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Trusted Answers

Fast and Secure Mass Information in an Open-Access Environment with the Agilent MassHunter WalkUp Software and the InfinityLab LC/MSD iQ Mass Selective Detector

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Introduction

Synthesis of an active pharmaceutical ingredient (API) requires many monitored reaction steps. To ensure that a reliable and cost-efficient synthetic pathway is established, each reaction step must be tested for overall purity and yield. Obtaining fast results is key to determining appropriate decisions for the next step. The same applies for drug discovery, process development, and quality control, where rapid access to analytical results leads to faster decisions in addition to saving time and money. The best way to meet these needs is with an open-access analytical instrument that is easy to use while providing fast, high-quality results.

This Technical Overview outlines the features and advantages of Agilent's open-access software, MassHunter WalkUp. WalkUp is an add-on control software package that sits above the regular data acquisition and data analysis software. It is designed and optimized for use in an open access configuration. In this type of configuration, there are two types of users: a system administrator and a sample submitter.

The administrator is the expert who manages the system. They create the methods that the submitters use to analyze their samples. Methods can be assigned to specific groups so that samples are submitted in a controlled environment. For the data analysis portion of the method, the data are processed using parameters defined by the administrator enabling consistency of the results. The results are automatically sent to the submitter after the run completes. The administrator can also assign certain privileges to the sample submitters so that they can be as self-sufficient as possible.

Sample submitters do not require knowledge of Agilent OpenLab CDS or how to operate the instrument. With WalkUp, a submitter can simply walk up to the instrument, submit a sample, and return to their desk to receive an emailed report with their analytical results (Figure 1). This allows laboratories to reduce training times and improve operational efficiency.
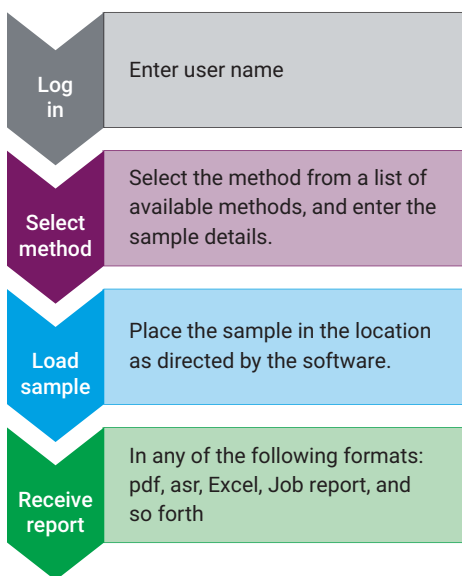
Log in	Enter user name
Select method	Select the method from a list of available methods, and enter the sample details.
Load sample	Place the sample in the location as directed by the software.
Receive report	In any of the following formats: pdf, asr, Excel, Job report, and so forth

Figure 1. A typical user workflow for the Agilent LC/MSD iQ WalkUp system.

Typical WalkUp system configuration

A typical Agilent InfinityLab LC/MSD iQ WalkUp system consists of the following modules:

- Agilent 1290 Infinity II High Speed Pump (G7120A)
- Agilent 1290 Infinity II Multisampler (G7167B) or Agilent 1290 Infinity II Vialsampler (G7129B)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
- Agilent 1290 Infinity II Diode Array Detector (G7117B)
- Agilent LC/MSD iQ (G6160A)

MassHunter WalkUp software

For this Technical Overview, the Agilent LC/MSD iQ WalkUp system used the following software packages:

- Agilent OpenLab CDS 2.4
- Agilent MassHunter WalkUp 4.0

Easy sample submission for users from any background

There are two ways to submit samples depending on the lab's intended use of the WalkUp system.

- **Rapid Sample Submission** is specifically designed for use with a sample purity or target mass confirmation workflow (Figure 2).
- **Classic Sample Submission** is used for more complex workflows requiring multiple methods, or for mass-based fraction collection (MBFC).

Both sample submission workflows require the same steps for submitters to follow:
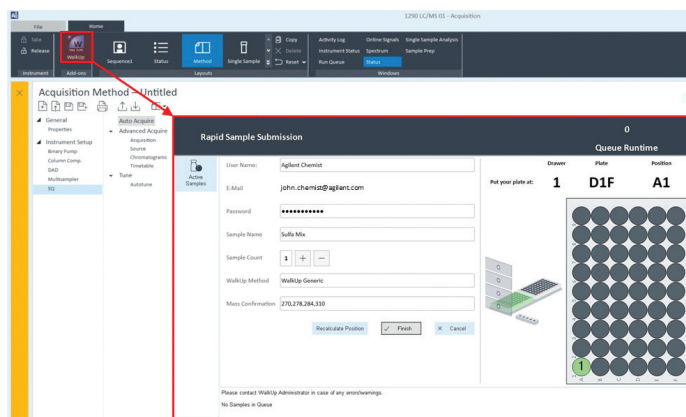

Figure 2. Agilent's MassHunter WalkUp is launched from the Agilent OpenLab CDS acquisition window. Once WalkUp is loaded, the submitter interfaces with the Agilent InfinityLab LC/MSD iQ through WalkUp, and does not need to use OpenLab CDS.

- Identify themselves to the WalkUp system, and enter an optional password.
- Input simple sample information.
- Choose from a list of analytical methods.
- Input target masses (optional).
- Load the sample into the sampler, as directed by the system.
- Receive the emailed results report.

Advantages and benefits for Lab Managers and Administrators

For the administrator of the system, some of the key benefits of WalkUp are:

- Secure mode which prevents the workstation from unauthorized modification

- Customizable sample submission screens

- Ability to import defined worklists with Classic Sample Submission

- Touch screen capability using Rapid Sample Submission

- External sample tray for uninterrupted sample submissions

- Powerful administrative tools that ensure open-access peace of mind

- Group and User management features

- Administration of how and where data are stored

- Automatic scheduling of system events

- Flexible method use with intelligent features

- Real-time sample queue and instrument status in small or large labs

- Verification of system configuration after changes have been made

- Convenient sample submission from a remote computer

- Email or SMS alerts when a problem occurs with the system

Secure Mode protects the Workstation

The system administrator has the option to lock the workstation to prevent submitter from modifying the method parameters by configuring WalkUp in Secure Mode. In this mode, the submitter interacts only with the sample submission screens (Figure 3). The submitters do not have access to any other features including the operating system. (Figure 3).

If the administrator decides to leave the workstation unlocked, there is an option to hide the OpenLab CDS software while still allowing the submitter to access other programs (such as a calculator or spreadsheet program) and the operating system.

Along with securing the workstation, the administrator can set User Group permissions (Figure 4), which enable:

- Creating import maps for multiple sample submissions

- Restricting methods to be used by certain groups or instruments

- Managing the sample queue to give certain users, samples, or methods different priority levels

- Defining data file naming and storage options with:

  - User-specific directories

  - Instrument-specific directories

  - Data files that stored locally, copied to multiple locations, or emailed upon run completion

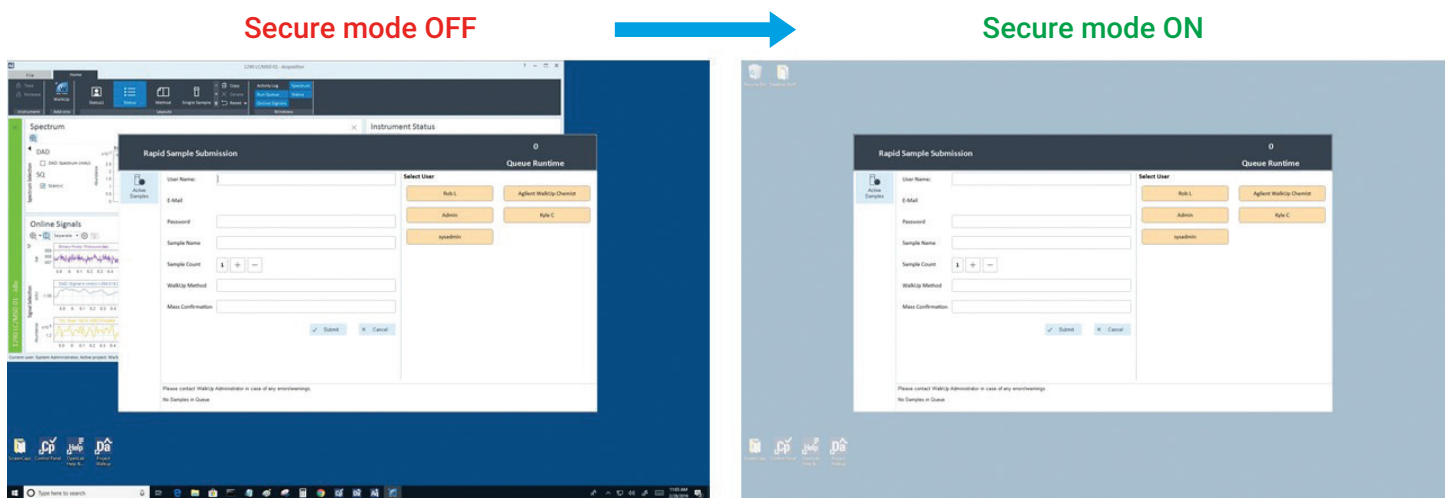- Restricting instruments or methods to specific workflows

Secure mode OFF → **Secure mode ON**

Figure 3. With Secure Mode off, a submitter can access Agilent OpenLab CDS and other programs on the computer. With Secure Mode on, the submitter is restricted to only WalkUp sample submission. All other programs and operating system commands are locked out.

Users And Groups

Figure 4. The group and user configuration window. The Users And Groups tab contains a list of all the groups and their users. Each group can be given a set of privileges and methods they can use.

Customizable sample submission screens

Using the WalkUp Rapid Sample Submission option further simplifies the sample submission process. When used with a touch screen monitor, the sample submission process is on one screen. With Rapid Sample Submission, the first thing the submitter must do is identify themselves to WalkUp. After selecting a user name and entering an optional password, the sample is given a name, and the method is selected. Then, the user inputs sample information, as shown in Figure 5. Finally, the target molecular masses are entered. WalkUp will select an appropriate position for the sample in the autosampler with a highlighted position in a picture.

Figure 5. Rapid Sample Submission: all inputs are made from this single screen. The right side shows the methods available for submitters; buttons for method selection are provided for touch screen use.

Classic sample submission with added options

Classic Sample Submission can be used for multiple samples in combination with multiple methods, as shown in Figure 6. As in Rapid Sample Submission, a submitter enters their name and password, if necessary (Figure 6A). After the submitter inputs the number of samples, and selects a workflow, a new screen appears allowing the submitter to select a method for each sample and input analyte masses (Figure 6B). Finally, WalkUp informs the submitter where to load the sample vials, and the analysis begins (Figure 6C).

In the Classic Sample Submission process, there is a timeout for sample submission that can be configured to ensure that no submitters accidentally hold up the queue (Figure 6D). This feature can be disabled by the system administrator. As with the Rapid Sample Submission, the administrator can configure WalkUp to email the data analysis report for each sample submitted.
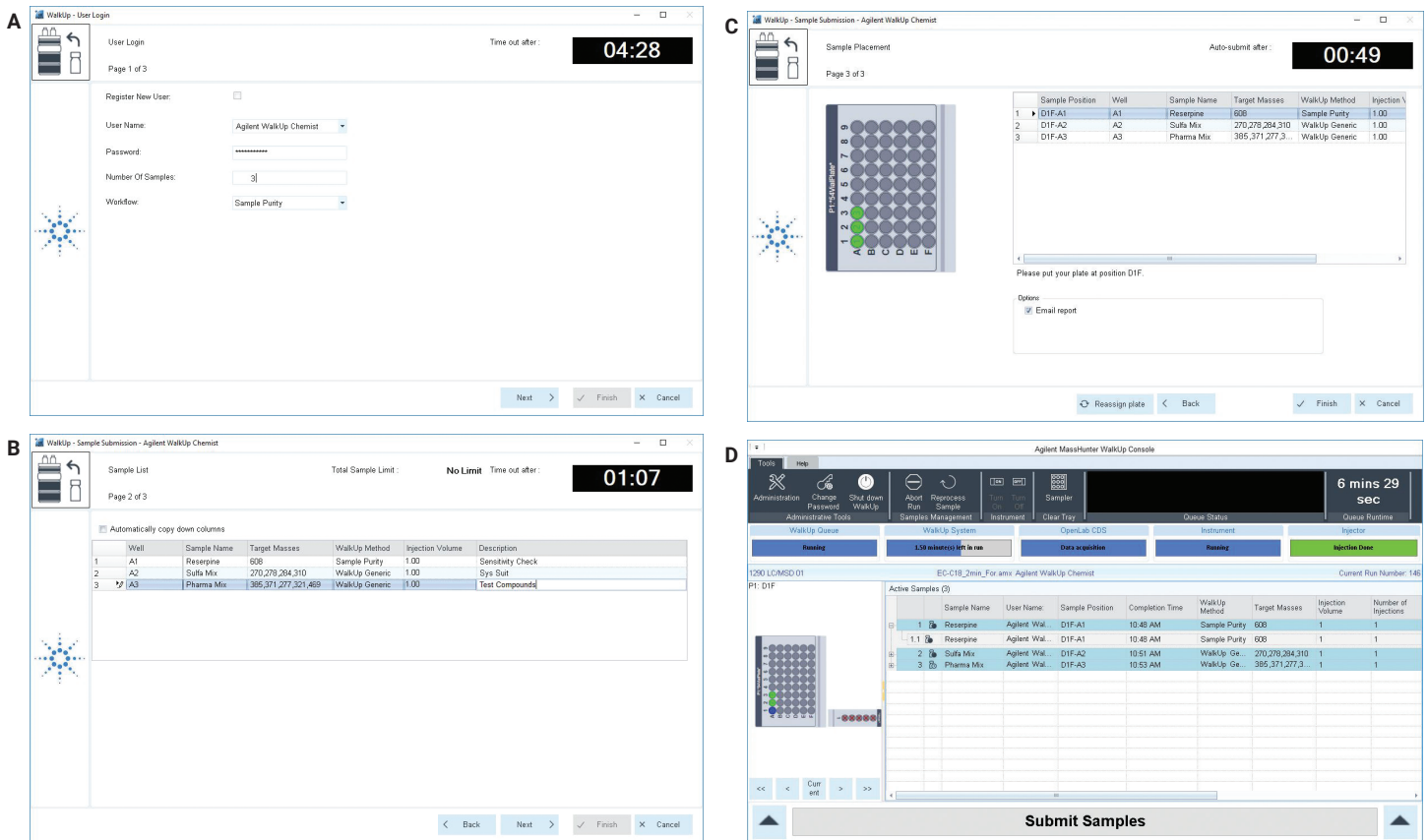

Figure 6. Classic Sample Submission screens. Multiple samples can be submitted at once with specific methods for each sample. An active sample queue shows which sample is currently analyzed with estimated times of completion.

External sample tray for uninterrupted sample submissions

An unique feature of the Agilent 1260/1290 Infinity II Vialsampler is an optional external tray. This allows the submitter to place their sample in the tray without opening the door of the Vialsampler. When a sample is submitted to the system, the submitter is instructed to place their sample in a specific external tray location. The Vialsampler arm will move the sample vial from the external tray into the sampler queue. After the run is complete, the Vialsampler can be set to discard the sample vial, freeing that position for another submitter. The end result is that the autosampler tray is always clear and ready to accept new samples. This reduces the administration overhead associated with maintaining an open-access system.
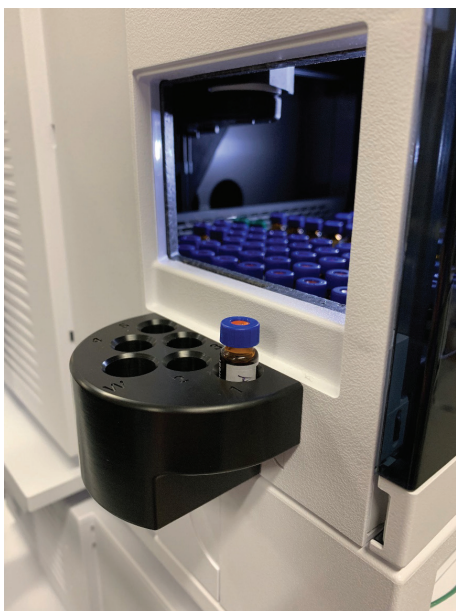

Figure 7. Optional external sample tray of the Agilent 1260/1290 Infinity II Vialsampler.

Group and user management features

Underneath the simplicity of sample submission screens are comprehensive administrative tools. These tools are not available to the submitter. The administrator decides the permissions of each group, configures the sample submission screens, acquisition methods are assigned, data are handled, and results are published. As an example, in Figure 8, a user is being added to the Agilent administrator group with email and SMS texting enabled. For the submitter user groups, data files and reports can be emailed to the user who submits a sample. Additionally, SMS texts can be sent for any WalkUp status notifications.
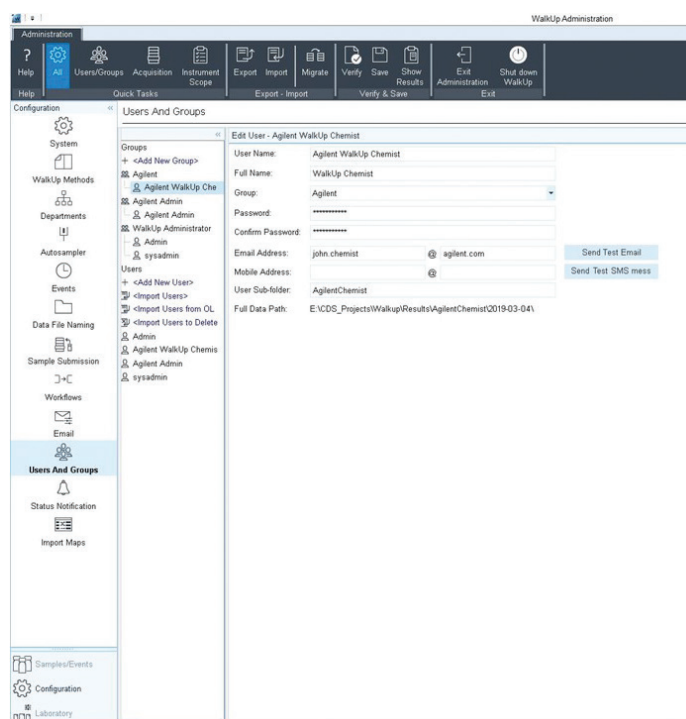

Figure 8. User configuration options in the Users And Groups configuration screen.

Administration of how and where data are stored

When samples are run using the MassHunter WalkUp software, the administrator controls how and where the data are stored. WalkUp always stores the data in the local file system. By default, each user gets their own data directory. However, custom data file paths and names can be configured based on a laboratory's naming scheme or convention. Within each user's directory, a subdirectory based on the date (Figure 9) enables users to quickly and easily locate their data. The same type of options exist for the naming of data files. Once the data have been acquired, the entire data file and path can be copied to multiple network drives or locations, ensuring maximum data security.
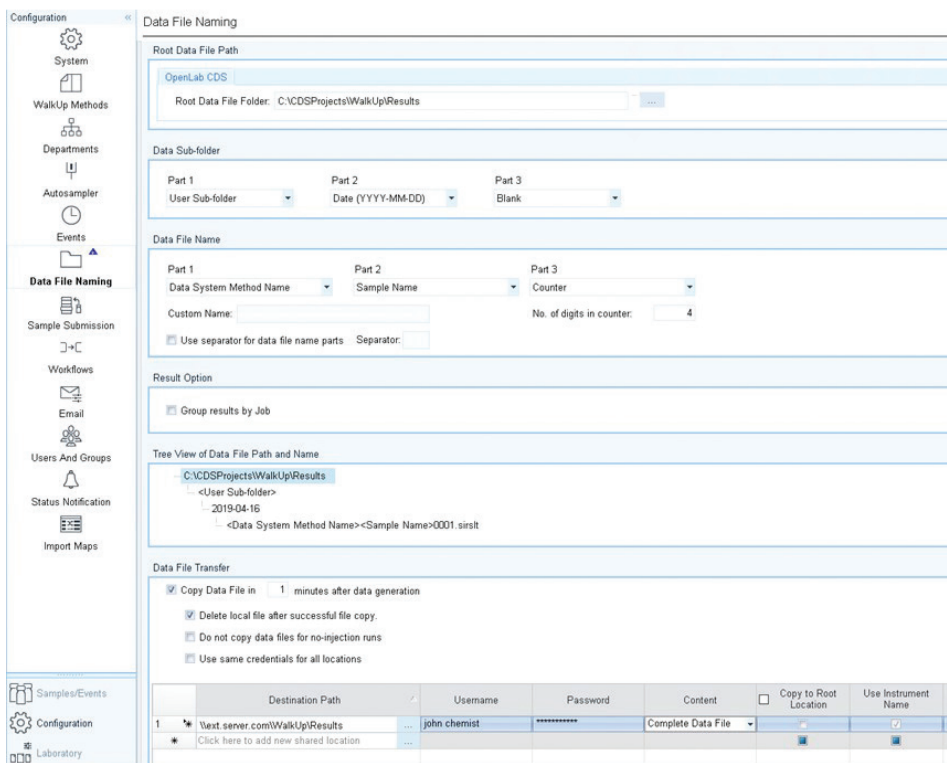
Figure 9. Data File Naming configuration tab. The root path is specified, and data file naming and storage options are configured. A destination other than the instrument workstation can be specified to multiple file server locations at the bottom.

Automatic scheduling of system events

System events can be scheduled on a time basis (for example, every day, week, or workday) or sample submission basis (for example, after a plate is complete, or after N runs) (Figure 10). Any number of WalkUp methods can be selected to run these events. Sample vials used by these events can be stored in autosampler locations that are not used by the standard sample queue.

Example events include:

- Running reference standards throughout the normal workday

- Calibration standards to ensure that reported amounts are on target

- System suitability check for instrument and column stability

- Blank runs to check for carryover or contamination

System events are intelligent and recognize that if a sample is currently being run, WalkUp will not interrupt that sample. It will wait for the current run to complete, then run the event.

Figure 10. A system suitability event is configured to run after 20 sample runs. An injection will automatically be made from Multisampler Tray location 3, which contains a system suitability test mixture.

Flexible method usage with intelligent features

WalkUp methods are created by the administrator using existing methods developed in OpenLab CDS. Specific workflows or instruments can be selected for each WalkUp method. Data analysis methods can be linked for automatic processing and reporting of data once a sample run is complete.

If the solvent conditions or column type changes require an equilibration method, the system can be configured to automatically execute an equilibration of the system. This is especially useful for HPLC configurations that use multiple column types that are selected depending on the method or sample type. This includes methods that have different pH. For instance, when methods are run with different pH

methods, column equilibration is automatically performed when the pH changes between methods. With the Agilent 1290 Infinity II Multicolumn Thermostat, up to eight individual columns can be selected by an internal valve. In addition, prewakeup and postrun methods can be executed before and after the sample run (Figure 11).

Workflows for any type of analysis

Workflows can be customized by the system administrator to suite different types of sample analysis such as sample purity or reaction monitoring (Figure 12). Parameters specified in the workflow will appear as columns in the Classic Sample Submission screen where the submitter can enter relevant information, such as a precursor mass and product mass to be monitored.

Figure 11. A WalkUp method for a sample purity analysis. The sample purity workflow is selected, which asks for the target mass of the compound of interest. A data acquisition method is selected from Agilent OpenLab CDS acquisition, and is linked to a data analysis method that will automatically process the data and generate a report.
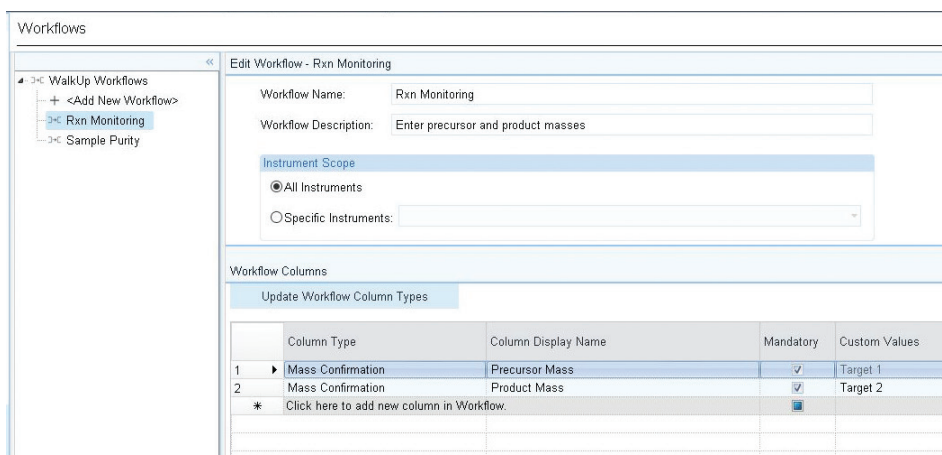
Figure 12. A workflow for reaction monitoring. Two columns are added to the sample submission screen for a precursor target mass and product target masses. These inputs have been selected to be mandatory, and those masses will be automatically extracted with their peak area ratios reported.

Data and reports emailed right to your inbox

Reports can be set up by the system administrator to be directly emailed to the analyst (Figure 13). Data are automatically processed after a sample run according to the data analysis method, and reports are created based on the report template in the OpenLab CDS. Figure 14 shows an example of a sample purity WalkUp report. This sample used the sample purity workflow, and the submitter entered a target mass of 733.9 g/mol, which was identified in the largest TIC peak. The UV chromatogram shows very little

absorbance of the API, and the concentration of the impurities is below the UV detection limit. By contrast, the impurities can easily be identified in the MS scan chromatogram. The first and third TIC peaks are impurities and the overall impurity of the sample was calculated to be 85.57% based on the target peak area over the total TIC integrated peak area. The report shows the sample purity, UV chromatogram, and the MS total ion chromatogram. The peaks are also labeled with the MS spectra base peak *m/z* for quick and easy mass confirmation.
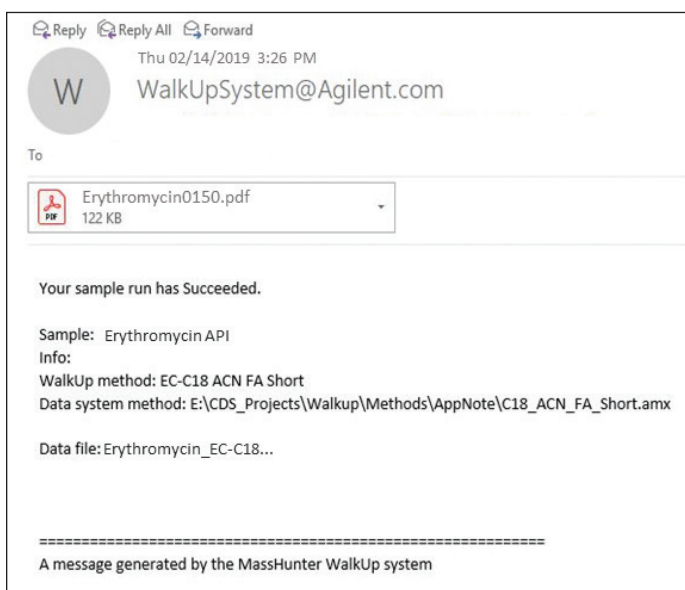

Figure 13. An email is sent to the submitter containing an attached sample purity WalkUp report in .pdf format.
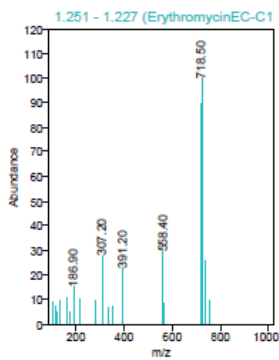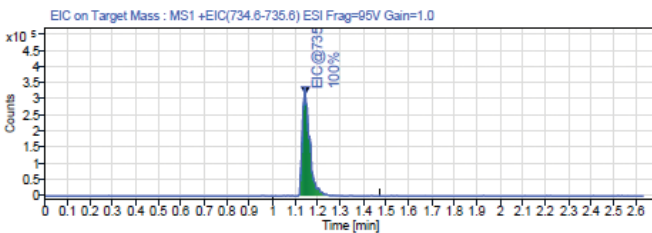
Sample Purity Report

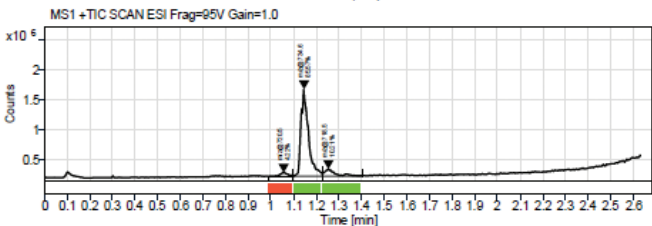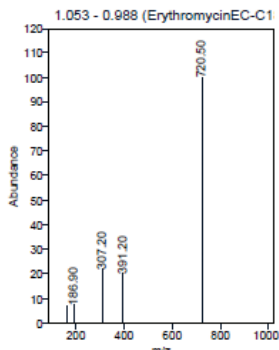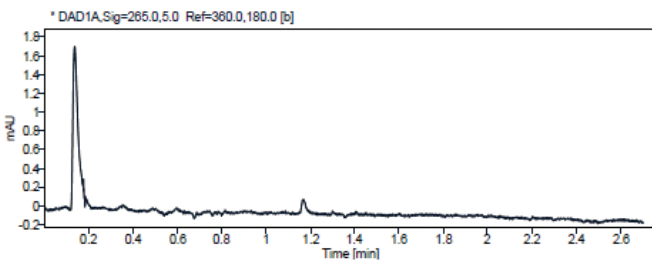Agilent Technologies

Data file:	ErythromycinEC-C18_3min_For_PosOnly_ MultiSampler.amx20190417 133931.dx
Sequence Name:	LCMSDiQ Sample Purity Day 2 Full Run 20190417 132342
Acquired on:	2019-04-17 13:40:08-07:00

Sample Purity Results

Target mass	Target found in analysis	Purity (%)	Purity result
733.9	Yes	85.57	Impure

* DAD1A,Sig=265.0,5.0 Ref=360.0,180.0 [b]

MS1 +TIC SCAN ESI Frag=95V Gain=1.0

EIC on Target Mass : MS1 +EIC(734.6-735.6) ESI Frag=95V Gain=1.0

1.053 - 0.988 (ErythromycinEC-C1

1.144 - 1.097 (ErythromycinEC-C1

1.251 - 1.227 (ErythromycinEC-C1
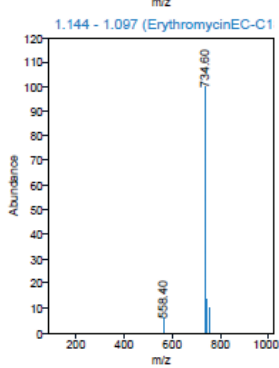
Peak Results (Area Percent at least 1%)

RT (min)	Signal	m/z	MS Purity (%)	Area	Area (%)
1.053	MS: Positive TIC SCAN	720.5	0.00	164786.48	4.22
1.144	MS: Positive TIC SCAN	734.6	100.00	3344767.54	85.57
1.251	MS: Positive TIC SCAN	718.5	100.00	399221.88	10.21

Figure 14. A sample purity WalkUp report shows the sample purity of target mass 733.9 g/mol (erythromycin) along with the UV chromatogram and MS total ion current. The colored bars below the TIC peaks show whether the MS peak is pure (green) or impure (red).

Powerful administrative tools that ensure open-access peace of mind

After any changes are made by an administrator using the Verify feature, all changes are verified The Verify feature is an automatic error-checking process that will go through the entire system configuration and return a table with any errors that must be corrected or warnings about missing information (Figure 15). Any error can be checked, which will send the administrator to the configuration tab where the error is occurring.
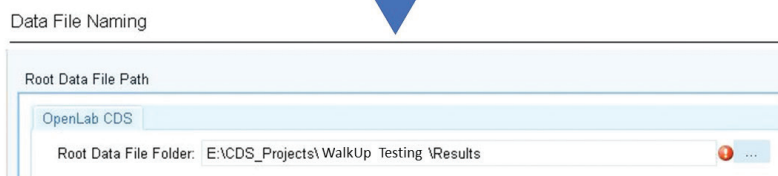

Figure 15. Verification log displaying an error with data file naming. If the system administrator clicks the error, it will send them to the data file naming tab and indicate, with a red exclamation mark, where the error is occurring.

Conclusion

With fast, flexible, and intuitive sample submission capabilities combined with flexible report-generation options, Agilent's MassHunter WalkUp software provides an open-access experience that is easy to work with for sample submitters.

Sophisticated administrative tools allow an administrator to configure workflows that can be executed by anyone with virtually no training required. A set of security features can be enabled to safeguard data. Reports are generated, and are automatically emailed to the submitters. In combination with an Agilent InfinityLab LC/MSD iQ which provides mass information for more definitive identifications, a true WalkUp experience is ensured, leading to fast results while saving precious time in labs where decisions must be made quickly.

Related information

The Agilent InfinityLab LC/MSD iQ single quadrupole-based system is exceptionally easy to use and maintain while still providing unbeatable robustness. For more information on the InfinityLab LC/MSD iQ, please see Agilent publication 5994-1011EN.

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