



# Optimizing Fraction Collection Triggered by the Agilent 1260 Infinity Evaporative Light Scattering Detector on the Agilent Purification Systems

## Technical Overview

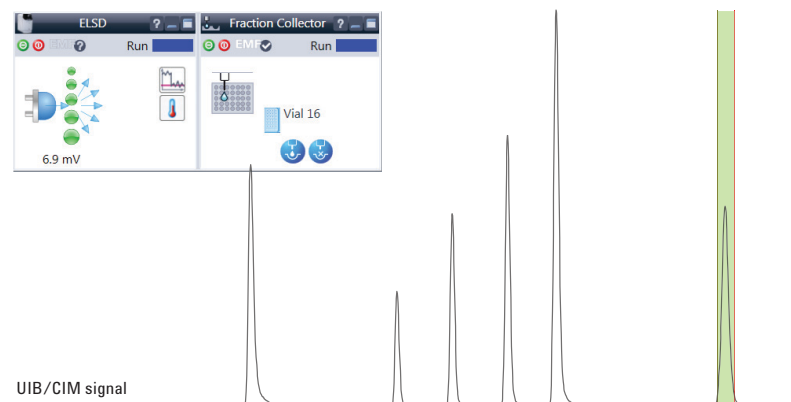
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### Abstract

On the Agilent OpenLAB CDS, fraction collection can be performed with a UV detector, or any additional detector, such as an Evaporative Light Scattering Detector (ELSD). In another Technical Overview<sup>1</sup>, we described a simple way to split the preparative flow on the Agilent preparative LC systems to trigger the collection with an ELSD.

This Technical Overview shows how to optimize peak-based fraction collection triggered by an ELSD. We describe the system setup, the delay calibration between detector and fraction collector, and the use of the variable light sensitivity of the Agilent 1260 Infinity Evaporative Light Scattering Detector. We also discuss the peak-based fraction collection settings required to achieve the best purity and recovery.



## Introduction

The evaporative light scattering detector (ELSD) is commonly used to detect compounds without a chromophore. Therefore, it is ideal for the purification of amino acids, peptides, carbohydrates, and lipids. Using the Agilent Splitter Kits corresponding to the required purification scale on the Agilent Purification System<sup>1</sup>, the signal from the ELSD can be used for peak-based collection of the target compounds. To ensure the best reliability and purity of the collected fractions, several parameters need to be optimized.

The delay time between the detector and the fraction collector needs to be measured. This delay is the difference between the time the compound is monitored in the detector, and the time when this compound reaches the diverting valve of the fraction collector. Correct measurement of this delay time is an important step of the system optimization. This procedure is automated on the Agilent 1260 Infinity Fraction Collectors using Agilent Lab Advisor Software.

This Technical Overview also describes how to improve parameters such as the light intensity of the ELSD, and the fraction collector settings.

## Experimental

### Instrument

Experiments were performed on the Agilent 1260 Infinity Preparative-scale Purification LC System:

- Agilent 1260 Infinity Preparative Pumps (G1361A, G1391A)
- Agilent 1260 Infinity Preparative Autosampler (G2260A)
- Agilent 1260 Infinity Multiple Wavelength Detector VL (G1365D) equipped with a Quartz flow cell 0.06-mm path length (G1365D#026)
- Agilent 1200 Series Column/Valve Organizer (G1383A)
- Agilent 1260 Infinity Preparative-scale Fraction Collector (G1364B)
- Agilent 1260 Infinity Evaporative Light Scattering Detector (G4260B)
- Agilent 1200 Infinity Series Universal Interface (G1390B),
- Agilent Splitter Kit 8–40 mL/min, Preparative (5023-2257)

## Generic system overview

ELSDs are destructive detectors, therefore, the preparative flow is split after UV detection. As shown in Figure 1, the main flow goes to the fraction collector, and the split flow to the ELSD.

To trigger the fraction collection with the Agilent 1260 Infinity ELSD, the signal output of the detector is connected to a Universal Interface Box (UIB) on an Agilent 1260 Infinity Purification System or a Control Interface Module (CIM) on the 218 and SD-1 Purification System. The UIB is an additional module connected to the LC system through a CAN cable. The CIM is a module part of the 218 and SD-1 pumps (more details in the splitter installation and configuration guide<sup>2</sup>).

### Column

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

### Software

- Agilent OpenLAB CDS A.01.05 or greater (ChemStation Edition)
- Agilent Lab Advisor Software, Rev. B.02.04

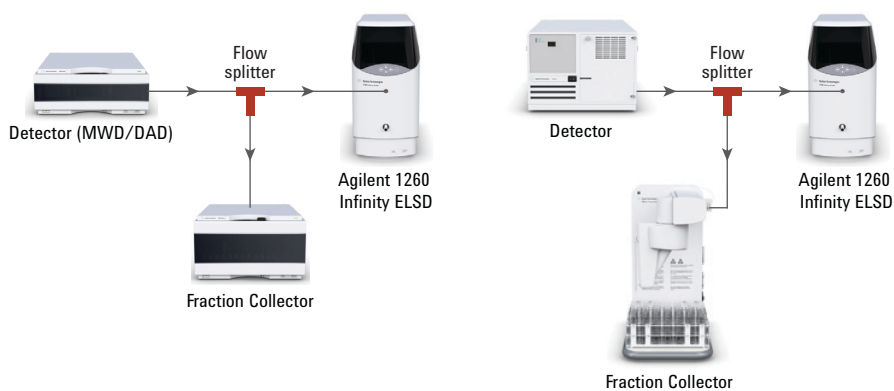


Figure 1. Schematic view of the splitter configurations of the Agilent 1260 Infinity Purification Systems, an Agilent 218 Purification System, and an Agilent SD-1 Purification System.

## 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- $\mu$ m membrane point-of-use cartridge (Millipak, EMD Millipore, Billerica, MA, USA).

## Results and Discussion

### Delay time calibration for ELSD peak-based fraction collection

The delay time is the time a compound needs to travel between the point of detection and the fraction collector. This delay is measured for each detector using a calibration mixture.

The delay time calibration on the Agilent 1260 Infinity Purification Systems is performed using the Agilent Lab Advisor Software<sup>3</sup>. With the Fraction Delay Sensor (FDS) of the 1260 Infinity Fraction Collectors, the delay time calibration is automated and measured during a simple and quick procedure. During this procedure, the delay time is measured between the signals coming from the IUB and the fraction collector FDS, and then converted into a delay volume. During a purification run, the resulting delay volume is recalculated into a delay time depending on the flow rates used.

After completion of the task, the resulting delay volume is displayed. For the system configuration used for this Technical Overview, the delay volume between the ELSD and fraction collector was 2,739  $\mu$ L.

On the Agilent 218 and SD-1 Purification Systems, the delay time calibration is performed during an OpenLAB CDS ChemStation run. The resulting delay time between the CIM signal and the fraction collector is saved into the method parameters for the operating preparative flow rate used during the purification run.

### Fraction collector and ELSD settings

The automated collection of the peaks can be performed on time-based fractionation mode, on peak-based mode, or a combination of both collection modes. The adjustment of the peak-based collection can be optimized with respect to the peak shapes of the compounds

purified and also considering the loading of the injected mixture. This step involves a preliminary work on the analytical column or by performing a pilot run to define threshold, upslope, and downslope.

Due to the high sensitivity of the ELSD, under high concentration, saturation of the detector can be observed.

As shown in Figure 3, the sensitivity could be decreased on the 1260 Infinity ELSD by reducing the light intensity. The upper threshold of the fraction collector can also be adjusted below the saturation of the detector to prevent slope triggering at the saturation of the detector. For the ELSD, as shown in Figure 3, an upper threshold of 1,000 mV can be used.

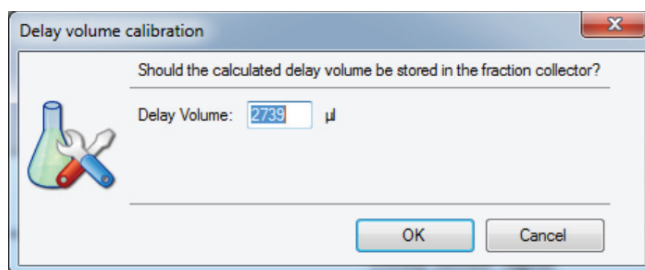


Figure 2. ELSD delay volume result shown on the Lab Advisor Software

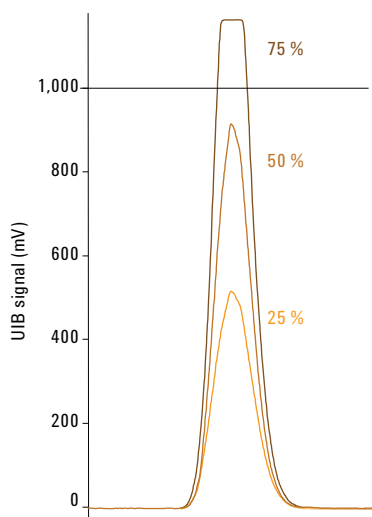


Figure 3. Overlay of the ELSD signals at 25 %, 50 % and 75 % of the maximal light intensity from three injections (250  $\mu$ L each) of the sample mixture.

In OpenLAB CDS A.01.05., online peak-based manual collection is possible (Figure 4). When the collection is triggered manually, the delay volume between the detector and fraction collector is considered (2,939  $\mu\text{L}$ ). Therefore, in this purification example at 29 mL/min, the fraction collector will start and stop the fractionation 5.7 seconds after the operator activates or deactivates the manual trigger.

## Conclusion

This Technical Overview shows how to optimize fraction collection with the Agilent 1260 Infinity Evaporative Light Scattering Detector. The delay calibration performed with the Lab Advisor Software provides a simple and quick method to calibrate the delay volume between detector and fraction collection. With the possibility of light intensity adjustment, the 1260 Infinity ELSD offers a flexible solution for preparative liquid chromatography by increasing or decreasing the sensitivity of the detector with respect to the sample concentration. These features ensure best reliability of the purification results.

## References

1. Penduff, P., Sample Purification Triggered with the Agilent 1260 Infinity Evaporative Light Scattering Detector, *Agilent Technologies Application Note*, publication number 5991-4041EN, March 2014.
2. Agilent Splitter Kits: Installation and Configuration Guide. *Agilent Technologies Kit Guide*, publication number 5974-0158EN, February 2014.
3. Agilent 1260 Infinity Analytical- and Preparative-scale Fraction Collectors, *Agilent Technologies User Manual*, publication number G1364- 90104, October 2013.

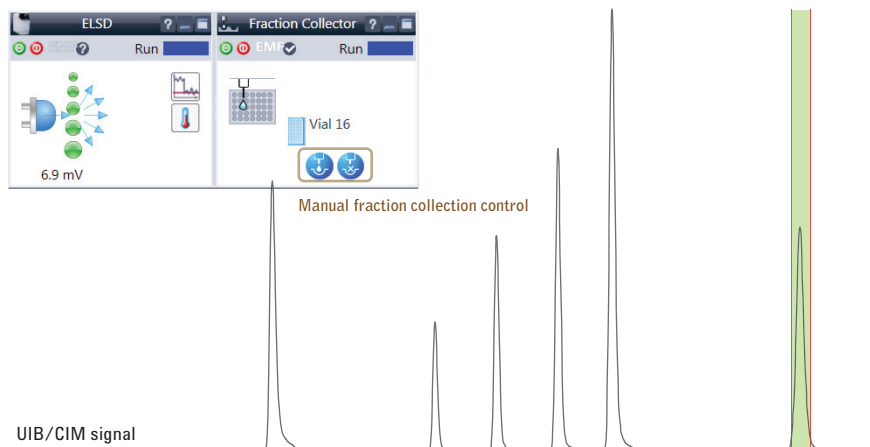


Figure 4. Manual collection on the ELSD signal peak based. This collection was triggered through the fraction collector user interface shown here.

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