Agilent 6120B Single Quadrupole Walkup System For Multi-user Labs

Confident LC/MS Analysis is Only a Few Clicks Away

Application Note

Drug Discovery & Development

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Abstract

Reliable and easy-to-use instrumentation for compound identification as well as purity checking are required during drug discovery and development. The Agilent 6120B Single Quadrupole hardware and software are ideal to support Walkup LC/MS methods for analyzing small molecules and biomolecules in a multi-user environment. A simplified software interface combined with robust hardware gives nonexpert users the confidence to perform high quality and reliable LC/MS analysis. Agilent MassHunter Walkup Software provides automated email reports, which allows the user to receive the results in an organized manner at their desk. This Application Note discusses key hardware and software features of the 6120B Single Quadrupole Walkup system, and its practical use for applications including the analysis of chemical libraries.

Agilent Technologies

Introduction

At each stage of the synthetic process, chemists need to confirm that compound synthesis has been successful, and estimate the purity or yield of the target compound. Fast results are necessary to make quick and appropriate decisions for the next synthetic step.

The user can easily perform complex analytical tasks using an Agilent 6120B Single Quadrupole Walkup System along with methods created by the administrator. The user does not need to be familiar with Agilent ChemStation or Agilent MassHunter software to operate the system. Instead, they need only learn a few simple sample submission steps. The system administrator can create detailed analytical methods for use by nonexpert users. Various administrative features and robustness evaluation of the 6120B Single Quadrupole Walkup System are described in Technical Overview 5991-6534EN. Using the Administrative features, one can customize the 6120B Single Quadrupole Walkup System for the user requirements. This Application Note presents the user-related features for Walkup LC/MS using the 6120B Single Quadrupole Walkup System.

Experimental Details

Chemicals and reagents

Methanol was LC/MS grade, and LC/MS eluent additive grade ammonium formate and ammonium acetate were used (Sigma-Aldrich, St Louis, USA). Milli-Q water was used in all experiments (Merck, Darmstadt, Germany). All other chemicals used for the study were purchased from Sigma-Aldrich (St Louis, USA).

Walkup instrumentation

The Agilent 6120B Single Quadrupole Walkup System consists of the following modules:

- Agilent 1260 Infinity Degasser (G1322A)
- Agilent 1260 Infinity Binary Pump (G1312B)
- Agilent 1260 Infinity Sampler (G1329B) with external tray (p/n G1313–60004) and waste tube (p/n G1313–27302)
- Agilent 1260 Infinity Column Thermostat (G1316A) with a 2-position/6-port valve
- Agilent 1260 Infinity Diode Array Detector (G4212B)
- Agilent 6120B Single Quadrupole LC/MS (G6120B) with multimode source enabled with fast polarity switching for positive and negative mode acquisitions (default 300 ms. However, this switching can be upgraded to 30 ms with resistive capillary, with an interval of 10 ms)

Walkup software

- Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS Systems, Rev. C.01.07
- Agilent MassHunter Walkup Software for LC/MS and LC systems, V: C.02.01
- Agilent MassHunter Analytical Studio Reviewer (ASR), V: G3772AA, B.02.01

Method parameters

Tables 1 and 2 list the LC and MS instrument parameters. The Walkup methods for various different tasks were prepared by the administrator. The user needs to select the method from the list according to the analytical requirements. In Tables 1 and 2, short (3-minute), and long (10-minute) methods are discussed. Two different mobile phases are available (pH 3.2 and pH 5.8) on A1 and A2 channels of an Agilent 1260 Infinity pump, offering multiple method possibilities for the user. The 6120B Single Quadrupole can perform mass acquisitions up to 2,000 Da. However, a mass range of 100–600 Da was selected for the analysis of small molecule library samples.

Table 1. Agilent 1260 Infinity LC method parameters.

Parameter	Short method	Long method
Column	Agilent Poroshell 120, EC-C18, 3 × 50 mm, 4 µm at 40 °C	Agilent Poroshell 120, EC-C18, 3 × 100 mm, 2.7 µm at 40 °C
Mobile phase A	A1) Ammonium formate buffer, pH 3.2 A2) Ammonium acetate buffer, pH 5.8	A1) Ammonium formate buffer, pH 3.2 A2) Ammonium acetate buffer, pH 5.8
Mobile phase B	Methanol	Methanol
Gradient	Time %B 0 40 0.2 60 0.7 95 3 95	Time %B 0 40 2 60 7 95 10 95
Detection	a) 254 nm b) Dual (ESI/APCI) Positive mode c) Dual (ESI/APCI) Negative mode	a) 254 nm b) Dual (ESI/APCI) Positive mode c) Dual (ESI/APCI) Negative mode
Injection volume	3 µL	3 µL

Methods used in this study

- **Short**: 3-minute gradient (50 cm, 4 µm column) with ammonium acetate and methanol for fast screening in positive and negative mode.
- **Short**: 3-minute gradient (50 cm, 4 µm column) with ammonium formate and methanol for fast screening in positive mode only.
- **Long**: 10-minute gradient (100 cm, 2.7 µm column) with ammonium acetate and methanol for purity checking, in positive and negative mode.
- **Long**: 10-minute gradient (100 cm, 2.7 µm column) with ammonium formate and methanol for purity checking, in positive mode only.

Agilent MassHunter Walkup workflow

Agilent MassHunter Walkup Software is intuitive, and does not require prior knowledge of LC/MS instrumentation or software. The sample sequence creation requires only a few simple mouse clicks, thus minimizing manual errors. Three easy steps shown in the workflow (Figure 1) lead to quick and customizable results and reporting. These steps include a simple user and application login, easy method selection, and sample loading, helping users create sample sequences effortlessly. Automated data analysis and email reporting enable fast streamlined productivity.

Table 2. Agilent 6120 Single Quadrupole parameters.

Parameter	Value
Peak width	0.02 minutes
Threshold	150
Step size	0.2
Fragmentor	70 V
Ionization mode	Multimode
Polarity	Positive and Negative
Drying gas flow	12 L/min
Nebulizer pressure	35 psi
Drying gas temperature	250 °C
Vaporizer temperature	200 °C
Capillary voltage Positive/Negative	2,000 V
Corona current, Positive/Negative	1 µA

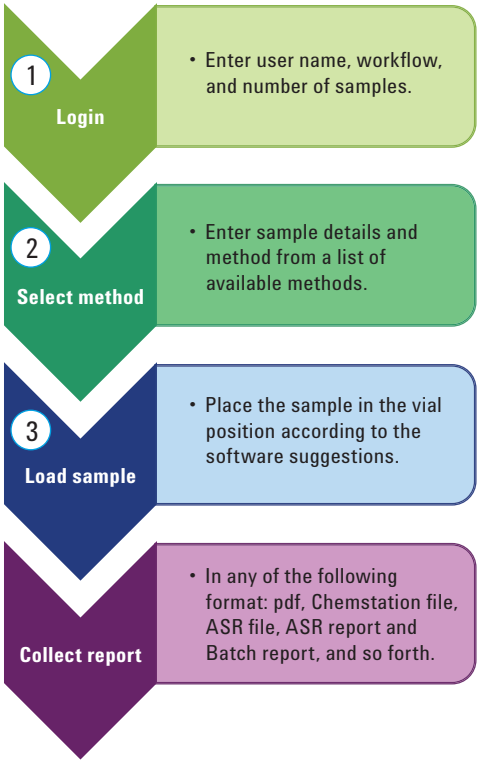

Figure 1. Typical user workflow for the Agilent 6120B Single Quadrupole Walkup system.

After logging on to the instrument with their user name and password, the user describes the number and types of samples they wish to submit, and selects a method from a list of available methods created by the administrator (Figures 2 and 3). The software displays specific locations to place the sample in the external tray (Figure 4). The sample is then loaded on the system, and queued for analysis and automated reporting.

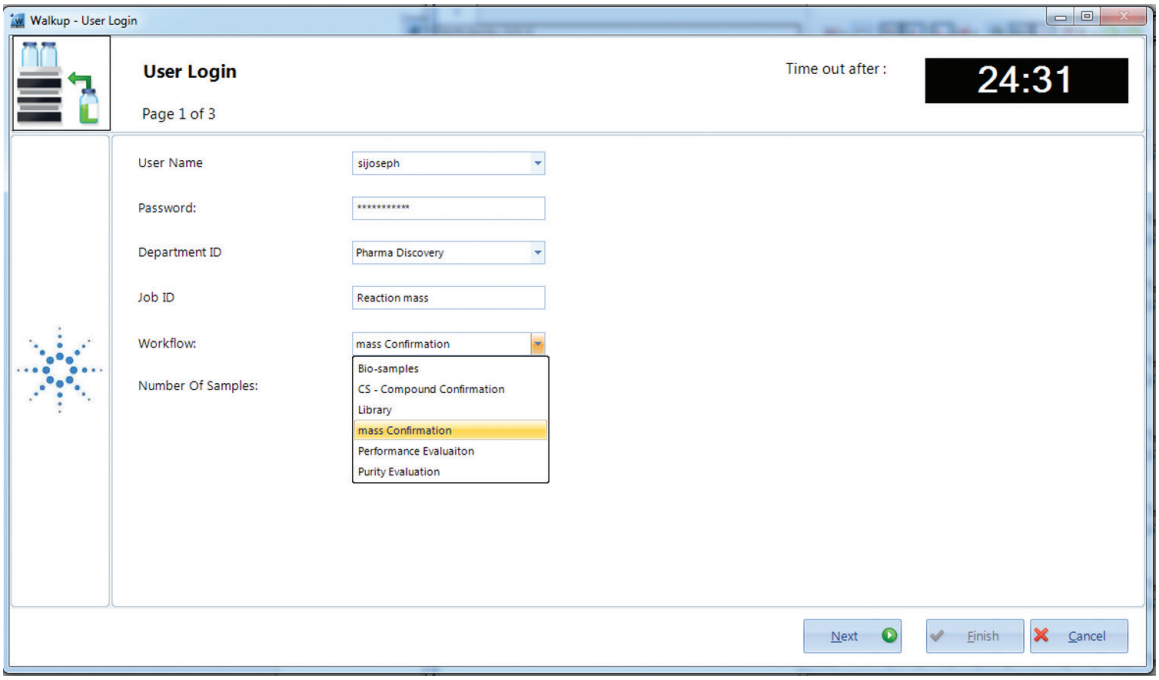

Figure 2. Step 1: User login page showing user name, password, selected workflow, and number of samples. In this example, mass confirmation workflow was selected.

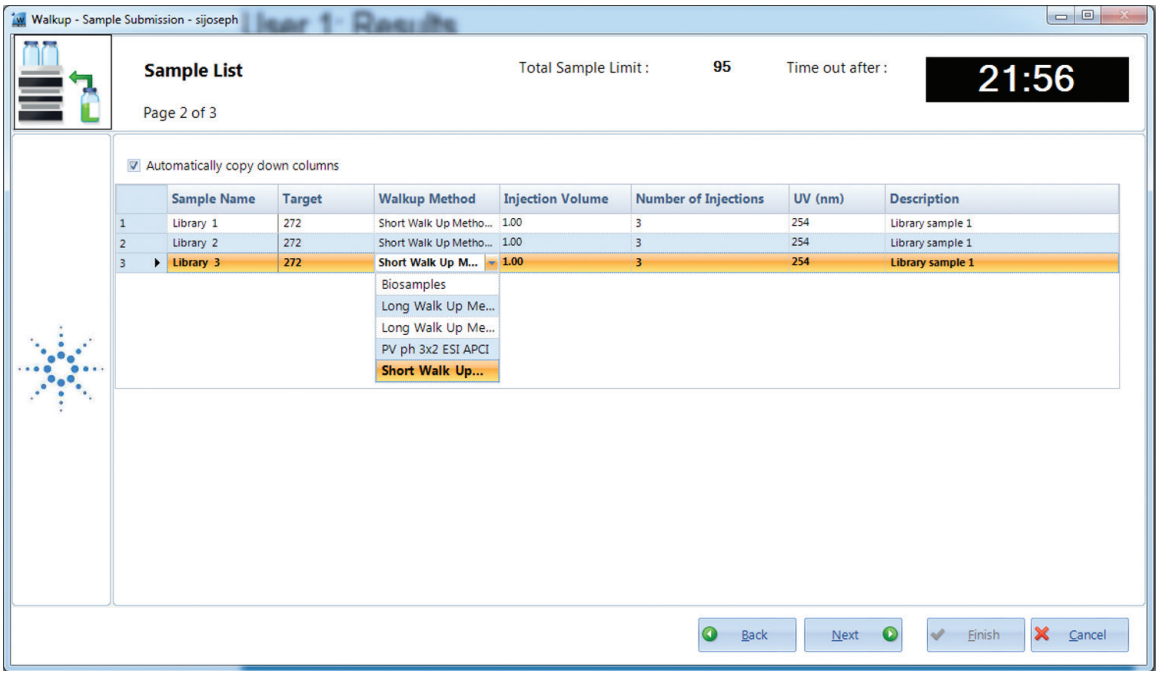

Figure 3. Step 2: Choose a method from a prepopulated drop-down list, and enter relevant sample details. In this example, the user selects a short method for quick gradient screen. The sample name and expected *m/z* are provided here.

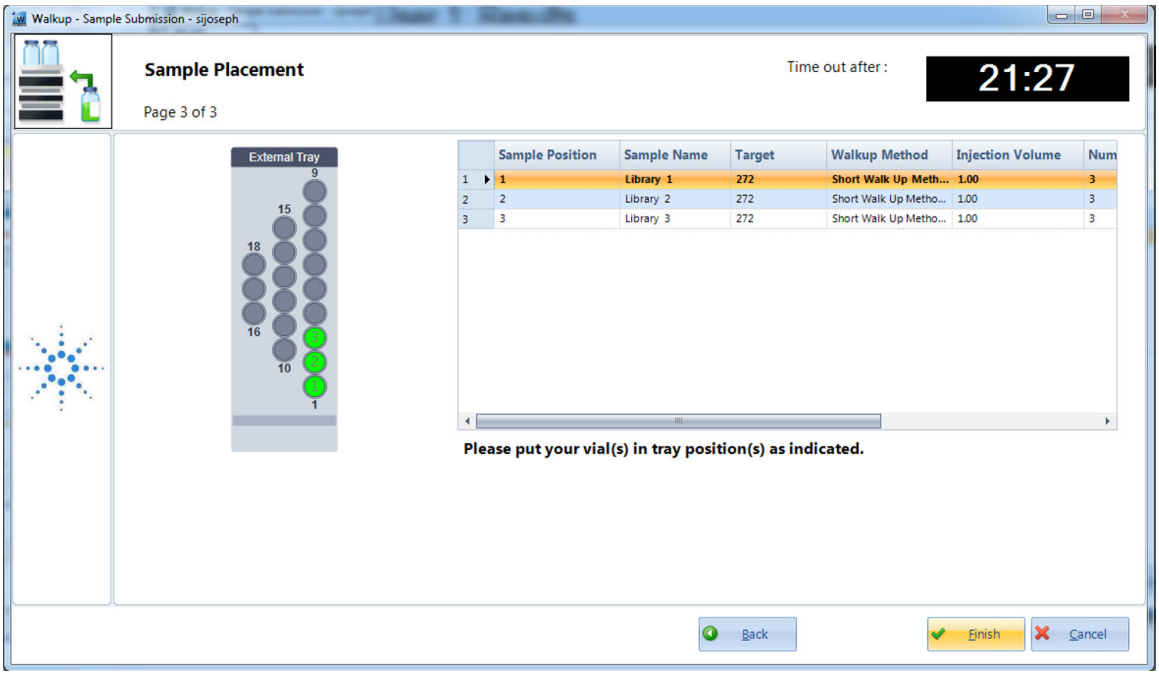

Figure 4. Step 3: Sample loading. The software displays the final sequence and vial positions in the external tray to facilitate sample loading.

The robotic arm of the Agilent Infinity autosampler places the submitted sample into the autosampler for injection (Figure 5). Various color codes displayed within the user interface enables the user to follow the course of the analysis.

Automatic notifications regarding any issues and alerts are sent directly to system administrators through email or SMS/text messaging.

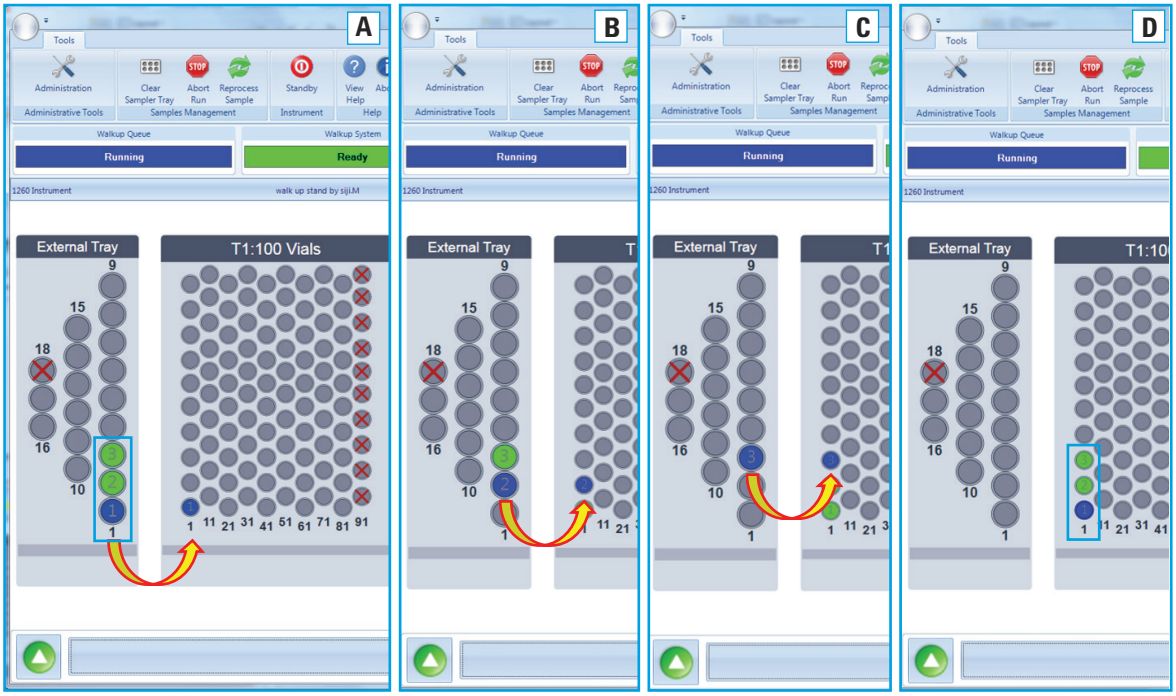

Figure 5. The user successfully placed three samples in the external tray. This figure demonstrates the sequential transfer of three sample vials from the external tray to the autosampler using the robotic arm of the autosampler (A, B, C, and D).

Results and Discussion

Applications of an Agilent MassHunter Walkup LC/MS workflow

The Agilent 1260 Infinity LC, combined with the 6120B Single Quadrupole Walkup System is analyte and application agonistic. The following are some examples of its use in a pharmaceutical research and development environment.

Compound library identification

A library of 30 compounds was used to demonstrate the methodology. A short 3-minute gradient method was used for fast compound confirmation. Both UV and MS detection were used to provide comprehensive detection. For the MS, a multimode source with positive and negative polarity switching was used to allow the detection of a variety of chemical compounds. The library samples were dissolved in dimethyl sulfoxide (DMSO), and directly injected into the LC-UV/MS system. Figure 6 shows the plate view results. The one-click purity view summarizes the results of the library analysis based on the user-defined settings.

Benefits of using complimentary detection

Using only a single method of detection can sometimes bias observed results, but the combination of MS with UV as used here provides a more confident identification of target compounds. Even if the compound of interest does not contain a strong UV chromophore, it may ionize in positive or negative mode in electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI), increasing the possibility of being detected.

Figure 7 shows the UV and MS traces of library sample 13. In this example, the main peak by UV detection elutes at 1.5 minutes, but the *m/z* value does not correspond to the target compound. MS detection confirmed the presence of the expected mass (*m/z* at 279) as a minor peak, which elutes at 1.7 minutes as shown in the UV trace. This clearly demonstrates the benefit of two modes of detection.

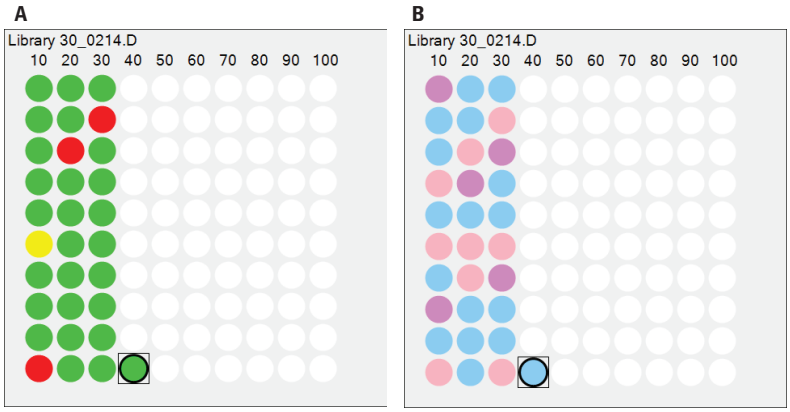

Figure 6. A) late view based on the presence of target *m/z* values. Green = One or more targets found, yellow = one or more targets found, but with low relative intensity, red = no targets found. Out of 30 library samples, two vials (library 17 and 21) did not show the expected target *m/z*. The remaining 28 vials contained the expected mass. The first vial is blank. B) The purity view based on set values: light orange = low purity, blue = medium purity, dark pink = high purity. In summary, eight library compounds are less pure, 17 of them are medium pure, and five are highly pure.

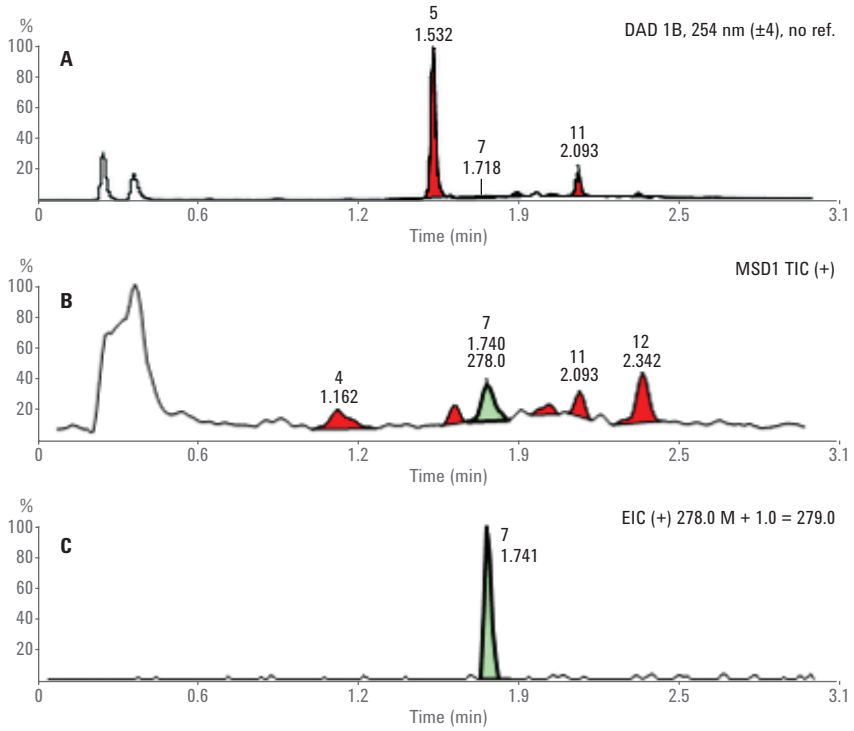

Figure 7. Result of library sample 13. The main peak as per UV detection (trace A) is not the expected target (*m/z* 279). Trace B is the Total Ion Chromatogram (TIC) in positive mode, in which the target mass is identified at 1.7 minutes. Trace C shows the Extracted Ion Chromatogram (EIC) of the target.

Compound library purity analysis

For purity analysis, a 10-minute gradient method was used to ensure chromatographic resolution. This method was developed using a 10-cm column. The aqueous mobile phase was either ammonium formate pH 3.2 (Channel A1 of the binary pump) or ammonium acetate pH 5.8 (Channel A2 of the binary pump) depending on the compound ionization behavior. Methanol was the organic mobile phase. A 2-position/6-port valve was used to switch between the short and long columns.

Having a short and long method in a Walkup setup is a good approach to first identify the presence of a target compound by confirmation of molecular weight. Subsequent purity analysis was performed using a longer LC method to ensure adequate chromatographic resolution. In Figure 8, UV analysis of library sample 10 using both short and long methods is displayed. The ASR report shows that the longer 10-minute method provides better chromatographic resolution, which helps accurate assessment of compound peak purity.

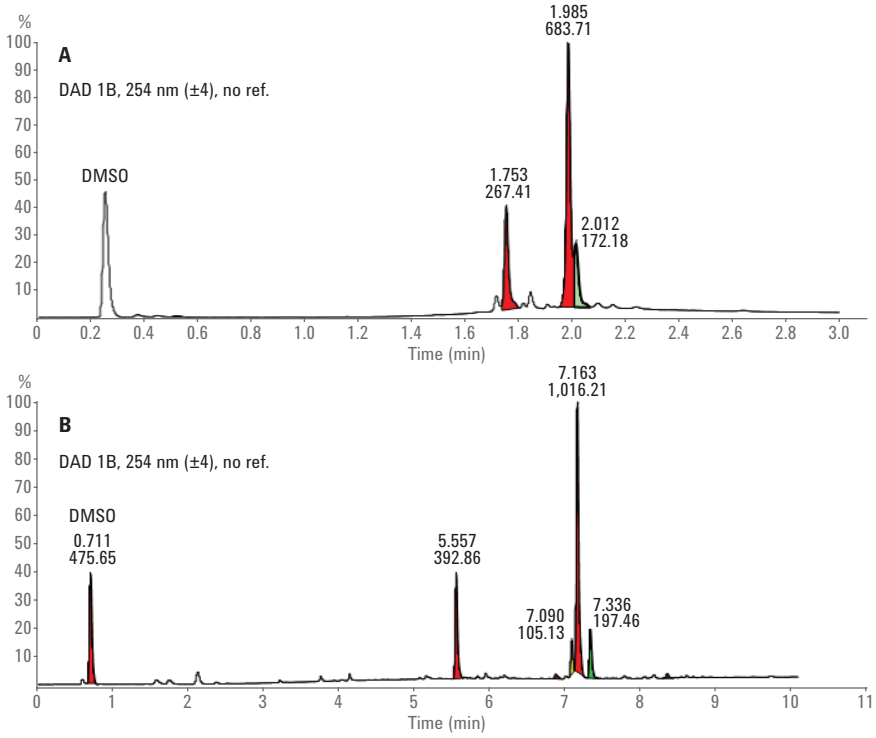

Figure 8. A UV trace of library sample 10 using the short, 3-minute method (A), and the long, 10-minute method (B). The main peak in the 3-minute method coeluted with an impurity, which made the purity assessment challenging. The target peak (retention time: 7.33) with the 10-minute method was clearly resolved, and MS detection confirmed the target mass value for the molecular weight of the compound.

Reaction monitoring for target compound analysis

Five different reactions were injected to monitor the formation of a compound with an expected molecular weight at *m/z* 282 using a short 3-minute LC method with methanol and ammonium acetate (pH 5.8).

Figure 9 shows the MS positive mode TICs of five different reaction trials for the synthesis of a compound. The flexible color coding provided in the ASR report helped to visualize results easily, and identify the target analyte. From this, it is evident that only the fifth trial showed the target *m/z* of 282.

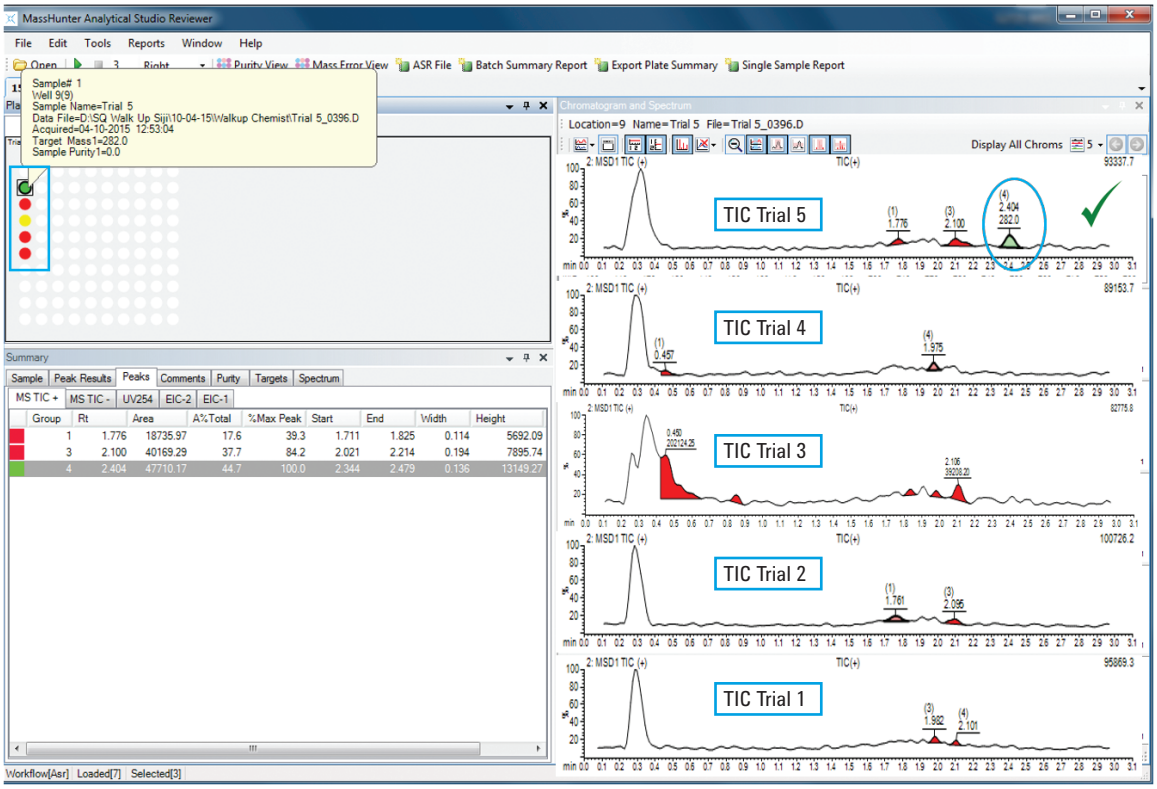

Figure 9. ASR report of five reaction monitoring samples. The TICs of all five samples are stacked on the right side of the figure. The vial plate view confirmed that trial 5 contained the desired target (green-colored well).

Reporting options

When the analysis has been completed, a report is automatically created and emailed to the user as specified in the preconfigured options set by the system administrator. Figure 10 is an example of an email report in PDF format.

Data analysis and results visualization of LC/MS data is facilitated by Agilent MassHunter Analytical Studio Reviewer (ASR). ASR is an intuitive user interface that enables quick LC/MS data review and results at a glance. ASR reports are highly customizable so that specific data is reported. These reports can be generated for a single sample or for a batch of multiple samples. An ASR results file can also be emailed directly to the user. These files can be opened with ASR software remotely, allowing the user to interactively browse the data, and quickly review the result summary (Figure 11).

The **purity view** feature in ASR software helps to quickly picture purity results based on either UV or MS detection.

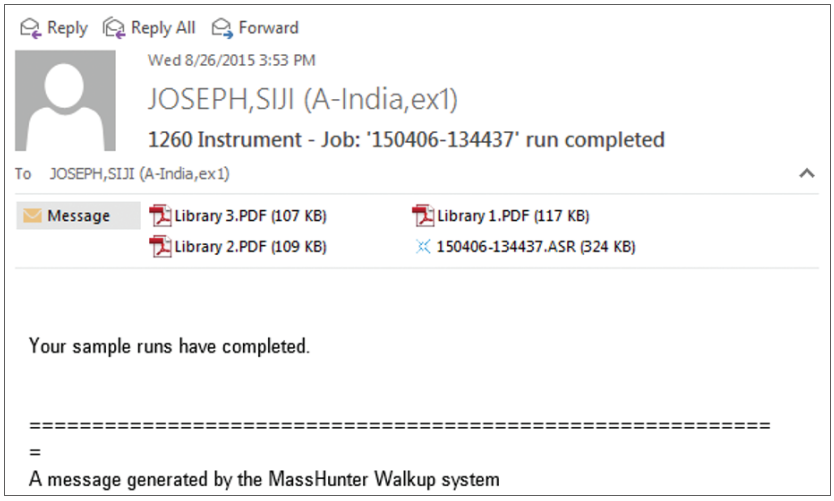

Figure 10. A typical email report after completing a given sequence analysis. The attachment includes individual pdf reports and ASR files for the batch.

Figure 11. Results view using Agilent MassHunter Analytical Studio Reviewer software. A) The ASR plate view, which helps to visualize the results based on the presence of target *m/z* values in each sample. Keeping the cursor on each well will display specific sample details. Upon clicking each well in the plate, corresponding chromatograms will be displayed in the right panel. B) UV and MS results summary pane for quick purity information. C) MS spectra of the selected peak will be displayed in this section. UV, MS (+), and MS (–) spectra are stacked in three different tabs. D) UV chromatograms, TICs, and EICs are displayed in this section.

Conclusion

Agilent MassHunter Walkup LC/MS software allows routine analysis of synthetic compounds in a multi-user environment. The robust Agilent 1260 Infinity LC coupled with an Agilent 6120B Single Quadrupole MS system offers reliable analysis of synthesized compounds covering a range of physiochemical properties. The complementary use of UV, together with MS detection, also facilitates identification of a wide range of target analytes. The Walkup software enables easy sample sequence creation and analysis, and does not require any specialized expertise with using the LC/MS hardware or software.

A wide range of reporting options are available within ASR software. Automated data interpretation using ASR ensures consistent and reliable results. Users automatically receive their customized results, helping them to make quick decisions about their chemical synthesis.

Reference

1. Joseph, S. Agilent 6120B single quadrupole Walkup System, *Agilent Technologies Technical Overview*, publication number 5991-6534EN, **2016**.

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This information is subject to change without notice.

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