Automated Purification of Compound Libraries Using the Agilent 1260 Infinity Automated LC/MSD Purification System

Technical Overview

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Abstract

Purifying a large number of compounds by liquid chromatography usually requires screening experiments and manual interaction during upscaling to preparative conditions. The Agilent Automated Purification software facilitates the workflow of analytical scouting to preparative purification by smart algorithms and calculated scale-up of separation gradients, all integrated in an intuitive interface. This Technical Overview demonstrates the purification of a compound library using the Agilent 1260 Infinity Automated LC/MSD Purification System controlled by the Automated Purification software.

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Introduction

Preparative liquid chromatography (LC) is a technique that is frequently applied in pharmaceutical chemistry or early stages of drug discovery. Depending on the compound of interest, optimum chromatographic conditions usually need to be determined by an analytical scouting run before large amounts of sample are purified. This process can be time-consuming, especially with the high number of samples typical in pharmaceutical chemistry.

The Agilent Automated Purification software facilitates this scale-up process, and supports analysts in speeding up their workflows, providing true automatic scale-up from analytical to preparative gradients. An intuitive interface guides the user along the complete workflow including analytical scouting, scale-up, focused gradient generation, fraction collection and review, and export of results. Smart algorithms check the validity of user inputs on-the-fly, and prevent the user from proceeding if conflicting parameters are entered. Two different views offer either the complete settings for method developers or a restricted set of parameters for operators or walk-up users (Easy Prep mode). Once all details of the task are entered, the workflow can be started either in fully automatic mode or with the option to manually change parameters and review analytical results before proceeding with the preparative runs.

The workflow in the Automated Purification software consists of an analytical scouting run, an (automatic or manual) evaluation step, and a preparative run with fraction collection. During the scouting run, diluted samples are analyzed by a generic gradient on the analytical flow path of the Agilent 1260 Infinity Automated LC/MSD Purification System. In the evaluation phase, the compounds of interest are identified by target masses specified in the analytical sequence, and aligned with the signal of the UV detector. These data can be imported from any analytical Agilent LC/MSD System. Based on the matching signals, the virtual elution point of each target compound is calculated. Software algorithms generate a focused gradient that is optimized for the calculated elution point of the target compound. The purification is then run on the preparative flow path of the system, applying the calculated focused gradient for each sample, enabling optimized separation and collection of the target compound.

This Technical Overview demonstrates the analytical-to-preparative workflow with the Automated Purification software using a sample library of eight compounds. Impure samples were separated and purified by peak-based fraction collection triggered by a combination of ultraviolet (UV) and mass selective detector (MSD) signals. Fraction purity and recovery of the injected sample amounts were measured as performance markers.

Experimental

Instrumentation

The Agilent 1260 Infinity Automated LC/MSD Purification System consisted of the following modules:

- Agilent 1260 Infinity Preparative Pump cluster (G1361A + G1391A)
- Agilent 1260 Infinity Dual-Loop Autosampler (G2258A)
- Agilent 1260 Infinity Diode Array Detector (G1315C) with 3 mm preparative flow cell (Option #022)
- Agilent 1290 Infinity II Preparative Open-Bed Fraction Collector (G7159B)
- Active Splitter (G1968F)
- Agilent 1260 Infinity Quaternary Pump (G1311B)
- Agilent 1260 Infinity Valve Drive (G1170A) with preparative 2-position/10-port valve head (G4730A)
- Universal Interface Box (UIB) II (G1390B)
- Agilent 6150 Single Quadrupole LC/MS (G6150B)

Columns

Analytical column

Agilent Prep C18 Scalar,
4.6 × 50 mm, 5 µm (p/n 446905-902)

Preparative column

Agilent Prep C18, 21.2 × 50 mm PrepHT cartridge, 5 µm (p/n 446905-102) with PrepHT end fittings (p/n 820400-901)

Software

All experiments were conducted using the Agilent Automated Purification software A.01.04 [038], an add-on to the Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS Systems, version C.01.07 SR2 [263], controlled by LC Drivers A.02.15 [026].

Solvents and samples

All solvents used 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). Acetaminophen, acetanilide, benzocaine, caffeine, ethylparaben, propylparaben, salicylic acid, sulfamerazine sodium salt, and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich, Taufkirchen, Germany. Samples were prepared in DMSO at a concentration of 10 mg/mL of the target compound and seven impurities at approximately 1 mg/mL each. Analytical scouting samples were diluted 50x in DMSO.

The analytical-to-preparative purification workflow in the Automated Purification software is called a task. Each task can hold a single sample or a sequence of multiple samples, and needs input of basic parameters such as sample name and location, target formula or mass, injection volume, and fraction start location. A generic analytical gradient is used for all scouting runs within a task; preparative gradients are calculated specifically for each sample after target identification.

Method settings

Table 1. Settings of the analytical base method for scouting runs.

Parameter	Description
Valve position	1: Analytical flow path
Mobile phase	A) 0.1 % Formic acid in water B) 0.1 % Formic acid in acetonitrile
Flow rate quaternary pump	1.5 mL/min
Flow rate preparative pump cluster	0.0 mL/min
Gradient quaternary pump	0.00 minutes – 2 %B 0.33 minutes – 2 %B 5.00 minutes – 98 %B 5.50 minutes – 98 %B 5.51 minutes – 2 %B
Stop time	6.50 minutes
Injection volume	5 µL, lower loop (50 µL)
Detection	Monitored signals: 230 nm, 254 nm, 270 nm Peak width >0.05 minutes (1 second response time) 5 Hz Data rate

Table 2. Settings of the preparative base method for purification runs.

Parameter	Description
Valve position	2: Preparative flow path
Mobile phase	A) 0.1 % Formic acid in water B) 0.1 % Formic acid in acetonitrile
Make-up solvent	D) 0.1 % Formic acid in methanol:water (70:30, v:v)
Make-up solvent flow rate	1.5 mL/min (100 %D)
Preparative flow rate	31.86 mL/min
Preparative gradient	0.00 minutes – 2 %B Gradient will be calculated by the Agilent Automated Purification software
Stop time	6.50 minutes
Injection volume	500 µL, upper loop (5,000 µL)
Detection/Trigger	230 nm/254 nm/270 nm (auto-selected by the software) Peak width >0.05 minutes (1 second response time) 5 Hz Data rate
UIB II Settings	Peak width >0.013 minutes (0.25 seconds response time) (50 Hz) Expected ERI mode: No mode check External contacts: Contact A: Closed
UIB II Timetable	0.00 minutes Change contacts Contact A state: Closed 0.01 minutes Change contacts Contact A state: Open
Fraction collection	Peak-based fraction mode Use MSD for mass-based fraction collection

Results and Discussion

Once all necessary parameters were entered, the task was started in fully automatic mode. After analytical scouting runs of each sample, the Automated Purification software was able to identify the target compound in each of the eight impure samples. Monitoring the extracted ion chromatogram (EIC) of the respective target mass in the MSD enabled alignment of the UV with the MSD signal even when peaks were not perfectly separated at baseline (Figure 1). Focused preparative gradients were created automatically (Figure 2), enabling scale-up by a factor of 5,000. Of the three monitored UV wavelengths, the software selected the one with the highest response of the target compound, ensuring most sensitive fraction triggering.

Fraction collection was triggered by a logical AND connection of the UV with the MSD signals. This combination enables highly selective collection of the target peaks only, which can reduce the total number of collected fractions, and enable more efficient use of the fraction collector capacity. After completion of all purification runs, the results are displayed in a comprehensive, interactive view. Collected fractions can be browsed in a table view, per sample, or by their location in the fraction collector. Clicking on a sample, fraction table entry, or fraction location highlights the corresponding peak in the UV chromatogram and EIC. Conversely, clicking on a peak displays the UV and mass spectra, and highlights the fraction location in the table and collector view (Figure 3). An export function enables the creation of .csv or Agilent OpenLAB CDS ChemStation Sequence files to process or re-analyze selected fractions.

Table 3. MSD Spray chamber and signal settings.

Parameter	Description
Spray chamber	Agilent Jet Stream electrospray
Signal 1	Positive scan 125–750 Fragmentor 125 V
Signal 2	Negative scan 125–750 Fragmentor 125 V
Nebulizer pressure	30 psig
Drying gas temperature	300 °C
Drying gas flow	12.0 psig
Sheath gas temperature	350 °C
Sheath gas flow	11.0 L/min
Capillary voltage	±1,300 V
Nozzle voltage	±2,000 V

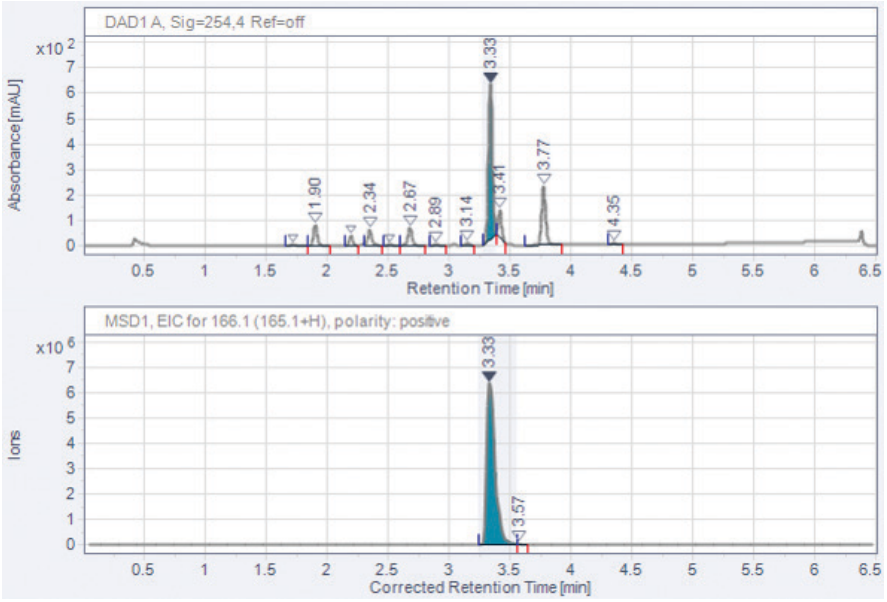

Figure 1. Automatic target peak recognition by alignment of UV and MSD signals.

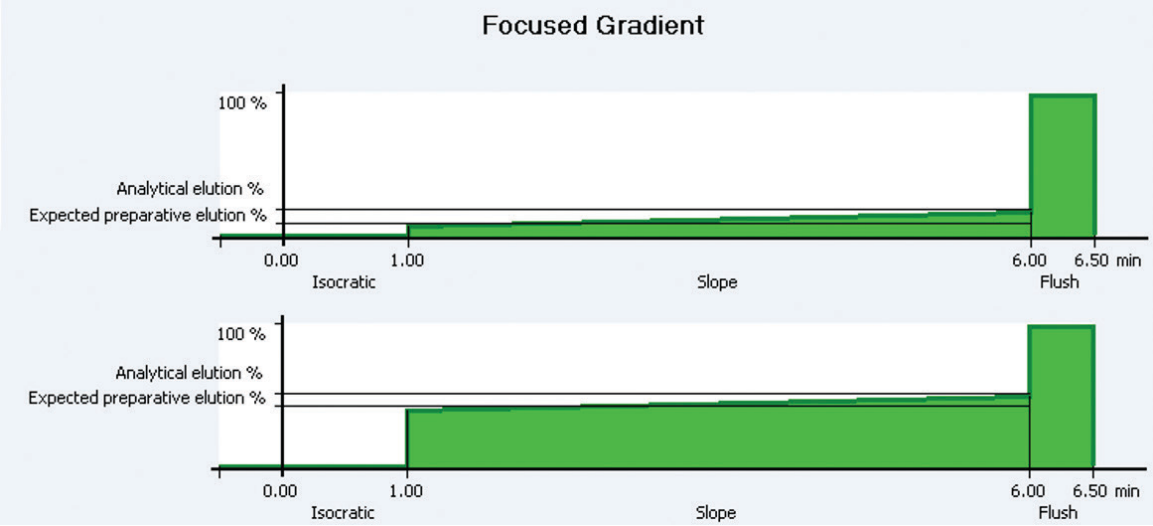

Figure 2. Sample focused gradients for early eluting (top) and strongly retained (bottom) compounds. Initial and flush phases are held constant, whereas the slope concentration start is adjusted to the elution point of each sample.

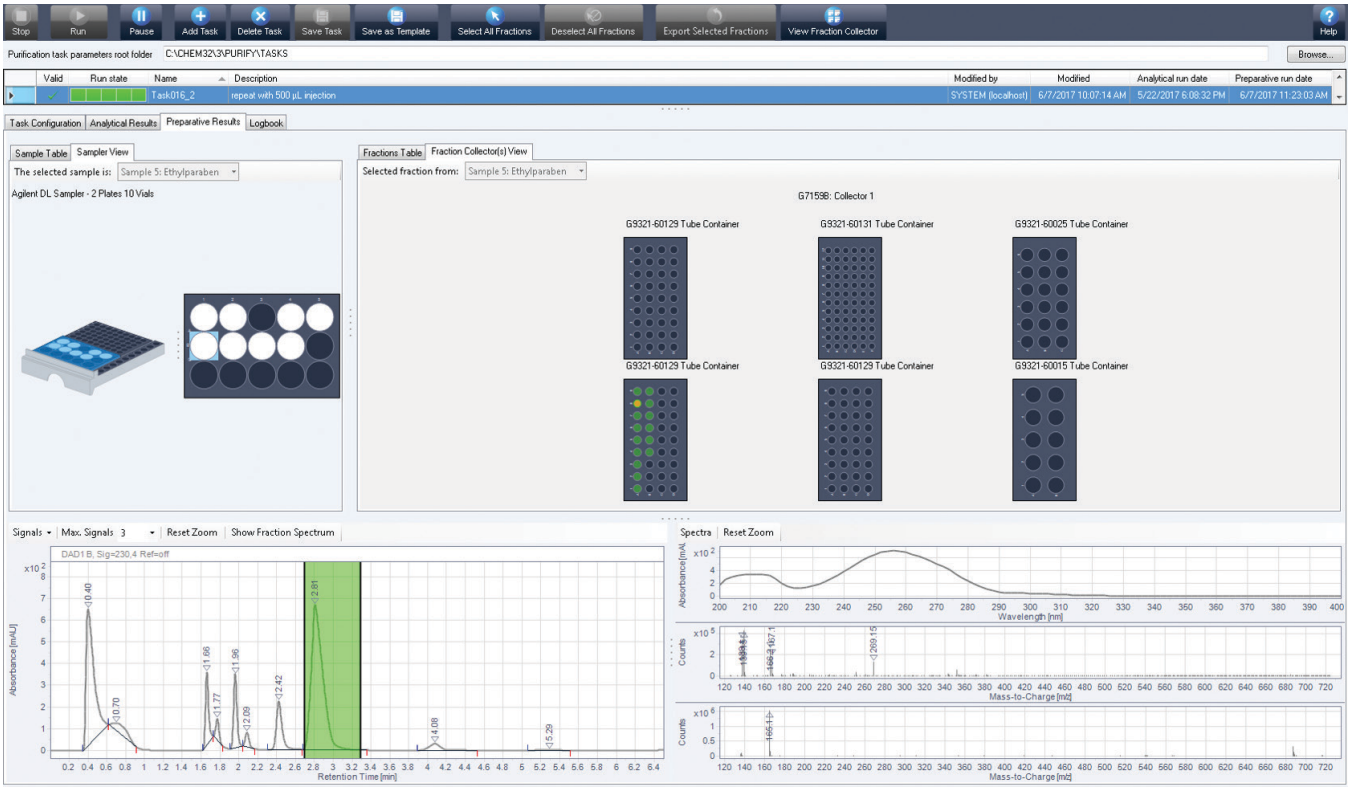

Figure 3. Preparative results view in the Agilent Automated Purification software. Sample information, fraction locations, UV and mass spectra, as well as the chromatogram of the preparative run are visible at a glance (clockwise starting top left).

All collected fractions were re-analyzed with respect to purity and recovery of the injected compound. Purity was 94 % or higher throughout all fractions. With the exception of one compound, all samples had a recovery of 91 % or higher (Table 4). This demonstrates that even in fully automated operation, the Automated Purification software produces pure and reliable results.

Table 4. Purity and recovery of collected fractions.

Compound	Purity	Recovery
Acetaminophen	>99 %	91 %
Acetanilide	>99 %	93 %
Benzocaine	99 %	92 %
Caffeine	94 %	96 %
Ethylparaben	97 %	92 %
Propylparaben	99 %	95 %
Salicylic acid	99 %	95 %
Sulfamerazine	99 %	81 %

Conclusion

The Agilent Automated Purification software is a versatile add-on to the Agilent OpenLAB CDS ChemStation that facilitates the workflows of purification laboratories. All crucial steps from analytical scouting to fraction collection and data review are combined in an intuitive user interface. With truly automated scale-up, on-the-fly calculation of focused gradients, and import and export functionalities, the Automated Purification software is a valuable tool to speed up the process of LC purification for experienced and novice users. This Technical Overview demonstrates the purification of a compound library on an Agilent 1260 Infinity Automated LC/MSD Purification System controlled by the Automated Purification software. Even in fully automated operation, fraction purity and recovery are typically above 90 %.

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Published in the USA, September 1, 2017
5991-8438EN

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