Agilent MassHunter Qualitative Data Analysis

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MassHunter Qualitative Analysis
Chromatogram Functions
MassHunter Qualitative Analysis Software B.07.00

Topics

• User Interface Configuration
• User Workflows
• Views
  - Navigator
  - Compound Details
• Methods
  - Unified Method Concepts
  - Method Explorer
  - Method Editor
• Working with Chromatograms
  - Anchoring and Scaling
  - Chromatogram Functions
  - Integrators
• Training Resources
• Define Qualitative Analysis
MassHunter Qual - Configurable Software

• One program for many instruments and types of data.
  • Single Quad (LC & GC) Unit resolution, Scan, SIM data
  • Triple Quad (LC & GC) Unit Resolution Scan, SIM, MRM (MS/MS) data
  • TOF (LC) High resolution, scan data
  • Q-TOF (LC & GC) High resolution MS/MS data

• Many software features can be used by all data types but many are only useful for a particular instrument type.

• MassHunter Qual MUST be configured to reduce complexity and hide unneeded and potentially misused features.

• Even when properly configured some features and parameters for MS/MS and accurate mass are still visible, ignore and avoid them.
User Interface Configuration

Separation types (Check GC or LC)

Unit Mass (Q, QQQ) Accurate Mass (TOF, QTOF)

Check Show Advanced Parameters

MS Levels: MS
MassHunter Qualitative Analysis Workflows

Depends Upon Software Loaded and Configuration Selected
Configure for Workflow

Configuration Changes Graphics, Table, and Method Layouts.
Tip: Load workflow’s default method and default layout.
Chromatogram Peak Survey Workflow

**General**

- Modify settings for chromatogram.

**Chromatogram Peak Survey**

- Modify settings for spectral extraction.

Specified workflow added.

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**Agilent Technologies**

MassHunter Webinar Series
MS Target Compound Screening Workflow

General

MS Target Compound Screening Workflow

- Extraction Data Format
- Chromatograms
- Mass Spectra
  - Find by Molecular Feature
  - Find by Formula
  - Identify by Database Search
  - Identify by Library Search
  - Identify by Formula Generation
  - Match Scoring
  - Compound Report
  - Automation
- Chromatogram
- Spectrum
- General
- Reports
- Find Compounds
- Find Compounds by Formula
- Identify Compounds
- Compound Automation Steps
- Worklist Automation
- Export

Agilent Technologies
Navigator View

Data Navigator

Navigator View

Chromatogram Results

Method Explorer

Method Editor

Spectrum Results
Data Navigator

The Data Navigator pane shows the data files which are loaded into Qualitative Analysis.

The user can selectively display the information associated with a data file (i.e. chromatograms, spectra, compounds) by selecting/deselecting a checkbox.

In the top drop-down, the user can choose to sort by Data File or Type (i.e. User Chromatogram, etc.)
Compound Details View

Compound List

Compound Chromatogram Results

Compound MS Spectrum Results

Compound Fragment Spectrum Results
Definitions

User Spectra are mass spectrum that the user creates.

Compounds are generated by one of the ‘Find by’ algorithms. Compounds are generated by the software.

User Spectra and Compounds are readily interchangeable through the context menu (right click on the User or Compound Spectrum in the MS Spectrum Results window).
Expose or Hide Windows as Needed

Menu

Toolbar
Docking & Undocking Windows
Restore Default Layout

- Complicated windows layouts can be restored to default layout.
- Preferred layouts can be saved and loaded.
- Layouts can be locked.
Restore Qual Setting

This may be a useful tool to restore the Qualitative Analysis settings if a configuration problem is suspected.

Or Desktop folder
Specify Compound Label Configuration

Configuration > Compound Label Configuration

Tip: Select Include all selected attributes that have values.
In this example, compounds shows the compound number, RT, compound and formula.
Compounds Labels Display in Data Navigator

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Labels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cpd 13: 1.020 169.0848; C7 H11 N3 O2; C7 H11 N3 O2; N[paj]-Methyl-L-histidine</td>
<td></td>
</tr>
<tr>
<td>Cpd 14: 1.039 103.9996; C5 H13 N O</td>
<td></td>
</tr>
<tr>
<td>Cpd 16: 1.068 161.1047; C7 H15 N O3</td>
<td></td>
</tr>
<tr>
<td>Cpd 17: 1.114 113.0586; C4 H7 N3 O; C4 H7 N3 O; Creatinine</td>
<td></td>
</tr>
<tr>
<td>Cpd 20: 1.146 115.0992; C6 H13 N O</td>
<td></td>
</tr>
<tr>
<td>Cpd 23: 1.193 85.0892; C5 H11 N</td>
<td></td>
</tr>
<tr>
<td>Cpd 24: 1.195 140.0581; C6 H8 N2 O2; C6 H8 N2 O2; Ethyl-imidazole carboxylate</td>
<td></td>
</tr>
<tr>
<td>Cpd 25: 1.215 170.0687; C7 H10 N2 O3; C7 H10 N2 O3; 2,3,4-Trihydroxybenzylhydrazide</td>
<td></td>
</tr>
<tr>
<td>Cpd 26: 1.232 228.1104; C10 H16 N2 O4</td>
<td></td>
</tr>
<tr>
<td>Cpd 27: 1.278 143.0945; C7 H13 N O2; C7 H13 N O2; Triparanol</td>
<td></td>
</tr>
<tr>
<td>Cpd 28: 1.318 137.0476; C7 H7 N O2; C7 H7 N O2; 2-Pyridylacetic acid</td>
<td></td>
</tr>
<tr>
<td>Cpd 29: 1.328 175.0955; C6 H13 N3 O3; C6 H13 N3 O3; Citrulline</td>
<td></td>
</tr>
<tr>
<td>Cpd 30: 1.346 202.1316; C9 H18 N2 O3; C9 H18 N2 O3; Ala Ile</td>
<td></td>
</tr>
<tr>
<td>Cpd 32: 1.420 85.0895; C5 H11 N</td>
<td></td>
</tr>
<tr>
<td>Cpd 33: 1.450 203.1164; C9 H17 N O4; C9 H17 N O4; L-Glutamic acid n-butyl ester</td>
<td></td>
</tr>
<tr>
<td>Cpd 34: 1.464 159.1257; C8 H17 N O2; C8 H17 N O2; DL-2-Aminoocctanoic acid</td>
<td></td>
</tr>
<tr>
<td>Cpd 35: 1.471 211.0948; C9 H13 N3 O3; C9 H13 N3 O3; Zalcitabine</td>
<td></td>
</tr>
<tr>
<td>Cpd 37: 1.499 145.0857; C5 H11 N3 O2; C5 H11 N3 O2; 4-di(methylethylideneamino)butanoic acid</td>
<td></td>
</tr>
<tr>
<td>Cpd 38: 1.539 216.1468; C10 H20 N2 O3; C10 H20 N2 O3; Val Val</td>
<td></td>
</tr>
<tr>
<td>Cpd 39: 1.613 268.1168; C11 H16 N4 O4; C11 H16 N4 O4; Isobutylglycine</td>
<td></td>
</tr>
<tr>
<td>Cpd 40: 1.623 244.0697; C9 H12 N2 O6; C9 H12 N2 O6; Uridine</td>
<td></td>
</tr>
<tr>
<td>Cpd 42: 1.646 192.0265; C6 H8 O7; C6 H8 O7; 2,3-Dioxogulonic acid</td>
<td></td>
</tr>
<tr>
<td>Cpd 43: 1.647 137.9956; C6 H2 O4</td>
<td></td>
</tr>
<tr>
<td>Cpd 44: 1.648 174.0159; C6 H6 O6; C6 H6 O6; Dehydroascorbic acid</td>
<td></td>
</tr>
<tr>
<td>Cpd 45: 1.655 180.0643; C7 H8 N4 O2; C7 H8 N4 O2; Theobromine</td>
<td></td>
</tr>
<tr>
<td>Cpd 46: 1.660 228.1470; C11 H20 N2 O3; C11 H20 N2 O3; Leu Pro</td>
<td></td>
</tr>
<tr>
<td>Cpd 47: 1.667 216.1223; C8 H16 N4 O3</td>
<td></td>
</tr>
<tr>
<td>Cpd 48: 1.685 169.0844; C7 H11 N3 O2; C7 H11 N3 O2; N[paj]-Methyl-L-histidine</td>
<td></td>
</tr>
<tr>
<td>Cpd 49: 1.685 141.0791; C7 H11 N O2; C7 H11 N O2; Ethosuximide</td>
<td></td>
</tr>
<tr>
<td>Cpd 51: 1.775 129.0425; C5 H7 N O3; C5 H7 N O3; Pyroglutamic acid</td>
<td></td>
</tr>
<tr>
<td>Cpd 52: 1.776 158.1415; C8 H18 N2 O</td>
<td></td>
</tr>
</tbody>
</table>
Open Data Files

Select multiple files at once for batch analysis, then click **Open**.

If **Load result data** is checked, the qualitative data manipulations previously saved will be loaded.

If **Run ‘File Open’ actions from selected method** is checked, automated processing is performed.

If neither **Load result data** or **Run ‘File Open’ actions from selected method** is checked, then a TIC is automatically extracted from the data files.
Tip

Every time a data file is loaded see 2 TICs.
Refresh Data File

Feature is useful when it is desired to view data as the data file is being acquired.

Initially use **Open Data File** as normal to view data file being acquired.

Then use **Refresh Data File** to update the view and add the most recently acquired data.

**Refresh Data File** is only active if the file is being acquired.

Similar application use for the GC/MSD ChemStation, where it is called SnapShot.
Let’s take a moment for chat questions on Configuration and Layouts.

Up Next:
Qualitative Methods
Demo on Configuration and Layouts.
Qualitative Analysis Methods

Qualitative Analysis Methods are stored in a .M folder.

Many application & instrument specific methods, generally use Default.m.

Default.M is read-only, after editing “Save As” to a customized method.
What is a Method? Unified Method Concept

Qualitative Analysis Methods are stored in a .M folder.
Quantitative Analysis Methods are stored in a .M folder.
Quantitative Analysis Reporting Methods are stored in a .M folder.
Unified method can now be automated to run from the sequence/worklist.
Method Explorer

Acts as a table of contents for the method.

Items in Method Explorer automatically display related Method Editor features.

Items are dynamic and controlled by the User Configuration and Workflow setting.
Method Editor

Display and Edit sections of the Method.

Tabs within the Method Editor further organize method parameters.

The “Run” icon executes the function associated with this part of the method.

In some cases the “Run” icon can have different actions, a drop down list will display them for selection.
Set parameters for action in Method Editor. Then, perform action. Note: The action will be performed on ALL selected (highlighted) items!
When you make a change to the current method the change is marked. In addition, all other functions that are affected by this change will be marked. Save the method to remove the icon.

An invalid value has been entered into a field. The field will reset to the last valid value it contained.

Additional information is required. The error must be fixed before the algorithm will execute.
Working with Chromatograms

- The power of Qualitative analysis is that you can have more than 1 data file open at a time.

- Extract Chromatograms from Data Files.

- Displaying Chromatograms
  - Selecting for display
  - Zooming
  - Scaling
  - Overlay / List mode
  - Anchoring
Define Chromatograms

Data Loaded & Displayed

Can Display:
- TOF Data
- QTOF Data
- QQQ Data
- SQ Data
- UV data
- FID Data
Extract Chromatogram

List of Chromatogram types is determined by data in file.
Selecting Chromatograms for Display

Items in the Data Navigator, like Chromatograms, will be displayed if checked and not displayed if unchecked.
Specify Number of Chromatograms Displayed

Maximum number of chromatograms to display in window, may be fewer.
Overlay vs. List Mode Chromatograms

List (Separated)

Overlaid
Anchoring

Setting an anchor keeps the anchored chromatogram displayed at all times.

Only one Chromatogram can be anchored at a time.

The anchor can be set and cleared from the context menu.
Zooming

Right-click and drag over the desired area or right-click in the axis and drag to zoom.

Autoscale

Normalization

Unzoom (multiple levels)

Linked Y-axis

Scale to largest in Each over Selected Range

Scale to largest in Each

Scale to largest in All

Scale Off
Chromatogram Display Options

Main Menu

Within Display

Customize Appearance of Chromatograms
Let’s take a moment for questions on Chromatogram Display Options.

Up Next:
Chromatogram Functions
Let’s take a moment for a demo on Chromatogram Display Options.

Up Next:
Chromatogram Functions
Chromatogram Functions

- Integrate (MS)
- Integrate (GC)
- Smooth
- Exclude Mass(es)
- Calculate Signal-to-Noise
- Define Chromatograms
- Extraction Data Format

Identify chromatographic peaks for further analysis.

Exclude certain masses when depicting chromatograms.

Extract chromatograms.
Extraction Data Format - Profile and Centroid

Data files may contain Centriod, Profile (Raw) or both data types. Settings determine which type is used to create chromatograms / spectra. Centroid data is the most commonly used, ~10 times smaller than Profile. Profile is useful for mass peak area comparisons such as when optimizing acquisition parameters, i.e. finding the mass defect or center of mass centroid.

How is Profile Data activated?
Extract Defined Chromatogram

Software extracts a list of chromatograms which are stored in the Extract Defined Chromatogram section of the method.

List of Chromatogram types is fixed list of all instrument types.
Extract Define Chromatograms

- Select MS Level based on acquisition scan type.

Types of Chromatograms

- **TIC** – Total Ion Chromatogram
- **BPC** – Base Peak Chromatogram
- **EIC** – Extracted Ion Chromatogram
- **SIM** – Selected Ion Monitor

Other Chromatograms – GC, DAD, ADC

Instrument Curve (LC) - %Comp., Temps, etc.

Triple Quad systems only

- **MRM** – Multiple Reaction Monitor
- **pNLC** - Precursor Neutral Loss Chromatogram

**Tip:** Select Change or Add.
Extracting GC, UV and other Non-MS Signals
Extract All Non-MS Chromatograms

Select Actions > Extract All Non-MS Chromatograms.
Extract Ion Chromatograms from Spectra

Double-click on spectrum ion and the EIC will appear.
Subtract Any Chromatogram

Right click in Chromatogram, select “Subtract Any Chromatogram”, the next chromatogram you click on will be subtracted from 1st one.
Integrate Chromatogram
Integrate Chromatogram

Independent Integrator for each configuration.
Integrator Types

**Agile2**
- Default Integrator, 3\textsuperscript{rd} generation parameterless integrator
- Better baselines, higher sensitivity to smaller peaks

**Agile**
- 2\textsuperscript{nd} generation parameterless integrator

**Universal**
- 1\textsuperscript{st} generation ChemStation integrator
- Familiar to ChemStation users

**General (RTE)**
- Familiar to MSD ChemStation users
- Areas in Universal are 10 time smaller than seen in ChemStation

**MS/MS and MS/MS (GC)**
- 1\textsuperscript{st} generation parameterless integrator intended for MS/MS systems, not recommended for SQ. Originally required 64 data points.

**ChemStation**
- 2\textsuperscript{nd} generation ChemStation
- Intended for UV
Integration Peak List

Right-click on the Peak List header to Add/Remove Columns.

Tip: Tables can be configured.
Integration Peak Tables (all tables)

Tables can be moved to different locations.

Tables can be split for easy viewing.

Columns can be added, removed, and moved.

Columns can be moved by Clicking and dragging on column header.
Manual Integration

Use mouse cursor to manually integrate peak.
Calculate Signal-to-Noise
Specific Noise Regions

• User defined specific noise regions.

• May be performed automatically when Chromatogram is integrated.
Calculate Signal-to-Noise Automatic Noise Region Detection

- Alternative to user defined specific noise regions in which the software seeks to locate a “noise region” between the peaks found by the integrator.

- User specifies a maximum length (desired) and minimum length of noise region and software locates an acceptable region if one exists.
Let’s take a few moments for questions on Chromatogram Functions.

Up Next:
Training Resources.
Time for a demo on Chromatogram Functions.

Up Next:
Training Resources
Training Resources

Training resources that are available.

Convenient Training

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