Sample Purification Triggered with the Agilent 1260 Infinity Evaporative Light Scattering Detector

Splitter solutions for columns with 4.6 to 75-mm ids

Technical Overview

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Abstract

This Technical Overview describes a solution to monitor and trigger the collection of preparative samples with the Agilent 1260 Infinity Evaporative Light Scattering Detector (ELSD) using a flow splitter. The Agilent 1260 Infinity ELSD is the detector of choice to monitor low UV-absorbing compounds or compounds with no UV-chromophore. Furthermore, this detection method is able to detect all compounds less volatile than the mobile phase, suitable for the detection of small molecule mixtures.

A drug-like sample mixture has been purified on an Agilent 1260 Infinity Preparative-scale Purification System by triggering the fraction collection with the Agilent 1260 Infinity ELSD. 99 % of purity and 91 % recovery were achieved.

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Introduction

For complex mixtures, natural products, small molecules, or low-UV-wavelength absorbing compounds, using an ELSD is a perfect solution to achieve reliable sample purification. In addition, this detection method allows adjustments of the different detector parameters such as light intensity, nebulizer temperature, gas flow, and temperature. This flexibility enhances the detector capabilities for preparative-specific applications. For example, in preparative liquid chromatography, the sensitivity of the detection method is an important criterium. By reducing or increasing the light intensity of the ELSD, the sensitivity of the detector can be adjusted to the concentration used for the sample purification.

In peptide synthesis, the remaining nonaromatic amino acids cannot be monitored by a conventional UV-detector, thereby requiring an ELSD or a MSD. The Agilent 1260 Infinity ELSD provides the simplest way to monitor or trigger the collection of the target compound without risk of collecting impurities with the collected target peptide fractions[1]. Another example is DMSO peak removal by increasing the gas flow or temperature to monitor the compounds underneath of the DMSO signal[2].

This Technical Overview describes an easy way to split the preparative flow after UV-detection to the ELSD and fraction collector for fraction collection triggering of the sample mixture.

Collecting the sample mixture can be done by ELSD or UV peak-based collection methods (Figure 1).

The split is achieved by flow restrictions generated by system backpressure after the column. Thus, for different flow rate ranges and systems, different splitter kits are required. Table 1 gives the different splitter configurations depending on purification scales and systems.

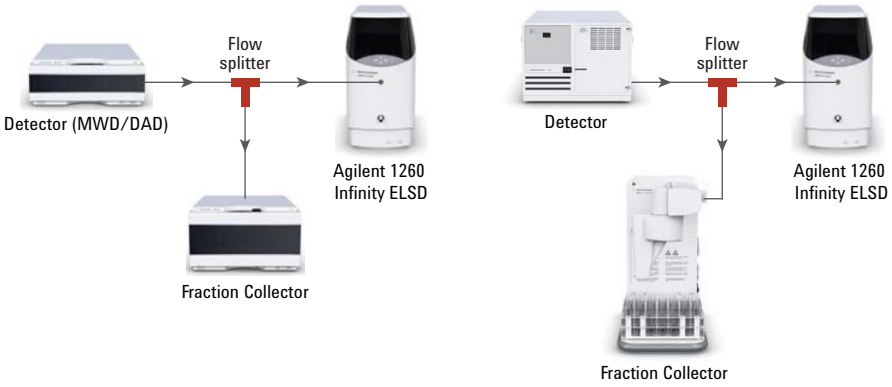


Figure 1. Schematic view of the splitter configurations on the Agilent 1260 Infinity Preparative-scale Purification System, the Agilent 218 Purification System, and Agilent SD-1 Purification System.

Table 1. Splitter and capillaries kits available for the different flow rate ranges of Agilent Purification Sytems.

	Analytical		Semipreparative	Preparative		Pilot
Productivity range	Micrograms	Milligrams		Grams		
Agilent 1260 Infinity Analytical Scale	Standard capillaries					
Agilent 1260 Infinity Preparative Scale*		Standard capillaries				
Agilent 218		Capillaries G9300-67030		Capillaries G9300-67031	Cap. G9300-67032	
Agilent SD-1		Capillaries G9300-67033			Cap. G9300-67034	
Column id	4.6 mm	½ inch (10 mm)	1 inch (21–25 mm)	(30 mm)	2 inch (50 mm)	3 inch (75 mm)
Splitter kits	1–5 mL/min 5023-2255	2–10 mL/min 5023-2256	8–40 mL/min 5023-2257	25–100 mL/min 5023-2258	75–300 mL/min 5023-2259	75–300 mL/min 5023-2259

Flow range extensions made possible by exchangeable pump heads

*Optional software available for automated analytical-to-preparative scale-up

Experimental

Instrument

Agilent 1260 Infinity Preparative-scale Purification System:

- Agilent 1260 Infinity Preparative Pump Gradient Extension (G1361A, G1391A)
- Agilent 1260 Infinity Dual-Loop Autosampler (G2258A)
- 1260 Infinity Multiple Wavelength Detector (G1365D) equipped with a Quartz flow cell 0.3-mm path length for multiple wavelength detection (G1365D#024)
- Agilent Column Organizer (G1383A)
- Agilent 1260 Infinity Preparative-scale Fraction Collector (G1364B)
- Agilent 1260 Infinity ELSD (G4260B)
- Agilent 1200 Infinity Series Universal Interface Box (G1390B)

Column

Agilent ZORBAX SB-C18, Prep HT Cartridge 21.2 × 100 mm, 5 µm (870100-902) with end fittings (820400-901)

Splitter

Depending on the operating flow rate, the splitter kit part numbers are given in Table 1.

Software

Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS Systems, Rev. C.01.05 [36]

Solvents and samples	
Solvent A)	Water + 0.1 % formic acid
Solvent B)	Acetonitrile + 0.1 % formic acid
Purification mixture for preparative runs	
Caffeine	5 mg/mL
Methyl 4-hydroxy-benzoat	2.5 mg/mL
Ethyl-4-hydroxy-benzoat	2.5 mg/mL
Propyl-4-hydroxy-benzoat	2.5 mg/mL
Benzyl-4-hydroxy-benzoat	2.5 mg/mL
Acetyl-protected sucrose	4.9 mg/mL in DMSO
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)	
Chromatographic conditions	
Gradient	1 minute isocratic hold at 2 % B, from 2 % B to 98 % B in 6 minutes and 2 minutes flush time at 98 % B
Flow rate	25 mL/min
Agilent 1260 Infinity Multiple Wavelength Detector (G1365D)	
Monitored wavelength	Signal A) 254 nm, bandwidth of 4 nm, Reference 360 nm, bandwidth 100 nm Signal B) 240 nm, bandwidth of 40 nm, no reference
Agilent 1260 Infinity ELSD (G4260B)	
Evaporator temperature	30 °C
Nebulizer temperature	35 °C
Gas flow rate	1.85 SLM
Output data rate	80 Hz
Smoothing	30
PMT Gain	1
LED Intensity	20 % for the six injections 3D-overlay (150 µL injection volume), and 4 % for the sample purification (750 µL injection volume)

Results and Discussion

In this Technical Overview, we purified a drug-like sample mixture containing a target compound without chromophore.

To prove the reliable performance of the splitter, the standard deviation of the retention time and peak area were calculated. The Agilent 1260 Infinity Preparative-scale Purification System, combined with the Agilent 1260 Infinity ELSD, gave highly reproducible purification results, with an average RSD of 0.02 % for the retention times, and 2.74 % for the average peak area precision (Figure 2).

The 21.2-mm id column was loaded with a higher volume to determine the typical recovery and purity of the system combined with the ELSD.

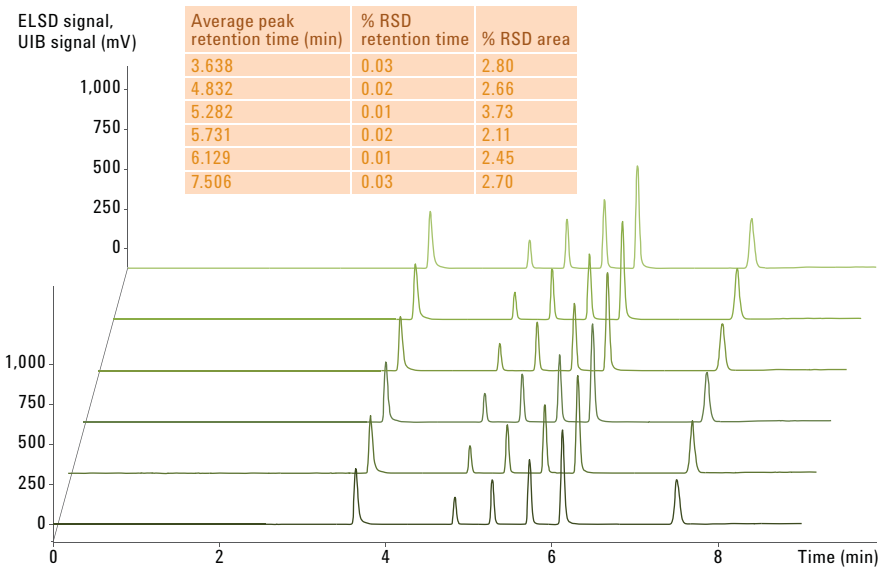
The collection was triggered by a Boolean or a combination of the UV and ELSD signal, each peak detector then triggered the collection on slope and threshold mode.

Figure 3 shows that 3.4 mg of the target non-UV chromophore compound was purified, achieving a recovery of 91 %, and a purity of 99 %.

Conclusion

This Technical Overview shows how a simple splitter solution was used to enhance the detection method for the purification of small molecules or non-UV chromphores with the combination of the Agilent 1260 Infinity Preparatives-scale Purification System with the Agilent 1260 Infinity ELSD.

The splitter kits were high reproducible concerning the purification results. Collection of the target compound was triggered by the ELSD, leading to a recovery of 91 %, and a purity of 99 %.



Average peak retention time (min)	% RSD retention time	% RSD area
3.638	0.03	2.80
4.832	0.02	2.66
5.282	0.01	3.73
5.731	0.02	2.11
6.129	0.01	2.45
7.506	0.03	2.70

Figure 2. UIB signals 3D-overlays of six injections, and resulting retention times and area precisions.

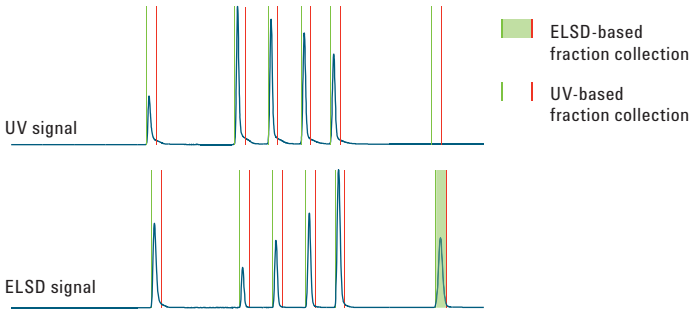


Figure 3. UIB/ELSD and UV signals 3D-overlays. Collection was triggered on both peak detectors. The highlighted green peak represents the fraction collected on ELSD peak-based collection, and the non-highlighted peaks represent the UV peak-based collection. The green and red tick marks represent the beginnings and ends of collection.

References

1. B. Schuhn "Performance Characteristics of the Agilent 1290 Infinity Evaporative Light Scattering Detector" *Agilent Technical Overview*, publication number 5991-2097EN (**2013**).
2. B. Schuhn "Optimizing the Performance of the Agilent 1290 Infinity Evaporative Light Scattering Detector" *Agilent Technical Overview*, publication number 5991-2176EN (**2013**).

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Published in the USA, August 1, 2015
5991-4041EN

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