



Walk-Up Liquid Chromatography System in Multi-User Environment Using Agilent MassHunter Walkup Software for LC and LC/MS Systems

Analysis of synthetic compounds using UV, MS and ELS detection

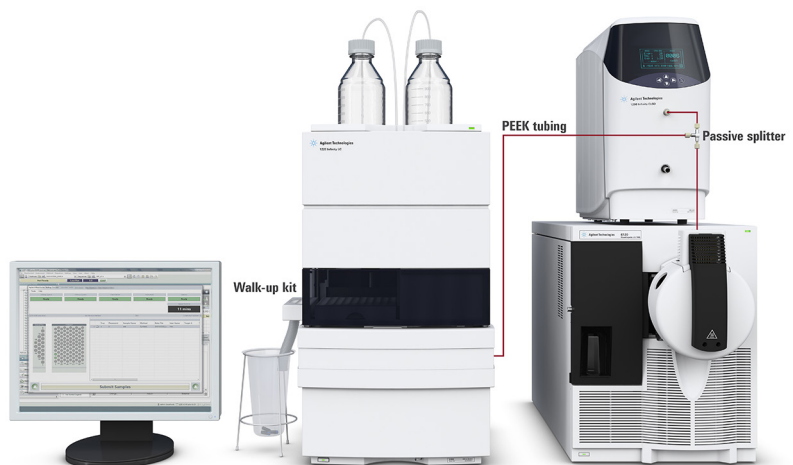
Technical Overview

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Abstract

This Technical Overview describes a LC system with three detectors in a multi-user environment operated with Agilent MassHunter Walkup Software for LC/MS and LC systems. The setup enables the highest coverage rate regarding impurity identification in a pharmaceutical or chemical synthesis laboratory. The combination of UV, Evaporative Light Scattering Detector (ELSD), and a Mass Spectrometric Detector (MSD) leaves no compound missed. The walkup software also enables inexperienced users to easily submit samples for subsequent analysis, and obtain their results remotely by email.



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Introduction

Chemists, in chemical synthesis laboratories synthesizing compounds for the pharmaceutical industry, usually have little or no experience in analytical procedures such as liquid chromatography (LC) or mass spectrometry (MS). Simplified analytical workflows using so-called walkup LC/MS systems can be used to provide easy access for inexperienced users. The Agilent MassHunter Walkup Software for LC/MS and LC systems allows the users to “walk up” with their samples^{1,2}. Simplified sample information input, method choosing, sample positioning, and information output, for example through email, enable easy sample submission and analysis. The software also offers administration features for super-users to provide chromatographic methods, keep LC/MS systems running and, in case of errors, be able to restart the system. In addition, the super-user can define different user profiles, passwords, and other system relevant parameters. Users or user groups with different chromatographic expertise can have different access levels.

To minimize the risk of missing impurities, a system setup was chosen consisting of a Diode Array Detector (DAD), an Evaporative Light Scattering Detector (ELSD), and a Mass Spectrometric Detector (MSD). The detectors complement each other³.

Because impurities might lack UV absorbance or have poor ionization capabilities, this setup provides the highest coverage regarding impurity identification.

Experimental

The system setup consisted of the following modules:

- Agilent 1220 Infinity LC System with DAD (G4294B) plus 1220 Infinity Walk-up Upgrade (G4291A)
- Agilent 1260 Infinity Evaporative Light Scattering Detector (G4260B)
- Agilent 6120 Single Quadrupole LC/MS (G6120B)

Software

OpenLAB CDS ChemStation Edition for LC and LC/MS Systems, Rev. C.01.05 [35]

Agilent MassHunter Walkup Software for LC/MS and LC systems C.01.00

Solvents

All solvents were LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22- μm membrane point-of-use cartridge (Millipak). Formic acid was purchased from Sigma-Aldrich, St.Louis, USA.

Equipment setup

A network hub was used to connect the PC, the LC, the ELSD, and the MS. The remote connection was set between the MS and the ELSD with the Agilent 1220 Infinity LC System, which is equipped with two remote connection ports. The in-series flow path connection between the 1220 Infinity LC System with DAD, ELSD, and the MSD was a passive flow splitter with PEEK tubing.

Results and Discussion

The MassHunter Walkup Software for LC/MS and LC systems enables simplified sample submission and status checking for inexperienced users. After logging onto the instrument by supplying a password, an optional security option, and the number of samples (Figure 1), the user describes the samples and assigns a method from a list (in this case called Synthesis), Figure 2.

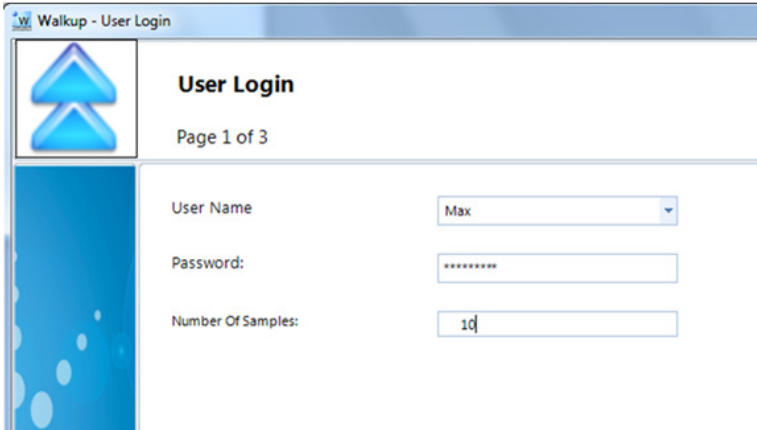


Figure 1. Agilent MassHunter Walkup User Login.

Chromatographic conditions		
Mobile Phase A	Water + 0.1 % formic acid	
Mobile Phase B	Acetonitrile + 0.1 % formic acid	
Column	Agilent ZORBAX RRHD Eclipse Plus C18, 3 × 50 mm, 1.8 μm (p/n 959757-302)	
Sample	Various synthesis test mixes	
T-Connector	60 cm/red PEEK capillary (0.13 mm id) in arm A (DAD to splitter), 60 cm/red PEEK capillary (0.13 mm id) in Arm B (splitter to MS) and 5 cm/yellow PEEK capillary (0.18 mm id) in Arm C (splitter to ELSD). Resulting in approximately 1.150 mL/min arriving in the ELSD and approximately 50 μL/min flowing into the MS.	
Gradient	Time	%B
	0.00	20.00
	5.00	95.00
	7.00	95.00
Post time	3 minutes	
Flow rate	1.2 mL/min	
Injection volume	10 μL	
Temperature TCC	30 °C	
DAD	254 nm/16 nm, Ref.: 360 nm/100 nm	
Data rate	> 0.013 minutes (0.25 second response time) (20 Hz)	
ELSD		
Evaporator temperature	40 °C	
Nebulizer temperature	40 °C	
Gas flow rate	1.2 SLM	
Data rate	40 Hz	
Smoothing	20	
PMT gain	1	
MSD scan		
Scan range	150–1,000	
Peak width	0.02 minute	
Threshold	150	
Step size	0.10	
Fragmentor	70	
MSD source		
Ionization mode	API-ES	
Polarity	Positive	
Gas temperature	350 °C	
Drying gas	10 L/min	
Nebulizer pressure	20 psig	

The methods have to be set up by the super-user in the administration screen, which is usually accessible by the super-user only. The software shows locations in the external tray of the autosampler where the samples should be placed. After finishing sample submission, the robot arm of the autosampler places the submitted sample into the autosampler for further processing, and analysis starts.

In this example, the user has submitted 10 different samples and the user interface shows the progression of sample analysis, displaying the remaining time for the actual run and the complete queue (Figure 3). The blue circles represent running samples, and the green circles represent samples waiting for analysis. The external tray is empty and new samples can be submitted by the next user. The samples can be automatically discarded after analysis using position 18 of the external tray (Figure 3). This position is connected to a waste tube, which ends with a waste container. This setting is found in the administration UI for the autosampler configuration.

A typical application for a walkup system is the implementation in a chemical synthesis lab, where different synthesized samples are analyzed for the right compound or impurities, for example, pharmaceutical compounds. Nine UV chromatograms of the 10 submitted pharmaceutical samples are displayed in Figure 4. Many of them show different impurities, resulting in the need for further purification or other polishing steps.

Three detectors were combined to reduce the chance of missing impurities that might not be seen by a single detector, for example, due to lacking UV absorbance or poor ionization capabilities. Figure 5 shows an example of a synthesized sample with impurities that cannot be seen using a single detector. The main peak in the DAD chromatogram is, for example, not visible or minimally visible with the ELSD or the MS, whereas the first peak in the ELSD is not visible or only marginal visible in the DAD.

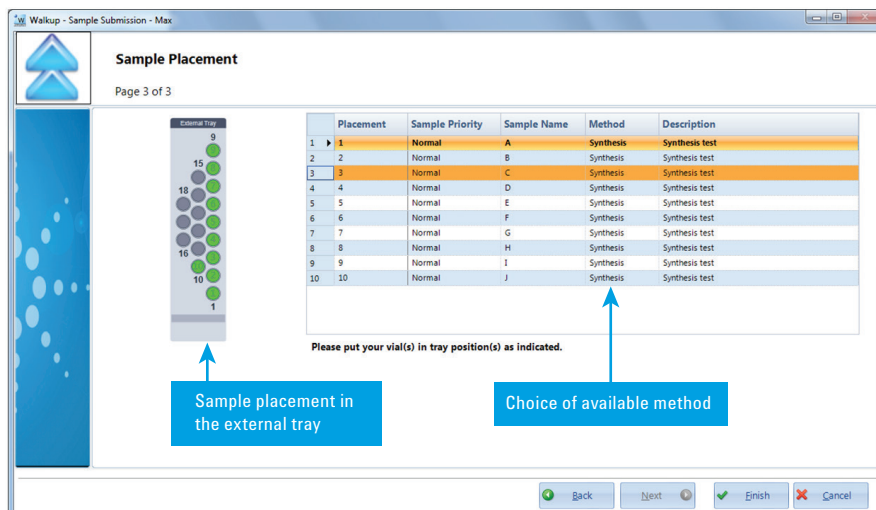


Figure 2. User interface for sample placement.

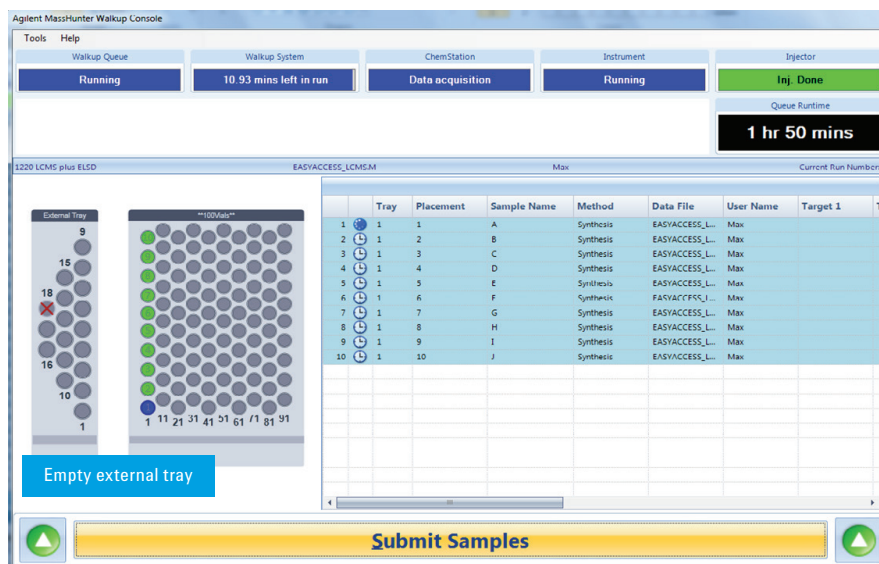


Figure 3. Agilent MassHunter Walkup Console User Interface – after sample submission and run started.

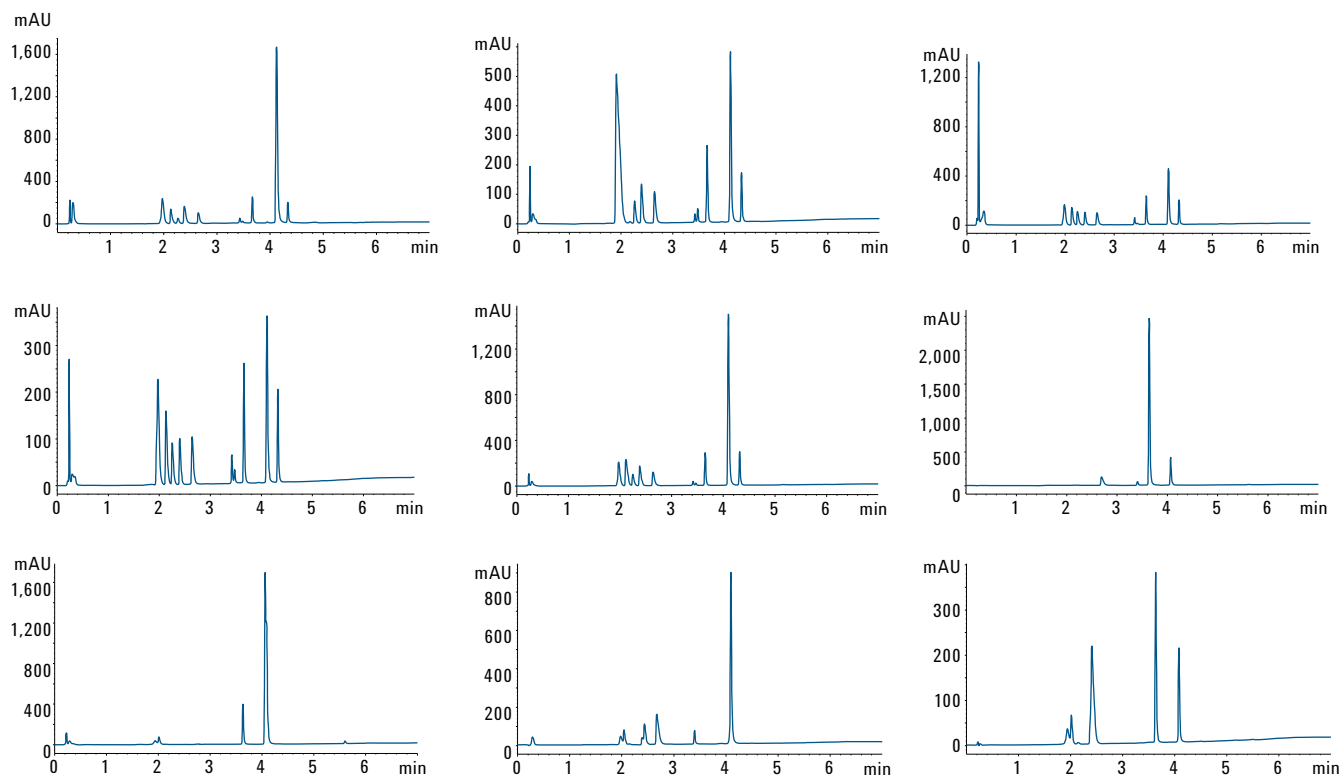


Figure 4. Synthesis check of nine different synthesized compounds for impurities with UV detection (DAD).

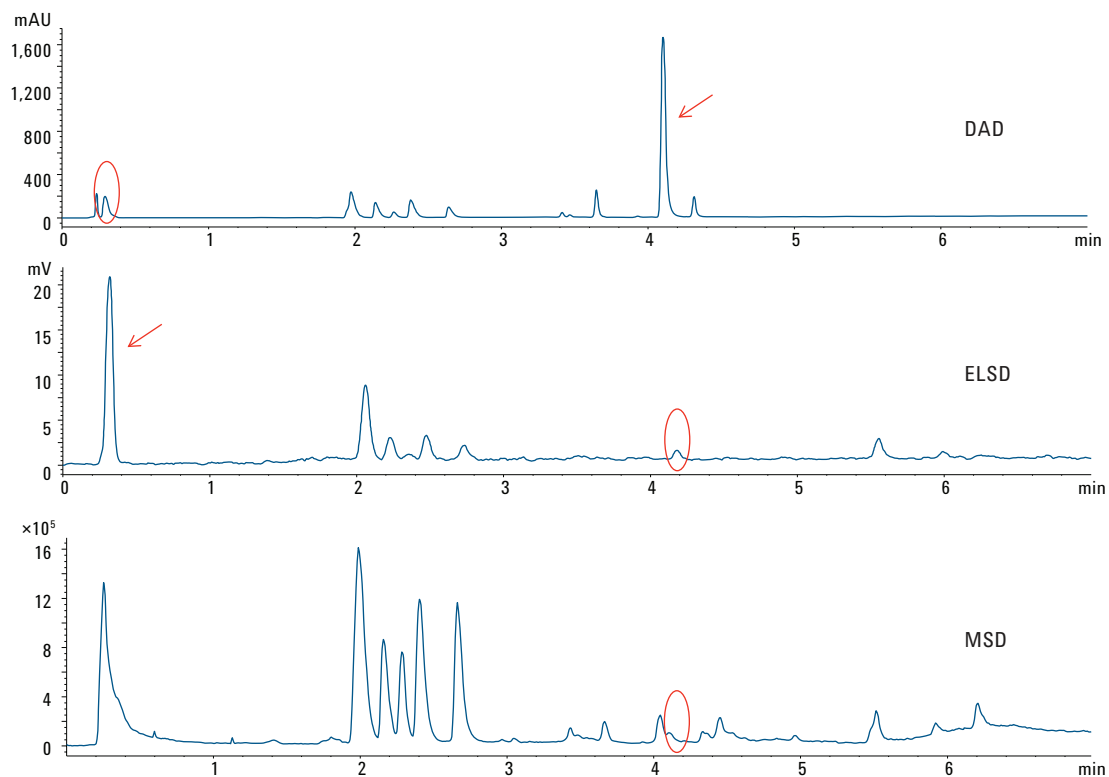


Figure 5. Synthesis check using three detectors types: DAD, ELSD, and MS.

Conclusions

The combination of the Agilent MassHunter Walkup Software for LC/MS and LC systems together with a three detector setup including DAD, ELSD, and MS enables the detection of most impurities in a chemically synthesized pharmaceutical compound. The three detector systems complement each other so that no compound or impurity is missed. The software also provides an easy sample submission and analysis for inexperienced LC/MS users. The user only describes the amount and type of samples, chooses a predefined method and can remotely obtain the results through email. The administration feature of the software allows method development in addition to instrument maintenance and user management.

References

1. "Agilent MassHunter Walkup System, Quick Start Guide" *Agilent publication* Publication number G2725-90016, July **2013**.
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3. "RRLC impurity profiling to detect non-UV absorbing compounds using diode array detection, single quadrupole MS and evaporative light scattering detection" *Technical Overview* Agilent Publication Number 5990-4980EN, **2009**.

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