



Agilent 6120B Single Quadrupole Walkup System

Administrative Features and Robustness Evaluation

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Technical Overview

Drug Discovery & Development

Abstract

Agilent 6120B Single Quadrupole Walkup instrumentation is a simple and efficient tool to perform complex LC/MS analysis in a typical multi-user environment. The administrator privileges within Agilent MassHunter Walkup Software enable the administrator to create Walkup methods, maintain instrument performance, and manage users. The administrator can add users and assign them different capabilities and access rights. MassHunter Walkup software can be integrated with Agilent OpenLAB services for maintaining confidentiality in data management and results distribution according to lab protocols. An administrator can prepare the Walkup software as a ready-to-use system to perform various advanced tasks. These tasks can include, for example, compound confirmation, purity checking, and identification of impurities, and can be performed by a non-expert user. This Technical Overview details the administrative features of the Agilent 6120B Single Quadrupole Walkup system. It also demonstrates method creation, easy sample loading, and report generation. The reproducibility of results produced by the 6120B Single Quadrupole Walkup instrumentation are also evaluated using an eight-compound test mix.



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Walkup Instrumentation

Agilent Single Quadrupole Walkup hardware

The Agilent 6120B Single Quadrupole Walkup System with robust hardware and simplified software features offers high-quality LC and LC/MS analysis in a multi-user environment. The 6120B 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 with multimode source enabled with fast polarity switching for positive and negative mode acquisitions (polarity switching time: default 300 ms, however, users can upgrade to 30 ms with resistive capillary, with an interval of 10 ms)
- 2-position/6-port valve included in column thermostat for flexibility to choose two columns in the flow path
- Sensitive UV detector with Agilent Max-Light Cartridge

The multimode source combines the functionality of both ESI and APCI in a single source. It can be operated in dedicated ESI or APCI modes, or a mixed ESI/APCI mode. Mixed mode ionizes the sample in both modes at the same time, for ionization of wider classes of compounds than possible in a single mode.

A few best-in-class specification features of the Single Quadrupole Walkup System are:

- **Single quadrupole sensitivity:** S/N > 75:1 (using 1 pg reserpine)
- **Single quadrupole mass range:** up to 2,000 m/z
- **Scan speed:** 10,000 Da/s

Agilent Single Quadrupole Walkup Software

Agilent MassHunter Walkup Software can be integrated with Agilent OpenLAB. An administrator can manage many of the systems remotely without having to be in the laboratory. The software package used for a Single Quadrupole Walkup setup consists of the following:

- 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
- Analytical Studio Reviewer (ASR), V: G3772AA, B.02.01.

Agilent MassHunter Walkup privileges

The MassHunter Walkup Software allows different user privileges including *administrator*, *MS chemist*, and *chemist*, each with different levels of accessible features. The administrator can:

- Access all features required, to create chromatographic methods
- Keep LC/MS systems running
- Assign regular monitoring of instrument performance using a test mix
- Clear errors
- Restart the system

An administrator can also define users or groups with different profiles of chromatographic expertise with different passwords and instrument parameters. Figure 1 summarizes the various privileges. Any administrative capabilities granted to a user or group require the use of a password to do those tasks.

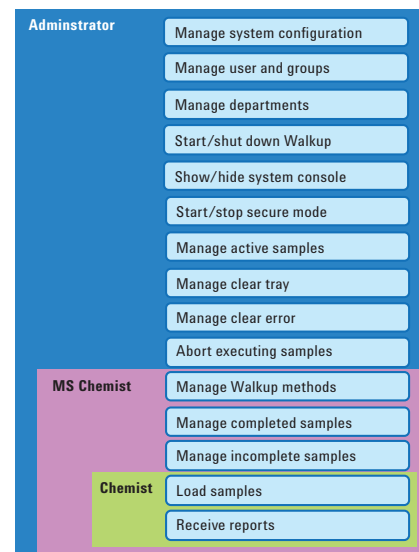


Figure 1. Summary of various privileges in Agilent MassHunter Walkup Software.

Agilent MassHunter Walkup administrator interface

Multiple MassHunter Walkup systems can be easily managed by adding an Agilent OpenLAB Shared Services Server. More details on this aspect are described in Technical Overview 5991-3786EN. The administrative interface includes many important and common functions related to instrument running, method selection, acquisition, and folder creations. These functions are systematically arranged for

easy navigation and operation. Using the **Samples/Events** tab, an administrator can navigate through the sample queue and clear the errors. Administrators can also view or edit the list of active and completed samples. They are also permitted to export the sample list (Figure 2). The **Samples/Events** tab also displays the **Run Events** pane. This pane allows them to run the scheduled but failed events. Using the **configuration** tab, an administrator can:

- Configure the system
- Create groups
- Configure the autosampler
- Schedule events, such as starting an equilibration method automatically
- Assign various file/folder naming patterns
- Set up automated email alerts
- Create workflows and methods, and assign them to users and groups

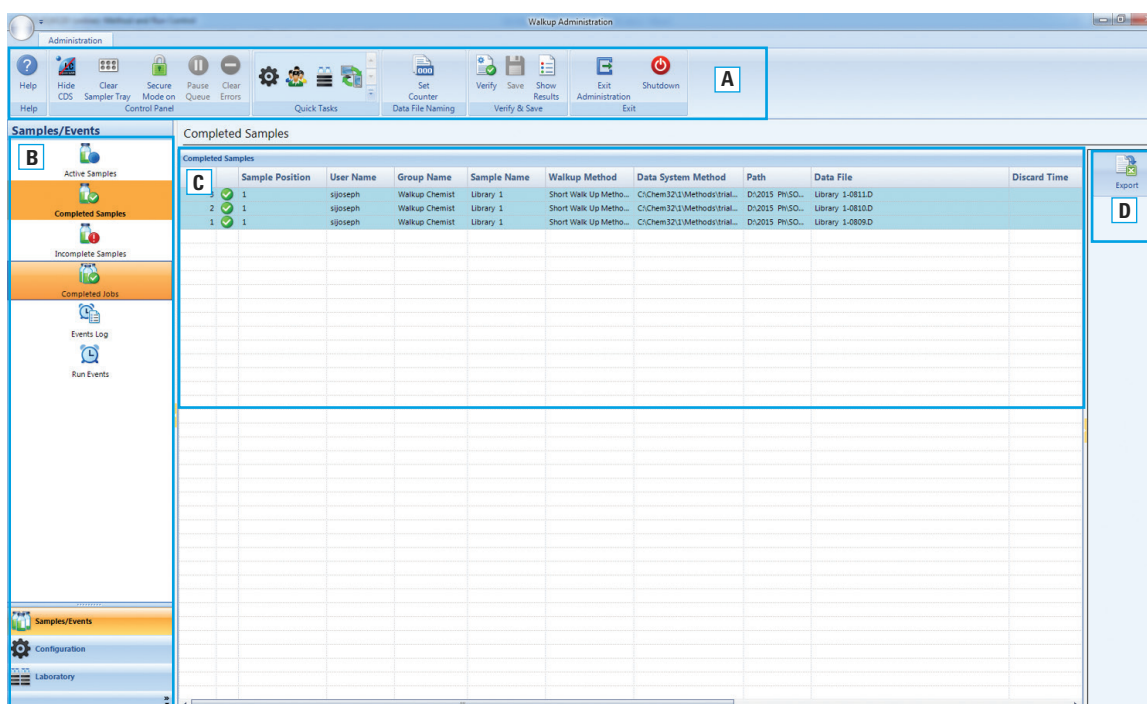


Figure 2. Typical administration interface in the **Samples/Events** tab. A) Administration control panel, B) navigation pane, C) list of samples as per the selected list, and D) option to export the selected sample list.

Administrative Features

Walkup method management

An administrator manages methods using **Walkup methods** in the **configuration** pane, and also defines which methods are available to each user. The Walkup administrator method setup page includes all the required fields for method selection, data analysis, and reporting settings directly in the user interface. The users do not need to navigate through other acquisition and

data analysis software (Figure 3). For the instrument robustness evaluation, using administrative privileges, a 10-minute method with methanol and ammonium acetate (pH 5.8) was created.

Chromatographic separation of an eight-compound mix was achieved using an Agilent Poroshell 120 EC-C18, 3 × 100 mm, 2.7 μm column. The data acquisition was performed using multimode source ionization with positive and negative polarity enabled.

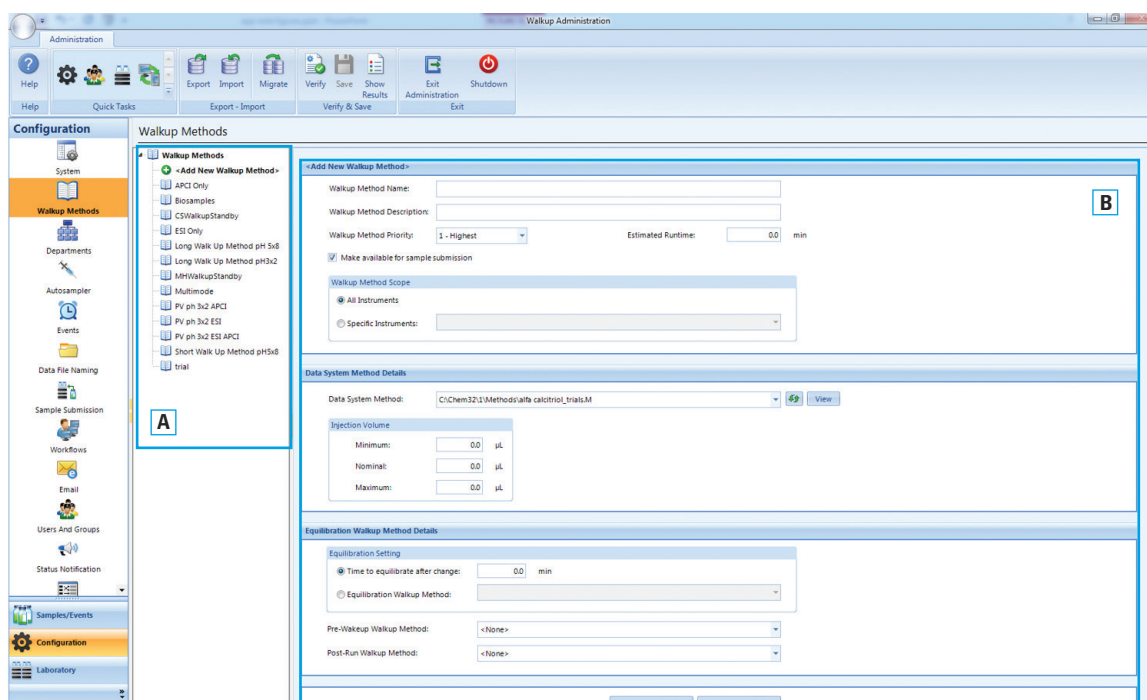


Figure 3. Typical Walkup method creation page within the configuration pane. A) List of walk up methods and B) space to assign and edit method parameters.

Walkup autosampler configuration

The Agilent MassHunter Walkup administration interface can be used to configure multiple autosampler options (ALS, HiPALS, HTC/HTS). The CTC sampler is a good choice for running many samples for extended periods without user intervention. While using an external tray (up to 17 vials), Walkup software indicates the vial positions in the external tray to load samples. A user

can load priority samples in the external tray, and the sampler arm moves them inside. The missing vial setup ensures continuous analysis, even if vials are found to be missing. It is also possible to **Enable Overlapped Injection** on this page (Figure 4). **Automatic discard** of injected vials (with optional delay) can also be configured through this page.

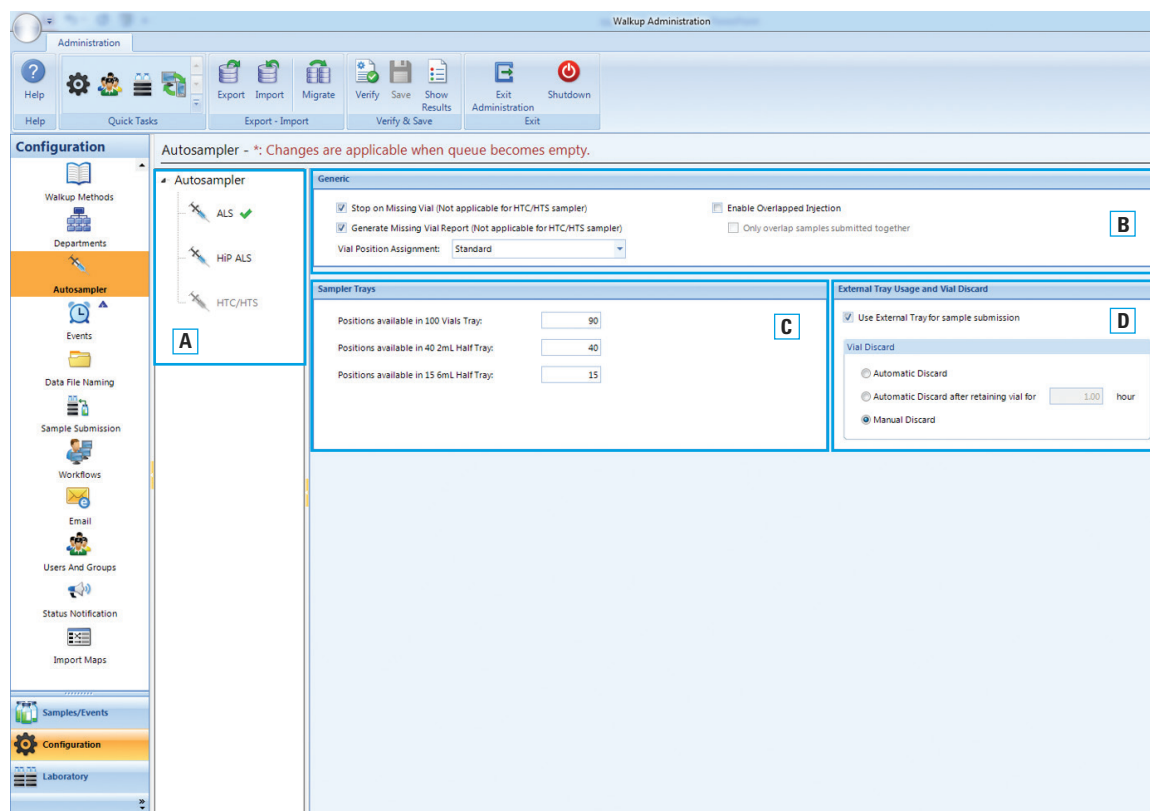


Figure 4. Typical autosampler setup page in the configuration tab within the administration screen. A) Place to assign sampler, B) location to keep missing vial/overlap injection, C) place to assign tray design and D) place to set up vial discard option.

Walkup report configuration

An administrator can configure the Walkup software to send the results of an analysis, including raw data files, to a selected user by email once the sample analysis is completed. Reports generated by Agilent ChemStation or Agilent MassHunter Analytical Studio Reviewer (ASR) can also be emailed. ASR is an intuitive user interface that allows users to quickly review LC/MS data. ASR creates single or batch reports, and can be customized to show the desired information as per lab requirements.

Walkup configuration certification

A MassHunter Walkup administrator can perform automatic verification or instrument compatibility checking of the set parameters. After verification, the Walkup software displays three types of notifications to the administrator:

- **Critical errors:** Which need to be resolved before the method can be saved
- **Warnings:** Potential areas of trouble
- **Messages:** Regarding the effects of recent changes (Figure 5).

The administrator can close the **Administration** screen and return to the Walkup Console, by clicking the **Exit Administration** button in the toolbar.

Robustness Evaluation of Single Quadrupole Walkup

A regular autotune is recommended to maintain MS system sensitivity over the mass range. By performing an autotune, the user can adjust the single quadrupole parameters to predetermined target values. However, in a multi-user Walkup environment, it is important to have regular procedures or checks to ensure the performance of the complete Walkup system. Optimal performance of the system can be checked by periodically testing a mix of compounds for a comparison of the UV chromatograms or MS results with standard results.

Configuration	Activity Result
1 System	System configuration is valid.
2 Walkup Methods	In Walkup method 'APCI', for assigned data system method 'C:\Chem32\1\Methods\APCI.M', selected reporting option must be 'Classical Reporting' for LCMS instrument.
3 Walkup Methods	In Walkup method 'ESI', for assigned data system method 'C:\Chem32\1\Methods\ESI.M', selected reporting option must be 'Classical Reporting' for LCMS instrument.
4 Walkup Methods	In Walkup method 'Method 1 Short pH32 Pos Neg', for assigned data system method 'C:\Chem32\1\Methods\Method 1 Short PHS45 ES APCI PosNeg.M', selected reporting option must be 'Classical Reporting' for LCMS instrument.
5 Walkup Methods	In Walkup method 'Method 2 Long pH 32 Pos Neg', for assigned data system method 'C:\Chem32\1\Methods\Method 2 Long PHS42 ES APCI PosNeg.M', selected reporting option must be 'Classical Reporting' for LCMS instrument.
6 Walkup Methods	In Walkup method 'Method 3 Long PHS45 Pos Neg', for assigned data system method 'C:\Chem32\1\Methods\Method 3 Long PHS45 ES APCI PosNeg.M', selected reporting option must be 'Classical Reporting' for LCMS instrument.
7 Walkup Methods	In Walkup method 'Method 4 performance verification', for assigned data system method 'C:\Chem32\1\Methods\Method 4 Short Performance Verification.M', selected reporting option must be 'Classical Reporting' for LCMS instrument.
8 Walkup Methods	In Walkup method 'Peptide high mass', for assigned data system method 'C:\Chem32\1\Methods\Method 5 PHS45 ES APCI PosNeg highmass.M', selected reporting option must be 'Classical Reporting' for LCMS instrument.
9 Walkup Methods	In Walkup method 'walk up stand by 59', for assigned data system method 'C:\Chem32\1\Methods\walk up stand by 59.M', selected reporting option must be 'Classical Reporting' for LCMS instrument.
10 Walkup Methods	Walkup Methods configuration is valid.
11 Departments	Departments configuration is valid.
12 Events	Events configuration is valid.
13 Data File Naming	Data File Naming configuration is valid.
14 Sample Submission	Sample Submission configuration is valid.
15 Workflows	Workflows configuration is valid.
16 Autosampler	Auto Sampler configuration is valid.
17 Email	Email configuration is valid.
18 Users And Groups	Users & Groups configuration is valid.
19 Status Notification	Status Notification configuration is valid.
20 Import Maps	Import Maps configuration is valid.
21 Walkup Administration	All configuration settings are valid. Click on Save button to save the settings.

Figure 5. Typical verification summary within the administration screen. A) List of errors, warnings, and alerts, B) summary of reports. There are no error messages shown in the given screenshot.

Test mix preparation

In this study, we used a mix of eight commercially available compounds. These compounds had a wide range of pKa values, and covered the typical range of hydrophobicity and molecular weights of pharmaceutical compounds. The use of a test mix for similar purposes has been described elsewhere¹. The compounds used for the instrument performance evaluation were:

- Metronidazole
- Glafenine
- Labetalol
- Hydrocortisone
- Dipyridamole
- Chrysin
- Disperse yellow
- Dipentyl phthalate

These compounds were purchased from Sigma-Aldrich (St Louis, MO, USA). A 5-ppm mixture of these eight standards was used as a test mix to evaluate the reproducibility of results from the instrument. In a similar fashion, the user can customize their own standard mix to evaluate the system performance based on their specific analytical needs.

Experimental details

Tables 1 and 2 list the LC and MS instrument parameters used for the performance evaluation. A mass range of 100–600 amu was used for the analysis of the test mix. Methanol was LC/MS grade, and an LC/MS eluent additive grade ammonium formate was used (Sigma-Aldrich, St Louis, MO, USA). Milli-Q water was used in all experiments (Merck, Darmstadt, Germany).

Table 1. Agilent 1260 Infinity LC method parameters.

Parameter	Value
Column	Agilent Poroshell 120, EC-C18, 3 × 100 mm, 2.7 μm at 40 °C
Mobile Phase A	Ammonium formate buffer, pH 3.2
Mobile Phase B	Methanol
Gradient	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
Injection volume	3 μL

Table 2. Agilent 6120B 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

Robustness evaluation

The instrument reproducibility evaluation monitoring can be assigned as a regular task at a customized interval. Creation of sample sequences using Walkup software is as simple as a few mouse clicks.

The administrator can simply describe the samples, select a reproducibility evaluation method from the list, and enter the number of replicates in the sequence creation step. The software displays the available sample positions in the plate or external tray, and indicates locations in the external tray into which the samples are placed. The robotic arm of the Agilent Infinity autosampler places the sample into the autosampler for injection. To evaluate the performance of the 6120B Single Quadrupole Walkup system, 3 μ L of the eight-compound mix was injected in six replicates. To assess robustness, the compound mix was injected at an interval of 200 injections, and the UV and 6120B Single Quadrupole (positive mode) traces were overlaid. The reproducibility of peaks was evaluated by comparing their resolution, height, and area.

Results and Discussion

Elution profile of the test mix

Figure 6 shows the UV elution profile of the test mix using a 10-minute method with methanol and ammonium acetate buffer (pH 5.8). The multimode, in positive mode, ionized all the compounds in the test mix, while in negative mode, only four compounds were ionized (Figure 7).

The chromatographic elution profile and ionization intensities of each component of the test mix were recorded. These initial data were used for the continual evaluation of instrument performance. This system suitability test can be repeated at regular intervals to compare and verify the chromatographic elution profile, multimode ionization, and fast polarity switching.

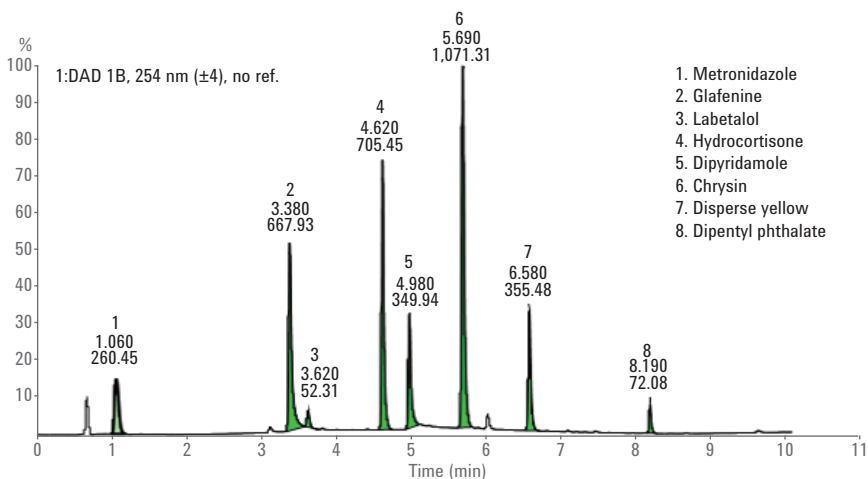


Figure 6. UV Elution profile of an eight-compound test mix.

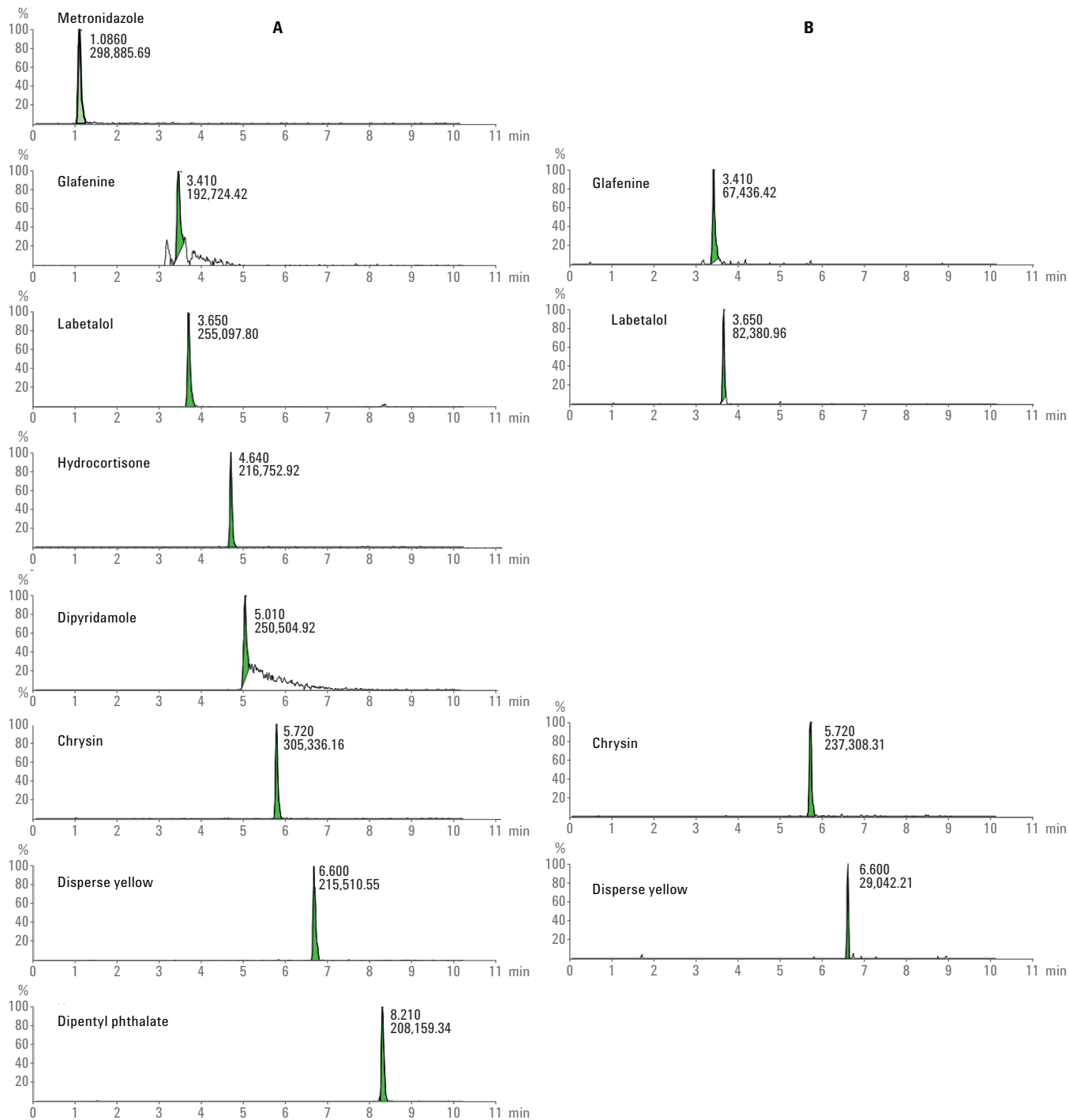


Figure 7. Extracted ion chromatograms (EICs) of individual components in the test mix. A) The EICs of test mix components in positive mode. B) The EICs in negative mode.

Agilent 6120B Single Quadrupole Walkup robustness evaluation

To evaluate the robustness, the test mix was injected before and after approximately 200 injections of other samples. The overlay of UV and Single Quadrupole TIC traces confirmed the reproducibility of the instrumentation (Figure 8). Thus, regular monitoring of instrument performance adds confidence to the results, and ensures maximum instrument uptime in a multi-user instrument.

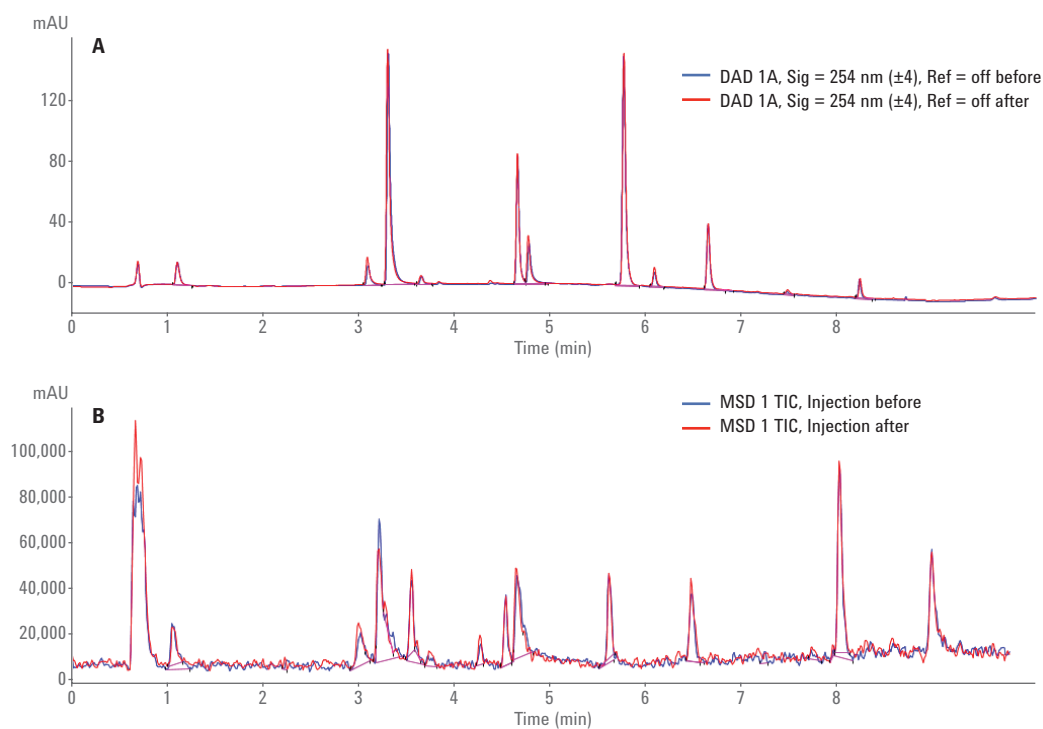


Figure 8. Reproducibility verification of Agilent 6120B Single Quadrupole Walkup instrumentation using an eight-compound mix. The top figure (A) is the overlay UV trace before and after 200 injections, whereas the bottom figure (B) is that of the MS TIC signal.

Conclusion

Agilent 6120B Single Quadrupole Walkup software administrator privileges are described and discussed in detail in this Technical Overview. This includes instrument configuration, method creation, sample loading, and report generation. Using these software features, an administrator can set up the 6120B Single Quadrupole Walkup system as an easy-to-operate and efficient analytical tool, for non-expert users. The reproducibility of the results produced by the LC/MS instrumentation is demonstrated using an eight-compound test mix.

References

1. Li, S.; *et al.* Enhanced Performance Test Mix for High-Throughput LC/MS Analysis of Pharmaceutical Compounds. *Chem.* **2006**, *8*, 820–828.
2. Walkup LC/MS Analysis. *Agilent Technologies Technical Overview*, publication number 5991-3786EN, **2014**.

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