Expanding Access to LC/MS to Everyone

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Software Product Manager
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Walkup LC/MS User Communities

**Medicinal Chemists**

Have I synthesized what I was supposed to?

Is my synthesized compound pure enough to use in the next step my company’s work (e.g., discovery libraries)?

**Biologists**

Have I expressed the correct protein (does it match the expected sequence)?

How does my protein/peptide/oligonucleotide compare to a reference?
Techniques available to answer those questions

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<td># of possible compounds</td>
<td>$\infty$</td>
<td>Hundreds</td>
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Technique abbreviations:
- MWD: Multiple Wavelength Detector
- DAD: Diode Array Detector
- SQ: Single Quadrupole
- TOF: Time of Flight
- Q-TOF: Quadrupole Time of Flight
What is the Benefit of Accurate Mass?

Confidence in Compound Identification!

Reserpine (C_{33}H_{40}N_{2}O_{9}) has a protonated ion at 609.28066

A single quad reports mass to +/- 0.1 = 165 ppm

Number of possible formulae using only C, H, O & N:

- 165 ppm: 209 possibilities
- 10 ppm: 13 possibilities
- 5 ppm: 7 possibilities
- 3 ppm: 4 possibilities
- 2 ppm: 2 possibilities

Accurate mass reduces risk of investing effort in the wrong molecule
Making these technologies accessible

A front-end to LC and LC/MS systems that allows investigators to submit samples without being experts in the technology

...with few instrument specialists who are responsible for multiple systems
Robust & Reliable Instrumentation

See more compounds in your sample!
- Choice of Ionization Sources: ESI, APCI, APPI or Multi mode (APCI+ESI)
- Fast Pos/neg switching for narrow UHPLC peaks
- Sensitive detection for low level components

Additional Features:
- Divert valve helps reduce contamination
- External tray for sample submission
- Automatically discard used vials

Robust and Reliable hardware you can trust
Feedback on MassHunter Walkup for LC/MS

“MassHunter Walkup makes our instruments “easy to use and hard to break”, says an Open Access Lab Administrator, at a major pharmaceutical company, where medicinal chemists and biologists come to MassHunter Walkup for LC/MS of small and large molecule early drug discovery in a large open-access environment. “MassHunter’s ease of use helps us bring complex assays done by experts to a Walkup method.”

“…offered the possibility to accept [protein] sequences by the end user at the time of sample submission, run through the data analysis and produce an annotated report automatically…we produce hundreds of [protein] sequences a year and that really removes a potential point-of-failure and stress point in our analytical workflow…huge time savings…usage has exploded! We [saved] more than 10 FTE weeks a year.”

ASMS 2014 talk on BioCompare website:
http://cnpg.comparenetworks.com/163768-Open-Access-Intact-Protein-LC-MS-in-a-Recombinant-Protein-Laboratory/
Intact Protein Analysis – the Manual Process

- **Data Acquisition**
- **MS Spectrum Deconvolution**
- **Zero-charge Mass Spectrum**
- **Compare Observed Mass with Expected**
- **Determine Post Translational Modifications**

- **Sample Preparation and Submission**
- **Reporting**

**Intact Protein Analysis Examples**
- 2\*G0F (NGA2F) (+1445.3580)
- 2\*G1F (+1607.5013)
- 1\*G1F/G2F (+3377.1458)
Intact Protein Analysis – the Automated Process

- Data Acquisition
- MS Spectrum Deconvolution
- Zero-charge Mass Determination
- Sequence Matching
- Determine Post Translational Modifications
- Reporting
- Delivery of Results

- 2*G0F (NGA2F) (+1445.3580)
- 2*G1F (+1607.5013)
- 1*G1F/G2F (+3377.1458)

Sample Preparation
Upload Sequence
Automation for Peptide Mapping

Data Acquisition → Compound Extraction → Sequence Matching → Sequence Coverage

- **Upload Sequence**
- **Sample Preparation**
- **Enzymatic Digestion**

Determine Post-Translational Modifications

- **2*G0F (NGA2F)** (+1445.3580)
- **2*G1F** (+1607.5013)
- **1*G1F/G2F** (+3377.1458)

Reporting

Delivery of Results

Agilent Technologies
Synthetic Compound Verification – the Automated Process

- Chromatographic Separation
- Peak Detection
- Assess Peak MS Spectra

608.273376 + 1
v.
609.2771

Compare expected monoisotopic mass to observed m/z considering adducts

m/z = 608.273376

Upload expected formula or molecular weight

Sample Preparation

Reporting

Delivery of Results
MassHunter Walkup: 3 Step Sample Submission

1

Enter username and number samples to be run.
Workflows eliminate unnecessary fields and mouse clicks

Page 1: Choose Workflow

Page 2: Fields change to reflect workflow selected
- Examples: Formula, Protein Sequence, Modifications, Dilution Factor
Sample Meta Data Written to Data File

<?xml version="1.0"?>
<Sample>
  <Version>2</Version>
  <Name>PFI_1234</Name>
  <Amount>0</Amount>
  <Multiplier>1</Multiplier>
  <Dilution>1</Dilution>
  <Description>Walkup method: 'Quick Confirm'
  Custom1: C12H14N4O25
  Custom2: 278.0837
  Custom3: 88
  Custom4: G10_B123
  Custom5: 14-Nov-09</Description>
  <ISTDs />
  <TargetMasses />
</Sample>
MassHunter Walkup: 3 Step Sample Submission

1. **Login**
   - Enter username and number samples to be run.

2. **Choose Method**
   - Enter sample information and select from a list of available methods.
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   Place sample in the position as directed by the software.
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4. **Receive Report**

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**Agilent Technologies**
New Ribbon
MassHunter Walkup C.02.01 for Sample Submitters

Non-sample submission actions
Status

Autosampler Tray

Queue shows priority and event-scheduled (e.g., clean up) samples

Time remaining in Queue
Sample Reprocessing

Common problem is incorrect formula or protein sequence entered, user wants to reprocess without rerunning

1. Select Reprocess
2. Enter search Criteria
3. Select Samples and modify input
Getting Results:
Email, Analytical Studio Reports, Raw Data or paper print-outs

Can e-mail:
• Reports from ChemStation, MassHunter – including BioConfirm, Analytical Studio Reviewer or Intelligent Reporter
• ChemStation or MassHunter Raw Data (*.D)
• Analytical Studio Reviewer file (*.asr)
Or Use Analytical Studio Reviewer to Browse Results at Chemist’s Desk
Benefits of MassHunter Walkup for the Sample Submitter

• Access to LC/MS capabilities without needing to be an expert in LC/MS

• Control over sample preparation and treatment and storage

• Many applications possible
  - Sample Purity
  - Compound verification
  - Protein Confirmation
  - Protein Coverage

• Consistent data analysis and spectral deconvolution allows for greater confidence during comparative studies
  - Example: pseudo 1st order rate constants
New Walkup Administration

Logical navigation for system setup

Common Tasks on Toolbar

Import/Export
Administration – One location for Method Setup

Brings up ChemStation (and ASR) method for reviewing/editing – no need to exit and go to ChemStation
Creating a Workflow to Identify Custom Columns
Administration: Verify Method Changes

Double clicking error takes you to area needing editing.
Remote Administration — Taking an Instrument Offline

- Administrator receives e-mail or SMS message that instrument needs attention
  Goes to his/her computer and places system offline

Existing queue completes, no new submission possible
Benefits of MassHunter Walkup for the Administrator

• View Instrument status
• Add/remove/modify users and update methods from any network computer
• Automatic notification of instrument errors via email or text (SMS)
• Remotely take an instrument offline (e.g., for maintenance)
  • Existing queue finishes
  • No new samples can be submitted
• Facilitated by OpenLAB Shared Services
OpenLAB Shared Services
Manage MassHunter and OpenLAB-controlled instruments

Administrator

Server

MassHunter Workstation Software
Qualitative Analysis
Version B.07.00
Agilent Technologies, Inc. 2014
www.agilent.com/chem
Agilent Technologies
MassHunter Walkup: Technology Progression

- **2012**: EasyAccess B.05.0x
- **2013**: Walkup C.01.00
- **2014**: Walkup C.01.01
- **2015**: Walkup C.02.01
- **2016**
MassHunter Walkup

Maximize uptime

Minimize training costs

Manage many systems remotely

Ensure quick response times

Eliminate human errors

Ensure consistent results
Walkup Method Development
5991-2868
APPENDIX
More on Information pMod Deconvolution

- Reduction of artifacts
- Spectral data that does not fit the model is rejected as noise
- Improved mass resolution in mixtures
- Uncertainty is represented as peak width