Proof of Performance

Time- and peak-based fraction collection with the Agilent 1220 Infinity LC System

Technical Overview

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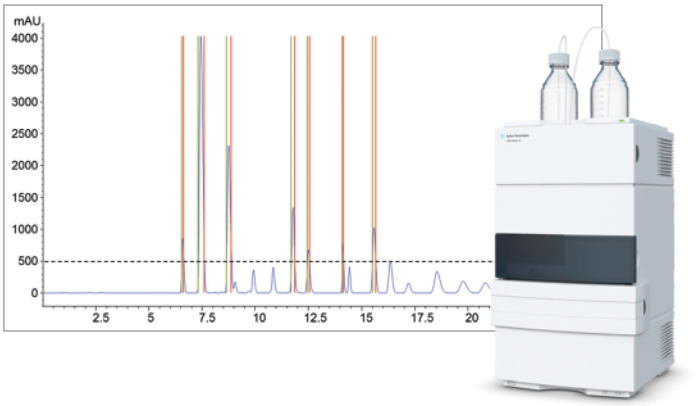

Abstract

Analytical-scale purification of compounds can be performed easily using standard HPLC or UHPLC equipment that is capable of delivering flow rates up to 10 mL/min. In this flow rate range, columns with ids from 4 up to 10 mm can be operated close to their *vanDeemter* optimum, facilitating the purification of crude compounds at amounts between 1 and 10 mg. In this Technical Overview, we demonstrate the combination of the Agilent 1220 Infinity LC System with the Agilent 1260 Infinity Analytical-scale Fraction Collector for time- and peak-based fraction collection using a generic compound mixture. Triggering of fractions is done based on retention time windows as well as based on the signal of the built-in variable wavelength detector (VWD) of the 1220 Infinity LC System. The collected fractions are re-analyzed to demonstrate the purity of the isolated compounds.

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Introduction

Preparative LC is not defined by column diameters or flow rates but by what happens to the compounds after they are separated on the column. While in analytical LC, the compounds are passed to waste after the last detector in the system, in preparative LC, they are transferred to a fraction collector. Based on selectable triggering criteria such as retention time windows or detector signals, the fraction collector transfers the desired parts of the chromatogram to dedicated fraction containers, for example, tubes, vials, or well plates. To ensure that only the desired part of the chromatogram such as a specific peak is collected with high purity and recovery, two prerequisites have to be fulfilled by the system:

- The first requirement is an accurate determination of the delay time of the system. When a compound is detected by the detector, the fraction collector has to wait until the compound has travelled to the fraction collector diverter valve. This delay time has to be determined beforehand. Manual procedures usually involve some sort of a dye and a stop watch — but they are usually very tedious, unreliable, and inaccurate. With the patented Agilent Delay Sensor[1] and a protocol executed within the Agilent LabAdvisor software, the delay calibration is a simple, fast, and accurate task.
- The second requirement is minimized system dispersion between the detector and the fraction collector. Unnecessarily long or wide capillaries lead to broadening of the peak travelling from the detector to the fraction collector due to dispersion effects. Since the time the diverter valve of the fraction collector remains in the collect position is determined by the width of the peak in the detector, excessive peak broadening can lead to decreased recovery and purity. The Agilent 1260 Infinity Analytical-scale Fraction Collector was especially designed to minimize system dispersion by using optimized connection capillaries and by positioning the diverter valve as close as possible to the fraction collection needle.

While both requirements have to be fulfilled for any preparative LC system, they are especially important when working at low flow rates such as used in analytical-scale preparative LC.

Experimental

Instruments and software

An Agilent 1220 Infinity LC Gradient System (G4290B), including a gradient pump (max. pressure 600 bar) with integrated degasser, autosampler, column oven, and variable wavelength detector with standard flow cell (10 mm path length) was used.

Fractions were collected using an Agilent 1260 Infinity Analytical-scale Fraction Collector (G1364C), equipped with a 4-well plate tray and four vial plates for 54 × 2 mL vials.

The system was controlled using the Agilent OpenLAB CDS ChemStation Edition Rev. C.01.03.

The delay calibration was performed using the Agilent Lab Advisor Software Rev. B.02.01.

Samples and solvents

SS EPA 610 PAH Mix in Methanol/ Methylene Chloride (1:1), (Supelco Analytical), containing the following PAHs: Naphtalene, Acenaphtylene, Acenaphtene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo(a)anthracene, Chrysene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Dibenzo(a,h)anthracene, Benzo(g,h,i)perylene, and Indeno(1,2,3-cd)pyrene

Acetonitrile was 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).

Chromatographic conditions

Parameter	Setting
Column:	Agilent ZORBAX Eclipse PAH 4.6 × 150 mm, 5 µm
Mobile phase:	A = Water, B = Acetonitrile
Gradient:	at 0 min 40% B
	at 12 min 80% B
	at 12.1 min 95% B
	at 25 min 95% B
Stop time:	25 min
Post time:	5 min
Flow rate:	1.5 mL/min
Injection volume:	Various
Column temperature:	25 °C
Variable wavelength detector:	230 nm

Results and Discussion

Delay calibration

The delay calibration procedure determines the delay time between the detector and the fraction collector in the system. The delay time is used to compensate for the time it takes for the compound to travel between the point of **detection** in the detector and the point of **collection** in the fraction collector. With the Agilent 1260 Infinity Fraction Collector, the delay calibration procedure is done using the fraction delay sensor (FDS), a very simple detector built in into the fraction collector.

Together with the signal from the Agilent 1220 LC Systems' UV detector the signal from the FDS facilitates determination of the delay time between detector and fraction collector as shown in Figure 1. The delay calibration procedure is a completely automated process using the Agilent Lab Advisor Software. The result is a delay volume, which is then recalculated into a delay time for any given flow rate in the actual fraction collection method. For the system setup used in this Technical Overview, the delay volume of the system was determined to be 83 µL as shown in Figure 2. This delay volume is stored in the fraction collector configuration.

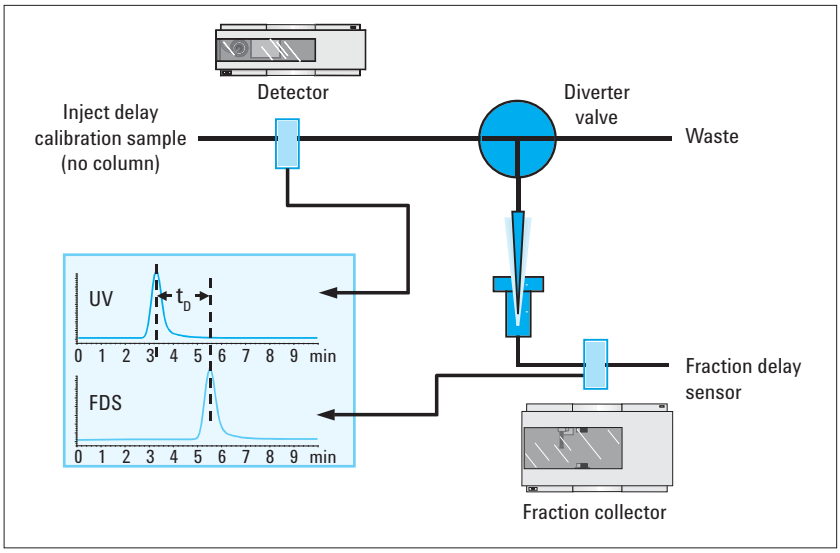


Figure 1
Delay calibration procedure.

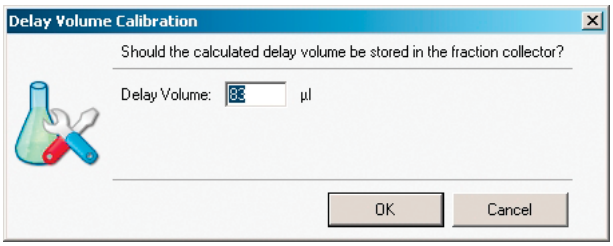


Figure 2
Result of the delay calibration procedure using the Agilent Lab Advisor Software.

Time-based fraction collection

Besides manually-triggered fraction collection, time-based fraction collection is the easiest method to collect fractions from a chromatographic run. In the experiment shown in Figure 3, fractions of 1 minute were taken between 6 and 16 minutes in the chromatogram.

Another possibility of time-based fraction collection with the Agilent 1260 Infinity Fraction Collector is to divide a time window into a certain number of equidistant fractions. Regardless of what mode of time-based fraction collection is applied, this triggering mode is very unspecific. In this example, this can be clearly seen when the collected fractions are re-analyzed. In the example from Figure 3, fraction number three contains only one, pure compound eluting at about 8.8 minutes (Figure 4a). Fractions number one and two (Figure 4b) contain both the compound eluting at 7.5 minutes. This is a typical problem when using time-based fraction collection, especially when retention times start to shift due to column aging.

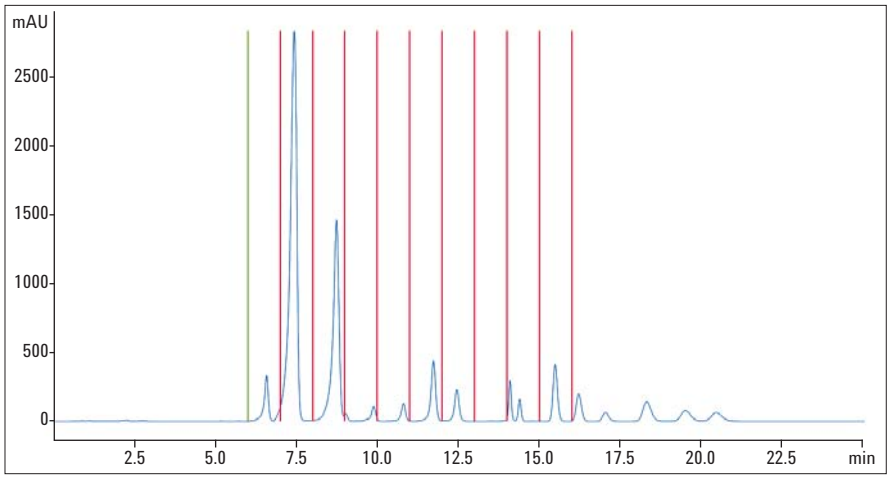


Figure 3
Result of time-based fraction collection.

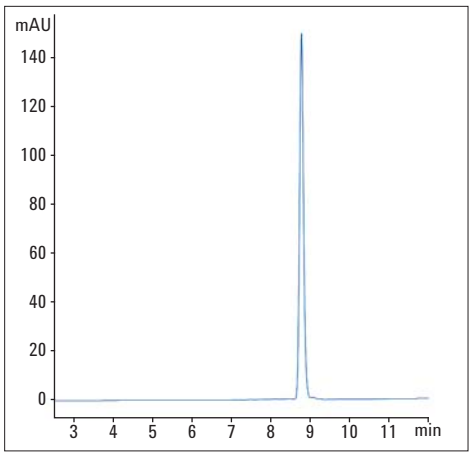


Figure 4a
Re-analysis of fraction 3.

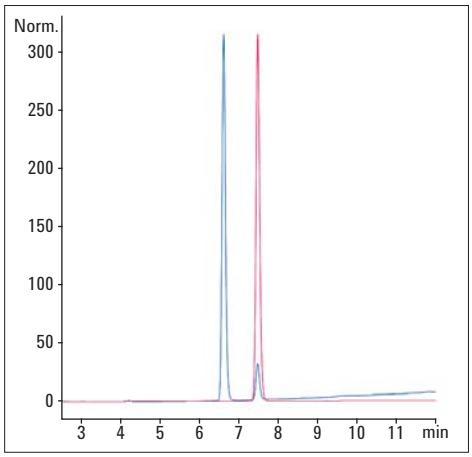


Figure 4b
Reanalysis of fractions 1 (blue) and 2 (red).

Peak-based fraction collection

Peak-based fraction collection is a more dedicated method to collect only the desired peaks and not unwanted baseline. To identify a peak and trigger the fraction collector, several parameters such as threshold, up slope, and down slope are available for the Agilent 1260 Infinity Fraction Collector[2]. In this experiment, simply a threshold of 500 mAU was set for peak identification. The resulting chromatogram and fraction collection results are shown in Figure 5.

As a result, only the major peaks are collected in dedicated fractions with high purity. This can be seen when re-analyzing the fractions, as shown for the first three fractions as an example in Figures 6 a–c.

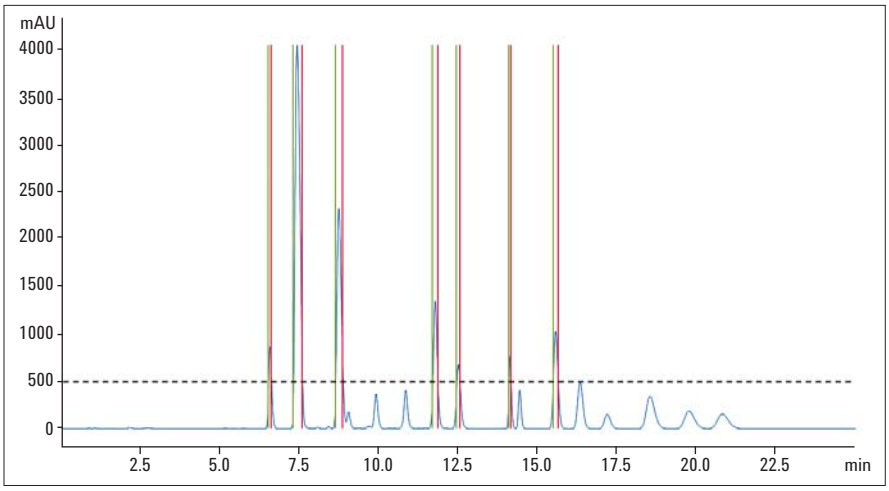
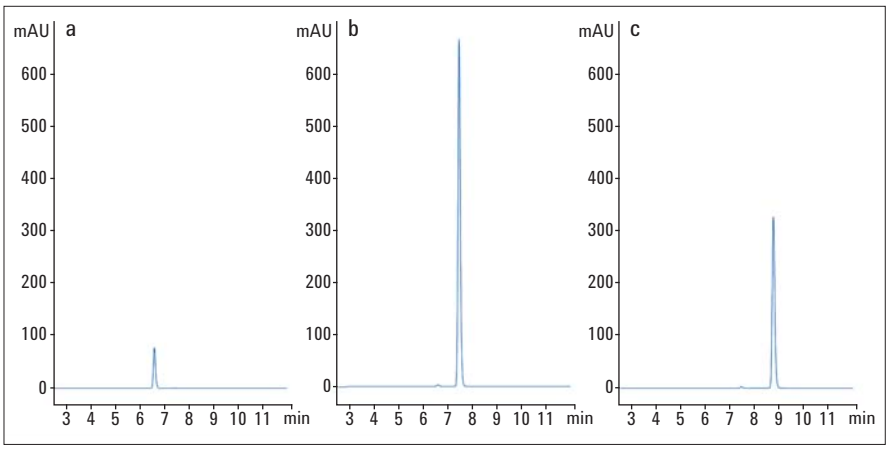


Figure 5
Peak-based fraction collection using a threshold of 500 mAU.

Conclusion

In this Technical Overview, we show the combination of the Agilent 1220 Infinity LC System and the Agilent 1260 Infinity LC Analytical-scale Fraction Collector as a simple and cost-effective system for analytical-scale preparative LC. Dedicated features such as the automated delay calibration procedure and the design of the fraction collector for lowest delay volumes, ensure infinitely better fraction collection leading to high purities and recoveries.



Figures 6 a –c
Re-analysis of fractions 1 – 3.

References

1.
"Principles in preparative HPLC" Agilent Technologies Primer, **2004**, publication number 5989-0652EN

2.
"Sophisticated peak-based fraction collection – working with up and down slope" Agilent Technologies Application Note, **2010**, publication number 5989-0511EN

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