Technical Overview

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Ensure Consistent and Reliable Results with Automated Daily System Suitability

Introduction

To ensure consistency, further reliability of our systems, and ease the burden on lab managers, Agilent codeveloped[1] a system suitability standard that enables universal monitoring of all LC/MS components—HPLC pump, autosampler, column, detectors, etc. The standard was designed to cover a wide range of compounds, including nonpolar, polar, pH sensitive, as well as both cations and anions. A completely automated end-to-end workflow was developed that only requires the user to keep a loaded standard vial in the autosampler using Agilent OpenLab CDS and Agilent WalkUp software. The instrument automatically checks the tune parameters, and injection events can be scheduled to be performed automatically, all the way to report generation.

Typical WalkUp LC/MS instrumentation

In this work, an Agilent InfinityLab LC Series with a single quadrupole mass spectrometer was used (Figure 1). The instrument configuration was as follows:

- Agilent 1290 Infinity II High-Speed Pump (G7120A)
- Agilent 1290 Infinity II Multisampler (G7167B)*
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
- Agilent 1290 Infinity II Diode Array Detector (G7117B)
- Agilent InfinityLab LC/MSD iQ (G6160AA)
- Agilent InfinityLab Flex Bench MS (G6015B)

*** For scheduled injection events, a multisampler is required that contains a hotel site for injection standards.**

OpenLab CDS software

Agilent OpenLab CDS is a modern chromatography data system that can operate and manage GC, LC, GC/SQ, and LC/SQ instruments with fully integrated data acquisition, processing, analysis, and reporting. OpenLab CDS can be configured for a networked environment with compliance features that support data integrity in accordance with US FDA 21 CFR Part 11, EU Annex 11, and other similar regulations. Most workflows can be fully automated with easy-to-set-up processing and reporting methods. Instrument uptime is maximized with early maintenance feedback (EMF), which gives real-time diagnostics of the instrument and its wearable parts and cues the user when certain parts are ready to be replaced. Instrument tuning is now automated with Tune Scheduler, where users can simply enter a date and time to automatically run the system tune or check tune. These features help inform users that their system is ready to use without the need of user input or knowledge. If all is green, then the instrument is ready to use. If something is wrong, like a clogged nebulizer, it will be highlighted in the EMF to quickly diagnose the problem and get the instrument back up and running.

Figure 1. Agilent LC/MSD iQ with an Agilent 1290 Infinity II LC.

WalkUp software

WalkUp is an open-access add-on control software that provides an extra layer of control over workflows and simplifies sample submission to a single screen.

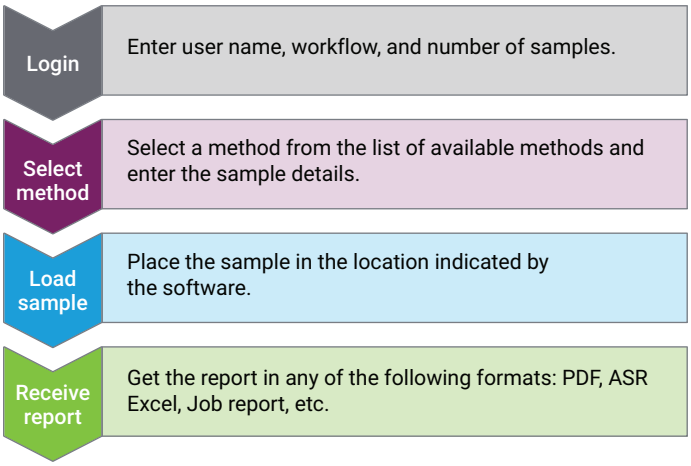


Figure 2. Typical user workflow for the Agilent LC/MSD iQ WalkUp system.

In an open-access system, control and simple sample submission are paramount. With WalkUp, results can be obtained from a simple workflow (Figure 2) that involves no more than walking up to the instrument, submitting the sample, and returning to your desk. All the methods can be locked to specific users or groups, and events such as system suitability can be automated.

OpenLab CDS with WalkUp is used in this work, but the same standard and method can also be used in Agilent MassHunter for LC/TQ and LC/Q-TOF, albeit without event scheduling and some minor differences in method setup and execution.

Agilent LC/MS 7-analyte system suitability standard

Table 1. Agilent LC/MS 7-analyte system suitability standards.

Image	Part Number	Description
	5191-4544	LC/MS 7-analyte system suitability standard, 1 × 1 mL vial
	5191-4546	LC/MS 7-analyte system suitability kit, 5 × 1 mL vials

The Agilent LC/MS 7-analyte system suitability standard (Table 1) has been tailored to check an entire LC/MS system, from column to mass detector. These compounds cover a wide range of hydrophobicity, contain positive and negative ions, are sensitive to pH, and have varied concentrations with a relatively stable shelf life. A single automated microliter injection is all that is needed every morning to check the quality of the instrument.

The standard contains the following seven compounds, shown in Figure 3.

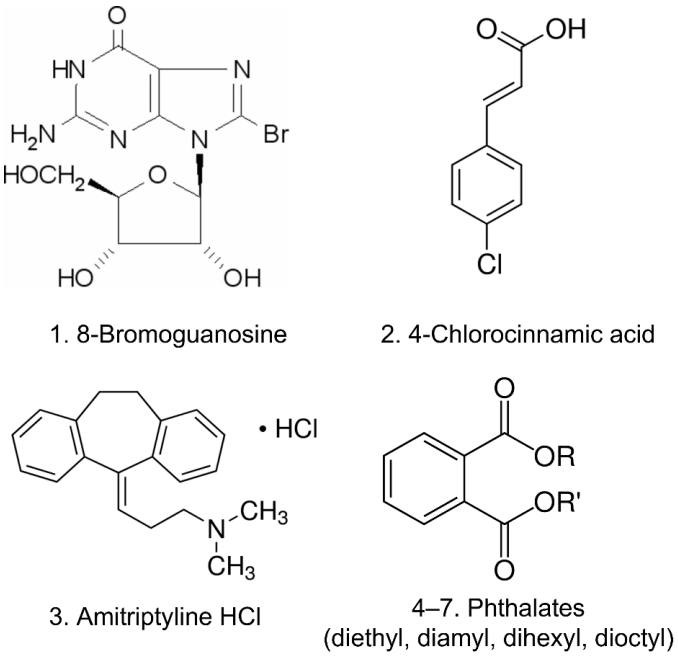


Figure 3. Compounds in the Agilent LC/MS 7-analyte system suitability standard.

The first compound, 8-bromoguanosine (8-BG), is very polar, elutes quickly from the column, and is used to test retention of polar analytes. In addition, it ionizes in both positive and negative mode and contains a bromine for confirmation of isotopic distributions. The next compound, 4-chlorocinnamic acid (4-CC), is acidic, only ionizes in negative mode, and tests whether the conditions are suitable to generate negative ions. The next compound, amitriptyline HCl (AMI), is basic and a representative pharmaceutical molecule that is very sensitive to the pH of the mobile phase. Shifts in amitriptyline's retention time (RT) give evidence that a buffer is failing, or the pH is changing over time. Amitriptyline is also notorious for oxidizing, which leads to the presence of a small, oxidized side peak—indicating that the standard is aging and needs to be replaced. The final compounds are all diphthalates of increasing chain size that test resolution and the elution and ionization of nonpolar compounds. The resolution is tested between the diethyl and diamyl phthalate (DEP and DAP) peaks while dihexyl phthalate (DHP) is 100x less concentrated than the other phthalates to test sensitivity. The final phthalate, dioctyl phthalate (DOP), is very nonpolar and is the final check that everything has eluted from the column.

System suitability workflow

LC/MS acquisition parameters and reference injection of standard

The Agilent LC/MS system suitability checkout method uses an Agilent InfinityLab Poroshell 120 EC-C18 column with an acetonitrile (ACN) and formic acid gradient. This method can also be optimized for high pH or buffered solutions with a variety of organic solvents such as methanol. The use of a short column and relatively fast flow rate allow for 3-minute run times with 30 seconds of equilibration time needed between injections.

A reference injection of the system suitability standard should be performed to set up the automated processing. This should be done on a clean, calibrated system as it will be the reference used for the daily reports. The LC/MS acquisition method (Table 2) was set up in OpenLab CDS data acquisition and a single sample injection was performed with 1 µL of the LC/MS 7-analyte system suitability standard.

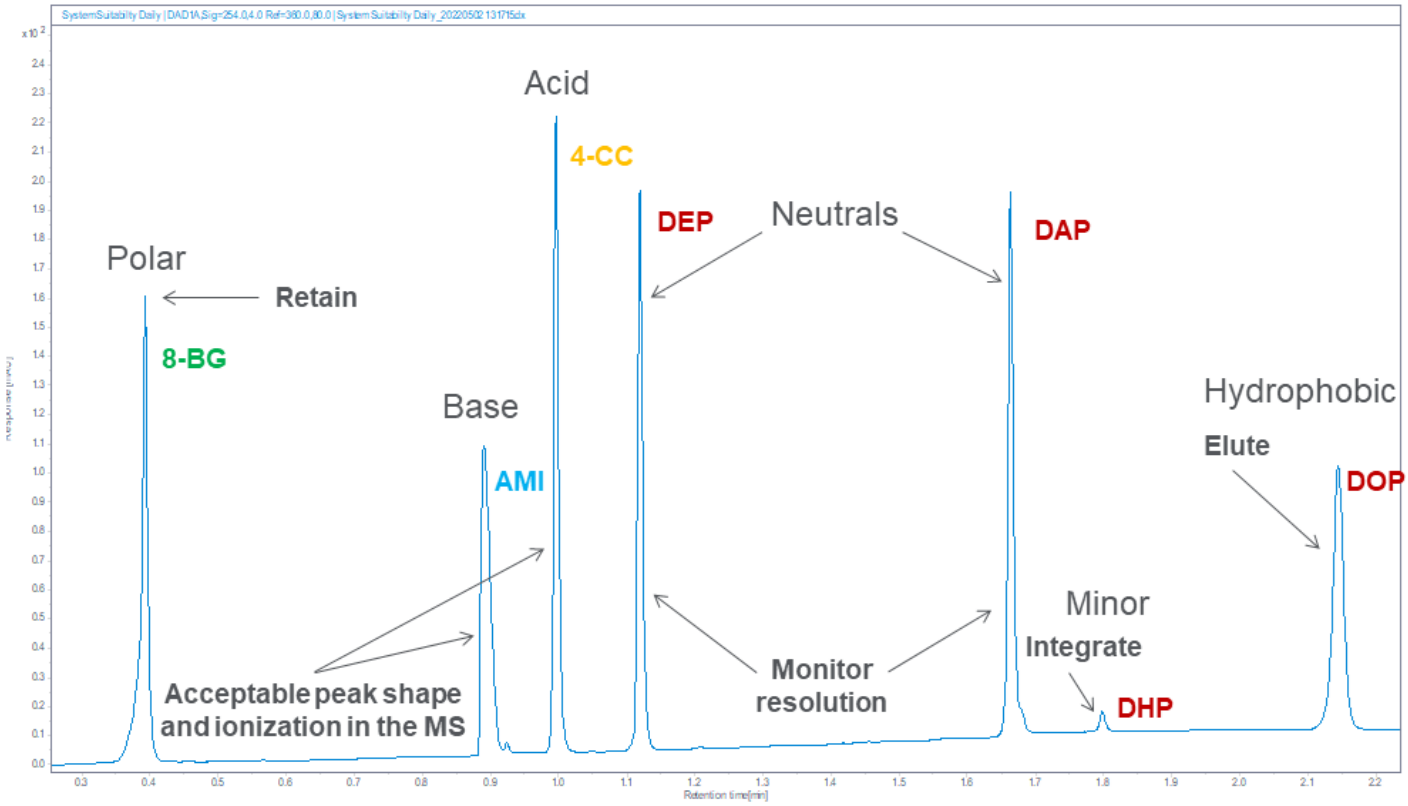


Figure 4. UV chromatogram of the Agilent LC/MS 7-analyte system suitability standard.

Table 2. Agilent LC/MS system suitability checkout method parameters.

Parameter	HPLC Set Value
Column	Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 1.9 µm at 40 °C (p/n 699675-902)
Solvent A	0.1% formic acid in water
Solvent B	0.1% formic acid in acetonitrile
Gradient	**Time (min) % B** 0.00 5 1.75 90 2.90 90 3.00 5
Postrun	0.5 minutes
Flow Rate	0.8 mL/min
Injection Volume	1 µL
UV	Sig = 254, 5 nm; Ref = 360, 80 nm
MS	Two scans, ESI+ and ESI–, *m/z* 50 to 600

OpenLab CDS processing method

The reference injection was processed using OpenLab CDS data processing. The initial processing method was an "MS" type method and integration parameters were set up using the Integration Optimizer tool. The positive and negative TIC chromatograms were checked to make sure that only the peaks of the standard were integrated (Figure 5); this ensures the automated compound update process will only add the peaks of the standard. The peaks in the standard were easily identified by the UV chromatogram, which contained strong absorbances for all the compounds. Compounds were added to the compound table for each respective UV peak by right-clicking the peak in the UV chromatogram and adding the peak as a compound to the method. Compound names were assigned according to the elution order in Table 3.

Table 3. Agilent LC/MS 7-analyte system suitability standard compound retention times (RTs).

Peak	Compound	UV RT (min)	MS Scan RT (min)
1	8-BG	0.403	0.423
2	4-CC	0.878	0.887
3	AMI	1.011	1.020
4	DEP	1.130	1.124
5	DAP	1.658	1.655
6	DHP	1.792	1.797
7	DOP	2.147	2.186

Compounds for the MS signal were added to the method automatically, using the **Add/Update MS compounds** feature, with a right-click on any of the MS chromatogram windows. **Add/Update MS compounds** will then automatically add compounds to the compound table for each integrated peak, add reference MS spectra and extract EICs for each compound, and set up qualifiers as configured in the method. The method now contains all the necessary information to produce a system suitability report.

The final step is to include a report template in the processing method and to indicate that the report should be printed to file according to the preferred file type (.docx, .pdf, .csv, .xslx, and .txt). All reports were generated as .pdf for this work. The processing method can now be saved and is ready for the WalkUp software and operation.

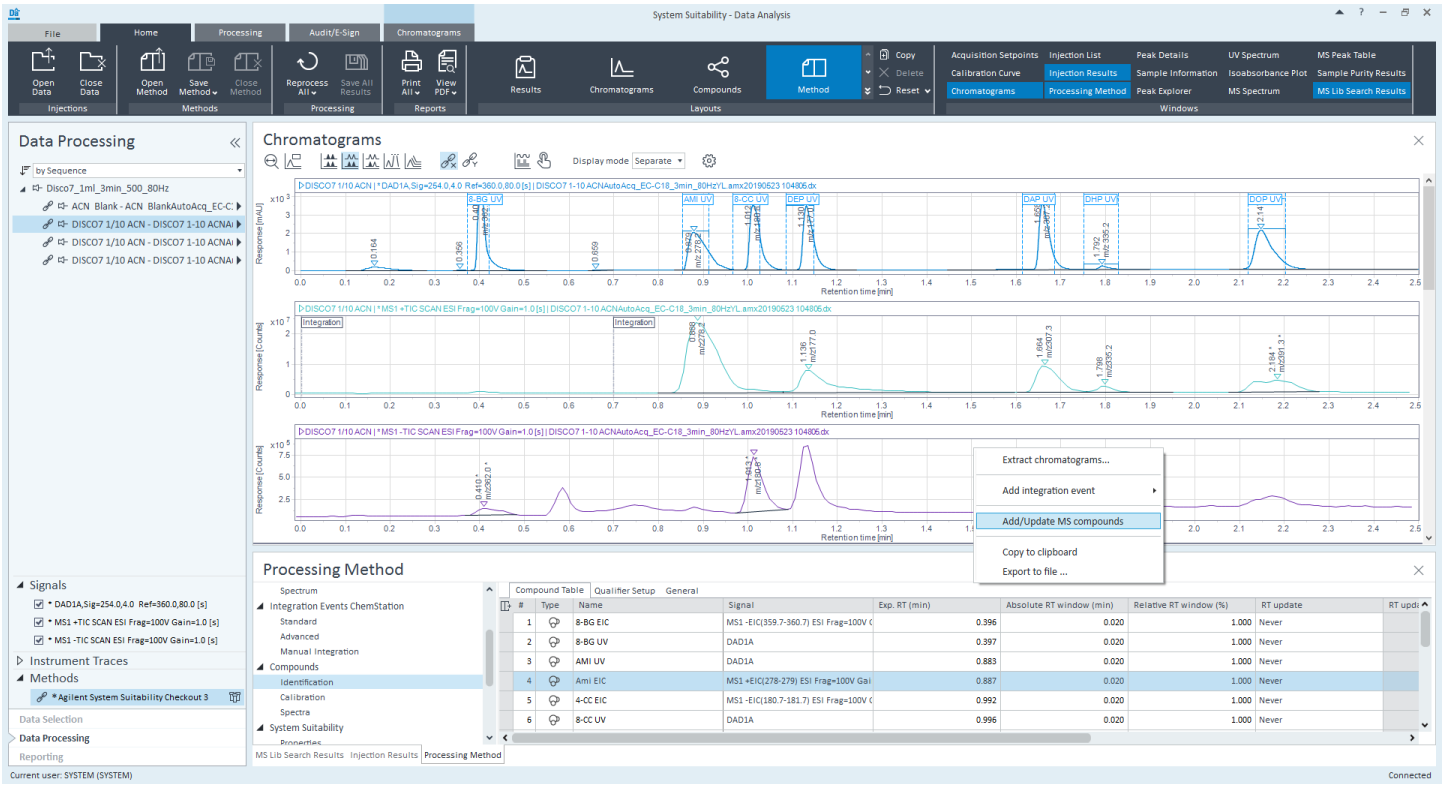


Figure 5. Agilent OpenLab CDS data processing: A 1 µL reference injection of the Agilent LC/MS 7-analyte system suitability standard was processed using the Add/Update MS compounds feature.

An OpenLab CDS processing method must first exist to make a WalkUp method. WalkUp uses the native data acquisition and processing, whether it be OpenLab CDS, OpenLab ChemStation, or MassHunter, to perform its tasks—so, the method must exist in the native software first. Therefore, any automation that is set up in the OpenLab CDS method will also be executed by WalkUp.

This allows for automatic reports to be generated by WalkUp without ever needing to access OpenLab CDS data analysis. Users simply need to only submit their samples through the WalkUp Rapid Sample Submission interface and WalkUp takes care of the rest. A report with the injection results will be automatically generated and sent to the user via email or stored on the system/server as configured (Figure 6).

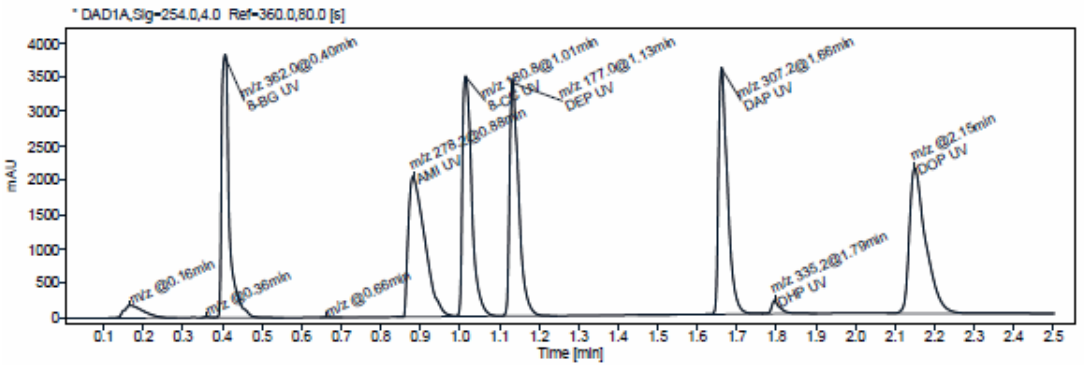
System Suitability Daily Report

Agilent Technologies

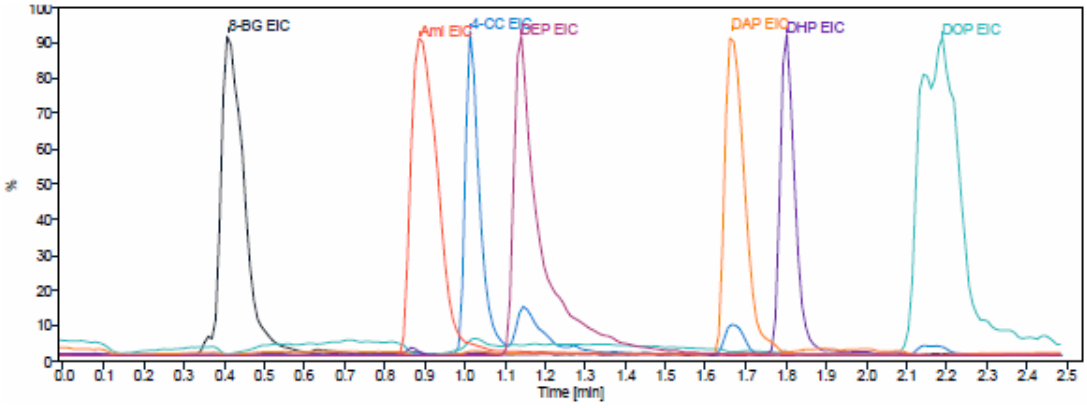
Project: *Test* **Acquired Date:** 2019-05-23 10:48:43-07:00

Instrument: 1290 Multisampler LC/MSD iQ

UV Chromatogram



Extracted Ion Chromatograms



UV Results

Name	RT [min]	Exp RT	Area	FWHM	Symmetry	Res. USP
8-BG UV	0.402	0.397	5225.03	0.018	0.564	1.769
AMI UV	0.879	0.883	5947.14	0.047	0.388	4.094
8-CC UV	1.012	0.996	5746.84	0.025	0.494	2.210
DEP UV	1.130	1.116	5461.25	0.024	0.473	2.862
DAP UV	1.658	1.650	5721.55	0.024	0.494	12.469
DHP UV	1.792	1.790	291.21	0.020	0.524	3.584
DOP UV	2.147	2.160	6114.87	0.042	0.470	6.966

MS Results

Name	RT [min]	Exp RT	Area	m/z	MS Match	MS Confirmation
8-BG EIC	0.410	0.396	147856.07	362.0	819.6	Confirmed
Ami EIC	0.887	0.887	82004858.79	278.2	993.2	Confirmed
4-CC EIC	1.013	0.992	720590.73	180.8	739.1	NotConfirmed
DEP EIC	1.137	1.114	4090605.44	177.0	833.3	Confirmed
DAP EIC	1.662	1.654	17116908.75	307.2	926.1	Confirmed
DHP EIC	1.798	1.795	3194597.06	335.2	874.4	Confirmed
DOP EIC	2.185	2.167	15548909.96	391.3	923.9	Confirmed

Figure 6. System Suitability Daily Report: The report checks the current system suitability run against the reference run in the method. The UV peaks are reported with peak width, symmetry, and resolution. The MS peaks are reported with their *m/z* and MS confirmation to the MS reference spectra. Peak shapes and retention times can be observed in the UV and EIC chromatograms.

WalkUp events

The WalkUp method was set up using the previously mentioned acquisition parameters (Table 2) and processing method saved in the OpenLab CDS project folder as a .amx and .pmx file, respectively. It was named "Daily System Suit", given a run time of 3 minutes and injection volume min/max of 1 µL. It is important to note that the project folder must be the same in WalkUp Admin as the project folder in OpenLab CDS containing the .amx and .pmx files. Otherwise, a conflict will be flagged when trying to save the WalkUp method, and the user will be pointed to the data file configuration tab to change the folder. WalkUp validates all changes in the Admin console before saving the configuration and will automatically highlight to the user where the conflict is occurring.

With the method set up, a WalkUp event can be configured to run the system suitability method automatically. Within the Events tab in the WalkUp Admin console (Figure 7), the event was named "SystemSuit" and given a run frequency of every 20 runs. The Daily System Suit method is selected and hotel #3 is being used for the sample. This means that after every 20th submitted run, the system will pause the queue, equilibrate the system if needed, and inject the system suitability mix with the selected method. Once the injection is complete, the system will restart the queue and once again equilibrate the system if needed. In most cases, the method used for the system suitability has the same solvents as the methods being submitted, so no equilibration is necessary.

The events can also be configured to run on certain times and days of the week, after a tray has completed in the queue, after waking up from idle, etc. Any method can be selected for the event, so it is not just limited to system suitability. For example, wash steps can be introduced or calibration curves updated. WalkUp has built-in logic that will equilibrate the system if needed, whenever a change in the method is detected. This is especially useful in open-access environments where the mobile phase may be changing constantly and different methods are being used for a variety of samples.

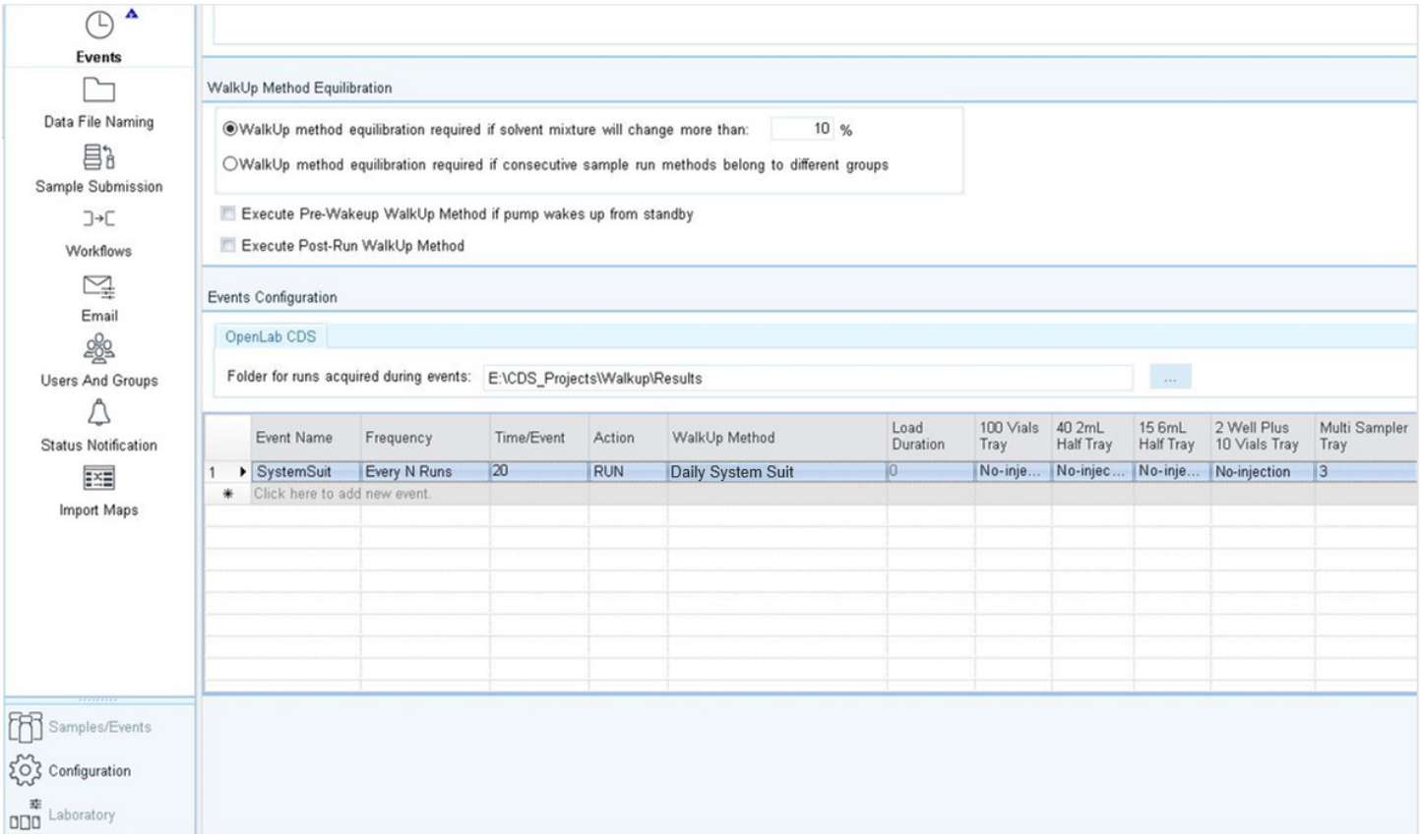


Figure 7. WalkUp events: An injection event of the system suitability standard is scheduled to run every 20 injections and take the standard from hotel #3 in the multisampler tray.

Custom reporting

Reports can be sent directly to the lab manager's email or securely to an external server in addition to storage on the instrument workstation. WalkUp reports are generated with the Intelligent Reporter templates (.rdl) created in OpenLab CDS data analysis. Therefore, any reports can be customized or created within OpenLab CDS data analysis and used for WalkUp. Custom system suitability workflows can be easily created by following the same steps outlined in this work with a custom system suitability mixture. The user only needs to collect a reference data set and process it in OpenLab CDS.

An example of custom reporting can be found in Figure 8. This report performs a statistical analysis of the peak retentions times and areas for the last 100 injections of the system suitability standard. The EIC chromatograms of the seven compounds are overlaid, and tables are given for the UV peaks and EIC peaks. The thresholds for the peak relative standard deviations (RSDs) are given, and the actual values are highlighted for each peak. Green is below threshold, yellow is less than 2% above threshold, and red is more than 2% above threshold. Charts plotting the peak areas for each injection are shown to the right of the tables. This is just one example of a custom report that can be generated using OpenLab CDS reporting.

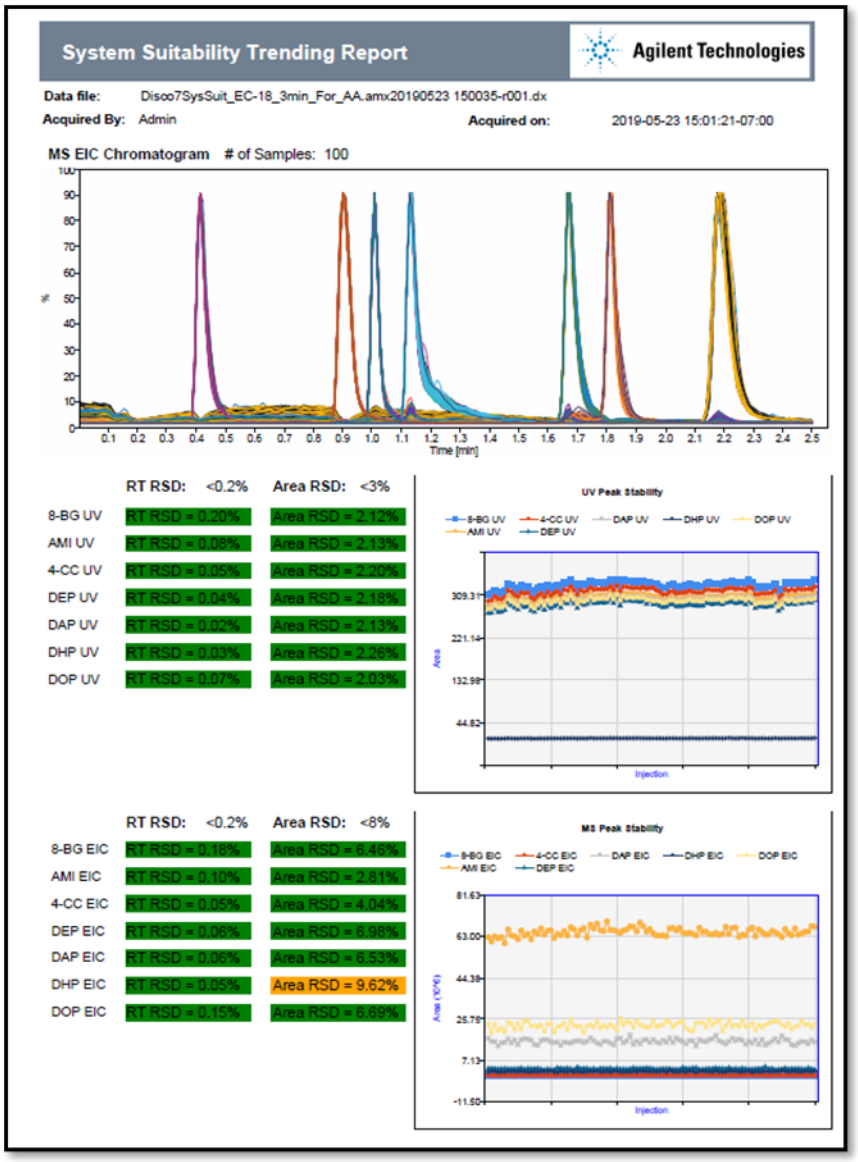


Figure 8. A custom System Suitability Trending Report: The report uses the daily system suitability injections to produce trending data that can be used to predict column health and troubleshoot issues.

Conclusion

Obtaining reliable results is of utmost importance in any laboratory and knowing something as simple as "is the instrument ready for analysis?" can be difficult to discern without the proper methods in place. System suitability methods check if an instrument is ready and can produce reliable results from a known standard.

Typically, labs must design their own system suitability standards and workflows to test their systems, which can be a complex and timely process. In open-access environments, this can be especially difficult because of the unknown nature and wide range of samples being analyzed. The Agilent LC/MS 7-analyte system suitability standard provides a universal and reliable standard that can be used to test an entire instrument—from the LC column to the mass spectrometer.

Agilent OpenLab CDS with Agilent WalkUp software can perform automatic workflows at predetermined times. With a WalkUp system suitability event configured, the system can automatically wake itself, run an injection of the system suitability standard, and generate a report of the system's suitability. The report can be emailed directly to the lab manager, notifying them if the system is ready for use. The event can be scheduled daily before the work hours or to run after a set of plates has been submitted to ensure the system is not dirty or uncalibrated. This complete workflow can help labs know if their instruments are ready and maximize uptime by scheduling events outside of normal working hours.

Reference

1. Mutton, I. *et al.* The Design and Use of a Simple System Suitability Test Mix for Generic Reverse Phase High Performance Liquid Chromatography–Mass Spectrometry Systems and the Implications for Automated System Monitoring Using Global Software Tracking. *J. Chrom. A.* **2011**, 3711–3717.

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