The MassHunter Software: Data Analysis Across the 6000 Series MS Platform

Featuring:
Agilent MassHunter Workstation

Agenda

Introduction
• Agilent 6000 Series LC/MS Platform.
• Agilent MassHunter Workstation Software.

Applications of the Agilent Mass Hunter Software in combination with the Agilent 6000 Series mass spectrometers
• Automated data processing of immunosuppressants in blood using Qualitative Analysis.
• Extraction of Molecular Features – Manual and automated analysis of complex mixtures.
• Metabolite Identification – The concept of “Find and Confirm.”
• The Quantitative Analysis software – drugs of abuse in oral fluid.

Questions
Evolving the Agilent 6000 LC/MS Series
A new and expanded portfolio for applications from life science to chemical analysis, combining exceptional performance and legendary Agilent reliability

LC/MSD
Single Quad
1996, 2006*

6300 Series Ion Traps
Intro. 2000

6210 TOF
Intro. 2003

New 6410 QQQ

New 6510 QTOF

New Agilent MassHunter Workstation Software

Instrument Control
Real-time monitoring
Method set-up
Autotune

Qualitative Analysis
Chromatographic results
Spectral results
Find compounds

Quantitative Analysis
User filters
Compound results
Calibration curve
MassHunter Workstation SW – design objectives

What are the biggest issues customers face in Mass Spec Software

- Learning Curve
- Ease of Use
- Productivity in Data Processing
- One Software for all Mass Specs
- Highly Customizable Reporting
- Import/Export of Data and Results

MassHunter Workstation SW – the solution

Addressing the Need

- MassHunter Data Acquisition
- MassHunter Quantitative Analysis
- MassHunter Qualitative Analysis
- MassHunter Metabolite ID (mid 2007)
- MassHunter Bioconfirm (end 2007)
- MassHunter Custom Reporting based on Excel 2003 & XML
- Spectrum Mill for MassHunter Workstation
- Genespring MS for MassHunter Workstation
Full integration: the lab, business applications, OpenLAB, the company network

The Lab
- GC/MS
- UV-vis
- ICP-MS
- Lab-on-a-Chip
- GC
- LC

Business Applications
- MS Office
- Legacy Data
- PDF
- ELN
- Images
- LIMS
- Research
- Drawings

Agilent OpenLAB (Laboratory Informatics)
- Intranet
- Internet

Agilent 6000 Series MS + MassHunter Workstation

<table>
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<tr>
<th>Instruments</th>
<th>Acquisition</th>
<th>File Format</th>
<th>Data Analysis</th>
<th>Reporting</th>
</tr>
</thead>
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<tr>
<td>6100 Series</td>
<td>Chemstation</td>
<td>Chemstation (.D)</td>
<td>GeneSpring MS for MassHunter WS</td>
<td>GeneSpring MS Reporting</td>
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<tr>
<td>6200 Series</td>
<td>Trap</td>
<td>Trap (.TEF)</td>
<td>MassHunter Qualitative Analysis</td>
<td>MassHunter Custom Reporting Excel 2003 Pro</td>
</tr>
</tbody>
</table>

AcqM ethR e p o rt
- Tune Report
- Qual Report
- Quant Report
- Bioconfirm
- EFC Report

GeneSpring MS
- MS Reporting
Agilent MassHunter Acquisition Software

Instrument Actuals/Status (configurable)

System Diagram

Real-Time Spec/Chro Display

Method Editor LC + MS

‘Flat’ User Interface
All Info At-A-Glance

Worklist (Sequence)

Autotune
Autotune and Calibration System
Built-in calibrant delivery system is unique to Agilent

Built-in calibrant delivery system used for fast auto-calibration

calibrant solutions

Triple (QQQ) Reporting (Excel) – tune
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Questions

MassHunter Workstation – Qualitative Analysis

• Compound-centric Data Mining & Data Navigation (Unique)
  • Find Compounds – Molecular Feature Extractor (MS level)
  • Find Compounds – Auto MS/MS
  • Find Compounds – Targeted MS/MS
  • Organize results in navigation tree and compound table
  • Dynamic link of compound information in navigation tree, chromatograms, spectra and tables

• Process and compare multiple data files (Unique)
  • Compare spectra and chromatograms between data files
  • Apply processing to multiple spectra, chromatograms, data files
  • Store all results including method parameters

• Superior algorithms (MFE + EFG)
• Flat user interface - All information ‘At-A-Glance’ (Unique)

=> Easy to Learn and Use, Highest Productivity!
MassHunter Workstation – Qualitative Analysis

- Nav Tree
- Compound Chromatograms
- Cmpd List
- Dockable Method Editor
- Compound Mass Spectra
- Mass List

Parameter-Free Integrator – Optimal for QQQ data

- All peak list level and peak level info available
- Visualization of baseline regression
- Handling of merged peaks
- Peak validation – Quality Factor and ‘Traffic Light Coloring’
- Accepted Peaks
- Discarded Peak
- Info messages
“MS-MS Integrator” (parameterless)

Parameter-less integrator w/ peak validation

- New parameter-less MSMS Integrator
  - does not have ANY parameters
  - discriminates between peaks and spikes
  - integrates correct from lowest to highest conc.
  - virtually eliminates manual integration
  - does NOT need smoothing

Performance over 4 orders of magnitude
Parameter-less integrator w/ peak validation

- New parameter-less MSMS Integrator validates Peak Quality based on
  - Peak height
  - Peak area
  - Peak width
  - Peak symmetry
  - Merged peaks to the right or left
  - Level of spikes on a peak
- Classifies peak quality as
  - Accepted
  - Inspect
  - Rejected
- Let’s you focus on peaks in question!

Qualitative Analysis – automated processing

#5 Data file automatically processed

... and integrates peaks in extracted chromatograms. Automatically extracts and integrates upon opening.
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MFE and EFG – fundamental algorithms

• Both algorithms are crucial for Agilent’s MassHunter SW
  • Used in several software applications for accurate mass LC/MS data
  • Very competitive and unique compared to other vendor’s offerings

• Molecular Feature Extractor (MFE)
  • Allows finding molecular features (= compounds) in an LC/MS run in the MS data domain (identify co-eluting and related mass signals)
  • Works on accurate mass MS data from TOF and Q-TOF.

• Empirical Formula Generator (EFG)
  • Allows determination of molecular formulas of unknown compounds using accurate mass TOF MS and Q-TOF MS/MS data.
  • Gets more specificity by using accurate mass MS/MS info (!)
  • Unique scoring algorithm
Molecular Feature Extractor (MFE) Software

List of all extracted molecular features defined and grouped by mass and retention time.

Detailed view of one molecular feature with all clusters, isotopes, measured accurate masses and mass errors.

Original and processed TIC.

2D plot of unprocessed and processed data by mass vs. retention time.

Processed and unprocessed MS spectra.

Persistent chemical background is removed and co-eluting interferences are resolved, while isotopic cluster recognition and grouping, charge state assignment and adduct recognition allows assignment of specific feature molecular weights while reducing data complexity and eliminating potential interferences.

Extraction of Molecular Features – Manual and automated analysis of complex mixtures

**Definition:** A feature is a discrete molecular entity defined by the combination of retention time and mass.

Extracted molecular feature from a complex natural product extract of Asian ginseng (Panax ginseng).
Contour plots of processed and unprocessed samples

2D contour plot of TOF spectrum of ginseng extract, retention time vs. mass.
A) Unprocessed 2D contour plot at retention time 24.8 – 28.0 min and mass 900 – 1250.
B) Contour plot around the unprocessed ginsenoside Rb1 ions.
C) and D) Contour plot of processed data.

Analysis of processed vs. unprocessed spectra for structure elucidation

A) Unprocessed mass spectrum at 26.3 min.
B) Processed mass spectrum at 26.3 min showing molecular features, which belong to the same group.

Structure of ginsenoside Rb1 obtained by interpretation of the processed mass spectrum and included molecular features.
Measured mass accuracies for ginsenoside Rb1

<table>
<thead>
<tr>
<th>Species</th>
<th>RT</th>
<th>m/z</th>
<th>Mass</th>
<th>Accuracy</th>
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<td>M+H+4</td>
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<tr>
<td>M</td>
<td>26.312</td>
<td>1108.6034</td>
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<td>-0.41 ppm</td>
</tr>
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</table>

Automated analysis of complex mixtures by molecular feature extraction

TIC of TOF MS:

Spiked Compound 2.9 min
Molecular formula generation report for spiked compound

<table>
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<tr>
<th></th>
<th>Formula</th>
<th>Mass, amu</th>
<th>Error, ppm</th>
<th>IRMS</th>
<th>Isotopic Match</th>
<th>Intensity, counts</th>
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<tr>
<td>2</td>
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<td>885.2965</td>
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<td>242</td>
<td>0</td>
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<td>4</td>
<td>C45H54N2O12S</td>
<td>886.2991</td>
<td>1.68</td>
<td>242</td>
<td>0</td>
<td>3.7e5</td>
</tr>
</tbody>
</table>

Automated molecular feature extraction - the proof of concept

XIC of +TOF MS: 869.3 to 869.3 amu Max. 3.7e5 cps

TOF MS, Mass accuracy -0.73 ppm, C₄₅H₅₄N₂O₁₂S
Automated Molecular Feature Extraction – Drug Screening

Clinical sample: Body fluids e.g. plasma, urine, CSF

Fast high throughput separation on 1200RR LC with alternating column regeneration of two 1.8 µm columns and TOF accurate mass measurement

Column switching under full temperature control

Two pumps for true alternating column regeneration

Data analysis with big pharmaceutical compound library

Report of identified pharmaceutical compounds - Identification of diuretic amiloride with 0.1 ppm.

Mass Profiling – A Powerful Workflow

Molecular Feature:
- a discrete molecular entity defined by the combination of retention time, mass and response in an LC/MS or GC/MS analysis
- typically represents a known or unknown compound

Mass Profiling:
- is used to find differences between sample sets
- originally developed for proteomics/metabolomics
- however can be applied to many analytical problems where finding differences between samples is important
- is done using statistical analysis

LC/MS or GC/MS Analysis
Find molecular features
Compare sample sets
Identification
Validation
See the difference - Comparison of molecular features by the Mass Profiler Software

The Mass Profiler operates on the extracted data files from MassHunter to let you investigate similarities and differences in features across multiple analysis by different plots and statistical methods.

Plot of mass vs. Retention time of a batch of BSA samples spiked with myoglobin and table for comparison.

Abundance Log/Log Plot of a batch of samples to identify up and down regulated components in the batches. Here myoglobin peptides in BSA.

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Questions
Metabolite ID the concept of Find and Confirm

<table>
<thead>
<tr>
<th>Compound Correlation</th>
<th>Isotopic Pattern Matching</th>
<th>Fragment Pattern Matching</th>
<th>EIC of expected masses</th>
<th>RAD Chrom.</th>
<th>UV Chrom.</th>
<th>Built-in Biotransf.</th>
<th>Metabolite Prediction</th>
<th>Molecular Formula assignment</th>
<th>Molecular structure elucidation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFE</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Table of Metabolites

- Find new metabolite candidate = add row to table
- Confirm existing metabolite candidate = add columns to table

MassHunter Metabolite ID – User Interface

- Overview table of identified Expected and unexpected Metabolites together with summary of algorithms and confirmation criteria.
- Dynamic Linking of Compound Info
- Metabolite Chros + Spectra
- Compound Correlation (Smpl/Ctr)
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MassHunter Workstation – Quantitative Analysis

• Batch-at-A-Glance View
  • Compound- OR Sample-centric orthogonal navigation (Unique)
  • Outlier flagging with high (red) and low (blue) (Unique)
  • Productivity tool: Calibration Curve Fit Assistant (Unique)

• Method Editor View
  • Method Task Editor allows to focus on sub-tasks
  • Import of compound info from acquisition method
  • Productivity tool: Concentration Setup Assistant (Unique)
  • Validation tool for quant method completeness (Unique)
  • Setup 20 different outlier criteria (Unique)

• Parameter-less integrator with built-in peak validation (Unique)
  • Does not require smoothing even for noisy data
  • Built-in integration validation allows focusing on problem peaks only

=> Easy to learn and use, unprecedented productivity for large multi-compound batches!
MassHunter Quant Analysis – Batch-At-A-Glance

- Filter by Sample Type
- Batch Table
- Context-located Tool strips
- Compound Information (Chroms + Specs)

View Results from Single or Multiple compounds

- Outlier Flagging + Filtering
- Sample- or Compound-Centric Orthogonal Navigation

Quantitative View - Method Editor

- Method Development Panel
- Components Panel
- Advanced Panel

- Context-located Tool strips
- Compound Information (Chroms + Specs)
Quantitative Analysis – CurveFit Assistant

Alternate choice

Try other curve types for better fits

Confirmation ions in Quantitative Analysis

THC retention time < 4.2 min.

D0 analyte quant

quant/qual overlay

D3 IStd quant

quant/qual overlay

THC Qualifier ion normalized
Calibration curve data point information

MassHunter Custom Reporting – Excel 2003 Pro

- Used across the MassHunter Workstation platform
  - Acquisition Method Report and Tune Reports
  - Qualitative Analysis Reports
  - Quantitative Analysis Reports
  - Metabolite ID Reports
- Output Flexibility
  - Always creates Excel report (can be password protected)
  - Optionally to printer and/or PDF
  - Optional e-mailing of reports
- Customization in familiar Excel environment
- Several levels of reporting customization
  - Comprehensive set of preconfigured report templates
  - Easy modification of report templates (add/remove elements, change header/footer, change precisions)
  - Import results from XML into LIMS systems
Easy Customization – via familiar Excel Features

- Add tables and graphics using custom tool buttons
- Drag and drop columns from XML results
- Use Conditional Formatting for Outlier Flagging
- Use Excel Number Formatting
- Print Preview
- Format Headers & Footers using Excel

Quantitation report

Quant Summary Report (STD)

Quant Calibration Report (STD)

Quant Sample Report (STD)
Summary – MassHunter Workstation
Meeting the needs

• Learning Curve
• Ease of Use
• Productivity in Data Processing
• One Software for all Mass Specs
• Highly Customizable Reporting
• Import/Export of Data and Results