Outline of MassHunter Software Session

1. Overview MassHunter Software Programs
2. From ChemStation to MassHunter (Optional)
3. MassHunter Qual and Quant (?)
4. MassHunter for LC/MS Demo
   - **Find by Methods:** MFE, FBF
   - **Identification:** MFG, Database, MS/MS Libraries
   - Molecular Structure Correlator
   - Profinder
5. Q and A....
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**

Agilent Technologies
Agilent MassHunter Core Programs

**Acquisition**
- ALL Agilent LCs, GCs, and Mass Specs
- 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
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.
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

PCD/Ls
• 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
Automated Data Mining in Qual

For GC/LC/MSD
  Find by Deconvolution and Integration

For High Resolution TOF and QTOF
  Untargeted Data Analysis: Molecular Feature Extraction
  Targeted Data Analysis: Find by Formula/Ion/Databases

For MS/MS Analysis
  Find by MRM (QQQ)
  Find by Auto MS/MS
  Find by Targeted MS/MS
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:**
- Being able to calculate molecular formulas for each fragment and the corresponding neutral loss which add up to the predicted formula for the precursor ion.

**Accurate mass information used:**
- From precursor ion
- From isotopes M+1, M+2
- From each fragment ion
- From each neutral loss
Mass Error < 0.1 ppm for L-Tryptophan C_{11}H_{12}N_{2}O_{2}

Scores
MS and MS/MS of Irgafos 168 Oxidized Form

<table>
<thead>
<tr>
<th>m/z</th>
<th>Formula</th>
<th>Height %</th>
<th>Diff (ppm)</th>
<th>Loss Mass</th>
<th>Loss Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>663.45262</td>
<td>C42 H63 O4 P</td>
<td>0.85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>607.3907</td>
<td>C38 H56 O4 P</td>
<td>9.28</td>
<td>0.61</td>
<td>56.0626</td>
<td>C4 H8</td>
</tr>
<tr>
<td>551.32881</td>
<td>C34 H48 O4 P</td>
<td>26.34</td>
<td>-0.61</td>
<td>112.1252</td>
<td>C8 H16</td>
</tr>
<tr>
<td>495.26613</td>
<td>C30 H40 O4 P</td>
<td>44.06</td>
<td>-0.53</td>
<td>168.1878</td>
<td>C12 H24</td>
</tr>
<tr>
<td>439.20341</td>
<td>C26 H32 O4 P</td>
<td>13.43</td>
<td>-0.32</td>
<td>224.2504</td>
<td>C16 H32</td>
</tr>
<tr>
<td>383.14125</td>
<td>C22 H24 O4 P</td>
<td>2.42</td>
<td>-1.51</td>
<td>280.313</td>
<td>C20 H40</td>
</tr>
<tr>
<td>327.07912</td>
<td>C18 H16 O4 P</td>
<td>2.21</td>
<td>-3.22</td>
<td>336.3756</td>
<td>C24 H48</td>
</tr>
<tr>
<td>57.07087</td>
<td>C4 H9</td>
<td>2.26</td>
<td>-17.42</td>
<td>606.3838</td>
<td>C38 H55 O4 P</td>
</tr>
</tbody>
</table>
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 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>
</tr>
<tr>
<td>Veterinary Drugs</td>
<td>Food / Forensics</td>
<td>1049</td>
<td>630</td>
</tr>
<tr>
<td>METLIN</td>
<td>Metabolomics</td>
<td>64,092</td>
<td>8040</td>
</tr>
</tbody>
</table>
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
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.
Agilent’s LC/MS Biopharma Tools

- TOF/Q-TOF mAb-Glyco chip
- SQ or TOF
- BioConfirm
  - Easy Access (Walk-Up MS)

Stages:
- Disease Research
- Biologic Discovery
- Preclinical Development
- Bioprocess Development
- Clinical Trials
- Regulatory Approval & Manufacturing
MassHunter BioConfirm

Simply the processing of biopharma samples such as intact proteins and peptide digests

- Mirror Plot to compare biosimilars
- pMod deconvolution to confirm intact proteins
- Localize PTMs using MS/MS data
- Compare protein digests to a reference sequence
- MassHunter Walkup for ease of use by anyone
- Interactive wizards speed up tasks
Spectrum Mill Proteomics
Fast and accurate protein identification

Spectrum Mill turns 6500 LC/Q-TOF proteomics data into answers

- Quickly identifies proteins and peptides using database searches and validation
- Locates modifications in a protein sequence and assigns a probability
- Integration with MPP for statistical analysis, visualization, and pathway analysis of proteomics samples

Peptides identified from human serum albumin
MassHunter Profinder – “NEW”
High-throughput compound finding

MassHunter Profinder is a productivity tool for processing multiple samples in metabolomics or proteomics analyses

- **Fast Compound Finding**
  - Untargeted using MFE
  - Targeted using Find by Formula

- **Visualize, review, and edit results by compound across many samples**

- **Higher quality results based on cross-sample processing**

Aligned EICs of six replicate injections
Mass Profiler Professional

Find differences in mass spec data sets and reach statistically valid conclusions.
Pathway Architect
Pathway analysis using multi-omic data

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
Sun isn’t setting on ChemStation, MassHunter for all MS
But with over 100,000 installed GC/MS instruments...

MassHunter Qual

MassHunter Quant

MassHunter GC/MS Acquisition
and Use Either
MassHunter Data Analysis
OR
MSD ChemStation Data Analysis

Both DA Applications preinstalled, Customer’s Choice!
# MSD Productivity ChemStation OR MassHunter?

<table>
<thead>
<tr>
<th>MSD ChemStation</th>
<th>MassHunter</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Established Agilent GC/MSD User</strong></td>
<td><strong>New to Agilent or GC/MSD</strong></td>
</tr>
<tr>
<td>- Adding instrument to routine production</td>
<td>- Competitive situation.</td>
</tr>
<tr>
<td>- Established workflows</td>
<td>- Looking for modern software</td>
</tr>
<tr>
<td>- Macros, Macros, Macros</td>
<td><strong>Superior Quantitation</strong></td>
</tr>
<tr>
<td><strong>DRS or Screener DB</strong></td>
<td><strong>21CFR11 &amp; Other Regulated Labs</strong></td>
</tr>
<tr>
<td><strong>EnviroQuant User</strong></td>
<td><strong>Multi disciplinary MS lab</strong></td>
</tr>
<tr>
<td>- Uses the US EPA workflow, especially reports and QA/QC criteria.</td>
<td>- Have Agilent Triple Quad or (Q-)TOF</td>
</tr>
<tr>
<td>- MassHunter EnviroQuant targeted for late 2013</td>
<td>- LC/MS, ICP-MS, &amp; GC/MS</td>
</tr>
<tr>
<td></td>
<td>- MS and MS/MS</td>
</tr>
</tbody>
</table>
Configure LC/MS ChemStation to automatically translate data files to MassHunter format at the end of each run. It is possible to optionally link a Qualitative Analysis method to be run after translation. Users can run a sequence that goes from injection to Qual report for each run!
Manually translate Single Quad LC/MS data files from ChemStation to MassHunter format—this allows using all the tools in Qual and Quant: extraction, integration, viewing, and reporting.
Manual Translation options for MSD ChemStation, WorkStation, and generic (.CDF) data files

MassHunter Quant

ChemStation (.D)

MassHunter Unknowns Analysis

WorkStation (.SMS)

MassHunter Qual

Generic (.CDF)
Reach New Peaks using Mass Hunter Qual/Quant
Qualitative Analysis

Chromatogram Display

Data Navigator

Results Table

Method Explorer

Method Editor

Spectrum Display

Agilent Technologies
Qual is used for All Data Type: MSD, QQQ, TOF
So Setup User Interface with Data File Type

Setting data file type automatically changes the method options, display and search capabilities

Accurate Mass
Both Levels
Show Advanced Parameters
Selecting Data Types Changes “Deconvolution”

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

### Accurate Mass Q(TOF)

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

### Unit Mass MSD/QQQ

- 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
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
Unsupervised Naïve data mining
Molecular Feature Extraction (MFE)

Finds Features in TOF/QTOF Data
Automated Data Reduction Software

3D Plot Before
Coeluting Features

Processed TIC

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

Over 280 Compounds Found in Coffee Spiked with Pesticide
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
Agilent’s Molecular Formula Generation Software

Scoring based on

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

Overall Score

Mass Match +

Abund. Match +

Spacing Match

= Overall Score
Isotope Pattern Matching

Red Boxes are Theoretical Isotope Pattern
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 dig” type data analysis
Saving Results...

The Method Defaults saves Minimal Amount of Graphics

Default Setting

Graphics not saved only compound list

Change to “Save Complete Results”

All Graphics Saved
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.
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
Agilent’s Quant Software for GC and LC
Like finding two pots of gold at the end of the rainbow
Quantitative Analysis

The **Quant** Batch screen looks like this.

- **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.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank-1</td>
<td>Blank</td>
<td>L1</td>
<td>11/11/2006 11:05 AM</td>
<td>2.500</td>
<td>2.141</td>
<td>638</td>
<td>-3.10</td>
<td>2.1151</td>
<td>2.1510</td>
<td>84.6</td>
<td>24.3</td>
<td>43.5</td>
<td>Infinity</td>
<td>2.128</td>
<td>1372</td>
<td>29.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calib-L1</td>
<td>Cal</td>
<td>L1</td>
<td>11/11/2006 11:10 AM</td>
<td>1.0</td>
<td>2.140</td>
<td>105</td>
<td>42.25</td>
<td>4.5770</td>
<td>4.5770</td>
<td>91.5</td>
<td>81.5</td>
<td>31.5</td>
<td></td>
<td>2.128</td>
<td>1288</td>
<td>48.46</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calib-L2</td>
<td>Cal</td>
<td>L2</td>
<td>11/11/2006 11:15 AM</td>
<td>1.0</td>
<td>2.134</td>
<td>267</td>
<td>107.28</td>
<td>12.6107</td>
<td>12.6107</td>
<td>100.9</td>
<td>67.1</td>
<td>146.8</td>
<td></td>
<td>2.121</td>
<td>1377</td>
<td>26.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calib-L3</td>
<td>Cal</td>
<td>L3</td>
<td>11/11/2006 11:20 AM</td>
<td>1.0</td>
<td>2.134</td>
<td>267</td>
<td>107.28</td>
<td>12.6107</td>
<td>12.6107</td>
<td>100.9</td>
<td>67.1</td>
<td>146.8</td>
<td></td>
<td>2.121</td>
<td>1377</td>
<td>26.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calib-L4</td>
<td>Cal</td>
<td>L4</td>
<td>11/11/2006 11:25 AM</td>
<td>1.0</td>
<td>2.134</td>
<td>267</td>
<td>107.28</td>
<td>12.6107</td>
<td>12.6107</td>
<td>100.9</td>
<td>67.1</td>
<td>146.8</td>
<td></td>
<td>2.121</td>
<td>1377</td>
<td>26.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calib-L5</td>
<td>Cal</td>
<td>L5</td>
<td>11/11/2006 11:30 AM</td>
<td>1.0</td>
<td>2.134</td>
<td>267</td>
<td>107.28</td>
<td>12.6107</td>
<td>12.6107</td>
<td>100.9</td>
<td>67.1</td>
<td>146.8</td>
<td></td>
<td>2.121</td>
<td>1377</td>
<td>26.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QC-L2</td>
<td>QC</td>
<td>L2</td>
<td>11/11/2006 11:35 AM</td>
<td>1.0</td>
<td>2.134</td>
<td>267</td>
<td>107.28</td>
<td>12.6107</td>
<td>12.6107</td>
<td>100.9</td>
<td>67.1</td>
<td>146.8</td>
<td></td>
<td>2.121</td>
<td>1377</td>
<td>26.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QC-L1</td>
<td>QC</td>
<td>L1</td>
<td>11/11/2006 11:40 AM</td>
<td>1.0</td>
<td>2.134</td>
<td>267</td>
<td>107.28</td>
<td>12.6107</td>
<td>12.6107</td>
<td>100.9</td>
<td>67.1</td>
<td>146.8</td>
<td></td>
<td>2.121</td>
<td>1377</td>
<td>26.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample-1</td>
<td>Sample</td>
<td>L1</td>
<td>11/11/2006 11:50 AM</td>
<td>1.0</td>
<td>2.143</td>
<td>104</td>
<td>80.65</td>
<td>3.7144</td>
<td>3.7144</td>
<td>30.5</td>
<td>70</td>
<td>54</td>
<td>129.91</td>
<td>2.130</td>
<td>1446</td>
<td>28.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample-2</td>
<td>Sample</td>
<td>L1</td>
<td>11/11/2006 11:55 AM</td>
<td>1.0</td>
<td>2.143</td>
<td>104</td>
<td>80.65</td>
<td>3.7144</td>
<td>3.7144</td>
<td>30.5</td>
<td>70</td>
<td>54</td>
<td>129.91</td>
<td>2.130</td>
<td>1446</td>
<td>28.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample-3</td>
<td>Sample</td>
<td>L1</td>
<td>11/11/2006 11:59 AM</td>
<td>1.0</td>
<td>2.143</td>
<td>104</td>
<td>80.65</td>
<td>3.7144</td>
<td>3.7144</td>
<td>30.5</td>
<td>70</td>
<td>54</td>
<td>129.91</td>
<td>2.130</td>
<td>1446</td>
<td>28.7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Quantitation Message(s)**
- Amp-d5: Integrator did not find any peaks
- Amp-d5: Qualifier M/Z = 124.4: Qualifier peak not found or does not match quantitation criteria
- Cocaine-d3: Integrator did not find any peaks
- Cocaine-d3: Qualifier M/Z = 85.0: Qualifier peak not found or does not match quantitation criteria
- Meth-d5: Qualifier M/Z = 121.4: Integrator did not find any peaks

**Outlier(s)**
- Blue = low / Red = high
- Amp: Qualifier ratio = 33.5 is outside the allowed range [21.2, 31.8]

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

<table>
<thead>
<tr>
<th>Amp Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT</td>
</tr>
<tr>
<td>-----</td>
</tr>
<tr>
<td>2.141</td>
</tr>
<tr>
<td>2.140</td>
</tr>
<tr>
<td>2.134</td>
</tr>
<tr>
<td>2.022</td>
</tr>
<tr>
<td>2.101</td>
</tr>
<tr>
<td>2.142</td>
</tr>
<tr>
<td>2.135</td>
</tr>
<tr>
<td>2.143</td>
</tr>
<tr>
<td>2.105</td>
</tr>
</tbody>
</table>
Batch Screen - Compound Information

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
- ISTD Qualifier Information
- Spectrum 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.

*View ISTD responses or display QC samples - click either the ISD or QC button.*

- **Change Curve Fit**
- **Zoom in/out button** (right click/drag)

Concentration can be set as relative (to ISTD) or actual.

**R^2.**

**Agilent Technologies**
Batch Screen - Curve Fit Assistant

Calculates all possible calibration curve combinations.
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 the 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.
Automatic Compound Detection

Automatic compound detection works with Scan data (Accurate Mass). Library information is used to add compound name and CAS# for scan data. The Quant and Qual ions and ratios are taken from the data file.

<table>
<thead>
<tr>
<th>Name</th>
<th>Data File</th>
<th>Type</th>
<th>Level</th>
<th>Acq. Method File</th>
<th>Acq. Date-Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cal_L07.d</td>
<td>Cal_L07.d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name</th>
<th>TS</th>
<th>Scan</th>
<th>Type</th>
<th>MZ</th>
<th>CAS#</th>
<th>Library Match Score</th>
<th>RT</th>
<th>Library RT</th>
<th>RI</th>
<th>Library RI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound_1</td>
<td>1</td>
<td>Scan</td>
<td>Target</td>
<td>44.0</td>
<td></td>
<td></td>
<td>3.833</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dichlorodifluoromethane</td>
<td>1</td>
<td>Scan</td>
<td>Target</td>
<td>75-71.8</td>
<td></td>
<td></td>
<td>95.6</td>
<td>4.249</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methane, chloro-</td>
<td>1</td>
<td>Scan</td>
<td>Target</td>
<td>50.0</td>
<td>74-87.3</td>
<td></td>
<td>89.3</td>
<td>4.493</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclobutane, ethyl-</td>
<td>1</td>
<td>Scan</td>
<td>Target</td>
<td>41.1</td>
<td>4806-61-5</td>
<td></td>
<td>69.3</td>
<td>4.790</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compound_6</td>
<td>1</td>
<td>Scan</td>
<td>Target</td>
<td>60.0</td>
<td></td>
<td></td>
<td>5.168</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methane, bromo-</td>
<td>1</td>
<td>Scan</td>
<td>Target</td>
<td>95.9</td>
<td>74-83-9</td>
<td></td>
<td>88.7</td>
<td>5.241</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aminomethanesulfonic acid</td>
<td>1</td>
<td>Scan</td>
<td>Target</td>
<td>64.0</td>
<td>13881-91-9</td>
<td></td>
<td>50.2</td>
<td>5.410</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trichloromono-fluoromethane</td>
<td>1</td>
<td>Scan</td>
<td>Target</td>
<td>100.9</td>
<td>75-69-4</td>
<td></td>
<td>91.0</td>
<td>6.098</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetone</td>
<td>1</td>
<td>Scan</td>
<td>Target</td>
<td>43.0</td>
<td>67-64-1</td>
<td></td>
<td>81.9</td>
<td>6.194</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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

Agile2 NEW
Our Applications Focused Software Sparkles
Personal Compound Database and Library (PCDL)

- 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
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 settable rules

- Use a metabolite database created by Pathways to PCDL
- Create Find by Formula method
- Chromatograms extracted and integrated
- Spectra scored from empirical formula
  - Isotope mass values
  - Isotope ratio

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?
Mass Profiler (MP) Differential Analysis Program

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

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

Group A (Impure)

Group B (Control)

Aligns Data
RT, Mass,
Abundance

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

Mass vs. Retention Time

What’s Unique!! Impurity Control
Graphical Displays

Mass vs. Retention Time

Log2 Ratio (SampleA3/SampleA6) vs. Retention Time

SampleA3 vs. SampleA6

71 Features present only in SampleA3
Mass Profiler – Reproducibility of Results

Reproducible Retention Time Stability

Mass vs. Retention Time

Spread in Chromatography RT

Spread of Peak in Time

Retention Time (min)
Mass Profiler – Reproducibility of Results

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

Outlier Sample or Feature
Blue Unique Group 2 and Red Unique Group 1

Mass vs. Retention Time

- Mass (Da)
- Retention Time (min)
Profinder

- 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
- Batch editing capability

Profinder

- Molecular Feature Extraction
- Find by Ion (using Find by Formula)

MPP Differential Analysis
**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**

Chromatograms displayed in overlaid mode

Agilent Technologies
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
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
Variety of Learning Methods

PDF Manuals
Pull Down “Help” in MH

Training Courses
Q-TOF LC/MS Techniques & Operation for Large Molecule Application

Training Videos
Recorded Webex E-seminars
MassHunter User Meetings

http://www.chem.agilent.com/search/?N=54&No=20&Nr=OR%28part_language%3Aen%2Cg_rec_type%3ASharePoint%29&Nrpp=10&Ntt=maintenance

Familiarization Guides..
Remember LC/MS and GC/MS Versions!
Updated Manuals/Training Materials Available on Website directly from the help page.

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

<table>
<thead>
<tr>
<th>Publ #</th>
<th>Title</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>5990-7061EN</td>
<td>QQQ LC/MS Bioanalysis Workflow Overview</td>
<td>9</td>
</tr>
<tr>
<td>5990-7060EN</td>
<td>QQQ LC/MS Bioanalysis Workflow Guide</td>
<td>40</td>
</tr>
<tr>
<td>5990-7063EN</td>
<td>Drug Discovery Screening Workflow Overview</td>
<td>12</td>
</tr>
<tr>
<td>5990-7062EN</td>
<td>Drug Discovery Screening Workflow Guide</td>
<td>50</td>
</tr>
<tr>
<td>5990-7065EN</td>
<td>TOF, QTOF LC/MS BioPharma Workflow Overview</td>
<td>12</td>
</tr>
<tr>
<td>5990-7064EN</td>
<td>TOF, QTOF LC/MS BioPharma Workflow Guide</td>
<td>50</td>
</tr>
<tr>
<td>5990-7068EN</td>
<td>TOF, QTOF LC/MS Metabolomics Discovery Workflow Overview</td>
<td>16</td>
</tr>
<tr>
<td>5990-7067EN</td>
<td>TOF, QTOF LC/MS Metabolomics Discovery Workflow Guide</td>
<td>168</td>
</tr>
<tr>
<td>5990-7069EN</td>
<td>TOF, QTOF LC/MS Screening of Pesticides Workflow Overview</td>
<td>12</td>
</tr>
<tr>
<td>5990-7072EN</td>
<td>TOF, QTOF LC/MS Screening of Pesticides Workflow Guide</td>
<td>84</td>
</tr>
<tr>
<td>5990-7074EN</td>
<td>QQQ LC/MS Quantitation of Pesticides Workflow Overview</td>
<td>8</td>
</tr>
<tr>
<td>5990-7073EN</td>
<td>QQQ LC/MS Quantitation of Pesticides Workflow Guide</td>
<td>54</td>
</tr>
<tr>
<td>5990-9886EN</td>
<td>QQQ LC/MS Peptide Quantitation with Skyline Workflow Overview</td>
<td>12</td>
</tr>
<tr>
<td>5990-9887EN</td>
<td>QQQ LC/MS Peptide Quantitation with Skyline Workflow Guide</td>
<td>54</td>
</tr>
<tr>
<td>5991-1644EN</td>
<td>Agilent Lipidomics Workflow Overview</td>
<td>14</td>
</tr>
<tr>
<td>5991-1643EN</td>
<td>Agilent Lipidomics Workflow Guide</td>
<td>66</td>
</tr>
<tr>
<td>5991-1910EN</td>
<td>Integrated Biology with Mass Profiler Professional Workflow Overview</td>
<td>18</td>
</tr>
<tr>
<td>5991-1909EN</td>
<td>Integrated Biology with Mass Profiler Professional Workflow Guide</td>
<td>124</td>
</tr>
<tr>
<td>5991-1995EN</td>
<td>Agilent All Ions MS/MS - Workflow Overview</td>
<td>8</td>
</tr>
<tr>
<td>5991-1994EN</td>
<td>Agilent All Ions MS/MS Workflow Guide</td>
<td>36</td>
</tr>
</tbody>
</table>

Any Other Questions?