



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)?

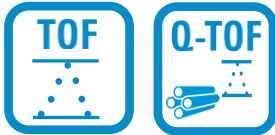


Biologists

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

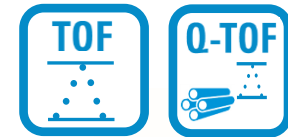
How does my protein/peptide/oligonucleotide compare to a reference?

Techniques available to answer those questions



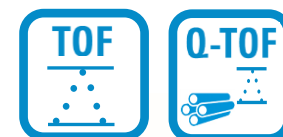
Technique	UV-based detection	Single Quadrupole (SQ)	Time-of-Flight (TOF/Q-TOF)
Mass Accuracy	N/A	Unit (165 ppm)	Accurate (1 ppm)

Techniques available to answer those questions



Technique	UV-based detection	Single Quadrupole (SQ)	Time-of-Flight (TOF/Q-TOF)
Mass Accuracy	N/A	Unit (165 ppm)	Accurate (1 ppm)
Application(s)	Sample Purity	Compound Verification	Discovery Protein Expression Verification

Techniques available to answer those questions



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Mass Accuracy	N/A	Unit (165 ppm)	Accurate (1 ppm)
Application(s)	Sample Purity	Compound Verification	Discovery Protein Expression Verification
# of possible compounds	∞	Hundreds	Single digits

What is the Benefit of Accurate Mass?

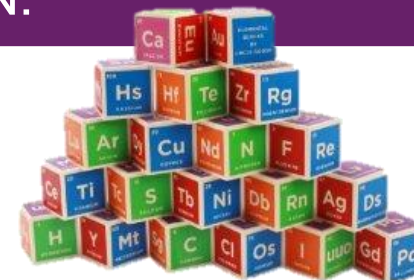
Confidence in Compound Identification!

Reserpine ($C_{33}H_{40}N_2O_9$) has a protonated ion at 609.28066

A single quad reports mass to $\pm 0.1 = 165$ ppm

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

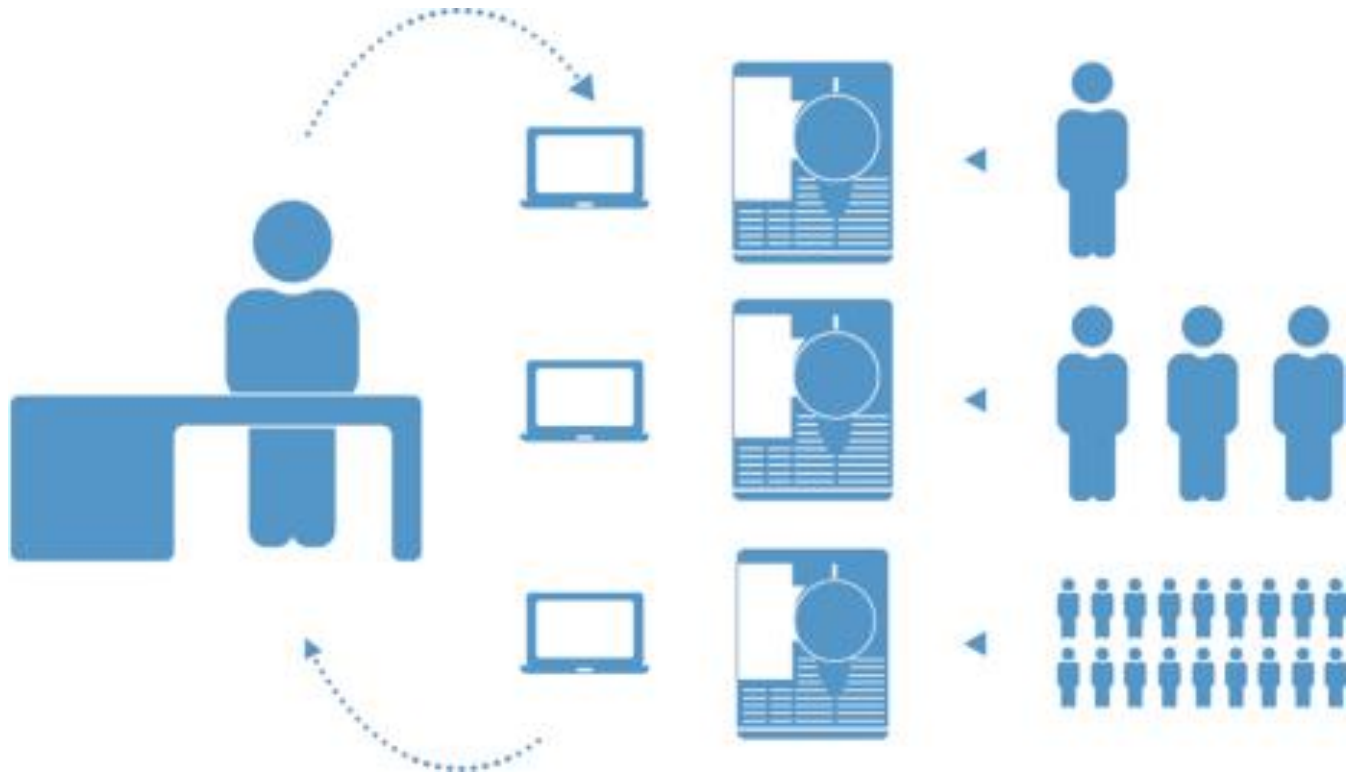
- | | |
|-----------|-------------------|
| • 165 ppm | 209 possibilities |
| • 10 ppm | 13 |
| • 5 ppm | 7 |
| • 3 ppm | 4 |
| • 2 ppm | 2 |



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

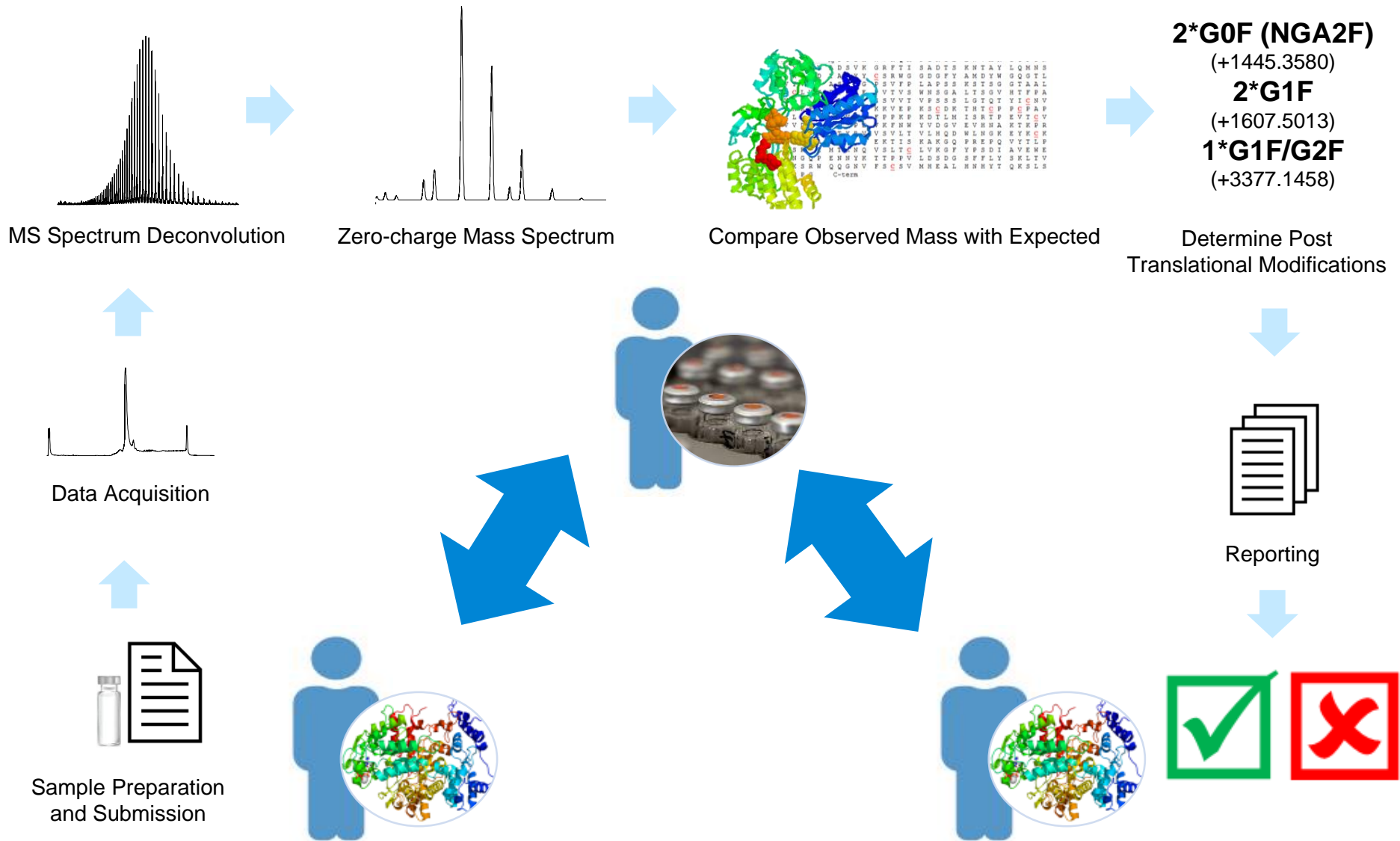
“MassHunter Walkup makes our instruments “**easy to use and hard to break**”, says an Open Access Lab Administrator, at a major pharmaceutical company, where medicinal chemists and biologists come to MassHunter Walkup for LC/MS of small and large molecule early drug discovery in a large open-access environment. “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.**”

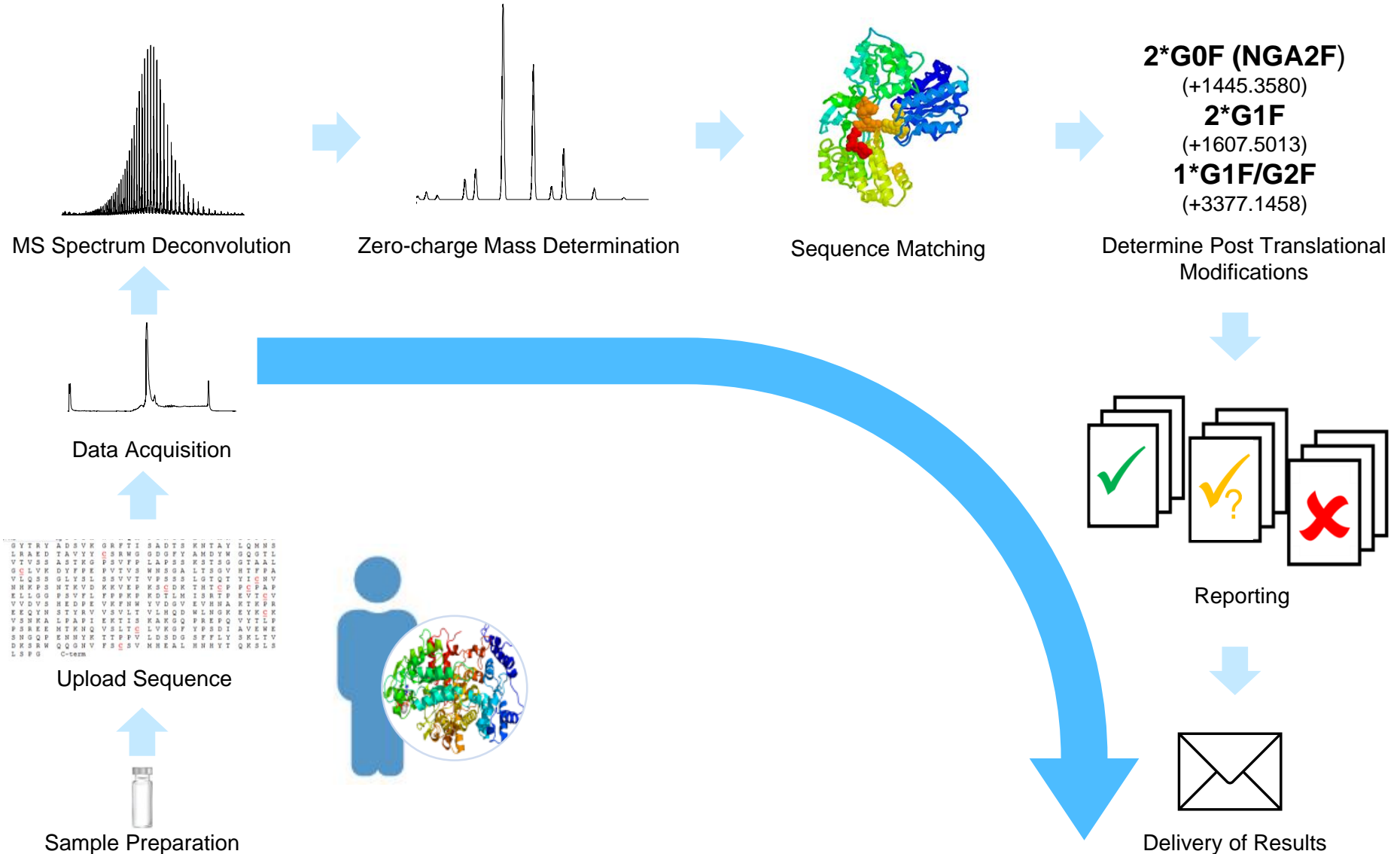
ASMS 2014 talk on BioCompare website:

<http://cnpg.comparenetworks.com/163768-Open-Access-Intact-Protein-LC-MS-in-a-Recombinant-Protein-Laboratory/>

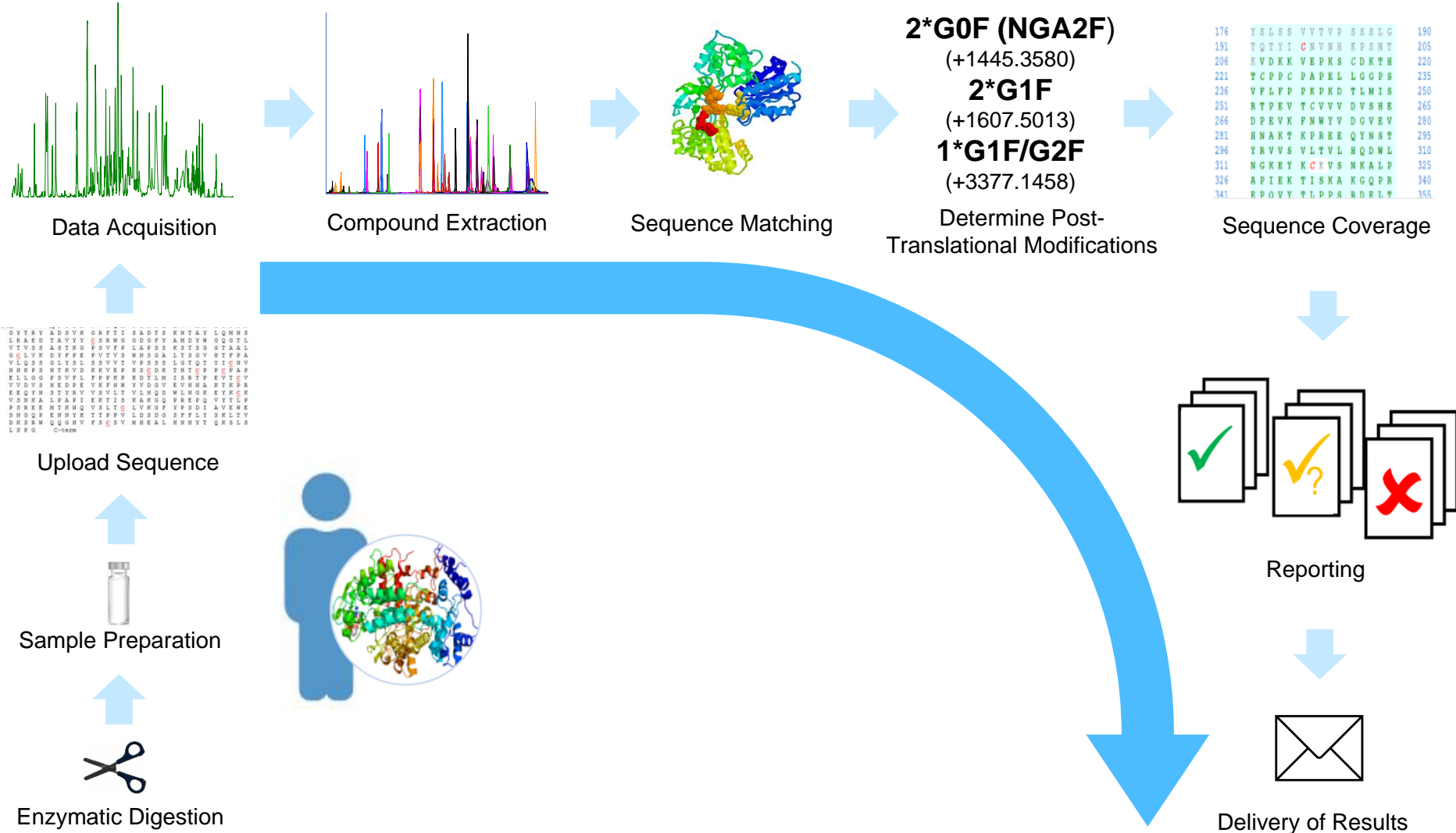
Intact Protein Analysis – the Manual Process



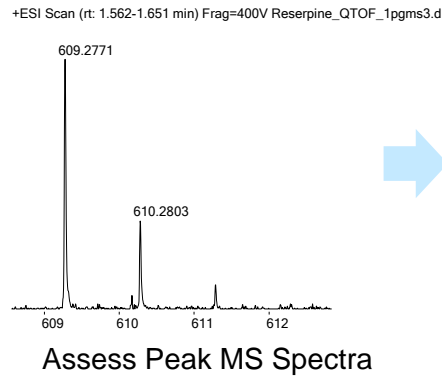
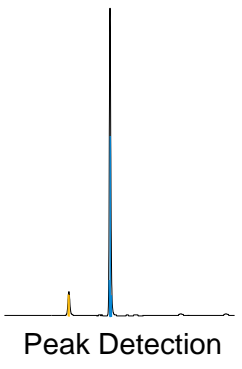
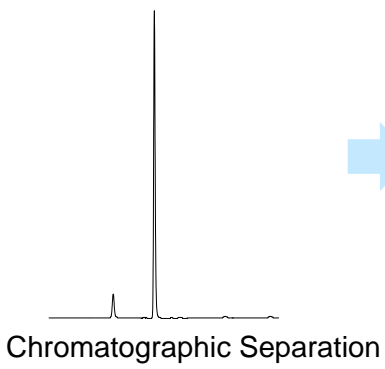
Intact Protein Analysis – the Automated Process



Automation for Peptide Mapping

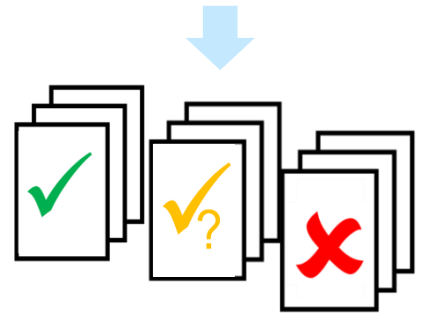


Synthetic Compound Verification – the Automated Process

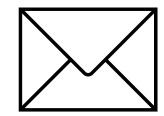


608.273376 + 1
v.
609.2771

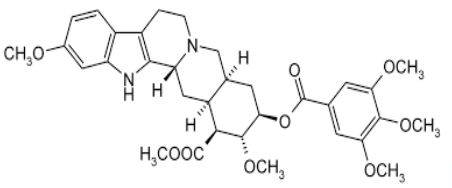
Compare expected monoisotopic mass to observed m/z considering adducts



Reporting



Delivery of Results



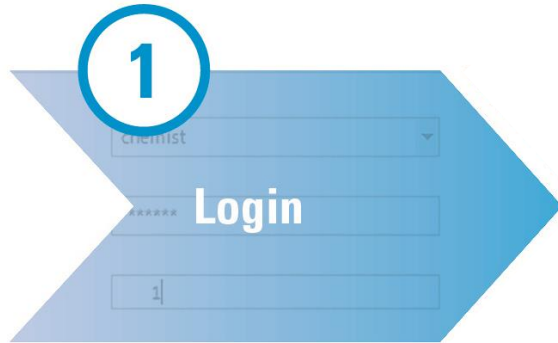
m/z = 608.273376

Upload expected formula or molecular weight



Sample Preparation

MassHunter Walkup: 3 Step Sample Submission



Enter username and number samples to be run.

Workflows eliminate unnecessary fields and mouse clicks

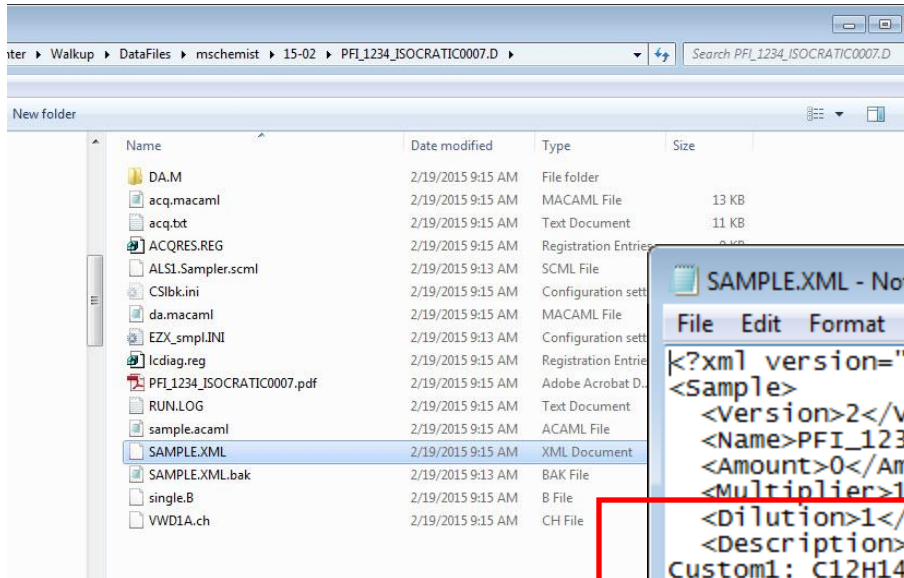
Page 1: Choose Workflow

	Notebook ID	Cutoff	Walkup Method	Sample Priority
1	batch41	0.2	Fast	No
2	batch42	0.2	Fast	No

	Notebook ID	Formula	Walkup Method	Sample Priority
1	compoundX	C6H12O6	Fast	Normal

Page 2: Fields change to reflect workflow selected
- Examples: Formula, Protein Sequence, Modifications, Dilution Factor

Sample Meta Data Written to Data File



```
SAMPLE.XML - Notepad
File Edit Format View Help
<?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: C12H14N4O2S
  Custom2: 278.0837
  Custom3: 88
  Custom4: G10_B123
  Custom5: 14-Nov-09</Description>
  <ISTDs />
  <TargetMasses />
  <RefDataFilePath></RefDataFilePath>
  <ACQMethodPath>C:\Chem32\1\Methods\_ISOCRATIC.M</ACQMethodPath>
  <DAMethodPath>C:\Chem32\1\Methods\_ISOCRATIC.M</DAMethodPath>
</Sample>
```

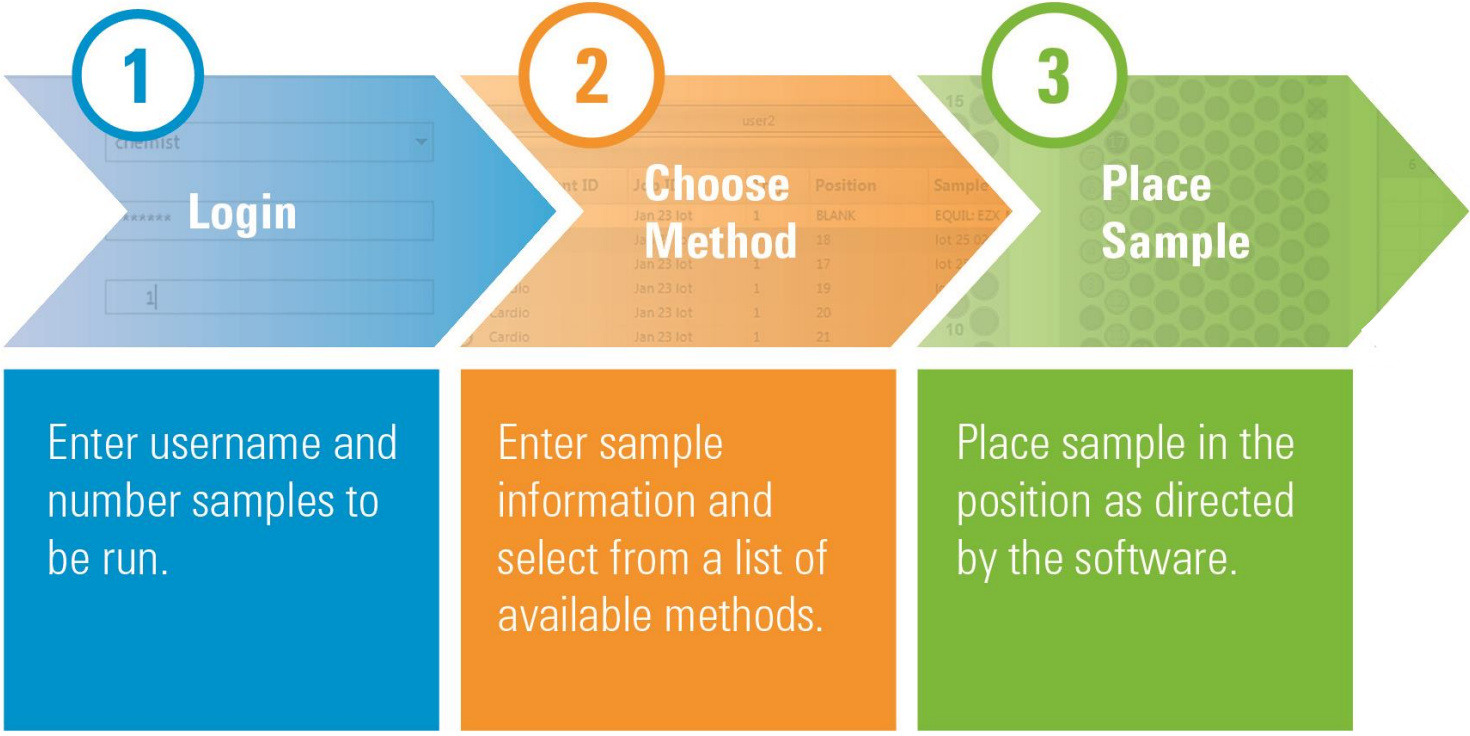

MassHunter Walkup: 3 Step Sample Submission



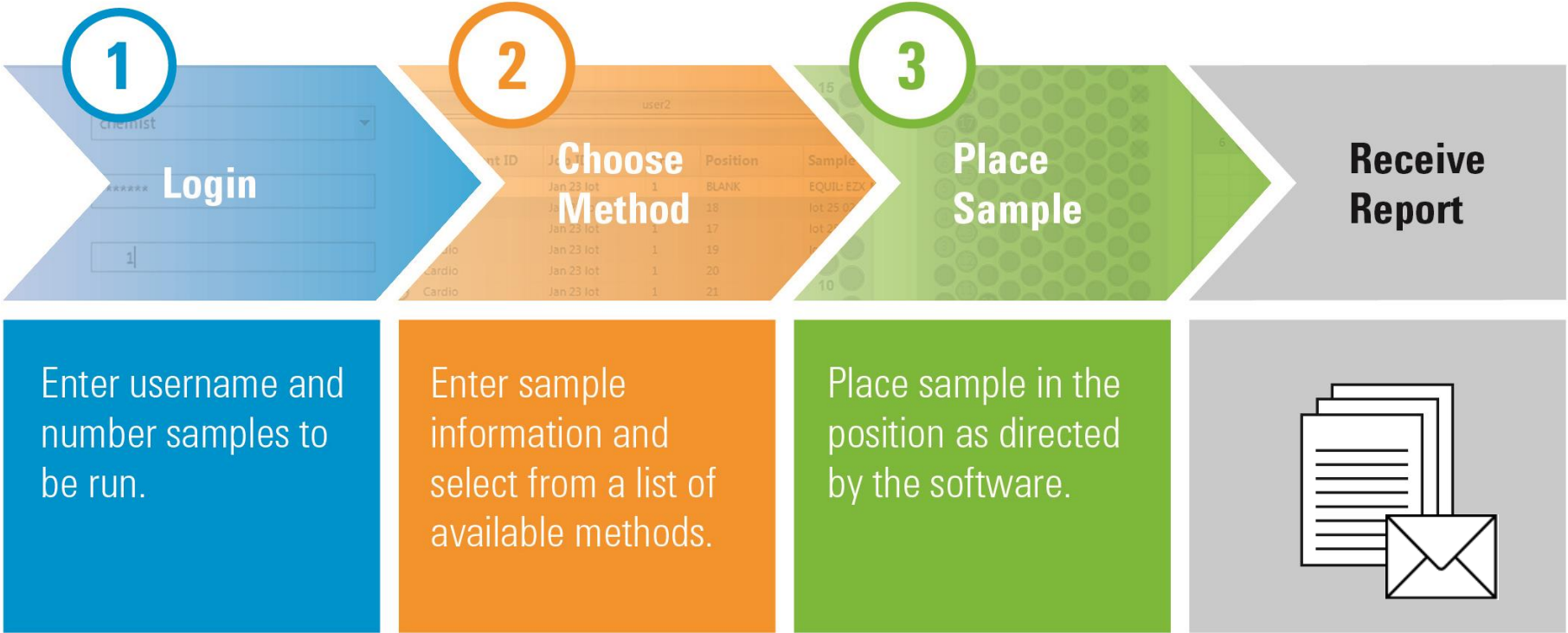
Enter username and number samples to be run.

Enter sample information and select from a list of available methods.

MassHunter Walkup: 3 Step Sample Submission



MassHunter Walkup: 3 Step Sample Submission



New Ribbon

MassHunter Walkup C.02.01 for Sample Submitters

Time remaining
in Queue

Non-sample
submission
actions
Status

The screenshot displays the Agilent MassHunter Walkup Console interface. At the top, the title bar reads "Agilent MassHunter Walkup Console". Below this is a "Tools" ribbon with several functional groups: "Administrative Tools" (Administration, Change Password), "Samples Management" (Clear Sampler Tray, Abort Run, Reprocess Sample), "Instrument" (Standby), and "Help" (View Help, About Help). A "QueueStatus" field shows "9 mins 30 sec" and a "QueueRuntime" field is also present.

Below the tools are five status panels: "Walkup Queue" (Running), "Walkup System" (Run is about to end), "ChemStation" (Data acquisition), "Instrument" (Running), and "Injector" (Inj. Done).

The main area is divided into three sections: "Walkup MS" (External Tray and T1:100 Vials), "mschemist" (a table of sample data), and "Current Run Number: 117".

The "External Tray" shows a vertical column of 18 vials, with vials 1, 10, 15, and 16 highlighted in green, and vial 18 marked with a red 'X'. The "T1:100 Vials" section shows a 10x10 grid of vials, with vial 1 highlighted in blue and vials 11, 21, 31, 41, 51, 61, 71, 81, and 91 marked with red 'X's.

The "mschemist" table contains the following data:

	Sample Name	Sample Position	Completion Time	Formula	Sample Time (min)	Injection Volu
1	compound1	1	1:11 PM	C8H12N2O2		2.5
2	form34z4	6	1:16 PM	C12H16O2		3
3	alpha23	3	1:18 PM		0	2.5
4	compound2	2	1:19 PM	C8H12N3O		2.5
5	alpha24	4	1:20 PM		5	2.5
6	alpha25	5	1:21 PM		30	2.5

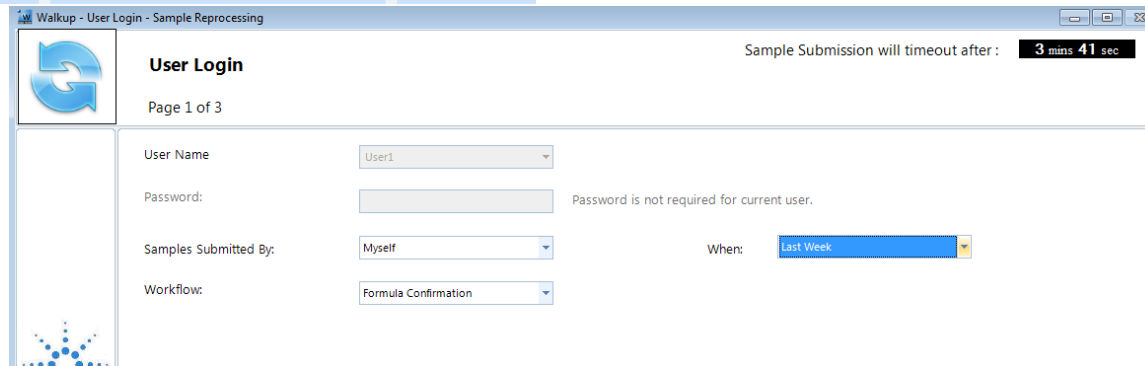
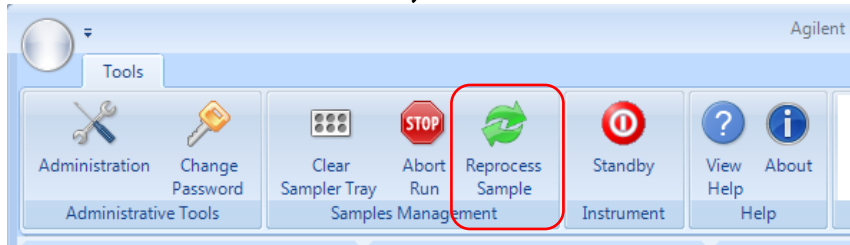
At the bottom of the interface is a "Submit Samples" button with a green play icon on the left and a green stop icon on the right.

Autosampler
Tray

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

Sample Reprocessing

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

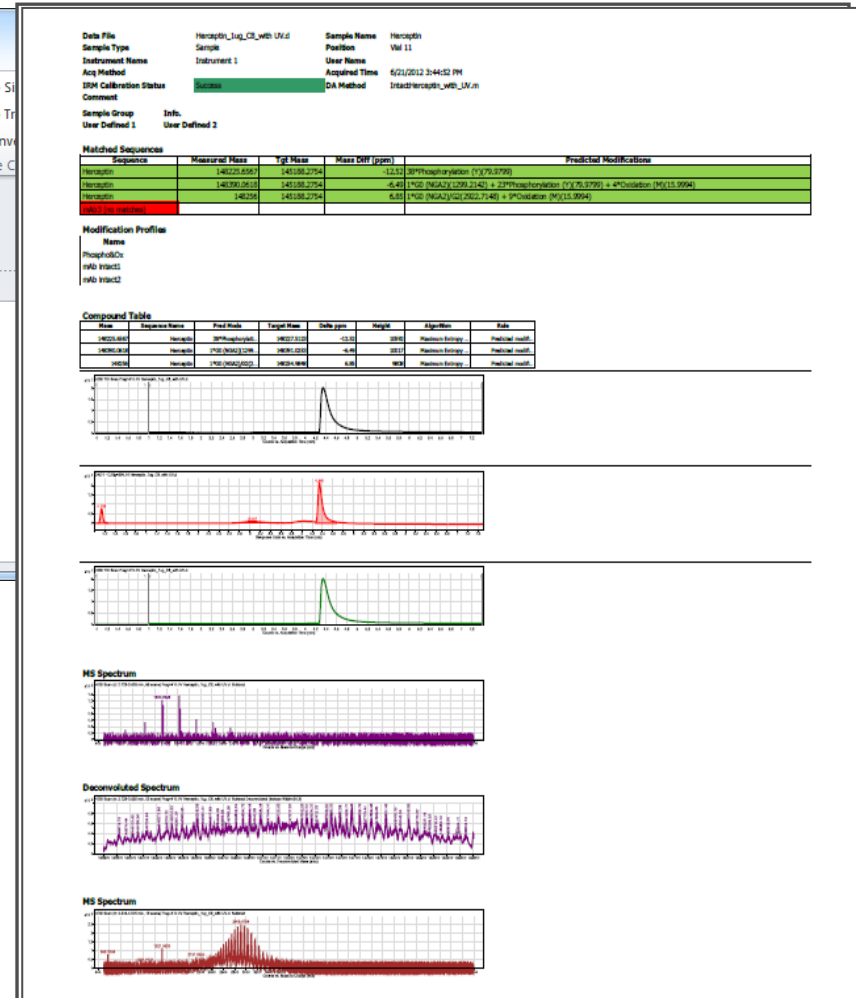
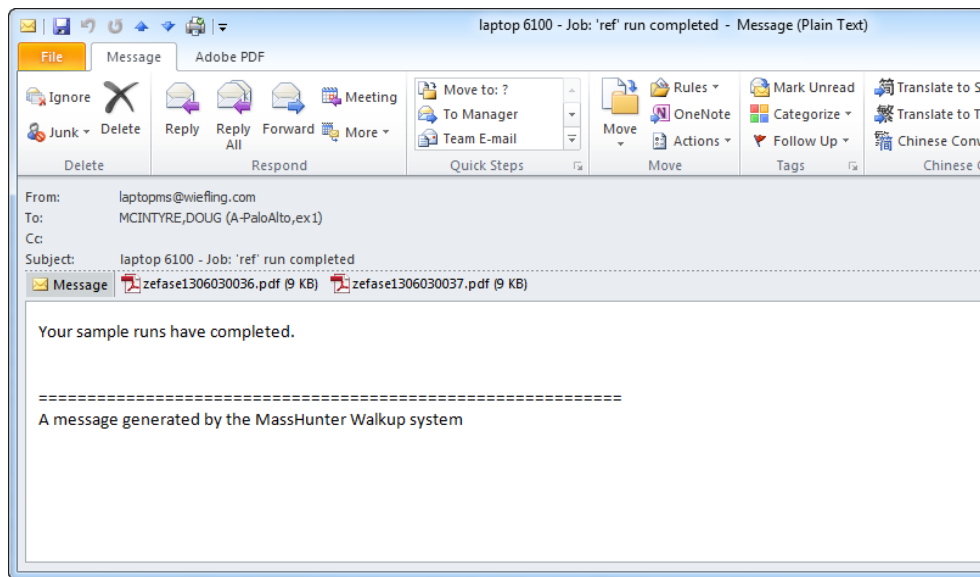


1. Select Reprocess
2. Enter search Criteria
3. Select Samples and modify input

Select	ID	MethodName	UserName	DataFile	StartTime	JobId	Formula
1	9781600051852	Fast	User1	978160005185...	07/31/2014	140730-165004	C6H12O6
2	1234	Fast	User1	1234201407300...	07/30/2014	140730-101732	C6H12

Getting Results:

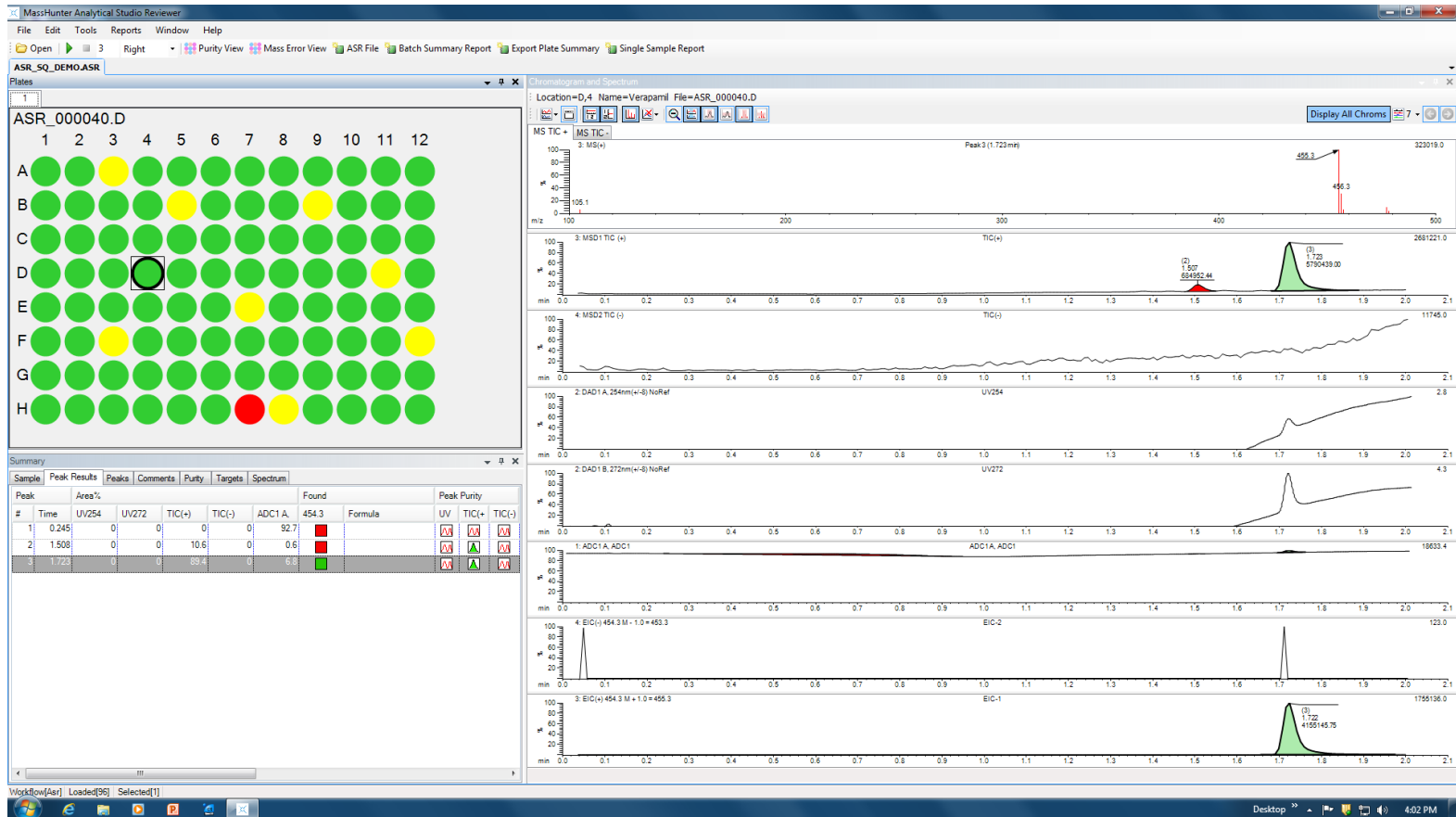
Email, Analytical Studio Reports, Raw Data or paper print-outs



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



pMod – An Advanced Protein Deconvolution Algorithm with Automated Peak Modeling for Charge Deconvolution of Mass Spectrometry Data

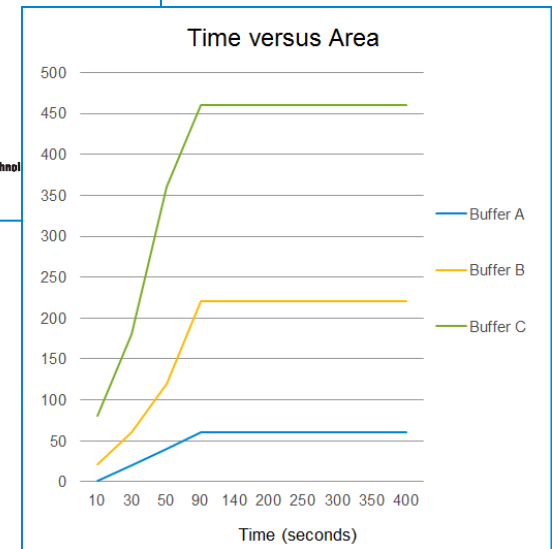
Application Note

Authors

Ning Tang, Xiaodong Wu, and Stephen Madden
Agilent Technologies, Inc.
Santa Clara, CA, USA

Introduction

The benefits of the widely-used maximum entropy deconvolution algorithm include transforming a multiple-charged spectrum into a zero-charged mass spectrum and significantly reducing the complexity of the results. However, this method can introduce many artifacts. To improve the deconvolution algorithm and deliver the most probable results that are virtually free of artifacts, Agilent Technologies has developed the peak modeling deconvolution (pMod) algorithm, an advanced data processing methodology. This application note describes the pMod method, compares it to the maximum entropy deconvolution methodology, discusses precision of zero-charge mass measurement, and shows enhanced resolution of protein peaks for precise analysis.



New Walkup Administration

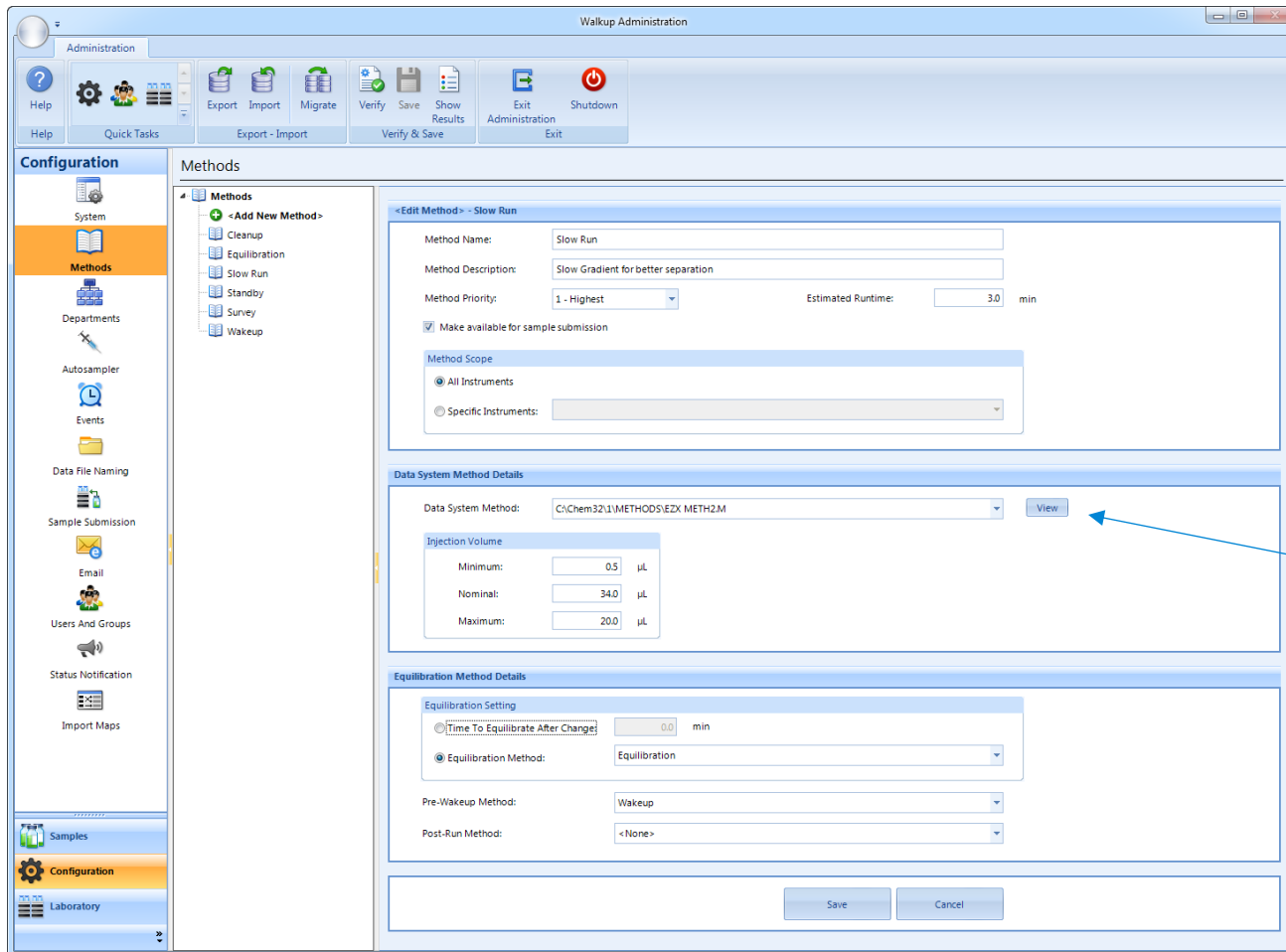
Import/Export

Common Tasks
on Toolbar

Logical
navigation for
system setup

The screenshot displays the 'Walkup Administration' application window. At the top, a toolbar contains icons for Help, Quick Tasks, Export-Import (Export, Import, Migrate), Verify & Save (Verify, Save, Show Results), Administration (Exit), and Shutdown. Below the toolbar is a 'Configuration' section with a left-hand navigation pane listing: System, Methods, Departments, Autosampler, Events, Data File Naming, Sample Submission, Email, Users And Groups, Status Notification, and Import Maps. The main configuration area is titled 'System - # indicates configuration applicable on Walkup restart; * indicates configuration applicable on queue empty.' and is divided into several sections:
1. **Startup#**: Includes checkboxes for 'Allow resizing of Walkup Console window', 'Center Walkup Console window', 'Secure Mode On', 'Hide Data System', 'Allow Clear Sampler Tray menu item in Walkup Console', and 'Allow tray to be cleared even when there are pending, running or incomplete samples'.
2. **System**: Includes checkboxes for 'Put instrument on standby when queue is paused', 'Delete completed sample records after 60 days', 'Generate Job Summary Report', and 'Enable Email Notification'.
3. **Sample Submission Settings**: Includes checkboxes for 'Allow automatic user registration, adding them to group', 'Allow samples to be submitted from import file', 'Cancel submission if no activity after 5 minutes', 'Restrict methods by user group permissions', and 'Save values for next submission'. A note states: 'On the last page of sample submission, automatically submit samples if no activity after above time-out'.
4. **Sample Priority***: Contains two sub-sections:
- **Sample Priority Settings**: Includes checkboxes for 'Allow high priority samples', 'Allow delayed samples', 'Only after (time) 1:00 pm move sample one at a time.', 'Allow plates to be submitted', 'Plates are submitted at Normal Priority', and 'Prioritize vials before plates'.
- **Queue Sorting Settings**: Includes a 'Sort by:' dropdown set to 'Maximum sharing', and three 'Sort by:' dropdowns set to 'MethodPriority', 'NA', and 'NA'.
At the bottom of the window, a navigation bar shows 'Samples', 'Configuration' (highlighted), and 'Laboratory'.

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

Configuration

- System
- Walkup Methods
- Departments
- Autosampler
- Events
- Data File Naming
- Sample Submission
- Workflows**
- Email

Workflows

- Walkup Workflows
 - + <Add New Workfl
 - Clearance
 - Compound Confir
 - CS - Compound Co
 - Permeability
 - Auto
 - Quant

Edit Workflow - Pfizer

Workflow Name:

Workflow Description:

Instrument Scope

All Instruments

Specific Instruments:

Workflow Columns

	Column Display Name	Column Type	Mandatory	Custom Values
1	▶ Formula	Custom Column	<input checked="" type="checkbox"/>	
2	Exact Mass	Custom Column	<input type="checkbox"/>	
3	Purity	Custom Column	<input type="checkbox"/>	
4	Location	Custom Column	<input type="checkbox"/>	
5	Last Check	Custom Column	<input type="checkbox"/>	
*	Click here to add new column in Workflow.		<input type="checkbox"/>	

Administration: Verify Method Changes

Administration

Verify and save - Allows to verify and save configuration settings.

Configuration	Verification Result
1 System	System configuration is valid.
2 Methods	In Walkup method "Cleanup", for assigned data system method "C:\Chem32\1\METHODS\EZX_CLEANM", report destination "File" is not selected. However, "Email" configuration is set to send following report formats through email: pdf
3 Methods	In Walkup method "Equilibration", for assigned data system method "C:\Chem32\1\METHODS\EZX_EQULM", report destination "File" is not selected. However, "Email" configuration is set to send following report formats through email: pdf
4 Methods	In Walkup method "Standby", for assigned data system method "C:\Chem32\1\METHODS\EZX_STANDBY.M", report destination "File" is not selected. However, "Email" configuration is set to send following report formats through email: pdf
5 Methods	In Walkup method "Wakeup", for assigned data system method "C:\Chem32\1\METHODS\EZX_WAKEUP.M", report destination "File" is not selected. However, "Email" configuration is set to send following report formats through email: pdf
6 Methods	Methods configuration is valid.
7 Autosampler	Auto Sampler configuration is valid.
8 Events	The "100 Vials Tray" field cannot be empty for Event : 1
9 Events	The "40 2mL Half Tray" field cannot be empty for Event : 1
10 Events	The "15 6mL Half Tray" field cannot be empty for Event : 1
11 Events	The "2WellPlus10VialsTray" field cannot be empty for Event : 1
12 Departments	Departments configuration is valid.
13 Data File Naming	Data File Naming configuration is valid.
14 Sample Submission	Sample Submission configuration is valid.
15 Email	Email configuration is valid.
16 Users And Groups	Users & Groups configuration is valid.
17 Status Notification	Status Notification configuration is valid.
18 Import Maps	Import Maps configuration is valid.
19 Walkup Administration	Configuration validation errors need to be resolved before they can be saved.

4 Errors

5 Warnings

10 Messages

4 Errors

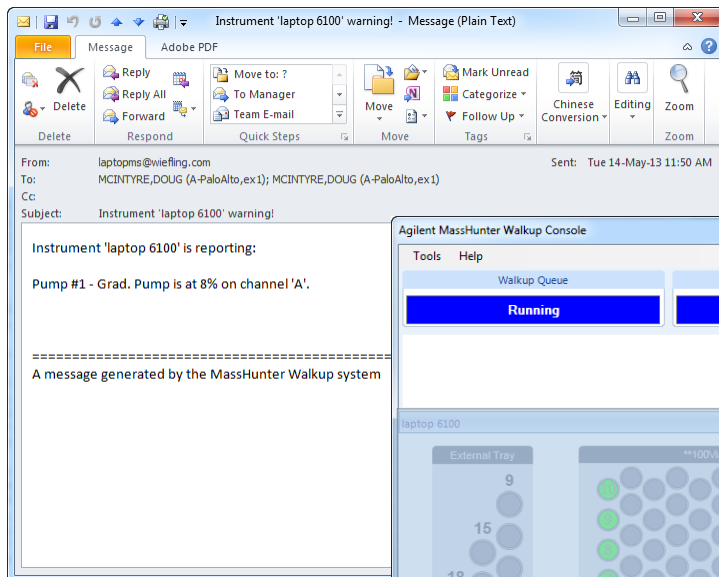
5 Warnings

10 Messages

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



Agilent MassHunter Walkup Console

Tools Help

Walkup Queue: Running

Walkup System: 1.25 mins left in run

ChemStation: Data acquisition

Instrument: Running

Injector: Inj. Done

Queue Runtime: 6 mins

laptop 6100 EZX-METH1.M chemist Current Run Number: 13

Department ID	Job ID	Tray	Position	Sample Name	Retain	Method	Data File	User Name
1	Cardio	lot 25	1 11	sample46	Yes	Survey	sample4613051...	chemist
2	Cardio	lot 25	1 12	sample47	Yes	Survey	sample4713051...	chemist
3	Cardio	lot 25	1 13	sample48	Yes	Survey	sample4813051...	chemist

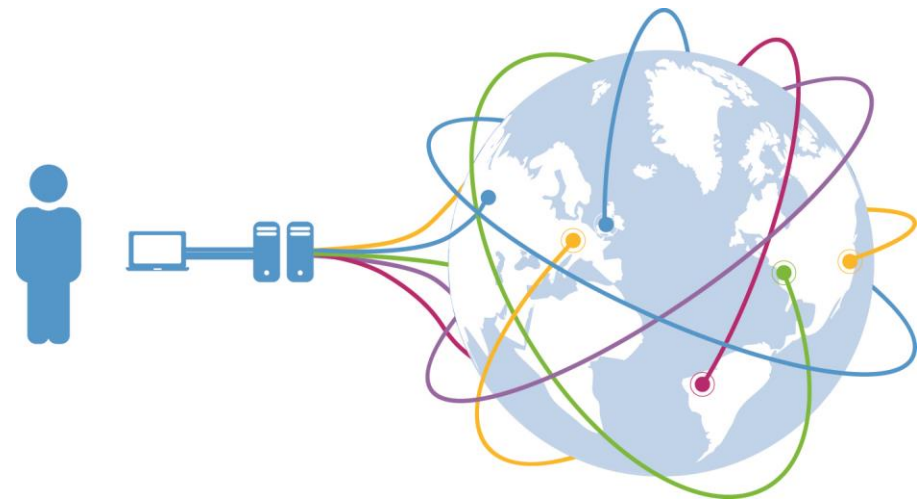
Maintenance scheduled...No new samples

Submit Samples

Existing queue completes, no new submission possible

Benefits of MassHunter Walkup for the Administrator

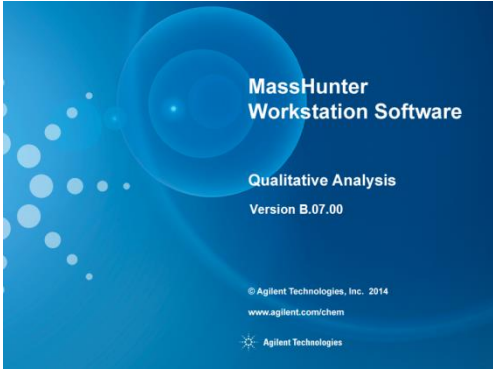
- View Instrument status
- Add/remove/modify users and update methods from any network computer
- Automatic notification of instrument errors via email or text (SMS)
- Remotely take an instrument offline (e.g., for maintenance)
 - Existing queue finishes
 - No new samples can be submitted
- Facilitated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



OpenLAB Shared Services

Manage MassHunter and OpenLAB-controlled instruments

Administrator



Agilent OpenLAB



MassHunter Walkup: Technology Progression

2012

2013

2014

2015

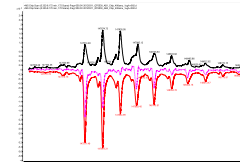
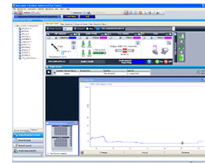
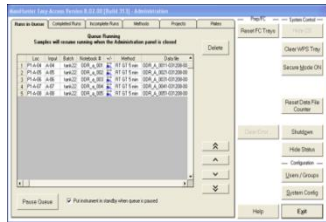
2016

EasyAccess
B.05.0x

Walkup
C.01.00

Walkup
C.01.01

Walkup
C.02.01



MassHunter Walkup

Maximize uptime



Minimize training costs



Manage many systems remotely



Ensure quick response times



Eliminate human errors



Ensure consistent results



Application Notes



LC Walk-up System Using the Agilent 1200 Infinity Series LC Method Development Solution and Agilent MassHunter Walkup Software

Test of reaction kinetics, column scouting, and impurity checks with one LC system and up to eight columns

Application Note

Drug Development

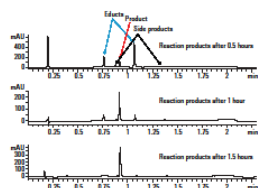
Authors

Angelika Gratzfeld-Hüsgen and Michael Frank
Agilent Technologies, Inc.
Waldbronn, Germany

Abstract

In chemical synthesis laboratories, different users are involved in the synthesis of new compounds. Typically, different projects are running for several groups or single users. The needs and analytical tasks for liquid chromatography in combination with mass spectrometers are:

- In-time reaction monitoring
- Control of reaction products
- Column scouting before preparative LC
- Purity analysis after preparative LC
- Fast and easy access to a running LC system
- Availability of columns of different selectivity
- Predefined chromatographic methods
- Fast reporting if sample has been analyzed

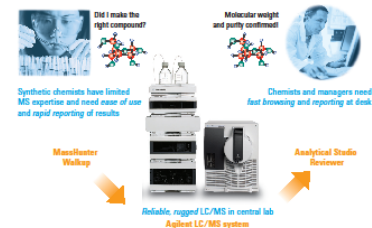


Walkup LC/MS Analysis

Software tools to enable chemists to easily confirm compound synthesis and sample purity

Technical Overview

Ensuring the success of any chemical synthesis requires the use of a variety of analytical tools including sophisticated instruments such as mass spectrometry and NMR. These techniques are routinely available to nonexpert users in academic, pharmaceutical, and industrial laboratories in support of their research goals. Agilent has become a leading provider of walkup LC/MS instrumentation including both single quadrupole and high resolution TOF instruments. The key to their widespread adoption has been the development of high performance, robust, and reliable instrumentation that is fully enabled with flexible software tools. These systems routinely provide information to confirm chemical syntheses, identify impurities, and isolate target fractions. The Agilent MassHunter Walkup Software also supports single and multiple instrument configurations that can adapt and scale to changing user requirements.

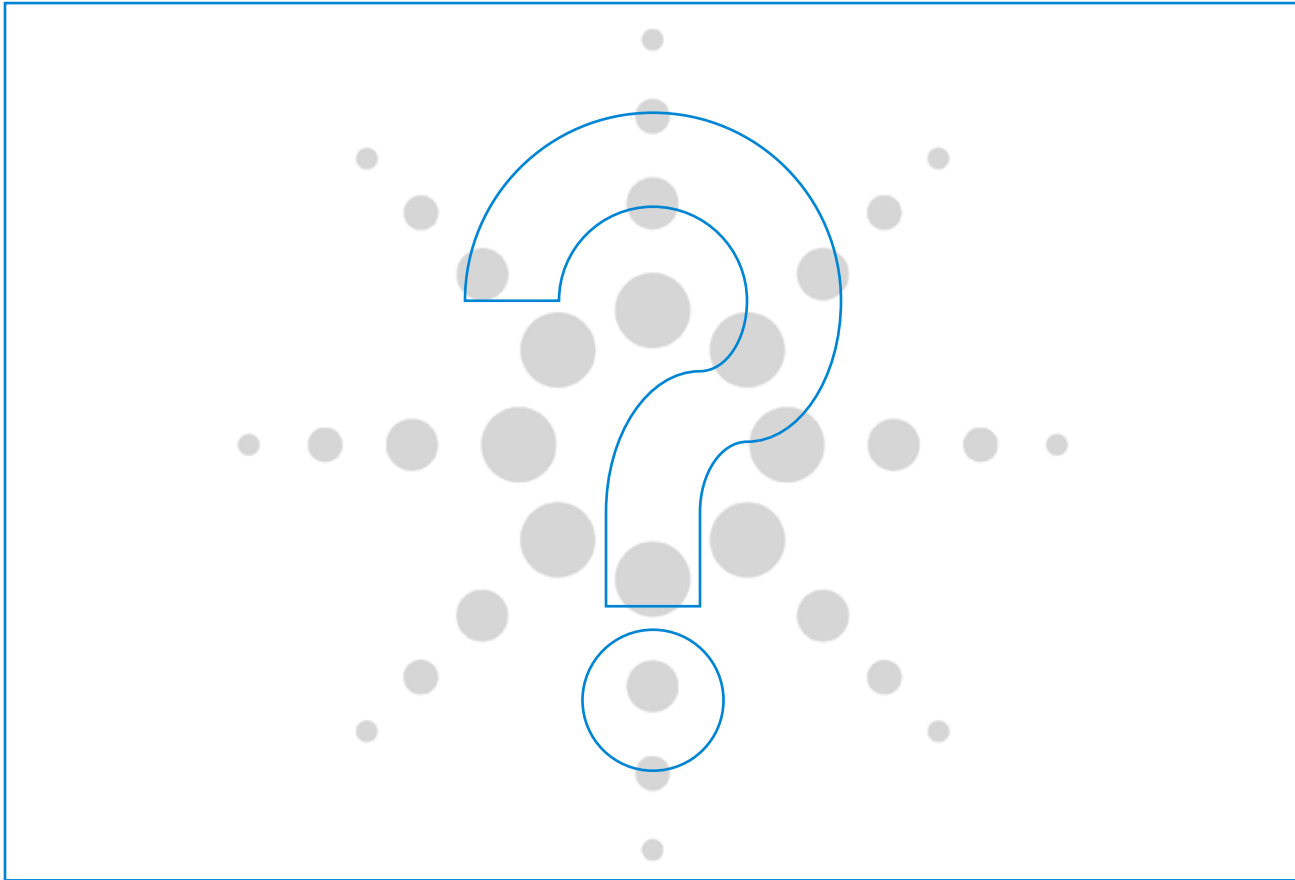


Synthetic and medicinal chemists in modern discovery labs need an analytical workflow that is easy to use and delivers clear and precise results.



Walkup Method Development
5991-2868

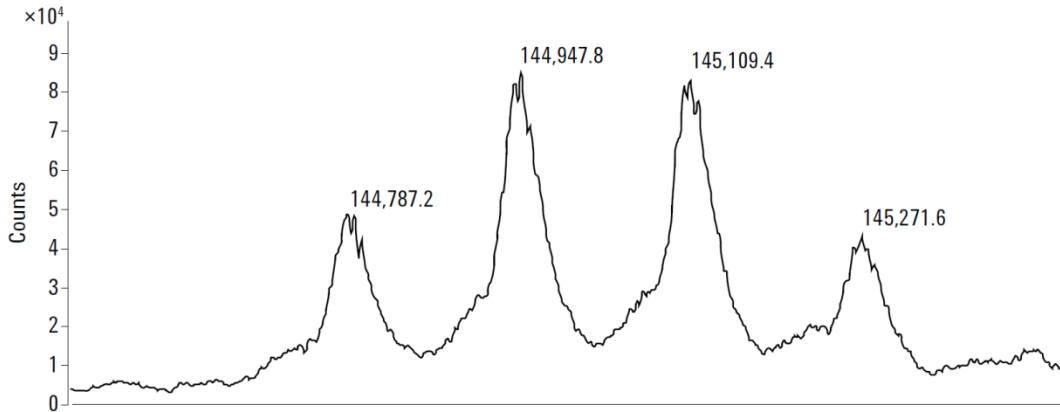
Walkup Technical Overview
5991-2868



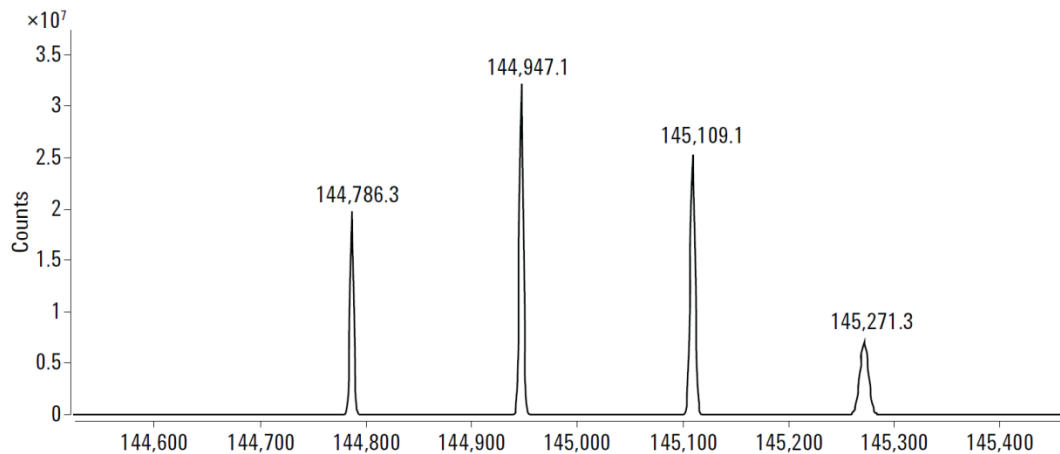
APPENDIX

More on Information pMod Deconvolution

Classical Maximum Entropy Deconvolution



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

