search criteria (.L, .xml)

Enable screening Adjust score

Generate Formulas from All Compounds ▾

Allowed Species Limits Charge State Fragment Formulas

Charge carrier to be assumed if not known

Positive ions: -electron +H +Na +K +NH4 +C2H5 +C3H5

Negative ions: +electron -H +Cl +Br +HCOO +CH3COO +CF3COO

MS ion electron state:

Group hits with same formula (but different charge carriers)

Elements and limits

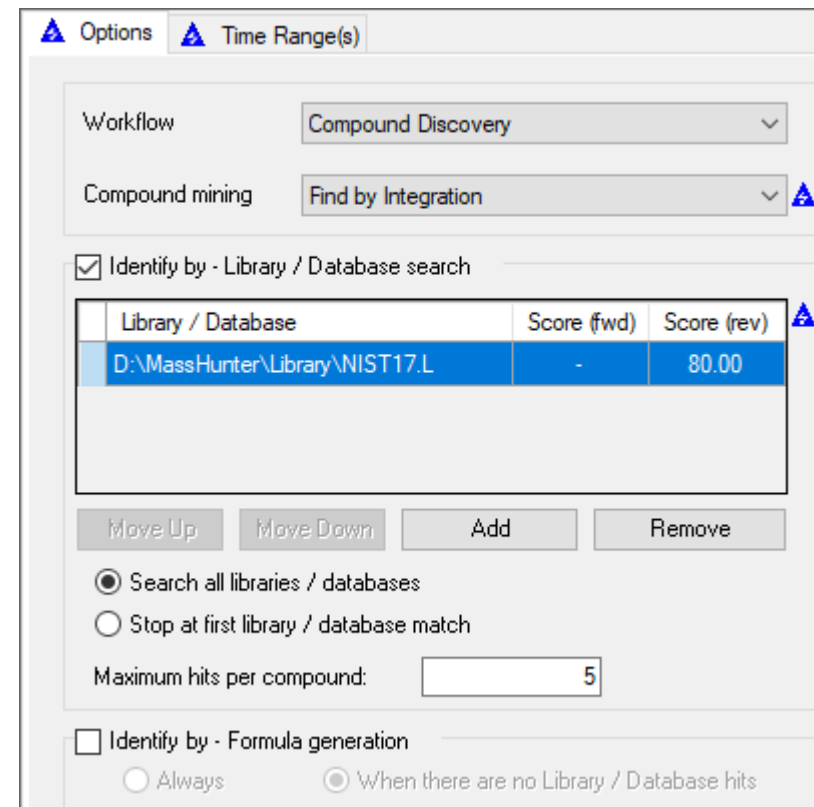
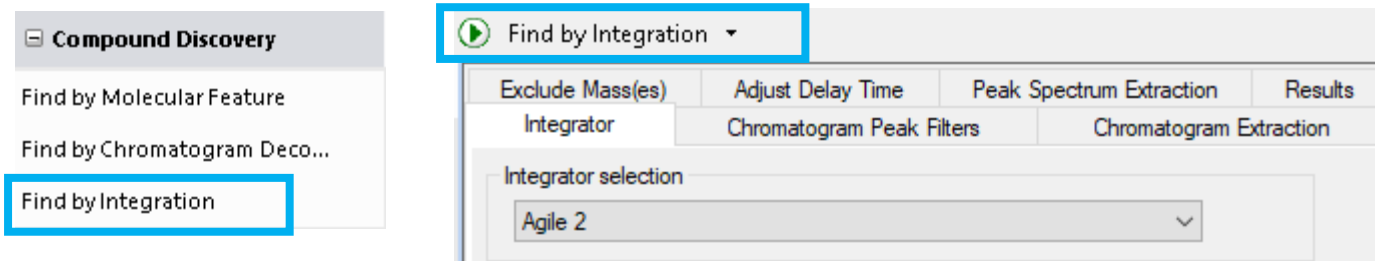
Element	Minimum	Maximum
C	3	60
H	0	120
O	0	30
N	0	30
S	0	5
Cl	0	3

- Component of the Method Editor
 - Establishes database or library to use.

Find by Integration

Example

- Load the data file Evaldemo2.D
 - Simple data file.
 - GCMS SQ data.
- Load the method Default-GCMS-SQ.M.
- Set the Workflow to Compound Discovery.
- Select Find by Integration for the Compound Mining.
- Add the NIST17.L.
- Review the Find by Integration parameters.



- Run Method Workflow or run icon.

Find by Integration

Results

Agilent MassHunter Qualitative Analysis Workflows B.08.00 - Default-GCMS-SQ.m

File Launch Edit View Find Identify Method Configuration Tools Help

Sample Table: evaldemo2.D

Results	Workflow	Acquisition
Result Summary	Saved Results Method Last Run Method	Workflow Target Source Sample Name FileName Sample Position Acquisition Method Acq
4 identified (4 found)	Default-GCMS- Compound Discovery	DA:MassHunter/Library/NIST17.L Sample A evaldemo2.D 1 evaldemo.M 12/27/2016 3:

Compound List: 4 found

General		Library Search		Compound Identification											
Formula	RT	Width	Height	Area	Score (Lib)	Lib/DB	Cpd	Name	Hits	CAS					
C12H26	5.253	0.087	3641422	3629101	99.66	57.1	Find by Integration	LibSearch	evaldemo2.D	99.66	NIST17.L	1	Dodecane	5	112-40-3
C12H10	6.671	0.094	4644713	5117592	99.58	154	Find by Integration	LibSearch	evaldemo2.D	99.58	NIST17.L	2	Biphenyl	5	92-52-4
C12H9Cl	7.963	0.06	4480633	4440001	99.37	188	Find by Integration	LibSearch	evaldemo2.D	99.37	NIST17.L	3	1,1'-Biphenyl, 4-chloro-	5	2051-62-9
C17H34O2	9.79	0.099	2204662	2512553	97.65	74	Find by Integration	LibSearch	evaldemo2.D	97.65	NIST17.L	4	Hexadecanoic acid, methyl ester	5	112-39-0

Compound Identification Results: Cpd 1: Dodecane; C12H26; 5.253

ID Techniques Applied: DBSearch-LibSearch

Best	Name	Formula	RT	Flags	Lib/DB
<input checked="" type="radio"/>	Dodecane	C12H26	5.253		NIST17.L
<input type="radio"/>	Dodecane	C12H26	5.253		NIST17.L
<input type="radio"/>	Dodecane	C12H26	5.253		NIST17.L
<input type="radio"/>	Dodecane	C12H26	5.253		NIST17.L
<input type="radio"/>	Hexadecane	C16H34	5.253		NIST17.L

Structure Viewer: Dodecane

Structure: CCCCCCCCCCCC

Sample Chromatogram Results

+ TIC Scan evaldemo2.D

+ TIC Scan evaldemo2.D

Compound Chromatogram Results

Cpd 1: Dodecane; C12H26; 5.253; + TIC Scan evaldemo2.D

Compound MS Spectrum Results

Cpd 1: Dodecane; C12H26; 5.253; + Scan (rt: 5.242-5.264 min, 5 sca...)

Method Editor: Workflow

Compound Identification Results: Cpd 1: Dodecane; C12H26; 5.253

Let's take a moment for questions on Workflows and Compound Mining Algorithms

Up Next:
Data Review



Results		Workflow		Acquisition					
Result Summary	Saved Results Method	Last Run Method	Workflow	Target Source	Sample Name	FileName	Sample Position	Acquisition Method	Acquisition Time
▶ 4 identified (4 found)	Default-GCMS-	Compound Discovery	D:\MassHunter\Library\NIST17.L	Sample A	evaldemo2.D	1	evaldemo.M	12/27/2016 3:18:37 PM (UTC-05:00)	

- Sample Table shows information about the sample.
 - Workflow column shows most recent workflow applied to data file.
- Results
 - Four compounds identified (4 found in library).
 - Saved Results Method.
 - Must be saved and shows Qualitative Analysis parameters.
- Workflow
 - Workflow used and library searched.

- Acquisition
Information about acquisition parameters.

- Toolbar

Add/Remove Columns

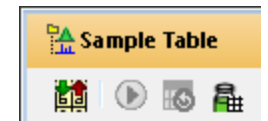
Run method workflow

Can run on multiple samples

Reprocess sample

Can reprocess single samples or reprocess with a different workflow.

Sample Information

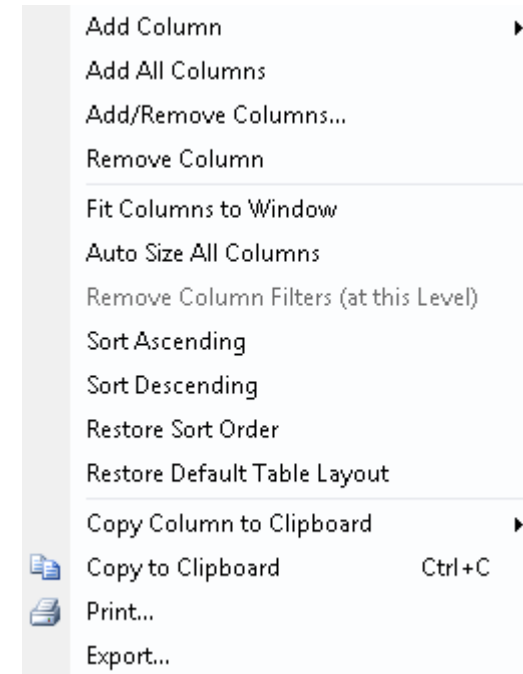
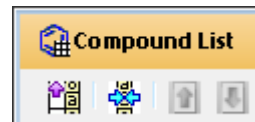


Data Review

Compound List

Compound List: 4 found															
General								Library Search			Compound Identification				
Formula	RT	Width	Height	Area	Score	Base Peak	Mining Algorithm	ID Source	File	Score (Lib)	Lib/DB	Cpd	Name	Hits	CAS
C12H26	5.253	0.087	3641422	3629101	99.66	57.1	Find by Integration	LibSearch	evaldemo2.D	99.66	NIST17.L	1	Dodecane	5	112-40-3
C12H10	6.671	0.094	4644713	5117592	99.58	154	Find by Integration	LibSearch	evaldemo2.D	99.58	NIST17.L	2	Biphenyl	5	92-52-4
C12H9Cl	7.963	0.06	4480633	4440001	99.37	188	Find by Integration	LibSearch	evaldemo2.D	99.37	NIST17.L	3	1,1'-Biphenyl, 4-chloro-	5	2051-62-9
C17H34O2	9.79	0.099	2204662	2512553	97.65	74	Find by Integration	LibSearch	evaldemo2.D	97.65	NIST17.L	4	Hexadecanoic acid, methyl ester	5	112-39-0

- Compound List shows 4 compounds
- Four compounds identified
- Context menu yields numerous options for formatting
 - Add/Remove Columns
 - Auto Size all Columns
 - Sorting
 - Copying, printing and exporting
- Toolbar
 - Hide any currently empty columns
 - Auto Size All Columns
 - Select previous and next compounds



Data Review

Compound List

The screenshot shows a software window titled "Compound List: 4 found". It contains a table with columns for General, Library Search, and Compound Identification. The first row is selected, and a context menu is open over it. The menu items are: Add/Remove Columns..., Select All, Identify Compound(s) (highlighted with a red box), Search Using NIST MS Program..., Add/Edit Manual Identification..., Send Spectra to PCDL, Clear Compound Identification Results, Delete (with a red X icon), Initiate Quantitation..., Copy to Clipboard (Ctrl+C), Print..., and Export...

General								Library Search			Compound Identification				
Formula	RT	Width	Height	Area	Score	Base Peak	Mining Algorithm	ID Source	File	Score (Lib)	Lib/DB	Cod	Name	Hits	CAS
C12H26	5.253	0.087	3641422	3629101	99.66	57.1	Find by Integration	LibSearch	evaldemo2.D	99.66	NIST17.				12-40-3
C12H10	6.671	0.094	4644713	5117592	99.58	154	Find by Integration	LibSearch	evaldemo2.D	99.58	NIST17.				1-52-4
C12H9Cl	7.963	0.06	4480633	4440001	99.37	188	Find by Integration	LibSearch	evaldemo2.D	99.37	NIST17.				051-62-9
C17H34O2	9.79	0.099	2204662	2512553	97.65	74	Find by Integration	LibSearch	evaldemo2.D	97.65	NIST17.				12-39-0

- Additional options are available in the context menu.
 - Identify Compound(s)
 - Initiates Identification workflow for selected compounds.
 - Search Using NIST MS Program
 - Add/Edit Manual Identification
 - Overwrites current Name column.
 - Send Spectra to PCDL database.
 - Initiate Quantitation
 - Useful with MRM data
 - Launches Quantitative analysis and transfers transitions and collision energies to the quantitation database.

Compound List

Super Header vs. Header

Compound List: 5 found

General											Library Search			Compound Identification			Database Search
Formula	RT	Width	Height	Area	Score	Base Peak	Mining Algorithm	ID Source	File	Score (Lib)	Lib/DB	Cpd	Name	Hits	CAS	RT Diff (DB)	
C12H26	5.278	0.097	325283	44275	99.39	57.1	Find by Integration	LibSearch	EVALDEMO.D	99.39	demo.I	1	Dodecane	1	112-40-3	27678.225	
C12H10	6.431	0.159	398482	69784	99.69	154.1	Find by Integration	LibSearch	EVALDEMO.D	99.69	demo.I	2	Biphenyl	1	92-52-4	1015.939	
C12H9Cl	7.737	0.167	347841	59098	99.27	188.2	Find by Integration	LibSearch	EVALDEMO.D	99.27	demo.I	3	4-Chlorobiphenyl	1	2051-62-9	1014.633	
C17H34O2	8.947	0.109	80492	15923	253.1	253.1	Find by Integration	LibSearch	EVALDEMO.D	98.89	demo.I	4	Methyl palmitate	0			
C17H34O2	9.772	0.153	373249	54842	98.89	74.1	Find by Integration	LibSearch	EVALDEMO.D	98.89	demo.I	5	Methyl palmitate	1	112-39-0	1012.598	

Super Headers can be moved as group.

Compound List: 5 found

Cpd	Compound Identification			General										Library Search			Database Search
Name	Hits	CAS	Formula	RT	Width	Height	Area	Score	Base Peak	Mining Algorithm	ID Source	File	Score (Lib)	Lib/DB	RT Diff (DB)		
1	Dodecane	1	112-40-3	C12H26	5.278	0.097	325283	44275	99.39	57.1	Find by Integration	LibSearch	EVALDEMO.D	99.39	demo.I	27678.225	
2	Biphenyl	1	92-52-4	C12H10	6.431	0.159	398482	69784	99.69	154.1	Find by Integration	LibSearch	EVALDEMO.D	99.69	demo.I	1015.939	
3	4-Chlorobiphenyl	1	2051-62-9	C12H9Cl	7.737	0.167	347841	59098	99.27	188.2	Find by Integration	LibSearch	EVALDEMO.D	99.27	demo.I	1014.633	
4		0			8.947	0.109	80492	15923	253.1		Find by Integration		EVALDEMO.D				
5	Methyl palmitate	1	112-39-0	C17H34O2	9.772	0.153	373249	54842	98.89	74.1	Find by Integration	LibSearch	EVALDEMO.D	98.89	demo.I	1012.598	

Sub Headers can be moved with the super header.

Compound List: 5 found

General											Library Search			Compound Identification			Database Search
Formula	File	RT	Width	Height	Area	Score	Base Peak	Mining Algorithm	ID Source	Score (Lib)	Lib/DB	Cpd	Name	Hits	CAS	RT Diff (DB)	
C12H26	EVALDEMO.D	5.278	0.097	325283	44275	99.39	57.1	Find by Integration	LibSearch	99.39	demo.I	1	Dodecane	1	112-40-3	27678.225	
C12H10	EVALDEMO.D	6.431	0.159	398482	69784	99.69	154.1	Find by Integration	LibSearch	99.69	demo.I	2	Biphenyl	1	92-52-4	1015.939	
C12H9Cl	EVALDEMO.D	7.737	0.167	347841	59098	99.27	188.2	Find by Integration	LibSearch	99.27	demo.I	3	4-Chlorobiphenyl	1	2051-62-9	1014.633	
	EVALDEMO.D	8.947	0.109	80492	15923	253.1		Find by Integration				4		0			
C17H34O2	EVALDEMO.D	9.772	0.153	373249	54842	98.89	74.1	Find by Integration	LibSearch	98.89	demo.I	5	Methyl palmitate	1	112-39-0	1012.598	

Data Review

Compound Identification Results

- Compound Identification Results more detailed information about compounds.
 - Detailed information about identified compound.
- May be tabbed window in default layout.
- Shows 5 library hits.
- Sub level shows additional information about identified compound.
- Context menu yields numerous options for formatting.
 - Add/Remove Columns
 - Auto Size all Columns
 - Sorting
 - Copying, printing and exporting
- Toolbar
 - Hide any currently empty columns
 - Auto Size All Columns

Compound Identification Results: Cpd 1: Dodecane; C12H26; 5.253

ID Techniques Applied

- DBSearch-LibSearch

Best	Name	Formula	RT	ID Source	Flags	Score	Lib/DB
<input checked="" type="radio"/>	Dodecane	C12H26	5.253	LibSearch		99.66	NIST17.L

Name	Lib/DB	Num Peaks	Reverse Score	Score (Lib)
Dodecane	NIST17.L	59		99.66

Best	Name	Formula	RT	ID Source	Flags	Score	Lib/DB
<input type="radio"/>	Dodecane	C12H26	5.253	LibSearch		99.32	NIST17.L
<input type="radio"/>	Dodecane	C12H26	5.253	LibSearch		99.12	NIST17.L
<input type="radio"/>	Dodecane	C12H26	5.253	LibSearch		96.87	NIST17.L
<input type="radio"/>	Hexadecane	C16H34	5.253	LibSearch		93.46	NIST17.L

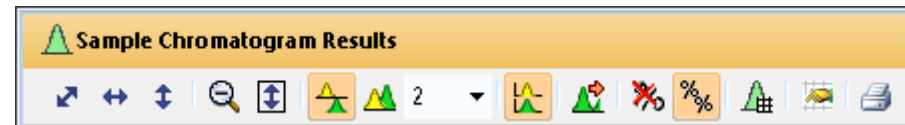
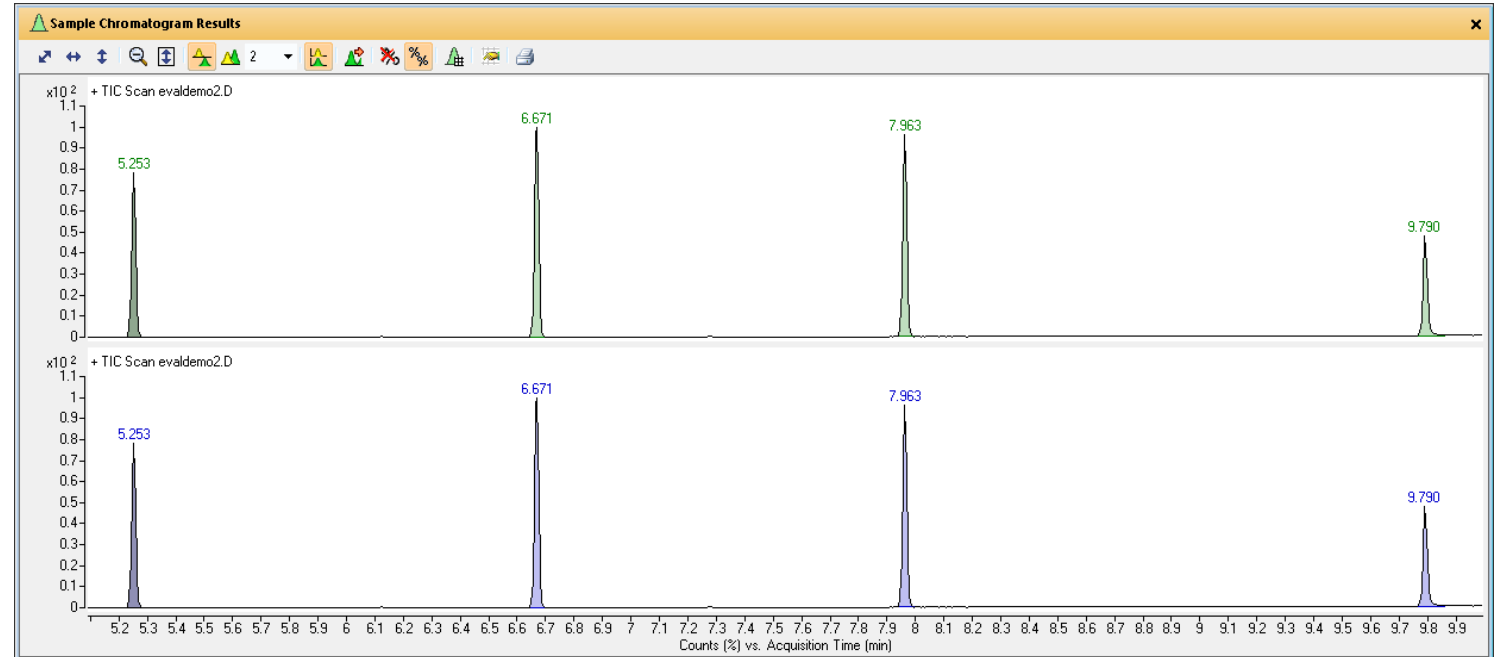
Method Editor: Find by Integration | Compound Identification Results: Cpd 1: Dodecane; C12H26; 5.2...

Compound Identification Results

Data Review

Sample Chromatogram Results

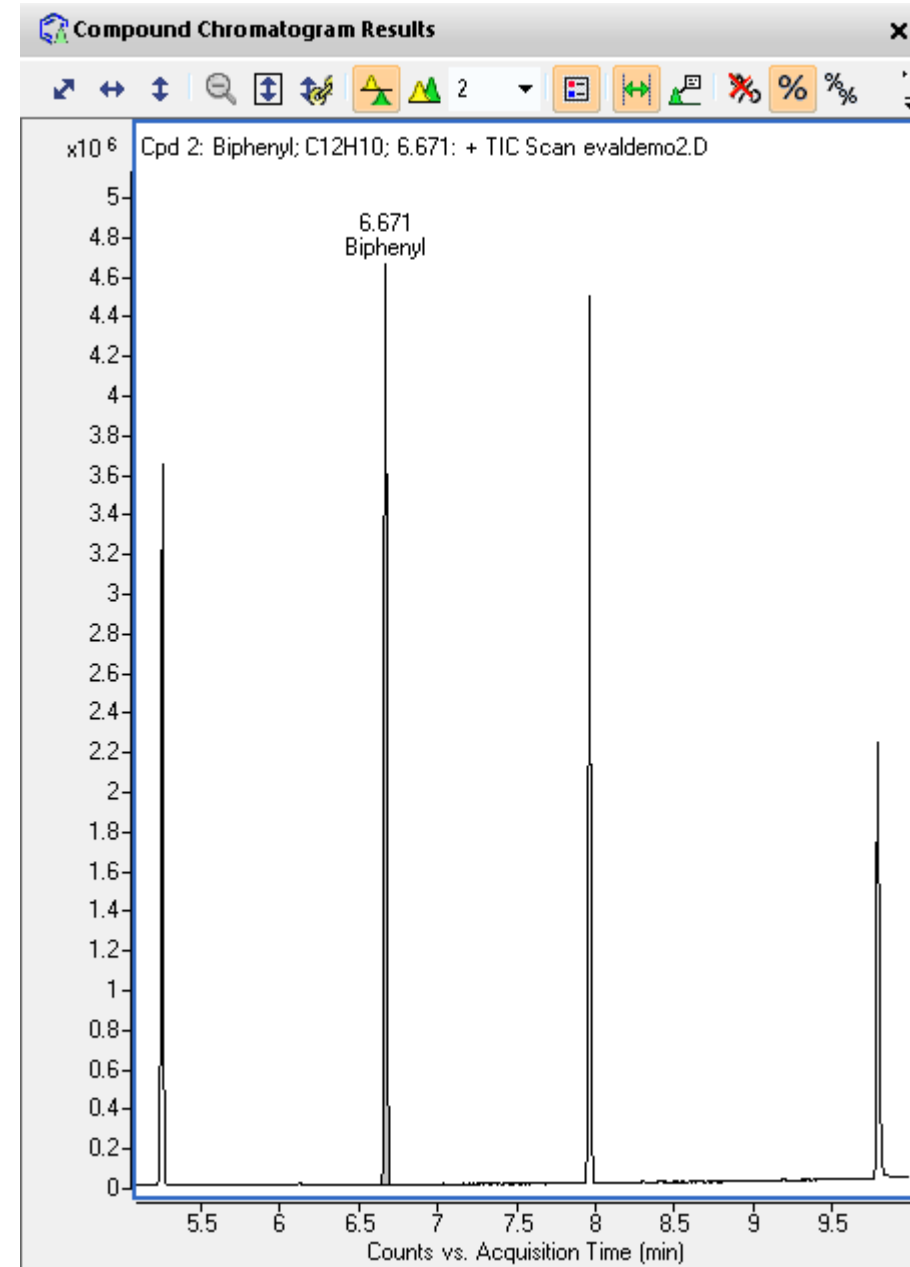
- Sample Chromatogram Results.
 - Can be overlaid or list modes
- Toolbar
 - Autoscale X-axis and Y-axis
 - Autoscale X-axis
 - Autoscale Y-axis
 - Unzoom (multiple levels)
 - Autoscale Y-axis during zoom
 - List mode or overlaid mode
 - Maximum number of panes
 - Compound overlay mode
 - Extract Chromatograms (allows TIC, BPC, EIC, etc. to be extracted).
 - Scale Chromatogram Off
 - Scale to Largest in Each Chromatogram
 - Integration Peak List
 - Chromatogram Display Options
 - Print



Data Review

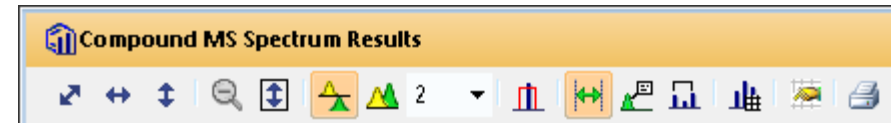
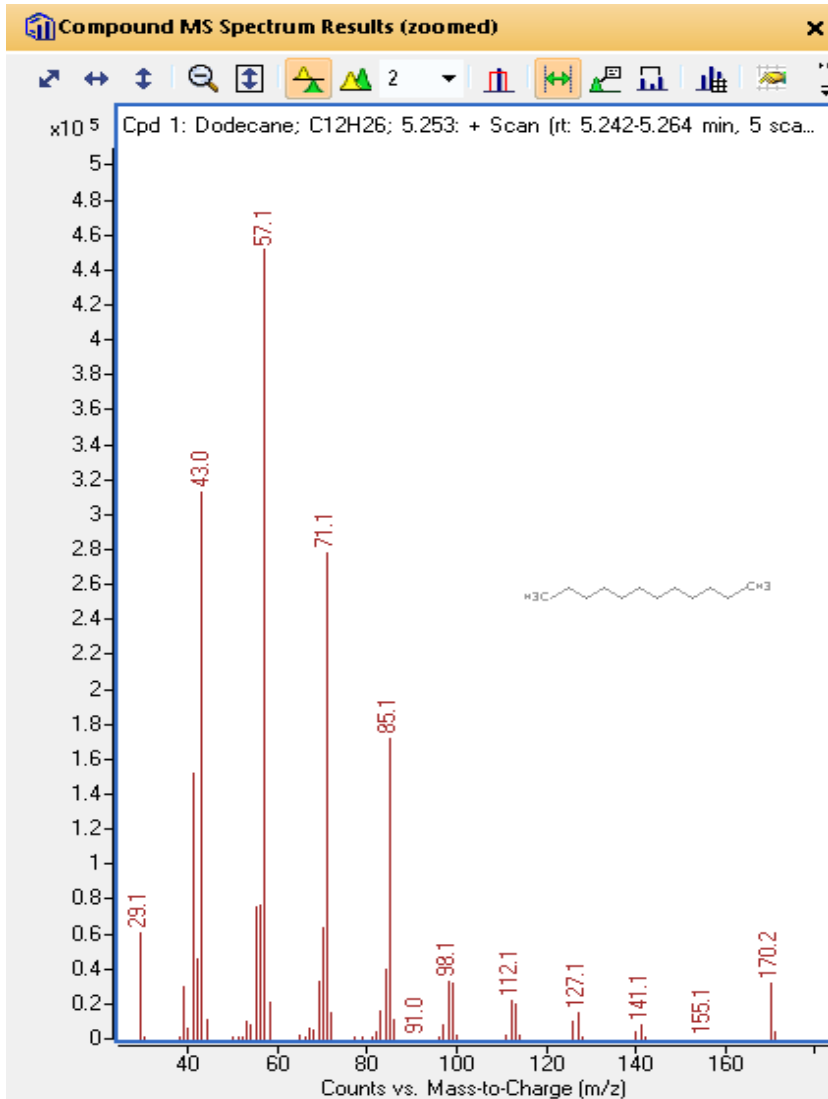
Compound Chromatogram Results

- Compound Chromatogram Results
 - Displays the TCC
 - Can be overlaid or list modes
- Toolbar
 - Autoscale X-axis and Y-axis
 - Autoscale X-axis
 - Autoscale Y-axis
 - Unzoom (multiple times)
 - Autoscale Y-axis during zoom
 - List mode or overlaid mode
 - Maximum number of panes
 - Show Legend in Overlaid Mode
 - Range Select tool
 - Annotation Mouse Tool
 - Scale to Largest in Each Chromatogram
 - Integration Peak List
 - Coelution Plot
 - Chromatogram Display Options
 - Print



Data Review

Compound MS Spectrum Results

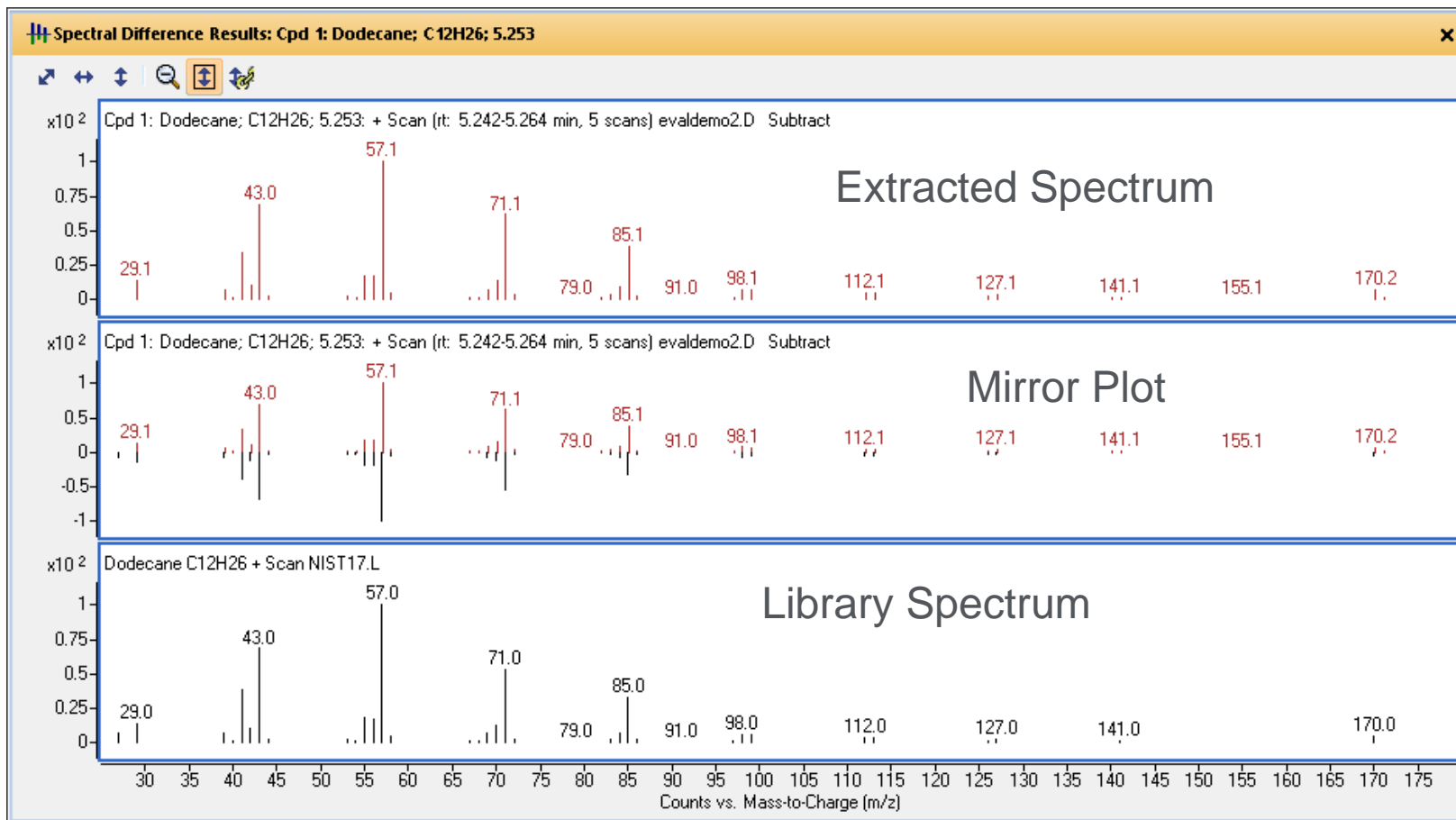


- Compound MS Spectrum Results.
 - Can be overlaid or list modes
- Toolbar
 - Autoscale X-axis and Y-axis
 - Autoscale X-axis
 - Autoscale Y-axis
 - Unzoom (multiple times)
 - Autoscale Y-axis during zoom
 - List mode or overlaid mode
 - Maximum number of panes
 - Show Predicted Isotope Distribution
 - Range Select tool
 - Annotation Mouse Tool
 - Delta Mass Caliper tool
 - Spectrum Peak List
 - Spectrum Display Options
 - Print

Data Review

Spectral Difference Results

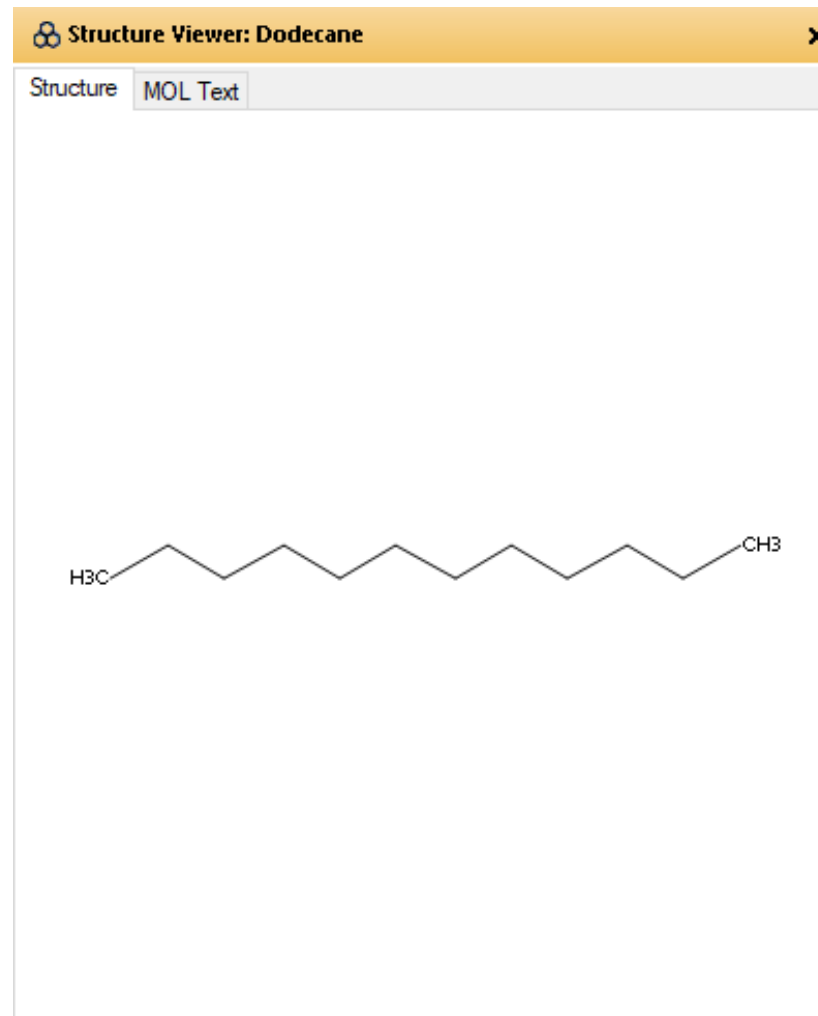
- Spectral Difference Results
- **View > Difference Results** activates this window
- Toolbar
 - Autoscale X-axis and Y-axis
 - Autoscale X-axis
 - Linked Y-axis mode



Data Review

Structure Viewer

- Composed of two tabs
- Structure tab
- MOL Text
 - Must be available in library
 - Easily copied to clipboard



Structure Viewer: Dodecane

Structure MOL Text

ID: C112403
Agilent 18022408542D 1 1.00000 0.00000
MassHunter Data Analysis software
12 11 0 0 0 1 V2000
0.4500 -1.0000 0.0000 C 0 0 0 0 0 0 0 0 0 0
-0.4500 -0.5000 0.0000 C 0 0 0 0 0 0 0 0 0 0
1.3000 -0.5000 0.0000 C 0 0 0 0 0 0 0 0 0 0
-1.3000 -1.0000 0.0000 C 0 0 0 0 0 0 0 0 0 0
-2.1500 -0.5000 0.0000 C 0 0 0 0 0 0 0 0 0 0
2.1500 -1.0000 0.0000 C 0 0 0 0 0 0 0 0 0 0
-3.0500 -1.0000 0.0000 C 0 0 0 0 0 0 0 0 0 0
3.0500 -0.5000 0.0000 C 0 0 0 0 0 0 0 0 0 0
-3.9000 -0.5000 0.0000 C 0 0 0 0 0 0 0 0 0 0
3.9000 -1.0000 0.0000 C 0 0 0 0 0 0 0 0 0 0
-4.7500 -1.0000 0.0000 C 0 0 0 0 0 0 0 0 0 0
4.7500 -0.5000 0.0000 C 0 0 0 0 0 0 0 0 0 0
1 2 1 0 0 0
3 1 1 0 0 0
4 2 1 0 0 0
5 4 1 0 0 0
6 3 1 0 0 0
7 5 1 0 0 0
8 6 1 0 0 0
9 7 1 0 0 0
10 8 1 0 0 0
11 9 1 0 0 0
12 10 1 0 0 0
M END

Copy to Clipboard Ctrl+C

Let's take a moment for questions on Data Review

Up Next:

Find by Chromatogram Deconvolution

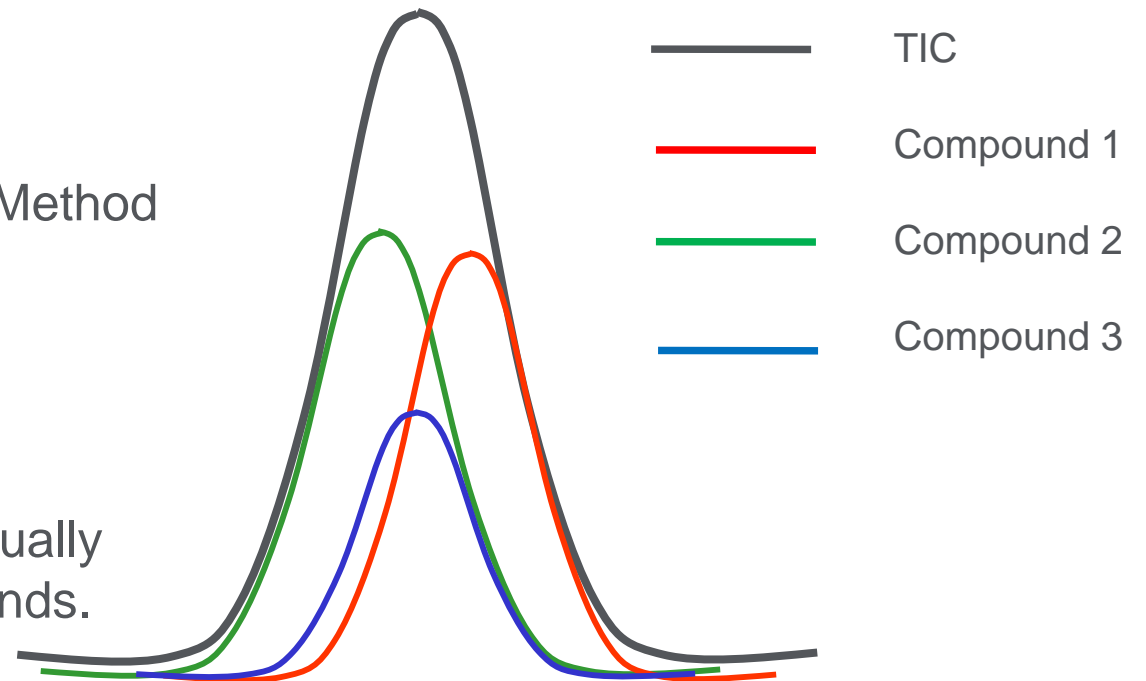


Find by Chromatogram Deconvolution

Case Study

- One of the 'Find by.....' algorithms.
- Useful to determine what is in a sample.
- Optional identification through the **Compound Identification > Identification Workflow** in the Method Editor.

One gaussian peak but actually composed of three compounds.



Find by Chromatogram Deconvolution

Case Study

An average spectrum is created over the entire or selected RT range.

An EIC is extracted for each mass in the average spectrum.

Each EIC is integrated using the Agile integrator and “EIC peaks” are created.

The “EIC peaks” are grouped together based on common RT and each set of “EIC peaks” become “Compounds”.

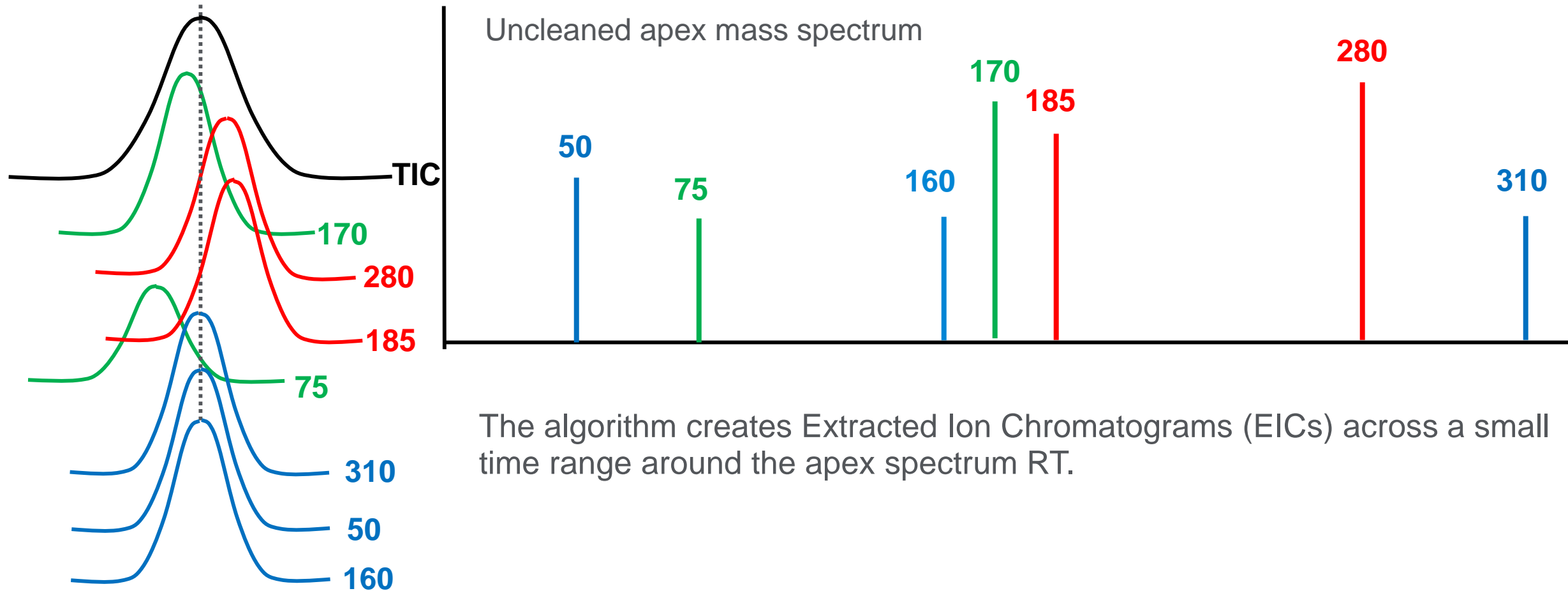
The “EIC peak” shape is used to create a Compound peak shape.

Filters can remove small ions and “compounds” with only a few small ions.

The relative intensity (area) of the “EIC peaks” and the mass of the EIC are used to create a clean spectrum of the Compound.

Find by Chromatogram Deconvolution

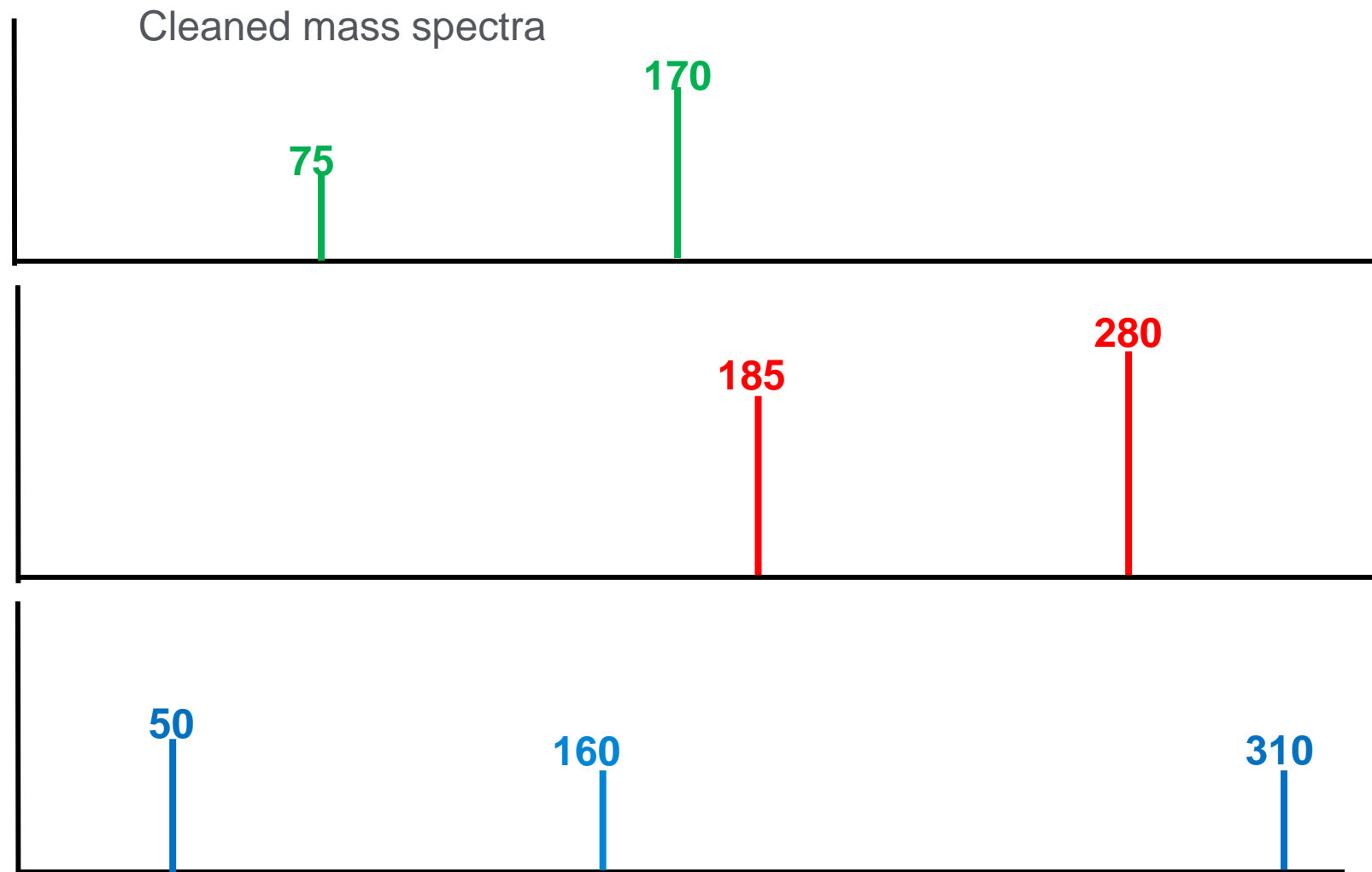
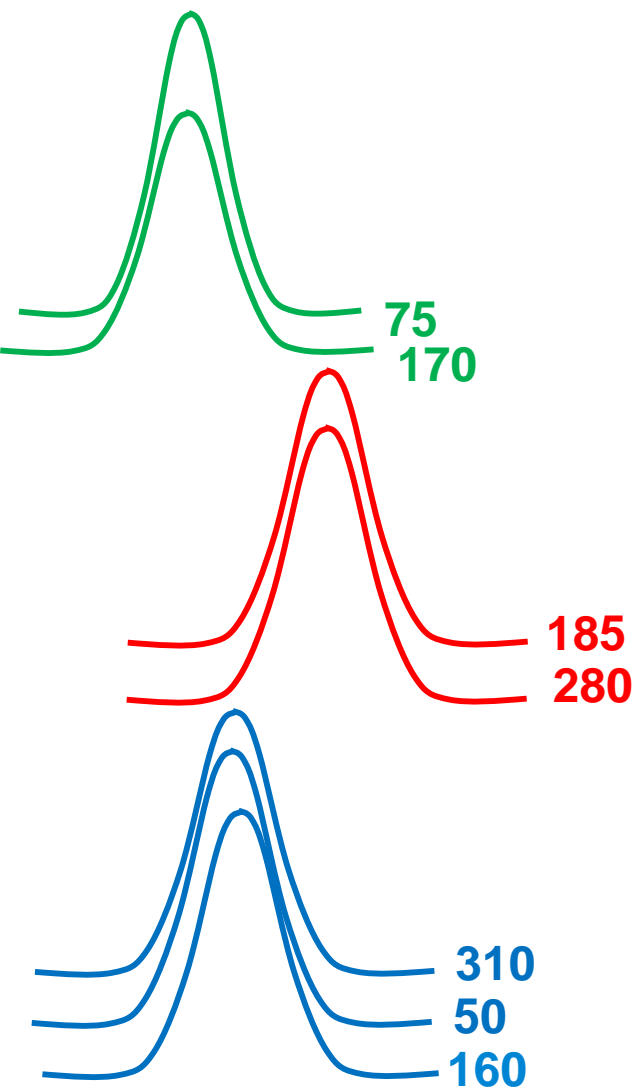
Case Study



The algorithm creates Extracted Ion Chromatograms (EICs) across a small time range around the apex spectrum RT.

Find by Chromatogram Deconvolution

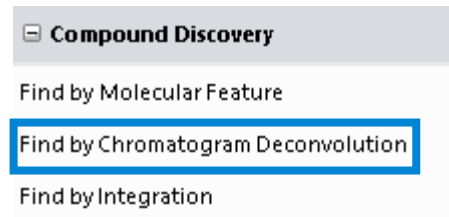
Case Study



Find by Chromatogram Deconvolution

Deconvolution Parameters

- RT window size factor
 - Key parameter that controls grouping
 - Smaller number → more compounds
 - Possible 'fake' compounds
- Excluded m/z
- Spectrum peak threshold
- SNR threshold
- Extraction window
 - Mass defect → -0.3 to +0.7 Low resolution
 - High resolution in ppm.
- Component shape
 - Upper 75% of peak is considered.



Settings | Mass Filters | Compound Filters | Results

Resolution:

RT window size factor:

Peak filter:

Excluded m/z:
example: 46,48

Spectrum peak threshold: %

SNR threshold:

Extraction window:

Left m/z delta:

Right m/z delta:

m/z delta units:

Component shape:

Use base peak shape

Sharpness threshold: %

Find by Chromatogram Deconvolution

Deconvolution Parameters

Settings | **Mass Filters** | Compound Filters | Results

Height filters

Absolute height >= 300 counts

Relative height >= 1.000 % of largest peak

- Mass filters are applied after the EICs are organized into compounds.
 - Remove small ions from the compounds based on absolute height or relative height of base peak or both.
- Compounds Filters are applied to the compounds based on absolute area or relative area of the largest compound or both.
- Results
 - Clear previous results is unchecked allows multiple algorithms to be applied.

Settings | Mass Filters | **Compound Filters** | Results

Area filters

Absolute area >= 500 counts

Relative area >= 1.000 % of largest peak

Settings | Mass Filters | Compound Filters | **Results**

Previous results

Clear previous results

New results

Highlight first compound

Highlight all compounds

Let's take a moment for questions on Find by Chromatogram Deconvolution

Up Next:
Molecular Feature Extraction



Find by Molecular Feature

Case Study

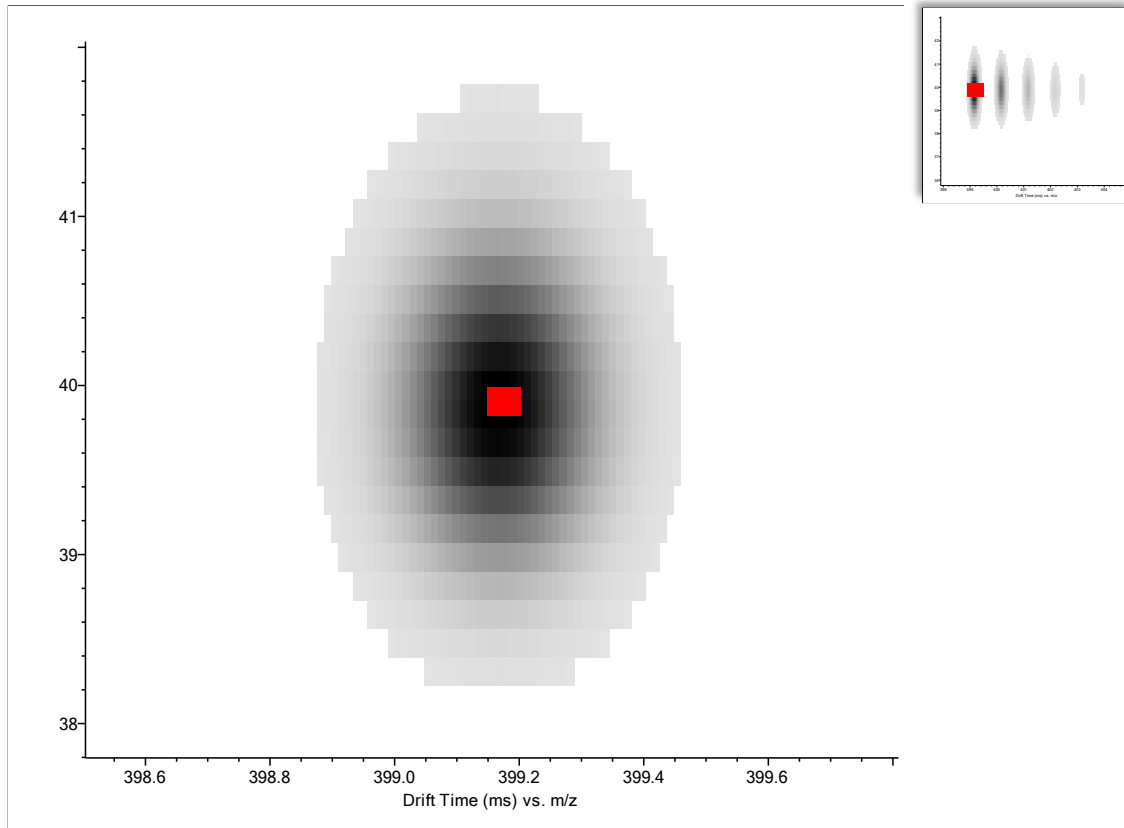
MFE (Molecular Feature Extraction)

- Untargeted analysis for a unique molecular entity based on three dimensional array of retention time, mass and abundance in GC or LC MS data. Operates on raw scan data.
 - Evaluates the data as a three dimensional array based on retention time, mass and abundance.
 - Removes persistent or slowly changing background.
 - Looks for masses with a common elution profile at approximately the same time.
 - Masses are grouped into compounds.
 - Co-eluting interferences are resolved.
 - Isotopic cluster are determined and grouped (accurate mass).
 - Charge state assignments and molecular adducts are recognized (multi-charged species and ESI).
 - 2D/3D visualization of data.
 - Chemical characterization and identification.
- **NEW FEATURE SUPPORTS UNIT MASS GC/MS CENTROID DATA.**

Find by Molecular Feature

Case Study

MFE (Molecular Feature Extraction)

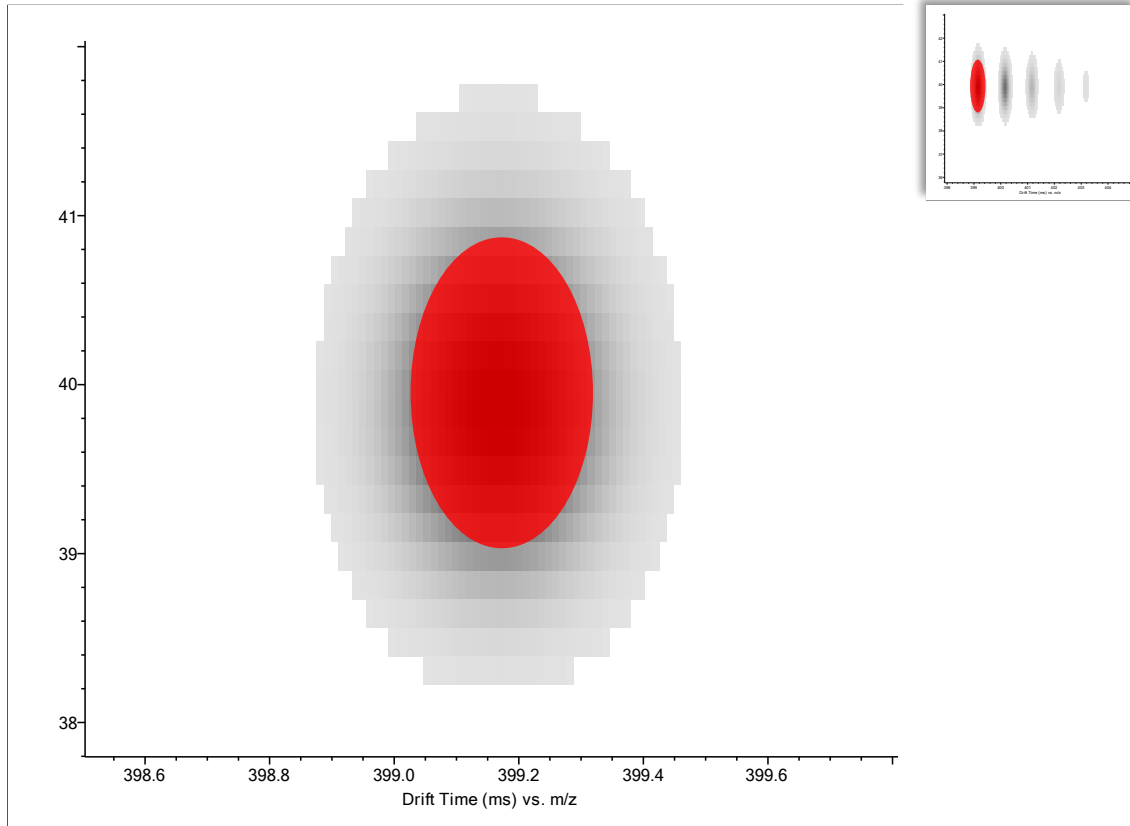


Identify the highest point in the three dimensional array.

Find by Molecular Feature

Case Study

MFE (Molecular Feature Extraction)



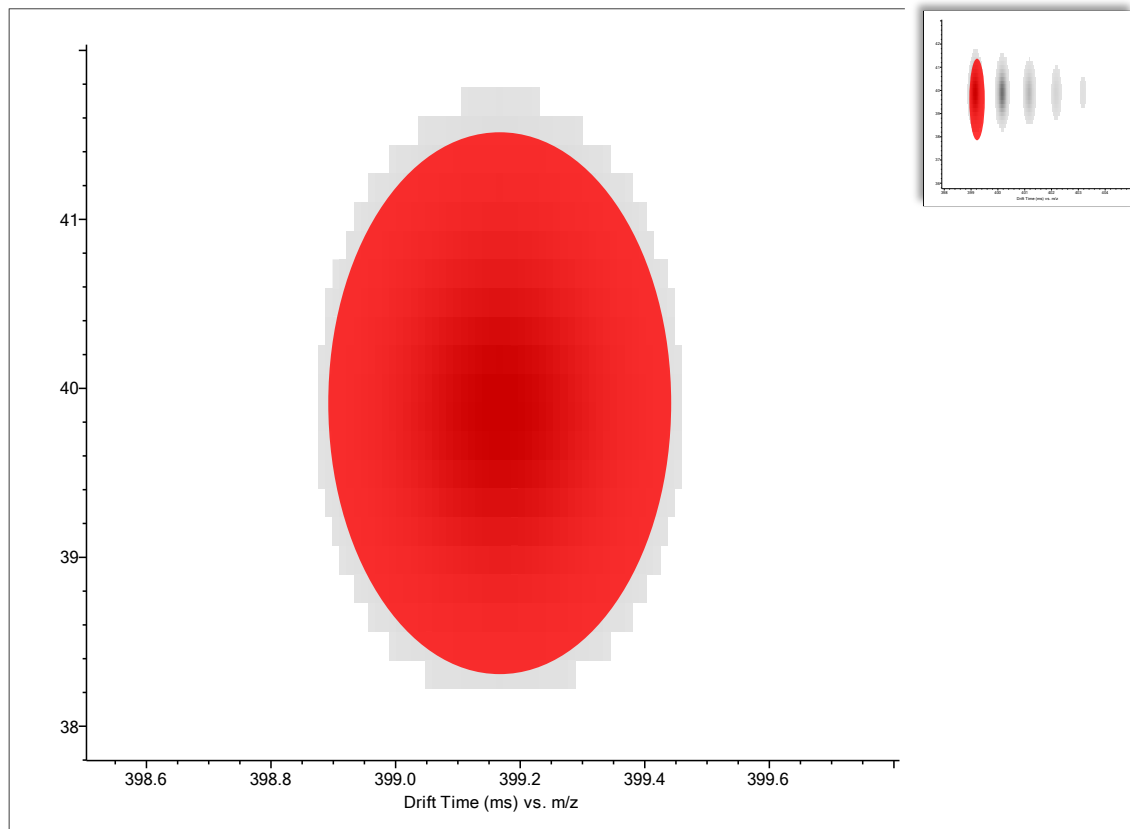
Identify the highest point in the three dimensional array.

Expand the array to include neighboring data points.

Find by Molecular Feature

Case Study

MFE (Molecular Feature Extraction)



Identify the highest point in the three dimensional array.

Expand the array to include neighboring data points.

Once the bounds of the array have been established, save the 3D peak array.

Resolve co-eluting interferences.

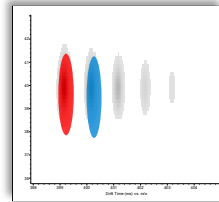
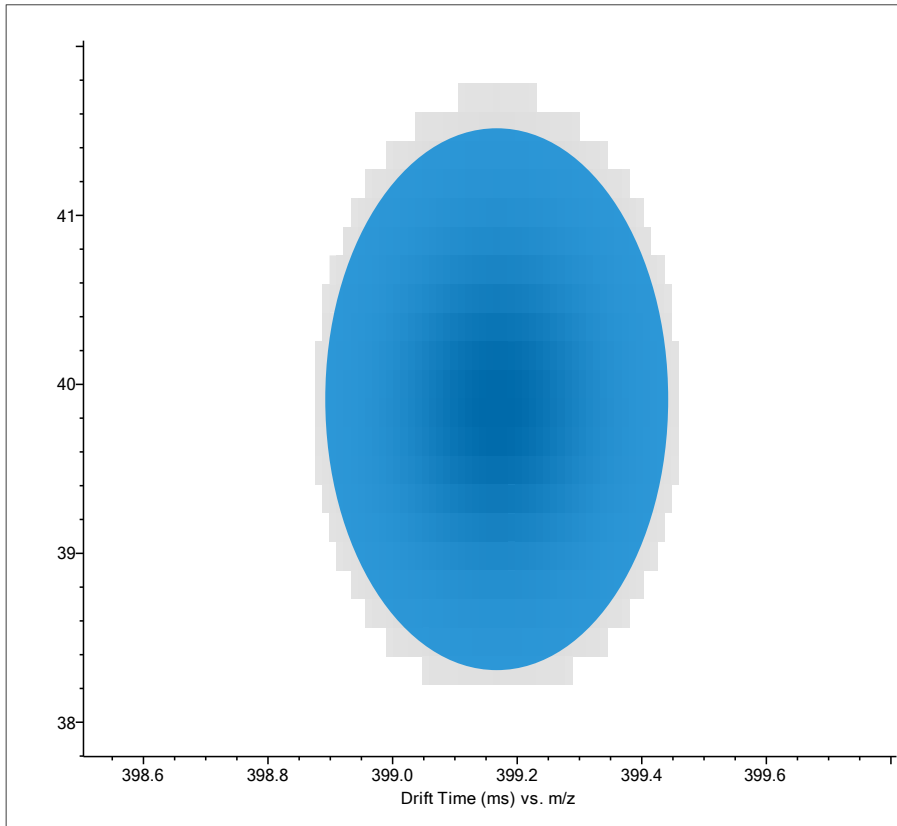
Chemical characterization and subsequent identification.

Reiteratively repeat the process.

Find by Molecular Feature

Case Study

MFE (Molecular Feature Extraction)



Identify the next highest point in the three dimensional array.
After completed, reiteratively process the next highest point in the array.

Find by Molecular Feature

Case Study

MFE (Molecular Feature Extraction)

- The algorithm effectively processes the three dimensional array based on retention time, mass and abundance.
- The algorithm does not evaluate the information based on spectra or chromatograms.
- The process is reiteratively applied to the entire data array until no peak remains above background.
- Noise is effectively removed.
- Highly computationally intensive but very effective in identifying compounds.



Find by Molecular Feature

Parameters (GC/MS)

- Method Editor > Compound Discovery > Find by Molecular Feature.
- Composed of 3 tabs.
- Limited to small molecules (chromatographic) for EI GC/MS.
- Quality score is the algorithm's estimation of quality of the feature.
- MS/MS Spectrum for GC QTOF data.

Extraction Compound Filters Results

Extraction algorithm

Target data type Small molecules (chromatographic)

Input data range

Restrict retention time to minutes

Restrict m/z to m/z

Peak filters

Use peaks with height \geq counts
(Profile and centroid spectra)

Extraction Compound Filters Results

Height

Relative height \geq %

Absolute height \geq counts

Limit to the largest compounds

Compound quality

Quality score \geq

Compound location

Restrict retention times to minutes

Charge states

Restrict charge states to Z

Extraction Compound Filters Results

Previous results

Clear previous results

New results

Highlight first compound

Highlight all compounds

Chromatograms and spectra

Raw spectrum

Prefer profile for raw spectrum, if available

Clip extracted raw spectrum

Asymmetric (m/z) - +

MS/MS spectrum

Extract separate MS/MS spectrum per collision energy

Extract average MS/MS spectrum for all collision energies

Precursor tolerance: +/- ppm

Deisotope MS/MS spectrum

Display limits

Display only the largest compounds

Find by Molecular Feature

Parameters (LC/MS) (1)

- Different parameters to accommodate the different data set.
- Composed of multiple tabs.
- Various target data types.
 - Choose applicable to data.
- Ion species
 - Choose expected adducts based on conditions.
 - Can add and delete additional adducts.

The screenshot shows the 'Extraction' tab of a software interface. It features several sections: 'Extraction algorithm' with a dropdown menu for 'Target data type' (currently set to 'Small molecules (chromatographic)'); 'Input data range' with checkboxes for 'Restrict retention time to' and 'Restrict m/z to'; and 'Peak filters' with a field for 'Use peaks with height' set to '>= 600 counts'. The 'Extraction' tab is highlighted with a blue border.

The screenshot shows the 'Ion Species' tab of the software interface. It is divided into three columns: 'Positive ions', 'Negative ions', and 'Neutral losses'. Each column contains a list of ion species with checkboxes. Under 'Positive ions', '+H', '+Na', and '+NH4' are checked. Under 'Negative ions', '-H', '+Cl', '+HCOO', and '+CH3COO' are checked. Under 'Neutral losses', 'H2O' and 'H3PO4' are listed but not checked. Below the lists are input fields and '+' signs for adding new species, and 'X' icons for deleting them. A checkbox at the bottom is labeled 'Salt dominated positive ions (M+H may be weak or missing)'. The 'Ion Species' tab is highlighted with a blue border.

Find by Molecular Feature Parameters (LC/MS) (2)

- Charge State

- Choice of 5 Isotope models.
- Choose model appropriate to dataset.
- Can set a range of charge states.

Extraction Ion Species **Charge State** Compound Filters Mass Filters Mass Defect Results

Isotope grouping
Isotope model: Common organic molecules

Charge state
 Limit assigned charge states to a range of: 1-2
 Report single ions or single-ion features with charge state z=1

Common organic molecules
Common organic molecules
Common organic (no halogens)
Peptides
Unbiased
Glycans

**Tip: If uncertain
select unbiased.**

- Compound Filters same.

- Mass Filters

- Targeted list.
- When checked, other items available to refine results.
- Can also create an exclusion list.

Extraction Ion Species Charge State Compound Filters **Mass Filters** Mass Defect Results

Mass filters
 Filter mass list 5.000 ppm
Include only these mass(es)

Source of masses
 These masses:
(type a comma-separated list of masses like "142.1012, 253.4003")
 Database
D:\MassHunter\PCDL\default.csv

Include only these mass(es)
Include only these mass(es)
Exclude these mass(es)

Find by Molecular Feature

Parameters (LC/MS) (3)

- Mass Defect tab
 - Not applicable to large molecules (proteins, oligos).
 - Useful for halogen containing and hetero atoms.
 - Compounds outside the mass defect tolerance are excluded from the results.

The screenshot shows the 'Mass Defect' tab in the software interface. It is divided into three sections: 'Mass defect filtering', 'Expected mass defect', and 'Mass defect tolerance'. In the 'Mass defect filtering' section, the checkbox 'Filter results on mass defects' is checked. In the 'Expected mass defect' section, a dropdown menu is set to 'Constant', and the input fields show '0.0000 Da + (0.0000 per 100.00 Da)'. A 'Calculate from formula' button is present. In the 'Mass defect tolerance' section, a dropdown menu is set to 'Constant (symmetric)' and the input field shows '+/- 0.0100 Da'. Two blue arrows point from the dropdown menus in the 'Expected mass defect' and 'Mass defect tolerance' sections to the corresponding dropdown menus in the adjacent panels.

Constant
Constant
Variable

Constant (symmetric)
Constant (symmetric)
Constant (asymmetric)
Variable

- Results tab is the same as before.

Find by Molecular Feature Results

Agilent MassHunter Qualitative Analysis Workflows B.08.00 - Default-LCMS.m

File Launch Edit View Find Identify Method Configuration Tools Help

Sample Table: sulfas_PosMS.d

Results Workflow Acquisition

Result Summary	Saved Results	Method	Last Run Method	Workflow	Target Source	FileName	Sample Position	Acquisition Method	Acquisition Time
0 Identified	4			Default-LCMS	Compound Discovery	D:\MassHunt\ sulfas_PosMS.d	P1-F1	sulfas_PosMSonly_demo.m	8/16/2008 11:29:01 PM (UTC-05:00)

Compound List: 4 found

General				Compound Identification		Molecular Feature Extraction								
m/z	Mass	RT	Width	Height	Base Peak	Ions	Mining Algorithm	File	Cpd	Hits	Vol	Vol %	Std Dev	Score (MFE)
271.0321	270.0248	0.333	0.052	761377	271.0321	4	Find by Molecular Feature	sulfas_PosMS.d	1	0	3772784	12.35	0	80
285.021	284.0136	0.526	0.054	384928	285.021	9	Find by Molecular Feature	sulfas_PosMS.d	2	0	1988835	6.51	0.0004	100
279.0907	278.0835	0.797	0.065	2767040	279.0907	7	Find by Molecular Feature	sulfas_PosMS.d	3	0	12131201	39.7	0.0006	100
311.0806	310.0735	1.231	0.062	2717021	311.0806	7	Find by Molecular Feature	sulfas_PosMS.d	4	0	12664198	41.44	0.0006	100

Sample Chromatogram Results

+ESI TIC Scan Frag=125.0V sulfas_PosMS.d

+ESI TCC Scan Frag=125.0V sulfas_PosMS.d

Compound Chromatogram Results

Cpd 1: 0.333: +ESI EIC[271.0323, 272.0346, 293.0140, 294.0153] Scan Fr...

Compound MS Spectrum Results

Cpd 1: 0.333: +ESI MFE Spectrum (rt: 0.309-0.582 min) Frag=125.0V...

Cpd 1: 0.333: +ESI Scan (rt: 0.325-0.566 min, 16 scans) Frag=125.0V...

Method Editor: Workflow

Run Method Workflow

Method Automation

Workflow: Compound Discovery

Compound mining: Auto-Select Compound Mining

Identify by: Library / Database search

Library / Database	Score (fwd)	Score (rev)
D:\MassHunter\PCDL\Sulfas_AM_PC...	25.00	80.00

Structure Viewer

No data to display.

Let's take a moment for questions on Molecular Feature Extraction (MFE)

Up Next:

Find by Formula and Compound Identification



Find by Formula

Parameters (Accurate Mass)

- Find by Formula
 - Targeted analysis.
 - Numerous tabs are available to refine parameters.
 - Requires a target source and optional RT matches.
 - Can confirm fragment ions -> Fragment Confirmation tab.
 - Uses MS scan data but can be used on MS/MS data but ignores MS/MS spectra.

EIC Peak Filters	Charge State	Fragment Annotation	Fragment Peak Filter
Target Source	Ion Species	Fragment Confirmation	EIC Integration
Target source (*.cdb, *.csv, *.cef, formulas, neutral masses)			
<input type="text" value="D:\MassHunter\PCDL\Sulfas_AM_PCDL.cdb"/>			<input type="button" value="..."/>
Values to match			
<input type="checkbox"/> Require RT match if database contains a RT for the target compound.			

Find by Formula Results

Agilent MassHunter Qualitative Analysis Workflows B.08.00 - junk.m

File Launch Edit View Find Identify Method Configuration Tools Help

Sample Table: sulfas_PosMS.d

Results Workflow Acquisition

Result Summary	Saved Results Method	Last Run Method	Workflow	Target Source	FileName	Sample Position	Acquisition Method	Acquisition Time
4 qualified (4 t			Default-LCMS	Target/Suspect Screening	D:\MassHunt\ sulfas_PosMS.d	P1-F1	sulfas_PosMSonly_demo.m	8/16/2008 11:29:01 PM (UTC-05:00)

Compound List: 4found

Formula	m/z	Mass	RT	Width	Height	Area	Score	Base Peak	Ions	Saturated	Mining Algorithm	ID Source	FV	CE	MS/MS Count
C12 H14 N4 O4 S	311.0808	310.0737	1.225	0.039	368125	10369	99.47	311.0808	6		Find by Formula	FBF	sul		
C10 H9 Cl N4 O2 S	285.0209	284.0135	0.518	0.04	627291	17560	99.73	121.0509	11		Find by Formula	FBF	sul		
C12 H14 N4 O2 S	301.0732	278.0836	0.791	0.044	318306	99973	99.6	279.0908	6		Find by Formula	FBF	sul		
C9 H10 N4 O2 S2	271.0321	270.0248	0.325	0.038	127375	33613	99.1	271.0321	6		Find by Formula	FBF	sul		

Sample Chromatogram Results

Compound Chromatogram Results

Compound MS Spectrum Results

Method Editor: Workflow

Method Automation

Workflow: Target/Suspect Screening

Compound mining: Find by Formula

Target source (*.cdb, *.csv, *.cef, *.formulas): D:\MassHunter\PCDL\Sulfas_AM_PCDL.cdb

Options

Require RT match if database contains a RT for the target compound:

Only report qualified compounds:

Structure Viewer

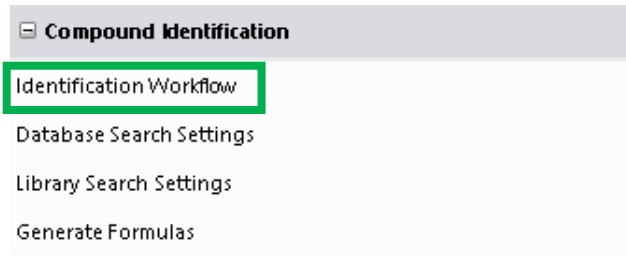
No data to display.

Compound Identification Results: Cpd 4: Sulfamethizole; C9 H10 N4 O2 S2; 0.325

Adduct confirmation

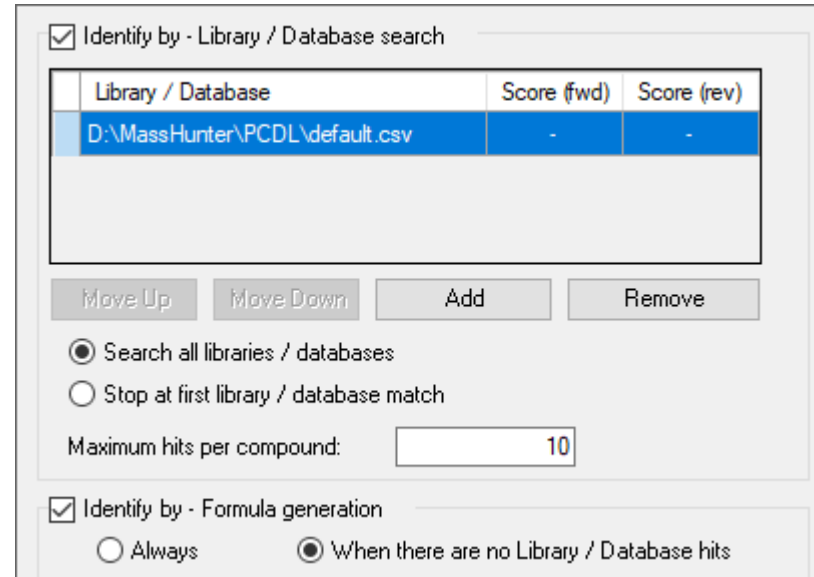
Compound Identification

Identification Workflow



Identification Workflow

- Identify by Library/Database search
 - Optional search
 - Select numerous libraries.
- Search all libraries / databases
 - Search all libraries or stop when hit is found.
- Identify by Formula Generation
 - Optional generation of formulas



Compound Identification

Database Search Settings

Compound Identification

Identification Workflow

Database Search Settings

Library Search Settings

Generate Formulas

- Numerous tabs
- Define database settings.

Search Criteria Peak Limits Positive Ions Negative Ions Search Results

Values to match

Molecular formula

Mass

Mass and retention time (retention time optional)

Mass and retention time (retention time required)

Match tolerance

Mass ppm

Retention time minutes

Search Criteria Peak Limits Positive Ions Negative Ions Search Results

Spectrum peak searches

Maximum number of peaks to search when peaks are not specified graphically:

Search Criteria Peak Limits Positive Ions Negative Ions Search Results

Search Results

Limit to the best

hits

Search Criteria Peak Limits Positive Ions Negative Ions Search Results

Charge carriers

-electron

+H

+Na

+K

+NH4

Neutral losses

H2O

Charge states, if not known

Charge state range

Aggregates

Dimers e.g., [2M+H]⁺

Trimers e.g., [3M+H]⁺

Search Criteria Peak Limits Positive Ions Negative Ions Search Results

Charge carriers

+electron

-H

+Cl

+Br

+HCOO

+CH3COO

+CF3COO

Neutral losses

H2O

Charge states, if not known

Charge state range

Aggregates

Dimers e.g., [2M-H]⁻

Trimers e.g., [3M-H]⁻

Compound Identification

Library Search Settings

Compound Identification

Identification Workflow

Database Search Settings

Library Search Settings

Generate Formulas

Library Search Settings

- Search Criteria
 - Adjust Score
- RT Scoring
 - Retention time or retention index.
- Peak Filters
- Tolerances
 - Accurate mass values

Search Criteria RT Scoring Peak Filters Tolerances

Search criteria (.cdb)

Restrict spectral comparison based on

Ionization mode

Instrument type

Collision energy +/- eV

Search criteria (.L, .xml)

Enable screening Adjust score

Search Criteria RT Scoring Peak Filters Tolerances

Match factor

Use RT match (.L, .xml)

RT penalty function

Trapezoidal

RT range sec

Penalty-free RT Range sec

Gaussian

Standard deviation sec

RT mismatch penalty type

Multiplicative

Additive

Max RT penalty:

RT Calibration file:

...

Search Criteria RT Scoring Peak Filters Tolerances

Height filters

Absolute height >= counts

Relative height >= % of largest peak

Maximum number of peaks

Limit (by height) to the largest

PCDL Manager

Installed from a stand-alone application

- Useful for high resolution MS/MS data
- Handles both CDB and CDBL
- Can have multiple spectra with various collision energies (CE).
- Works with ion mobility data.

The image displays two software windows from Agilent's MassHunter suite. The left window is the PCDL Manager, showing search criteria for 'Atrazine' and a list of compounds with their properties. The right window is the Spectra Viewer, showing three mass spectra for Atrazine at different collision energies (10, 20, and 40 eV).

MassHunter PCDL Manager - Pesticides

Spectra search criteria: Enter one standard name or ion species per line. Examples: Standard Name (M+Mg2)+2

Additional search parameters: Ion polarity: (Any), Ionization mode: (Any), Precursor ion species: (Any), Instrument type: (Any)

Spectra for compound: Atrazine

Compound Name	Ion Species	Precursor Ion	CE	Polarity	Ionization
Atrazine	(M+H)+	216.10105	10	Positive	ESI
Atrazine	(M+H)+	216.10105	20	Positive	ESI
Atrazine	(M+H)+	216.10105	40	Positive	ESI

Spectra for compound: Atrazine

Name	Formula	Mass	Retention Time	Cation	Anion	Link
Fenuron (N,N-Dimethyl-N-phenylurea)	C9H12N2O	164.09496	4.076	<input type="checkbox"/>	<input type="checkbox"/>	101-
Crimidine	C7H10CIN3	171.05633	4.917	<input type="checkbox"/>	<input type="checkbox"/>	535-
IPC / Propham	C10H13NO2	179.09463	5.564	<input type="checkbox"/>	<input type="checkbox"/>	122-
Chlorpropham (Chlorpropham)	C10H12ClNO2	213.05566	8.282	<input type="checkbox"/>	<input type="checkbox"/>	101-
Atrazine-desethyl (Desethylatrazine)	C6H10CIN5	187.06247	4.27	<input type="checkbox"/>	<input type="checkbox"/>	6190
Simazine	C7H12CIN5	201.07812	5.564	<input type="checkbox"/>	<input type="checkbox"/>	122-
Metamitron	C10H10N4O	202.08546	3.973	<input type="checkbox"/>	<input type="checkbox"/>	4139
Isoproturon	C12H18N2O	206.14191	6.69	<input type="checkbox"/>	<input type="checkbox"/>	3412
Chlorotoluron (Chlorotoluron)	C10H13ClN2O	212.07164	6.328	<input type="checkbox"/>	<input type="checkbox"/>	1554
Monolinuron (phenylurea)	C9H11ClN2O2	214.05091	6.574	<input type="checkbox"/>	<input type="checkbox"/>	1746
Metribuzin	C8H14N4OS	214.08883	5.719	<input type="checkbox"/>	<input type="checkbox"/>	2108
Atrazine	C8H14CIN5	215.09377	6.548	<input type="checkbox"/>	<input type="checkbox"/>	1912
Methabenzthiazuron	C10H11N3OS	221.06228	6.237	<input type="checkbox"/>	<input type="checkbox"/>	1869
Metoxuron	C10H13ClN2O2	228.06656	5.228	<input type="checkbox"/>	<input type="checkbox"/>	1993
Sebutylazine	C9H16CIN5	229.10942	7.686	<input type="checkbox"/>	<input type="checkbox"/>	7286
Terbuthylazine (TERBA)	C9H16CIN5	229.10942	7.466	<input type="checkbox"/>	<input type="checkbox"/>	5915

Spectra Viewer

Acquired spectra table:

Compound Name	Ion Species	Precursor Ion	CE	Polarity
Atrazine	(M+H)+	216.10105	10	Positive
Atrazine	(M+H)+	216.10105	20	Positive
Atrazine	(M+H)+	216.10105	40	Positive

Library spectra table:

CompoundName	Precursor Ion	CollisionEnergy	IonPolarity
Atrazine	216.10105	10	Positive
Atrazine	216.10105	20	Positive
Atrazine	216.10105	40	Positive

Graphics / Mass Lists

Acquired spectrum: Abundance vs m/z (0-95)

+ESI MS2 QTOF FV=120 CE=10 (M+H)+: Abundance vs m/z (40-240). Peaks at 96.05563 (1.56), 174.05409 (20.04), 216.10104 (100.00).

+ESI MS2 QTOF FV=120 CE=20 (M+H)+: Abundance vs m/z (40-240). Peaks at 96.05563 (26.76), 132.03230 (19.07), 174.05409 (100.00), 216.10104 (41.13).

+ESI MS2 QTOF FV=120 CE=40 (M+H)+: Abundance vs m/z (40-240). Peaks at 68.02432 (100.00), 104.00100 (62.37), 132.03230 (7.76), 174.05409 (2.55).

Compound Identification

Generate Formulas

Compound Identification

Identification Workflow

Database Search Settings

Library Search Settings

Generate Formulas

Generate Formulas

- Allowed Species
 - Elements and limits.
 - Use for narrowing possible hits.
 - Can add elements.

Allowed Species Limits Charge State Fragment Formulas

Charge carrier to be assumed if not known

Positive ions:

- electron
- +H
- +Na
- +K
- +NH₄
- +C₂H₅
- +C₃H₅

Negative ions:

- +electron
- H
- +Cl
- +Br
- +HCOO
- +CH₃COO
- +CF₃COO

MS ion electron state: allow both even and odd

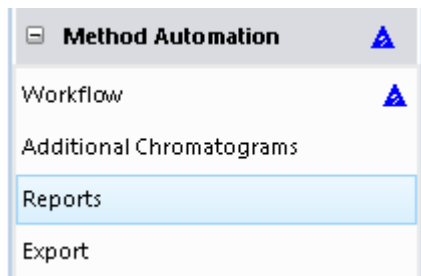
Group hits with same formula (but different charge carriers)

Elements and limits

Element	Minimum	Maximum
▶ C	3	60
H	0	120
O	0	30
N	0	30
S	0	5
Cl	0	3

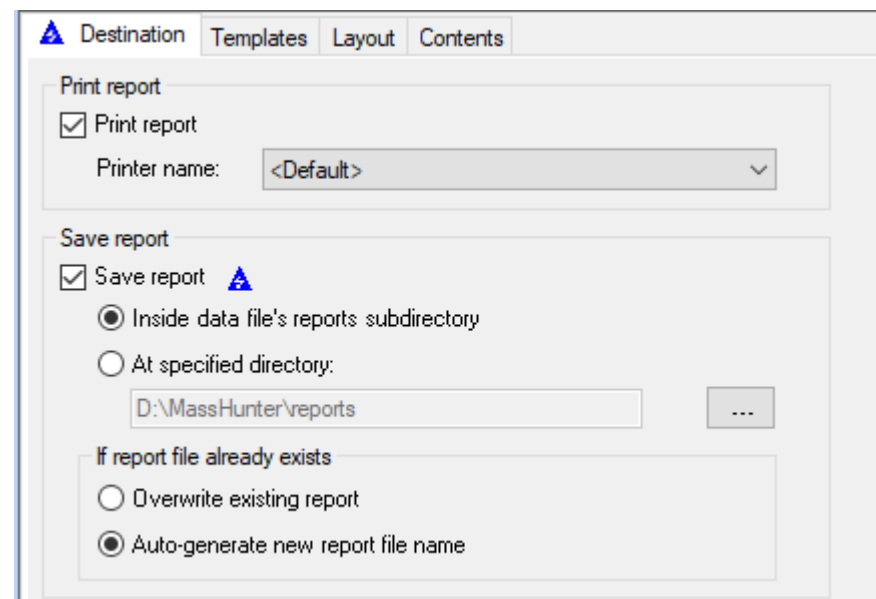
Reports

Generating Compound Reports



Method Automation > Reports

- Printed or electronic format available.
- Report can be saved to data file or directory.
- Auto generate file name.



Destination Templates Layout Contents

Use Microsoft Excel® for reporting
 Use PDF-based reporting

Report template folder
D:\MassHunter\Report Templates\Qual\B.08.00\en-U

Report templates

Target screening report template :
TargetCompoundScreeningReport.xslt

Compound Discovery report template :
CompoundReportWithIdentificationHits.xslt

Sample purity report template :
FomulaConfirmationReport.xslt

Compound report template :
CompoundReport.xslt

Qualitative method report template :
QualitativeMethodReport.xslt

Acquisition method report template :
AcqMethodReport.rdlc

TargetCompoundScreeningReport.xslt

CompoundReportWithIdentificationHits.xslt

TargetCompoundScreeningReport.xslt

CompoundReport.xslt

FomulaConfirmationReport.xslt

CompoundReportWithIdentificationHits.xslt

CompoundReportWithIdentificationHits.xslt

TargetCompoundScreeningReport.xslt

CompoundReport.xslt

FomulaConfirmationReport.xslt

FomulaConfirmationReport.xslt

CompoundReportWithIdentificationHits.xslt

TargetCompoundScreeningReport.xslt

CompoundReport.xslt

FomulaConfirmationReport.xslt

CompoundReport.xslt

CompoundReportWithIdentificationHits.xslt

TargetCompoundScreeningReport.xslt

CompoundReport.xslt

FomulaConfirmationReport.xslt

QualitativeMethodReport.xslt

QualitativeMethodReport.xslt

AcqMethodReport.rdlc

AcqMethodReport.rdlc

Templates

- Excel Reporting or PDF based reporting.
- Various templates are available.
- Organized based on workflows.

• **Tip: Use PDF-based reporting.**

Destination | **Templates** | **Layout** | **Contents**

General

Page orientation: Portrait Landscape

Page size: A4 Letter

Show sample information

Peak table limits (include all peaks unless limits are specified)

Chromatogram peaks largest peaks per table

Mass spectrum peaks largest peaks per table

Custom plot limits (autoscaled unless limits are specified)

<input type="checkbox"/> Chromatograms	<input type="text" value="2.000-10.000"/>	min
<input type="checkbox"/> Compound chromatograms	<input type="text"/>	min
<input type="checkbox"/> MS spectra	<input type="text" value="100.0000-1200.0000"/>	m/z
<input type="checkbox"/> MS/MS spectra	<input type="text"/>	m/z
<input type="checkbox"/> Deconvoluted spectra	<input type="text" value="15000.00-35000.00"/>	Da
<input type="checkbox"/> UV spectra	<input type="text" value="240-330"/>	nm

Destination | **Templates** | **Layout** | **Contents**

Compounds

Show compound table

Sort by:

Sort order:

Exclude details for unidentified compounds

Chromatograms

Show user chromatogram(s)

Show compound chromatogram(s)

Overlay compound chromatogram(s)

Compound spectrum (MS)

Show MS spectrum Show MS peak table

Show predicted isotope match table

Show MS spectrum (zoomed in on special peaks)

Zoom padding: - + m/z

Overlay predicted isotope distribution

Compound spectrum (MS/MS)

Show MS/MS spectrum Show MS/MS peak table

Library search results

Show library spectrum Show difference spectrum

Layout & Contents

- Allow further customization of the report.

Let's take a moment for questions on Compound Identification and Reporting

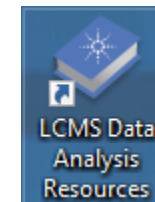
Up Next:

Training Resources



Training Resources

LCMS Data Analysis Resources DVD



LC/MS Data Analysis Resource App



B.08.00

Resources

Below is a list of resource information for LC/MS Data Analysis.

▼ Qualitative Analysis Guides

[Familiarization Guide for LC/MS](#)

[PCDL Manager Quick Start Guide](#)

▼ Quantitative Analysis Guides

[Familiarization Guide](#)

▼ BioConfirm Guides

[BioConfirm Quick Start Guide](#)

[BioConfirm Familiarization Guide](#)

▼ Training

[Qualitative Analysis Training Videos](#)

▼ Familiarization

[Qual eFamiliarization for TQ](#)

[Qual eFamiliarization for TOF/Q-TOF](#)

[Quant eFamiliarization](#)

Help

- Online Help
- Contents
- Searchable

MassHunter Qualitative Analysis Workflows Help

Agilent Technologies

MassHunter Qualitative Analysis Workflows Help

Qualitative Analysis <ul style="list-style-type: none">Developing MethodsCustomizing LayoutsFinding CompoundsIdentifying CompoundsWorking with other applicationsTools	Getting Started <ul style="list-style-type: none">NEW What's newAvailable Manuals
Workflows <ul style="list-style-type: none">Workflows OverviewRun a Target/Suspect Screening WorkflowRun a Sample Purity workflowRun a Compound Discovery workflowRun a Custom workflowReprocess Samples in Sample Table	Basic Software Tasks <ul style="list-style-type: none">Open a data fileClose one or more data filesZoom in / Zoom outCreate one or more rangesAnnotation Tasks
User Interface <ul style="list-style-type: none">Main WindowMethod Editor TabsDialog BoxesQualitative Analysis Tools	Reference <ul style="list-style-type: none">Change, Error and Warning IconsAlgorithmsColumn DefinitionsExample ReportsGraphicsAdditional Topics

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Training Resources

Available Training Resources

Convenient Training

In our classrooms, at your site or online.

From a team of industry experts that deliver a high quality learning experience.

Classroom Training

Introductory level to in-depth, hands-on for laboratory instrumentation and software.

Customized On-Site Training

Effective learning environment designed to achieve operational excellence and employ development without the need to travel.

Online

Offerings from foundation level to expert delivered at your own pace.

Agilent University

Access From Home Page

Upgraded customer experience

Search and find courses that meet your interests and needs in the format they require.

Introduce new eLearning capabilities

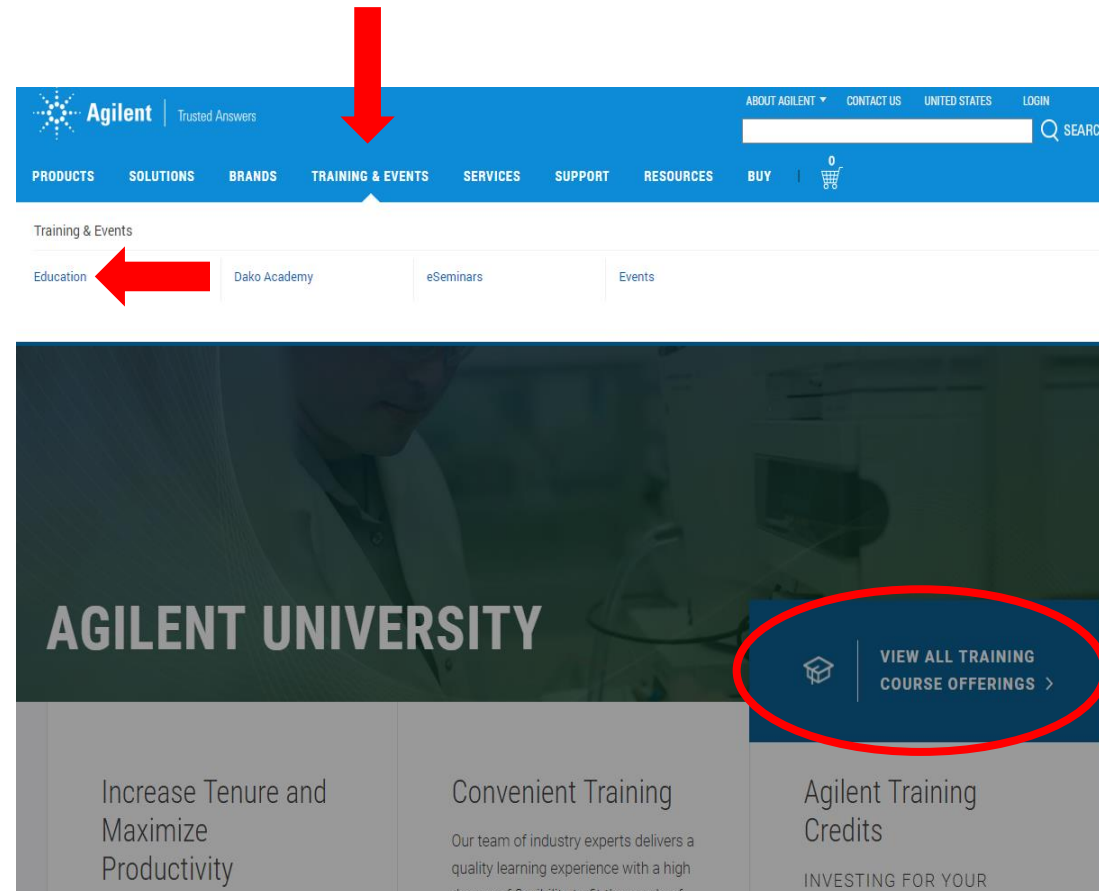
Recorded and video-based learning
Virtual online classes

Expanded portfolio

Foundational subjects
Intermediate subjects
Advanced subjects
Workflow and applications

Helping customers

Educate your employees on Agilent instruments and software.
From new hires to the most seasoned scientists.



Agilent Community



Collaborate - Ask and answer questions.

Connect - Interact with other Agilent users.

Discover - Find relevant discussions, documents, and videos.

Share - Contribute your insights.

