Automated Workflow Solution for Preparative Chromatography From Analytical Scouting to Fraction Reanalysis

Agilent Automated Purification Software on the Agilent 1260 Infinity Preparative-Scale LC/MS Purification System

Technical Overview

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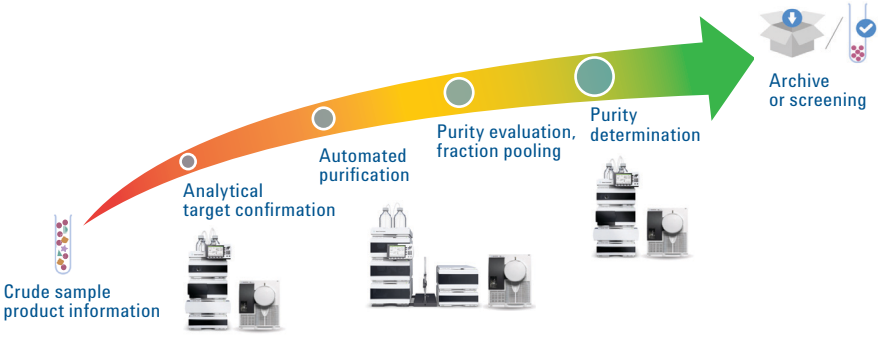
Abstract

The purification of a valuable product or product library after a long and complex synthesis is a very important process. The Agilent Automated Purification Software introduces an Open-Access-based solution for isolating substances, with automated focused gradient to simplify the pre- and post-purification steps.

This Technical Overview describes the easy steps to follow, from crude sample analytical scouting submission to pure target product archiving or screening. For example, a purification sample containing a target peak overlapped by an impurity was purified on the Agilent 1260 Infinity Preparative-Scale LC/MS Purification System using the Automated Purification Software. This purification produced a pure target compound with 88 % recovery, saving 40 % of solvent and runtime compared to a standard purification method.



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Introduction

In synthetic chemistry, automation of the purification process is one of the keys for increasing the productivity of a research group. Scaling-up a compound separation performed on an analytical system to a preparative liquid chromatography system requires an optimization step on the analytical column. This step concerns the development of the gradient method for the isolation of the target compound with the best balance between purity, throughput, and time. For these perspectives, a focused gradient profile offers the best solution for the purification of the target compound. To enhance the fine focused gradient profile, the user must have a good knowledge of preparative liquid chromatography and method development (more detail about the analytical to preparative scale-up process in the Agilent Technical Overview, 5991-2013EN[1]).

The Agilent Automated Purification Software combines the performance of the focused gradients and automation of the preparative chromatography workflow. This add-on to the Agilent OpenLAB CDS Chemstation gives users privileges in an Open-Access work environment.

To that end, the software provides two different user modes:

- In the Expert Mode, users can easily choose to use the default automated purification settings or use a customized method profile.
- In the Easy-Prep mode, the operators follow the defined easy-to-use user interface.

The method used in the Easy-prep mode can only be fully managed and customized by the administrator.

This Technical Overview describes the purification steps in the Easy-Prep mode, described from the simplified operator view.

Experimental

Instrument

Analytical

Agilent 1260 Infinity Binary LC/MS System comprised of:
Agilent 1260 Infinity Binary Pump (G1312B)
Agilent 1260 Infinity High Performance Degasser (G4225A)
Agilent 1260 Infinity High Performance Autosampler (G1367E)
Agilent 1260 Infinity Thermostatted Column Compartment (G1316A)
Agilent 1260 Infinity Diode Array Detector (G1315C) equipped with the standard flow cell (G1315C#018)
Agilent 6140 Single Quadrupole Mass Spectrometer

Preparative

Agilent 1260 Infinity Preparative-scale LC/MS Purification System comprised of:
Agilent 1260 Infinity Preparative Pumps (G1361A, G1391A)
Agilent 1260 Infinity Preparative Autosampler (G2260A)
Agilent 1260 Infinity Diode Array Detector (G1315C) equipped with a Quartz flow cell 0.06-mm path length (G1315C#026)
Agilent 1200 Series column organizer (G1383A)
Agilent 1260 Infinity Fraction Collector PS (G1364B)
Agilent 6120 Single Quadrupole Mass Spectrometer

Column

Agilent ZORBAX RRHT StableBond SB-C18, 2.1 × 50 mm, 1.8 µm, (825700-902) comprised of:
Agilent ZORBAX RRHT Cartridge Hardware Kit, 50-mm (820555-901)
Agilent ZORBAX Prep HT SB-C18, 21.2 × 100 mm, 5 µm (870100-902) with end fittings (820400-901)

Software

Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS Systems, Rev. C.01.05 [40]
Agilent Automated Purification Software Add-On, Rev. A.01.01

Solvents and samples

Solvents A: Water + 0.1 % formic acid
Solvents B: Acetonitrile + 0.1 % formic acid
Purification mixture for analytical and preparative runs: Drug-like sample mixture
All solvents used were LC grade, not degassed. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22-µm membrane point-of-use cartridge (Millipak, EMD Millipore, Billerica, MA, USA).

Chromatographic and spectrometric conditions

Analytical generic gradient method	
Flow	0.800 mL/min
Column temperature	40 °C
Isocratic hold at 2 % B for 0.2 minutes	
Gradient slope from 2 to 98 % in 4 minutes	
Purge at 98 %B for 0.8 minutes	
Injection volume	0.5 µL
UV wavelength	230 nm, 40 nm bandwidth, no reference
Preparative focused gradient method	
Automated focused gradient	Generated on-the-fly from the analytical scouting run. Desired compound identification from the molecular formula/mass.
Flow rate	29.3 mL/min (automated flow rate from the Automated Purification Software)
Injection volume	350 µL
UV wavelength	230 nm, 40 nm bandwidth, no reference
Mass spectrometric conditions	
Scan/fragmentation parameters	
API-ES ionization mode, positive/negative switching	
Percent cycle time/polarity	50.00 %
Scan mass range (negative and positive)	125–725
Fragmentor	70
Gain EMV	1.0
Threshold	150
Step-size	0.20
MSD spray chamber	
Gas temperature	350 °C
Drying gas	12.0 L/min
Nebulizer pressure	50 psig
Quadrupole temperature	350 °C
VCap (positive)	3,500 V
VCap (negative)	3,000 V

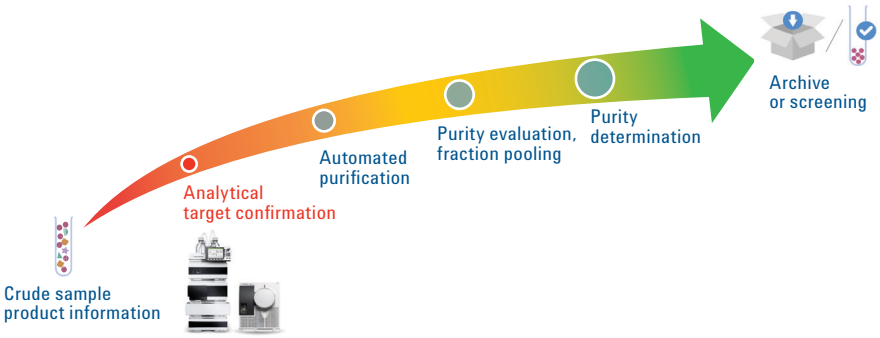
Results and Discussion

Analytical target confirmation

Task creation

After the completed reaction has been monitored on any Agilent analytical system, such as a 1260 Infinity Binary LC/MS System, the sample is submitted for purification. The Easy-Prep user interface of the Automated Purification Software is divided into two different sections, a task progression section and a task configuration and results section (Figure 1).

In the task progression section, the user is invited to create a task with a batch name or sequence name to save the task results (analytical and preparative runs), for example, into a work group folder. This task is created with a combination of systems, one for the analytical run, and one for the preparative run.



The resulting combination is then displayed and summarized **in the task configuration and results section** under the System Selection tab. Each system description includes predefined characteristics such as dwell volumes, column specifications, flow rates, delay time between UV and MS, or delay time between detectors to fraction collectors.

This system selection assigns the administrator a predefined configuration of the preparative gradient profile, which needs to be applied for a specific scale-up process.

In this particular case, the system characteristics from the 1260 Infinity Binary System located in Laboratory 2 and the Open-Access 1260 Infinity Preparative-Scale LC/MS System were assigned for the purification process. A gradient profile was generated from the resulting combination of systems, taking into consideration the different parameters of the scale-up process.

Task progression section

1. Task creation
2. Batch/task name

Task configuration and results section

3. System selection overview

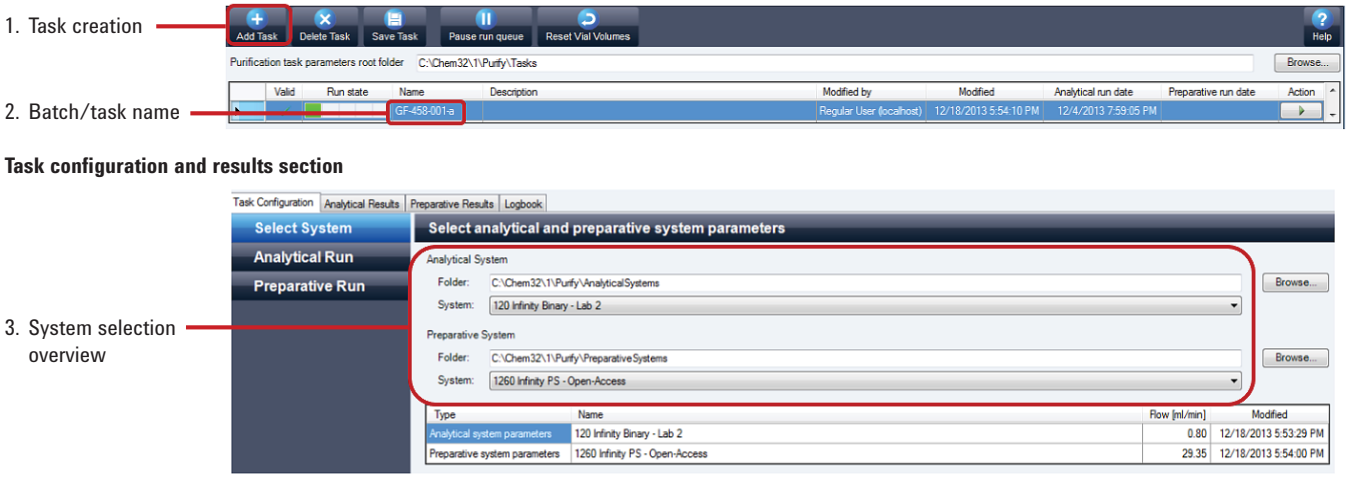


Figure 1. Agilent Automated Purification Software user interface under an Easy-Prep user view.

Analytical target confirmation

Analytical run(s) selection and preparative task submission

To complete the task submission, the user has to browse the analytical result under the analytical data root folder, and specify the preparative sample tray locations and injection volumes.

Analytical mass-spectral data provides the required information for identification of the target peak. By extracting the target compound ion chromatogram, the software automatically identifies the target peak for the preparative run.

The chromatographic conditions of the analytical data characterize the elution of this target peak. The preparative gradient is generated to focus on this elution area.

The preparative task is submitted to the run queue from the Task Progression Section. Depending on the administrator defined user rights, at this step, the Easy-prep user submits the task and reviews the automated peak identification or starts the purification process directly.

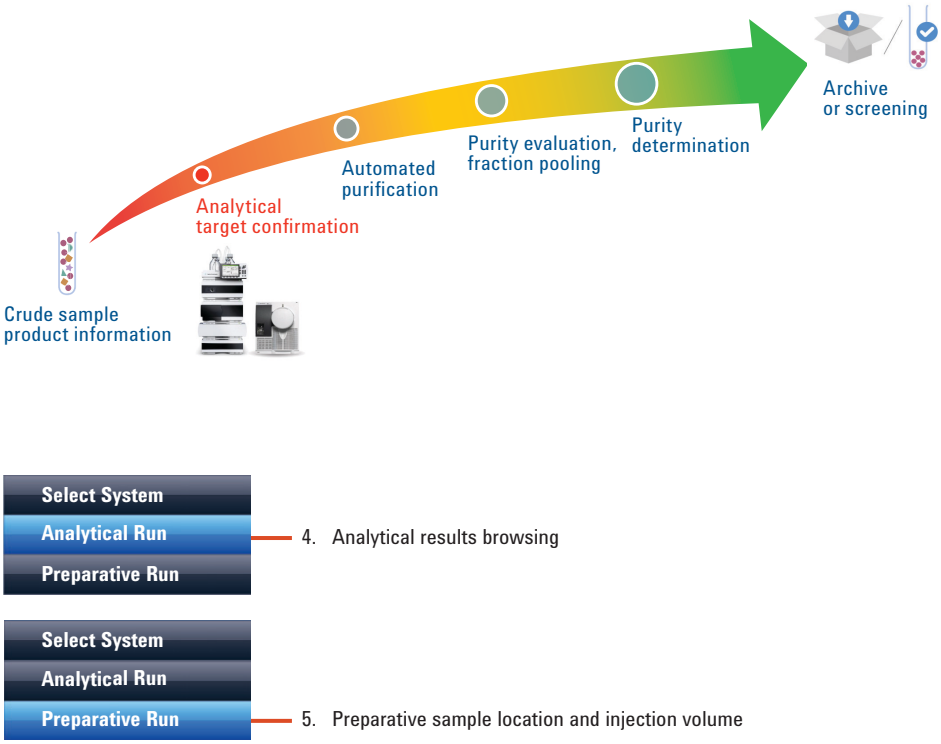


Figure 2. Analytical data selection and preparative sample(s) location and injection volume(s).

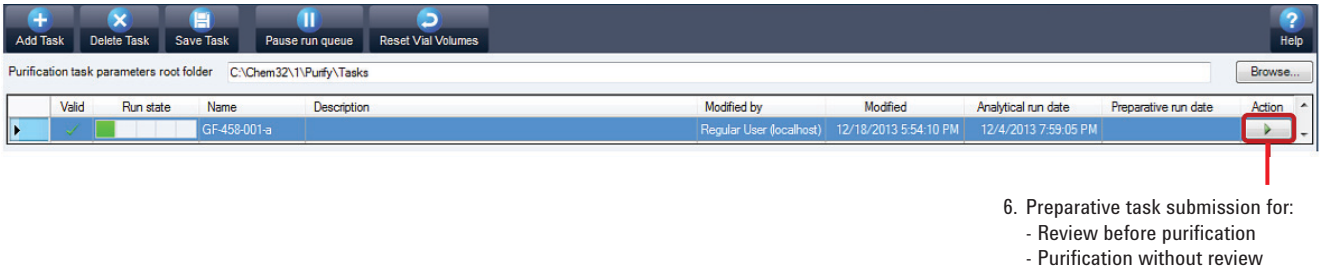


Figure 3. Preparative task submission.

Automated purification

Target peak and gradient review from the analytical run (Optional)

If the Easy-Prep user is allowed to review the automated target compound identification; the analytical chromatogram appears with a highlighted identified target peak as shown in Figure 4. If the highlighted peak does not match the desired target compound, another target peak can be manually assigned.

Then, the gradient profile will automatically be updated to the new elution area of interest, and modify the automated gradient profile used for the preparative run (Figure 5).

From the system characteristics and the elution area of the target peak, a focused gradient was generated to increase the chromatographic selectivity to the target peak and reduce the run time of the preparative run (Figure 5).

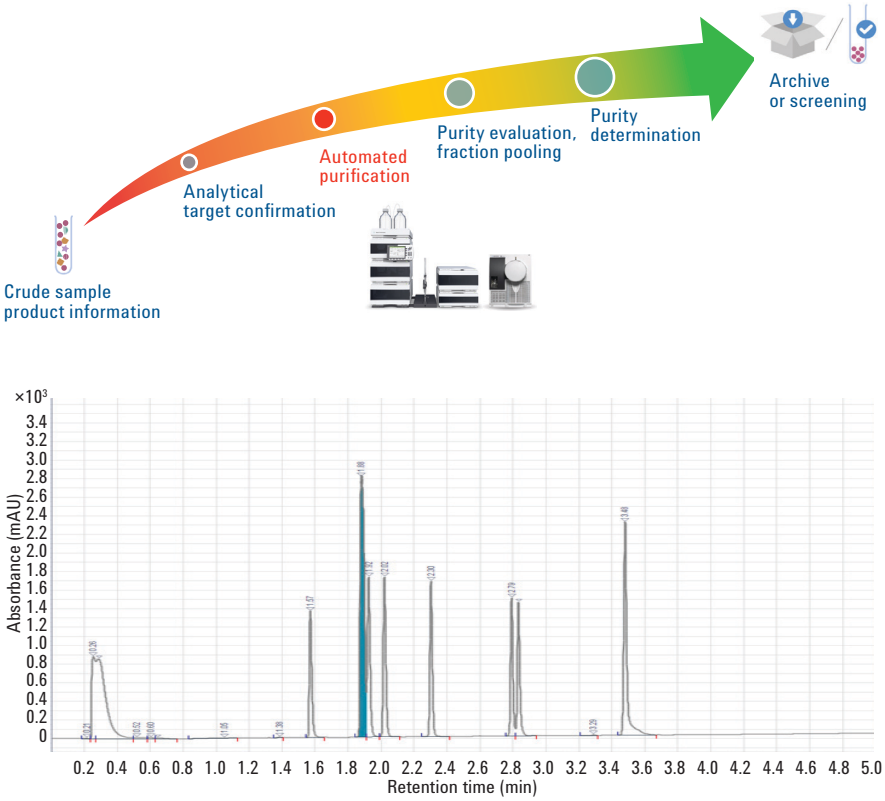


Figure 4. Automated target peak identification from the analytical data and target mass.

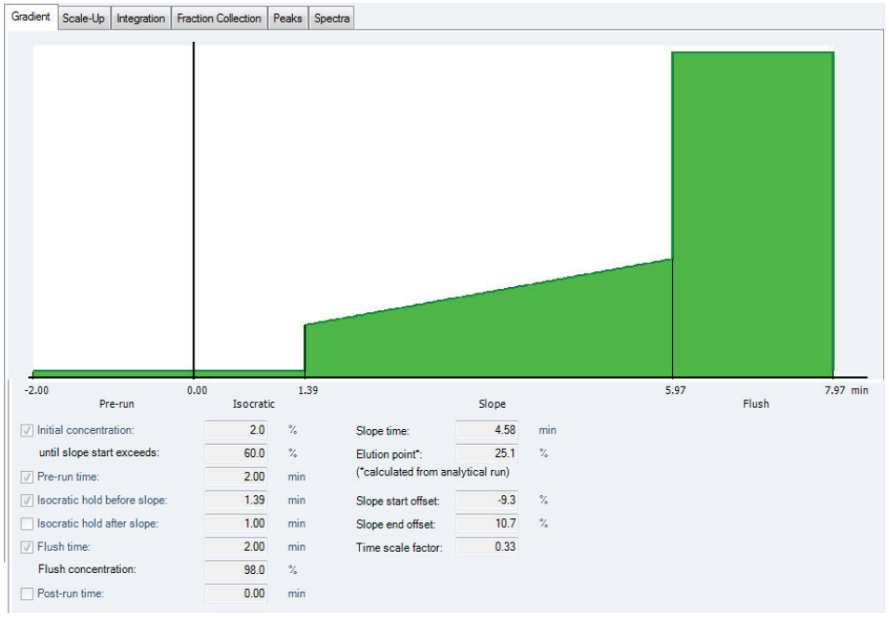


Figure 5. Automated focused gradient generated from the elution of the target peak.

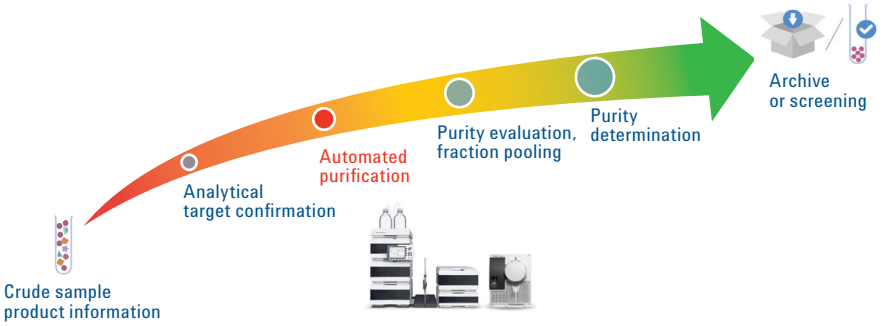
Automated purification

Preparative run review

After submission and execution of the purification run, a summary of the preparative result can be reviewed in the Task Configuration and Results section (Figure 6). This tab shows the:

- Preparative sample(s) location from the autosampler tray (top left)
- Collected fraction(s) location(s) and their crude sample mixture(s) belonging (top right)
- Preparative chromatogram of the selected sample mixture purification (bottom left)
- Average spectral data of the selected fraction (bottom right)

This information can be exported for pooling of fraction or reformatting the sample location for fraction reanalysis, for example.



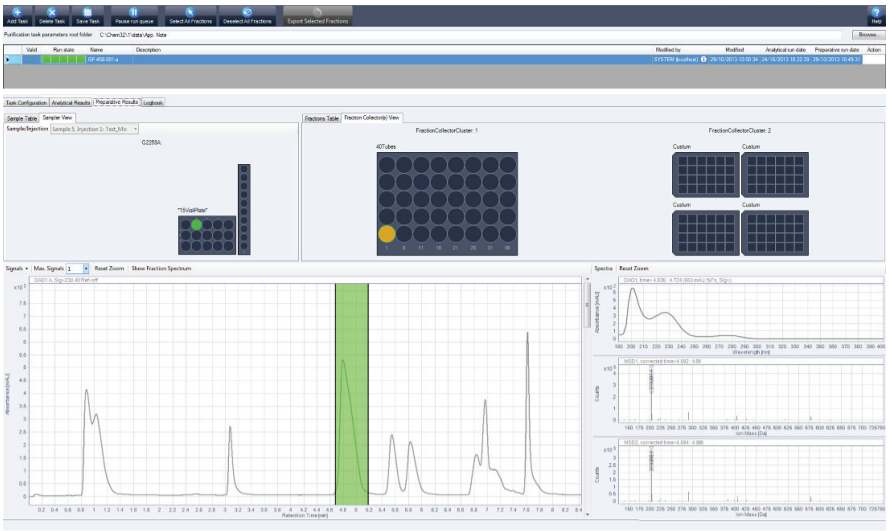


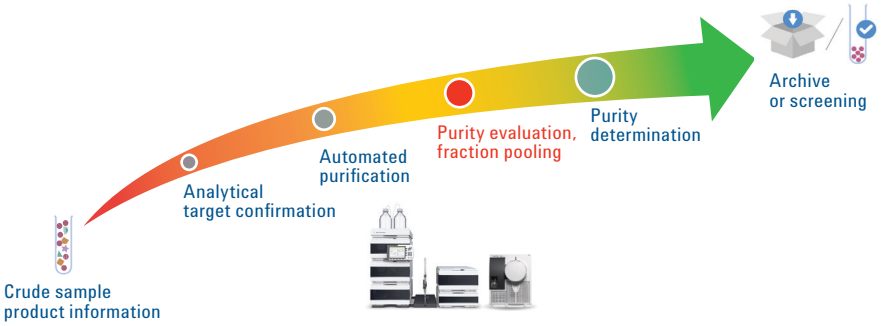
Figure 6. Preparative results review.

Fraction purity evaluation and pooling

The preparative results data, and the average spectral data of the selected fraction, give a good evaluation of the fractions to select for fraction pooling, solvent evaporation, and conditioning or biological screening.

In this example, the resulting fraction spectral data are shown in Figure 7.

The purification results tab provides an easy way to export the selected fractions to a liquid handler or an analytical LC system (Figure 8).



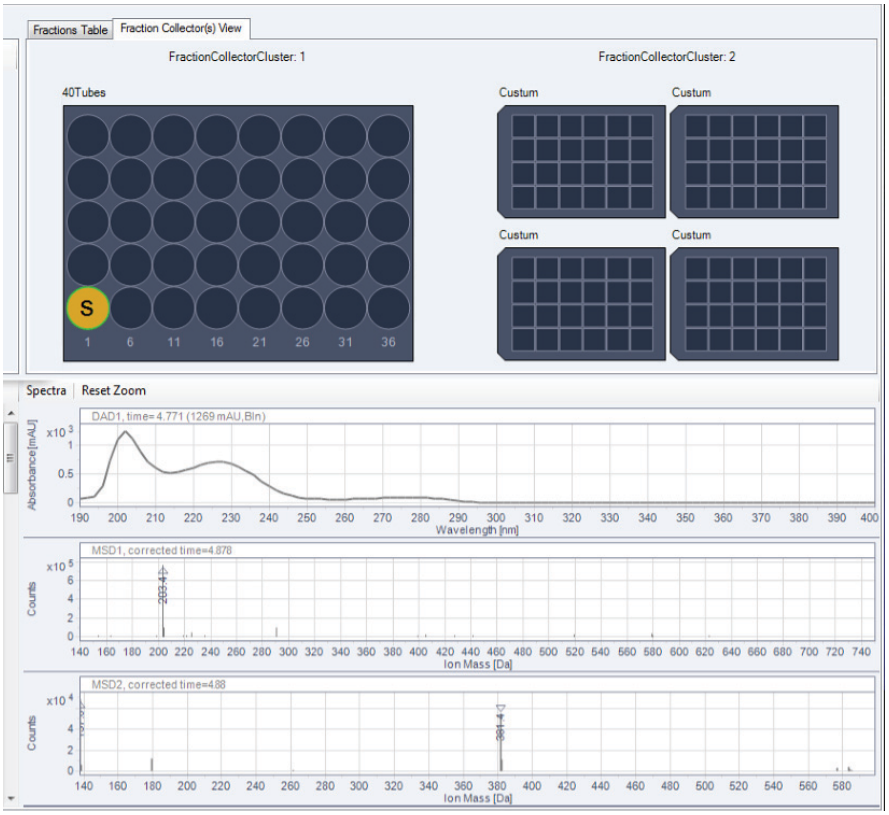


Figure 7. Selected collected fraction and average spectral data.

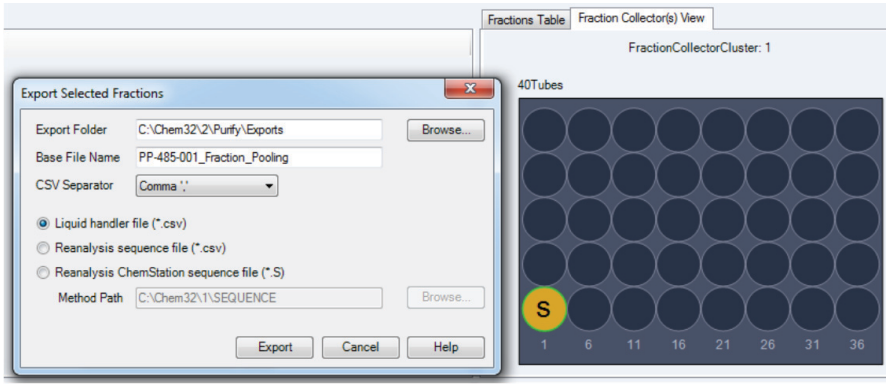


Figure 8. Exporting selected fraction (marked S) for pooling of fraction and fraction reanalysis.

Purity determination

After purification, the collected fraction was reanalyzed on the 1260 Infinity Binary LC/MS system. In this example purification, 88 % recovery of a pure target compound (Figure 9), was achieved after purification of the crude mixture corresponding to 54 mg of pure target product.

Conclusion

The Agilent Automated Purification Software Add-On coupled with the Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS Systems offers an automated solution to achieve higher throughput.

With an automated on-the-fly gradient generation, based on the target mass or formula of the desired product, the chromatographic selectivity is increased on the automated identified target peak. Thus, the optimized purification method allows shorter run time and decreases the solvent consumption by 40 %.

Furthermore, with the Easy-prep mode of the Automated Purification Software, preparative method development is no longer required, opening access of the purification system to nonexpert users with diverse chromatographic experience.

This Technical Overview demonstrates how 54 mg of a targeted compound was purified using the Agilent 1260 Infinity Preparative-Scale LC/MS Purification System, with 88 % recovery and 100 % purity.

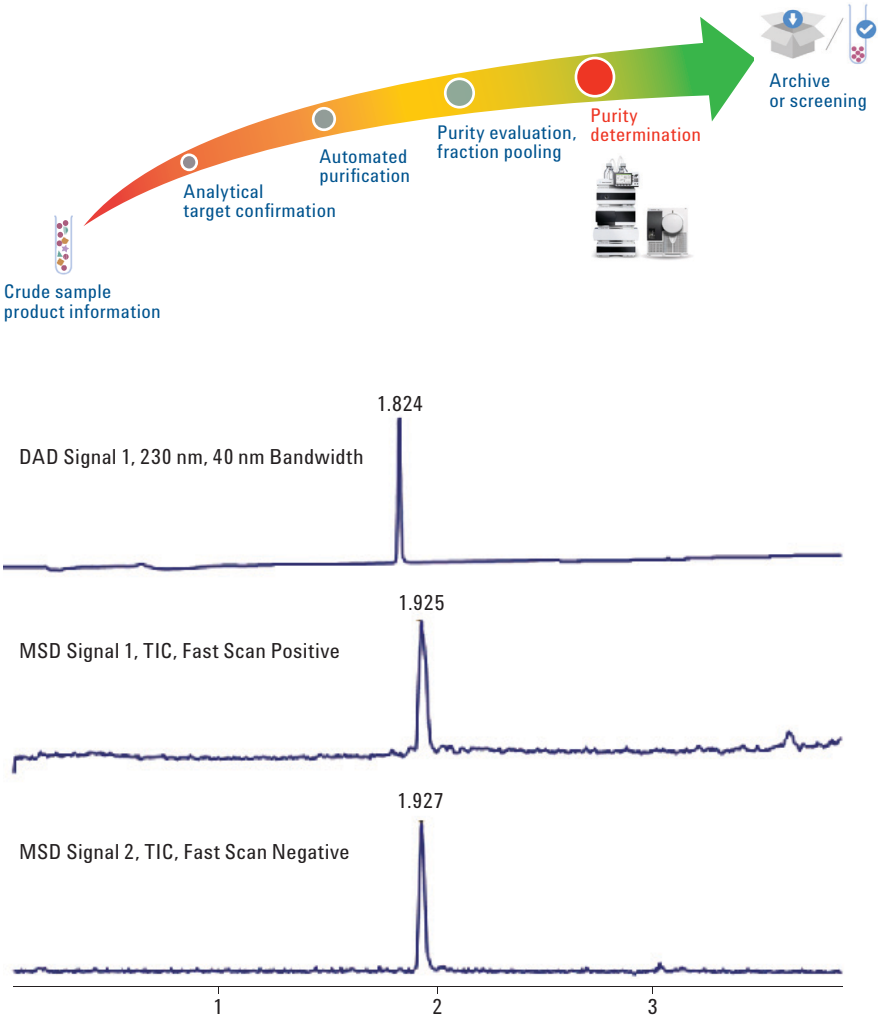


Figure 9. Agilent OpenLAB CDS ChemStation data analysis of the collected fraction.

Reference

1. Penduff, P., Analytical to Preparative HPLC Method Transfer: An easy way to scale up from UHPLC to preparative HPLC using focused gradients, *Agilent Technologies Technical Overview*, publication number 5991-2013EN, **2013**.

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