MassHunter Software Overview, Tips, & Tricks

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ASTS – Vancouver, BC May 8th
La Jolla, CA May 20th
MassHunter Workstation
One software for all your Agilent mass specs

Minimize the learning and optimize the use of software in your lab across different mass spec instrument platforms

- Control and data processing for Agilent GC/MS, LC/MS, and ICP-MS instruments
- From GC and LC Single Quad to Accurate Mass QTOF’s
MassHunter Software for your Key Applications

- **Qualitative Analysis** for confident identifications and to set up methods
- **Quantitative Analysis** for fast and accurate results
- **Acquisition** for innovative techniques such as tMRM to confirm compounds
- **Personal Compound Database and Libraries (PCDLs)** for rapid identification

- **Food**
- **Pharma**
- **Veterinary Drugs**
- **Forensic Toxicology**

- **Spectrum Mill** for accurate identification of proteins
- **BioConfirm** for characterization of intact proteins, peptides, and monoclonal antibodies
- **Mass Profiler Professional (MPP)** for differential profiling
- **Pathway Architect** for bringing together genomics, proteomics, and metabolomics

**Proteomics**

**Biopharma**

**Metabolomics**

**Integrated Biology**

- **Proteomics**
- **Biopharma**
- **Metabolomics**

**Agilent Technologies**

**May 20, 2014**

**ASTS - La Jolla**
Agilent MassHunter Core Programs

**Acquisition**
- Support for all Agilent LCs, GCs, and mass spectrometers
- Superior compound detection (LC/MS) tMRM, All Ions MS/MS, and Ion Mobility

**Qualitative Analysis**
- Application Focused Solutions Kits: Software, Libraries, Methods
- Patented Data mining & identification software algorithms formula/structure

**Quantitative Analysis**
- High-throughput quantitation of target compounds
- Easily visualize results using the Batch Table or Compounds-at-a-Glance from ALL Agilent LCs, GCs, and Mass Specs
MassHunter Suite of Software:

- Qualitative Analysis (Qual)
- BioConfirm
- Spectrum Mill B.04.01
- Molecular Structure Correlator (MSC)
- PCDL Manager
- Profinder
- Pathways to PCDL
- Mass Profiler (MP)
- Mass Profiler Professional (MPP)
- SimLipid (Premier Biosoft)

Fully integrated workflows to enable you to identify, plan, and execute your next experiment

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MassHunter Software Window 7

Current Versions (May 2014)

- MassHunter Acquisition for QQQ B.07.00 NEW
- MassHunter Qualitative Analysis B.06.00 SP1*
- MassHunter Quantitative Analysis B.07.00 NEW
- MassHunter BioConfirm B.06.00
- MassHunter PCDL Manager B.04.00 SP1
- MassHunter METLIN Metabolite PCDL B.05.00
- MassHunter Mass Profiler Professional 12.65 NEW
- MassHunter Profinder B.06 NEW
- Accurate Mass Libraries Pesticide/Vet Drugs/Forensics
- Pathway to Database Creator Software
- ChemStation SQ (LC/MS and GC/MS) to MassHunter file translator

All run on Windows 7 Pro 64 bit with Excel 2013
Keep Your MassHunter Current!

Online service patches and updates found at www.agilent.com


General Software Technical Support with Patches and Updates

The MassHunter Qualitative Workflow
Identify with confidence

**Acq**
- High resolution
- Accurate mass
- LC/TOF and Q-TOF

**Qual**
- Find compounds
- Characterize
- Identify

**PCDLs**
- Market-focused databases and libraries
- Application Kits for quick startup

Agilent provides a complete suite of tools, consumables and consulting to set up for the rapid identification of unknowns
Qualitative Analysis

Data Navigator

Results Table

Method Explorer

Method Editor

Spectrum Display

Chromatogram Display
Qual is used for All Data Type: MSD, QQQ, TOF
So Setup User Interface for Data File Type

Setting data file type automatically changes the method options, display and search capabilities
Selecting Data Types Changes Display

**TIP**: To use low res libraries you must have the GC checked
And Changes Compound Identification

**Accurate Mass Q(TOF)**

- Identify Compounds
  - Search Database
  - Search Accurate Mass Library
  - Generate Formulas
  - Combine Identification Results

**Unit Mass MSD/QQQ**

- Identify Compounds
  - Search Accurate Mass Library
  - Combine Identification Results

**Tip:** The low resolution *.L file should be copied to the X:\MassHunter\Library\ subdirectory and one can use the NIST library if you have a license
Chromatogram Display

Comprehensive Display Options

• Display **multiple** Chromatograms, from one or many data files.
• Extract **multiple signal types**, i.e. TIC, EIC, UV, FID, Instrument curves, etc.
• Annotate peaks or chromatograms with text or images

Definition of chromatograms can be stored in the method and used with automation.
Improved Parameter-less Integrators
MS/MS and Agile what’s the difference?

The Agile integrator requires
75% fewer data points for Integration
typically 15 points minimum

Agile2 Now in QUANT B.07
For Accurate Mass Systems

Use Advanced Tab to Narrow Extraction Limits (10 ppm)

Set to Exclude Reference and Background Ions
Spectra Display

Extract Spectra via:
- Manual spectra selection
- Integrated peaks
- **Multiple Find-by-Methods**
  - By Deconvolution
  - By Integration
  - By Molecular Feature
  - Find by Formula (Ion)

Identify from Library Searching GCMS
- Multiple Libraries (*.L)
- Link to NIST MS Search
How to Set Labels on MS and MS/MS Spectra

Multiply Charged Species

In Profile Mode Resolution of MS Peak
Using Extract Peak Parameters – Saturation Correction

- Change 10% to 5%
- Change 40% to 10%

For Narrow Peaks
Remember to Check Never Return an Empty Spectrum
Unsupervised Naïve data mining

Molecular Feature Extraction (MFE)

Finds Features in TOF/QTOF Data

Data Reduced sum intensities of isotopes, adducts, clusters and multiply charges ions together.

3D Plot Before Coeluting Features
Graphical Results Displayed

Over 280 Compounds Found in Coffee Spiked with Pesticide

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Choose Isotope Model to Match Application

TIP:
For Compounds containing metals or elemental such as B, Li, Si select **Unbiased**

Check Limit Assigned Charge States Maximum Values

For Small Molecule Applications: Set to 2
For High Molecular Weight Apps: Uncheck or Max 10
### Compounds Labels Display

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Data</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cpd 13: 1.020 169.0848; C7 H11 N3 O2; C7 H11 N3 O2; N(pap)-Methyl-L-histidine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cpd 14: 1.039 103.0996; C5 H13 N O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cpd 16: 1.068 161.1047; C7 H15 N O3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cpd 17: 1.114 113.0586; C4 H7 N3 O; C4 H7 N3 O; Creatinine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cpd 20: 1.146 115.0992; C6 H13 N O</td>
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<td></td>
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<tr>
<td>Cpd 23: 1.193 85.0892; C5 H11 N</td>
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<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>
<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>
<td></td>
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<tr>
<td>Cpd 26: 1.232 228.1104; C10 H16 N2 O4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cpd 27: 1.278 143.0945; C7 H13 N O2; C7 H13 N O2; Triparanol</td>
<td></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>
<td></td>
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<tr>
<td>Cpd 29: 1.328 175.0955; C6 H13 N3 O3; C6 H13 N3 O3; Citrulline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cpd 30: 1.346 202.1316; C9 H18 N2 O3; C9 H18 N2 O3; Ala Ile</td>
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<td></td>
</tr>
<tr>
<td>Cpd 32: 1.420 85.0895; C5 H11 N</td>
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<td></td>
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<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>
<td></td>
</tr>
<tr>
<td>Cpd 34: 1.464 159.1257; C8 H17 N O2; C8 H17 N O2; DL-2-Aminoacetoic acid</td>
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<tr>
<td>Cpd 35: 1.471 211.0948; C9 H13 N3 O3; C9 H13 N3 O3; Zalcitabine</td>
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<tr>
<td>Cpd 37: 1.499 145.0857; C5 H11 N3 O2; C5 H11 N3 O2; 4-(diaminomethylideneamino)butanoic acid</td>
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<td></td>
</tr>
<tr>
<td>Cpd 38: 1.539 216.1468; C10 H20 N2 O3; C10 H20 N2 O3; Val Val</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cpd 39: 1.613 268.1168; C11 H16 N4 O4; C11 H16 N4 O4; Isobutyglycine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cpd 40: 1.623 244.0697; C9 H12 N2 O6; C9 H12 N2 O6; Uridine</td>
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<td></td>
</tr>
<tr>
<td>Cpd 42: 1.646 192.0265; C6 H8 O7; C6 H8 O7; 2,3-Dioxogulonic acid</td>
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<td></td>
</tr>
<tr>
<td>Cpd 43: 1.647 137.9956; C6 H2 O4</td>
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<td></td>
</tr>
<tr>
<td>Cpd 44: 1.648 174.0159; C6 H6 O6; C6 H6 O6; Dehydroascorbic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cpd 45: 1.655 180.0643; C7 H8 N4 O2; C7 H8 N4 O2; Theobromine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cpd 46: 1.660 228.1470; C11 H20 N2 O3; C11 H20 N2 O3; Leu Pro</td>
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<td></td>
</tr>
<tr>
<td>Cpd 47: 1.667 216.1223; C8 H16 N4 O3</td>
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<td></td>
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<tr>
<td>Cpd 48: 1.685 169.0844; C7 H11 N3 O2; C7 H11 N3 O2; N(pap)-Methyl-L-histidine</td>
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<td></td>
</tr>
<tr>
<td>Cpd 49: 1.685 141.0791; C7 H11 N O2; C7 H11 N O2; Ethosuximide</td>
<td></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>
<td></td>
</tr>
<tr>
<td>Cpd 52: 1.776 158.1415; C8 H18 N2 O</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Specify Compound Label Configuration

Highlight Parameter in list and use > and < to move to and from **Selected**.

Check **Include all selected attributes that have values** to display all attributes in table.
Targeted Data Mining: Find by Formula (FBF)

• **Sources from:**
  - Chemical formulae
  - CEF file
  - PCD/PCDL

• Takes input formula, calculates mono-isotopic mass and isotope pattern filtering

• Extracts and integrates EICs from the data, extracts peak spectra

• Calculates score based on accurate mass, isotope abundance pattern, and isotope spacing

• **Retention Time Matching Optional**
Analysis of MS/MS Data: Multiple Options

Depends on how the data was acquired:

- **MRM for QQQ Data**
- **MFE Extracting MS/MS Data**
- **Auto MS/MS for QTOF**
- **Targeted MS/MS for QTOF**
  
  - Extracts chromatogram for each targeted mass listed in the acquisition method
  
  - Each compound can have an associated EIC, MS spectrum and MS/MS spectra either an average of collision energy or separated by collision energy
MFE Extracting MS/MS Data

**Benefit:**
- Identifies Adducts and Groups Them Together
- MS/MS Spectra separated by CE or Combined
- Deisotope MS/MS Spectrum
- Identifies Compounds in which MS/MS Missed
- Easy to Setup MS/MS Inclusion List

**Disadvantage:**
- No MS/MS Fragment ion Filtering
- Compound List Larger with MS and MS/MS

Results Tab

Remember to set MS/MS Peak Filters
Database Searching

- After feature extraction, search for identifications
- Search Database: searches .csv file or PCD
- Search Accurate Mass Library: searches PCDL
- Scoring based on
  - Accurate mass match
  - Isotope abundance
  - Isotope spacing
  - Retention time (if selected)
  - Dot product scoring of MS/MS spectral match
- Forward and/or reverse scoring
Molecular Formula Generation

Figure 1. Agilent’s molecular formula generation software uses multiple dimensions of information to generate and score lists of possible molecular formulas. It has been optimized for analysis of accurate-mass data from Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS systems.
Agilent’s Molecular Formula Generation Software

Scoring based on

- Monoisotopic mass (varies in ppm)
- Isotope distribution (varies in %)
- Isotope spacing (varies in ppm)

Mass Match +
Abund. Match +
Spacing Match =

Overall Score

| m/z | Formula (N) | Calc m/z | Score | Cross Score | Mass | Calc Mass | Diff (ppm) | Abs Diff (ppm) | Spacing Match | Abund. Match | Mass Match | m/z | DBE |
|-----|-------------|----------|-------|-------------|------|-----------|------------|---------------|---------------|-------------|------------|-----------|-----|-----|
| 285.021 | C10H9ClN4O2S | 285.0208 | 99.56 | 284.0137 | 284.0135 | 0.71 | 0.71 | 99.19 | 99.29 | 99.63 | 285.021 | 8 |
| 265.021 | C7H12N2O6S2 | 265.0176 | 77.28 | 264.0137 | 264.0137 | 0.01 | 0.01 | 99.54 | 1.93 | 1.00 | 265.021 | 3 |
| 265.0241 | C7H13ClN4O2S2 | 285.0241 | 75.57 | 284.0137 | 284.0168 | 11.12 | 11.12 | 99.87 | 98.87 | 46.22 | 285.021 | 3 |
Isotope Pattern Matching

Red Boxes are Theoretical Isotope Pattern
Molecular Formula Generation

Isotope Pattern Matching and MS/MS for Formula confirmation

MS Score based on:
- Mass main Isotope
- Isotope Pattern
- Mass M+1 and M+2

MS/MS Score based on:
- Ability to calculate molecular formulas for each fragment & the corresponding neutral loss which add up to the predicted formula for the precursor

Accurate mass information used:
- Precursor ion
- Isotopes M+1, M+2
- Fragment ion
- Neutral loss

Identify Compounds
Annotate and Document

Annotate chromatograms and spectra using graphics (*.jpg), text, and chemical structures (.mol)

Use Mass Caliper to document fragmentation and losses in spectrum

Export or use copy & paste to add graphics or results into presentations and documents
Workflows in Qual

- Workflows guide users through Qual functions needed for specific tasks
  - Load via Configuration Menu on Toolbar
  - Default method with default parameters and report templates
  - Specified layout of user interface limited to needed windows
  - Section at the top of the Method Explorer grouping together the relevant functions

- Helpful as a starting point for new users and as a preliminary analysis before “deep dive” type data analysis
Saving Results

The Method default saves Minimal Amount of Graphics

Change to “Save Complete Results”
Automation - File Open Actions & Post Acquisition Worklist Processing

Automate common file open actions in manual review
- Standardize review processes
- Automate common actions, i.e. extract EIC.

Define Qualitative methodology for sequence computation.
- No user interaction required.
Compound Details View

- Compound List
- Individual Compound Results
- FBF Spectrum
- Raw MS Spectrum
What is a .cef file?

- **Compound Exchange Format file**
- Agilent-specific file format that moves compound information back and forth between Qual, MPP, and other Agilent software
- XML-based – the amount of information contained depends on what the user has extracted
  - Accurate mass, retention time
  - Formulas
  - Spectra
- Can be edited manually
Questions about MassHunter Qual?
The MassHunter Quantitative Workflow

Quantify with confidence

- Acq
  - Study Manager
  - tMRM
  - LC/QQQ

- Quant
  - Rapid method setup
  - Easy review
  - Confirmation of IDs

- Reports
  - Fast PDF reporting
  - Upload to customer LIMS in their format

Agilent provides fast setup and running of quantitative methods from acquiring samples to the final report
Quantitative Analysis

The Quant Batch screen:

- **Results Information** for the batch can be displayed. Visual guides highlight specific data that fall outside specific, predefined conditions.

- **Compound Information** displays graphical representation of the peak, qualifier information, spectral information, and the ISTD. Visual guides help identify associated data problems.

- **Batch Information** provides easy visualization and customization of relevant desired data.
Batch Screen – Sample & Results Information

In **Results Information**, the Quantitation Message contains a list of error messages that result from a compound’s quantitation.

- Blue = low
- Red = high

“Outliers” define and specify results of known problem samples/substances that fall outside predefined conditions.

These icons filter outliers in the display.
Results Information: Outlier Options

More than 40 quality checks can be specified to highlight outliers in results.

- Set high and low limits.

Custom Calculations can be added for User defined quality checks.
Compound information displays all relevant information in one view.

- Compound Integration
- ISTD Integration (Quantitation: Part of Agilent’s Parameterless Integration)
- Qualifier Information
  - Uncertainty Bands
  - Actual Ratio
  - Manual Integration
- Spectrum Information

ISTD Qualifier Information

ISTD Spectrum Information
Batch Screen - Library Reference Spectra

• Confirmation of Compound Identification
• Visual comparison of Sample and Library Spectra
• Seen in Batch-at-a-Glance and on Reports
• Extracts Spectra from Library by matching on CAS number based in Quant method and creates a small reference library (reflibrary.xml)
Batch Screen – Calibration Curve

An example of **Batch Information** is this view of the Calibration Curve. The Curve fit can be changed and data can be updated instantaneously.

To view ISTD responses or display QC samples click either the ISTD or QC button.

Change Curve Fit

$R^2$

Concentration can be set as relative (to ISTD) or actual.
Compounds-at-a-Glance

Compounds-at-a-Glance allows you to view multiple traces of compounds at a single glance.

- View up to 10 x 10 chromatograms
- Overlay Target with ISTD
- Overlay Quantifier with Qualifiers
- View chromatograms across 100 samples
Compounds-at-a-Glance

You can also view compound outliers at-a-glance.
Compounds-at-a-Glance

You can perform manual integration of compounds-at-a-glance.
Simplify Quantitation Method Creation

Easily create a target method from acquired data (SIM or Scan).

- Compound Information from a data file - populates Compound Name, Retention Time, Quant and Qual ions, and ion ratios automatically.
Quantitate Using 2-D Signals (GC and LC)

In addition to Scan and SIM data, MassHunter can also quantitate with 2-D signals, such as FID and UV. Set-up and Quantitation of GC and LC signals is the same as for GC/MS or LC/MS data.
Compound Specific Integration Choice: Including parameterless integration
Questions about Quant?

Look for “Quant Schema” on your Quant install disc for very detailed help and descriptions!
Personal Compound Databases and Libraries
Confident identification using your mass spec

PCDs (accurate mass database) and PCDLs (MS/MS libraries) allow you to search your data to identify compounds using accurate mass against reference spectra.

<table>
<thead>
<tr>
<th>PCD/PCDL</th>
<th>Market</th>
<th>Compounds</th>
<th>Compounds with MS/MS Spectra</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pesticides</td>
<td>Food Safety</td>
<td>1669</td>
<td>733</td>
</tr>
<tr>
<td>Forensics/Tox</td>
<td>Forensics/Tox</td>
<td>9008</td>
<td>3019</td>
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<tr>
<td>Veterinary Drugs</td>
<td>Food / Forensics</td>
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<tr>
<td>METLIN</td>
<td>Metabolomics</td>
<td>64,092</td>
<td>8040</td>
</tr>
</tbody>
</table>

Identify Compounds
PCDL Manager

- Allows user to view, edit, and rearrange PCDs and PCDLs
- Can create custom PCDs and PCDLs, including addition of MS/MS spectra from acquired data
- Custom Databases: Polymer Additives, Coffee, Lipids, HMDB, Natural Products
PCD versus PCDL

• Personal Compound Database (PCD)
  - Required: name, accurate mass
  - Optional: structure, retention time, formula, structure, CAS or other ID numbers

• Personal Compound Database Library (PCDL)
  - All of the above, plus MS/MS spectra

• Scoring based on
  - Accurate mass match
  - Isotope abundance
  - Isotope spacing
  - Retention time (if selected)
  - Dot product scoring of MS/MS spectra match
    • Forward and/or reverse scoring
Pathways to PCDL for Metabolomics

Convert pathway metabolite information into Agilent personal compound databases

- Pathway database source - WikiPathways, BioCyc and KEGG
- Select one to many pathways
- Removes redundant metabolites
- Adds compound information – Formula, Compound ID(s), Name, Structure

Can link to METLIN PCDL to add compound information

- Retention time or MS/MS spectra
Power of Pathway Directed Data Mining

Mine Data Using Find by Formula and Database

- Extract chromatogram and spectra using empirical formula and user-set rules
  - Use a metabolite database created by Pathways to PCDL
- Create Find by Formula method
- Chromatograms extracted and integrated
- Spectra scored from empirical formula
- User specifies match criteria threshold
  - Spectra score
  - Retention time (optional) increases specificity
- User can review and edit results
- Produce a CEF file for import into MPP

Targeted Data Mining of Qualitative Data for Greater Specificity
Questions about PCDL Manager or Pathways to PCDL?
Molecular Structure Correlator (MSC)

- Utilizes centroid MS/MS data to help elucidate structures for unknown compounds that are not found in PCDs or PCDLs by:
  - Calculating formulas for a precursor, fragment ions, and neutral losses
  - Can Search Online Databases: Chemspider for possible structures
  - Matches experimental fragments those from proposed structure by a systematic bond breaking, and displaying the best matches
Questions about MSC?
Mass Profiler (MP) Differential Analysis Program

- Feature alignment
- Single-variate statistics for binary experiments
- IDBrowser for compound identification

GPC-TOF Example: Polyols

Clearly see repeating units of 44 and 58 m/z
Mass Profiler

• Impurity Profiling/Differential Analysis Software
• Graphical Displays
  - Mass vs Retention Time
  - Log2 Ratio (Group1/Group2) vs Retention Time
  - Log2 Abundance Group 2 vs Log2 Abundance Group 1
  - Unique to Group 1 vs RT and Unique to Group 2 vs RT (New)
• Feature Identification
  - Molecular Formula Generation and AMRT database
  - Single Feature or Batch Processing (summary report)
  - Web Internet Database Searching
Mass Profiling Software

Group A (Impure)

Group B (Control)

Aligns Data
RT, Mass, Abundance

What’s Changed? Mass/RT > 2 Fold Change

What’s Unique? Impurity Control
Mass Profiler – Reproducibility of Results

Reproducible Retention Time Stability

Mass vs. Retention Time

Shift of Peak with Time
Mass Profiler – Reproducibility of Results

Log2 Ratio (Single Sample / Group Average) vs. Retention Time

Outlier Sample or Feature
Unique Group 1 and Unique Group 2

Mass vs. Retention Time

Retention Time (min)

Mass (Da)

Average feature
- Colored by feature
- Colored by sample
- Colored by group
Questions about Mass Profiler?
Untargeted Data Acquisition & Analysis

Untargeted

Separate & Detect
LC-TOF/QTOF

Feature Finding
Profinder

Alignment & Statistics
Mass Profiler Professional

Identify
ID Browser

Pathways
Pathway Architect

Pathway Targeted

Untargeted

LC-TOF/QTOF

Profinder

Mass Profiler Professional

Pathway Architect

Agilent Technologies
Mass Hunter Profinder - NEW

- Batch based or “project” based logic
- Extracts and aligns features prior to statistical analysis in MPP
- Can replace previous recursive workflow in Qual
- More efficient, fewer manual steps
- Visualize, review, and edit results across the batch for higher quality results
Profinder Workflows

1. **Batch Molecular Feature Extraction**
   - Reduces False Positives, No Editing
   - MFE → rMFE

2. **Batch Recursive Feature Extraction**
   - Reduces False Negatives, Allows Editing
   - MFE → rMFE → Find by Ion

3. **Batch Targeted Feature Extraction**
   - Uses database targets, Allows Editing
   - Find by Formula
## Profinder Results Navigation

- **Compound Groups Table**
- **Chromatogram Results**
- **Spectrum Results**

### Chromatogram Results
Chromatograms displayed in overlaid mode.

### Compound Details Table

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May 20, 2014
Questions about Profinder?
DA Reprocessor
Use this, it saves memory and works in the background!

Run Qual method on a batch of samples

Right click upper left corner to add one or multiple samples

Populates automatically

Open your Qual method
Questions about DA Reprocessor?
Mass Profiler Professional
Find differences in mass spec data sets and reach statistically valid conclusions

Get from data to answers using **statistical tests** such as PCA

Dive deeper into results using **visualizations** e.g. heat maps

**Accurate identifications** using ID Browser

Use data from LC/MS, GC/MS, ICP-MS, and NMR

Run QC assays using the Sample Class Predictor

**Bring genomics, proteomics, and metabolomics together**
Mass Profiler Professional (MPP)

- Compound alignment
- Filtering
- Single- and multi-variate statistics
  - t-test, ANOVA, clustering, fold change, PCA
- IDBrowser for compound identification
- Sample Class Prediction
- Pathway Architect
- Multiple data types and sources
  - Metabolomics, lipidomics, proteomics, other small molecule profiling
  - LC-MS, GC-MS, ICP-MS, generic import
  - Targeted, untargeted
ID Browser

Searches against csv or PCD files to identify compounds
ID Browser

Searches against csv or PCD files to identify compounds

Measured and theoretical isotope ratios

Ion species and isotope abundance values

Compound structure

Database match score

Number of hits

Database match or MFG formula

MFG score

Overall score

May 20, 2014

ASTS - La Jolla
SimLipid from PREMIER Biosoft International

One way to think of SimLipid is like “ID Browser for Lipids”
SimLipid vs. METLIN PCD

METLIN PCD has 31,011 lipid compounds. It is suited in lipidomics for customers who:

- Are agreeable to an **MS-only workflow** (i.e. database search)
- Want to use Agilent’s ID Browser for identifications (part of MPP) instead of SimLipid
- Want to have the other ~33,000 metabolite compounds in METLIN
- Lower cost solution – perpetual license

SimLipid has 36,224 lipids in its database. It is suited for customers who:

- Want to identify lipids using **MS/MS pattern matching** (in addition to database search)
- Have instruments other than Agilent’s
- Are familiar with or already own SimLipid for lipid identifications
- Requires an annual subscription
The MPP Lipidomics Workflow

- SimLipid 3.3 and higher supports import and export of Agilent’s Compound Exchange Format (.CEF) file. Compounds found in Qual and exported as .CEF can be
  - annotated in SimLipid as lipid compounds using
    - MS database searching
    - MS/MS pattern matching for features that have MS/MS
  - export annotated results in .CEF file format
  - import annotated .CEF file into MPP,
    - use Pathway Architect to give biological contextualization.
Overview of SimLipid Process

- Import CEF files into SimLipid
- Run High Throughput Search on CEF files
- Load HTP Results from SimLipid server
- Generate HTP Report (optional)
- Export annotated CEF files for analysis in MPP
Import Agilent CEF Files

Choose the Agilent icon on the SimLipid menu bar and select either Import up to 3 files or Batch Mode.

Select your CEF files.
Run High Throughput Search on the CEF Files

Select High Throughput Lipid Search (“HTP”) in the menu bar

Select CEF files to run High Throughput search on and click OK.
Run High Throughput Search on the CEF Files

Select the compounds to run HTP search on and the specific search parameters, then press the Search button.

2000 compounds (aka “profiles” in SimLipid) can be searched at one time
Run High Throughput Search on the CEF Files

The High Throughput search runs and then you are given a search ID (even on individually installed instance, i.e. non-server)
Load HTP Results

Go to the HTP icon on the menu bar and select to load your HTP search request.

A message log will return how many of the compounds were identified (e.g. 162 out of 1000)

Searches are cumulative—the next time you search it will automatically select the second 1000 compounds.
Generate High Throughput Report (optional)

Select the Generate High Throughput Report icon in the menu bar

Select CEF files to generate High Throughput report on and click OK.
Generate High Throughput Report (optional)

Select the compounds (aka profiles) to include in the report and the parameters to be displayed.
Export Annotated CEF files for Analysis in MPP

Select the CEF icon in the menubar.

Select CEF files to export and click OK.
LC/MS/MS Identification of 1,2-dipalmitoyl-sn-glycero-3-PC

Mass Spectrum: m/z versus Intensity

S.No. | m/z
---|---
1   | 104.1074
107 | 734.5721
1   | 166.0638
18  | 184.0745
11.1| 166.0638
2   | 104.7271
3   | 108.1006
4   | 113.1999
5   | 124.7027
6   | **125.0003**
7   | 125.0562
8   | 125.1304
9   | 125.5472
10  | 142.1619
12  | 167.8584
13  | 171.3158
14  | 183.4557
15  | 183.4651
16  | 183.5501

Agilent Technologies

ASTS La Jolla MassHunter Session
May 28, 2014
LC/MS/MS Identification of 1,2-dipalmitoyl-sn-glycero-3-PC

Counts vs. Mass-to-Charge (m/z)

Cpd 869: 1,2-dipalmitoyl-sn-glycero-3-PC: +ESI Product Ion (20.53 min) Frag=100.0V (734.5726[z=1] -> **) Female-Serum-MSMS-C18-PosESId

Cpd 869: 1,2-dipalmitoyl-sn-glycero-3-PC: +ESI Product Ion (20.53 min) Frag=100.0V CID@10.0 (734.5726[z=1] -> **) Female-Serum-MSMS-C18

1,2-dipalmitoyl-sn-glycero-3-PC

MS/MS @ 40

Metlin Spectral Library

Female Serum

Male Serum

Counts vs. Acquisition Time (min)
Ordering SimLipid

SimLipid is available from PREMIER Biosoft International through their website:

http://www.premierbiosoft.com/lipid/index.html

They can be contacted through sales@premierbiosoft.com

Phone: +1-650-856-2703

On their website, they offer temporary licenses for customers to try out the product before buying.
Pathway Architect
Pathway analysis using multi-omic data

Start here

Finish here

Legend:
- Hydrogen
- Adenosine triphosphate
- Oxygen
- NADH
- Coenzyme A
- NADPH
- Glucose
- Pyruvate dehydrogenase
- Enzyme

Agilent Technologies
May 20, 2014
Pathway Architect

Customers can take the value of their data to another level. Pathway Architect helps them make sense of the data by:

- Visualizing it on
  - Wikipathways
  - BioCyc/MetaCyc
- Overlaying genomics, proteomics and metabolomics data
- Designing their next experiment for MS/MS analysis or custom microarrays

TCA Cycle
Starting Pathway Architect

Transcriptomics results

Pathway Organism

Metabolomics results

Pathway database
Mass Hunter Qualitative Analysis and Its “Accessories”

- Qualitative Analysis (Qual)
- PCDL Manager
- Pathways to PCDL
- Profinder
- Mass Profiler (MP)
- Mass Profiler Professional (MPP)
- ID Browser
- SimLipid (Premier Biosoft)
- Molecular Structure Correlator (MSC)

Fully integrated workflows to enable you to identify, plan, and execute your next experiment
How can you learn more about MassHunter software?

- Read the PDF manuals and guides that shipped with your instrument or software
- Watch the videos that shipped with your instrument or software
- Read the Workflow Overviews and Workflow Guides specific to your application area
- Use the Help feature in the software!
  - Especially good for unfamiliar terms or software features
- Attend a training class – contact your Account Manager if interested
  - QTOF operation
  - QQQ operation
  - MPP
- View eSeminars – search Agilent website
Familiarization Guides
Remember LC/MS and GC/MS Versions!

Agilent MassHunter Workstation Software
Qualitative Analysis
Familiarization Guide
Agilent Technologies

Agilent MassHunter Workstation Software
Qualitative Analysis
Familiarization Guide for GC/MS
Agilent Technologies
Training: Learn about Liquid Chromatography Online Primers and Basic Concept Guides

http://www.chem.agilent.com/search/?N=68+4294964917&Nr=OR%28part_language%3Aen%2Cg_rec_type%3ASharePoint%29&Ntt=primers
Updated Manuals/Training Materials Available on Website directly from the Help Page

Visit our Web Site

Searching Literature Mass Spectrometry Manuals Last 6 Months PDF’s of new manuals
Application Specific LC/MS Workflow Guides:

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Any Other Questions?