Technical Overview

Spotting Fraction Impurities with More Confidence Using the Agilent 1290 Infinity II Preparative Open-Bed Sampler/Collector

Authors

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Abstract

The Agilent 1290 Infinity II Preparative Open-Bed Sampler/Collector combines sample injection and fraction collection in a single module. This feature facilitates purification workflows such as analytical scouting and preparative scale-up, or fraction collection with subsequent fraction reanalysis. To ensure highest confidence in fraction reanalysis and reduce manual interactions to a minimum, reanalysis after purification is supported by automated homogenization. This technical overview demonstrates the importance and functionality of the homogenization feature for two fractions containing two compounds each. Fraction reanalysis was performed with and without the automated homogenization step. The comparison of chromatographic results shows significant differences, and proves that successful determination of fraction impurities requires homogenization prior to reanalysis.

Introduction

The 1290 Infinity II Preparative Open-Bed Sampler/Collector is the cornerstone of each 1290 Infinity II Preparative LC system. It combines the capacity and flexibility of an open-bed fraction collector with sampling capabilities that stretch across the entire fraction bed. This feature enables workflows that typically require manual interaction to be carried out automatically with fewer or no manual steps. Dilution of the sample, analytical scouting before scale-up to preparative conditions, as well as reanalysis of collected fractions, are easier and fully integrated into the Agilent OpenLab CDS ChemStation chromatography data system.

The integration of a dedicated analytical flow path in the 1290 Infinity II Preparative Open-Bed Sampler/Collector enables reanalysis of collected fractions to determine fraction purity. During the collection of fractions, compounds are not homogeneously distributed in the fraction vessel. The reanalysis of inhomogeneous samples can cause a false assessment of the sample purity. It is important to mix the collected fractions before reanalysis. In a classic approach, fractions are typically mixed by hand, then transferred to sample vials to be used in a separate LC instrument.

This technical overview demonstrates the homogenization feature of the 1290 Infinity II Preparative Open-Bed Sampler/Collector. Two fractions, each containing one target compound and one unintentionally collected impurity, are reanalyzed. One analysis is done without prior homogenization, a second with homogenization. Peak areas of the target compound and impurity are compared.

Experimental

Instrumentation

In this study, the Agilent 1290 Infinity II Preparative LC System consists of:

- Agilent 1290 Infinity II Preparative Binary Pump (G7161B) with 200 mL pump heads (option #206)
- Agilent 1290 Infinity II Preparative Open-Bed Sampler/Collector (G7158B)
- Agilent 1290 Infinity II Preparative Column Compartment (G7163B)
- Agilent 1260 Infinity II Diode Array Detector WR (G7115A) with 0.06 mm preparative flow cell (option #086)
- Agilent 1260 Infinity II Diode Array Detector WR (G7115A) with 10 mm analytical flow cell (option #018)
- Agilent 1260 Infinity II Quaternary Pump (G7111B)

Columns

For purification: Agilent ZORBAX SB-C18 PrepHT, 21.2 × 50 mm, 5 µm (p/n 870050-902) with PrepHT end fittings (p/n 820400-901)

For reanalysis: Agilent ZORBAX Eclipse XDB-C18 4.6 × 50 mm, 5 µm (p/n 946975-902)

Software

Agilent OpenLab CDS ChemStation edition for LC and LC/MS Systems, version C.01.10 [195]

Solvents

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). Butylparaben, Sudan Orange G, and amcinonide were purchased from Sigma-Aldrich Corp., St. Louis, USA. Valerophenone was purchased from Honeywell Fluka, Morristown, USA.

Method settings

Parameter	Analytical Runs	Preparative Runs
Mobile Phase	A) 0.1% formic acid in water B) 0.1% formic acid in acetonitrile	
Flow Rate	1 mL/min	20 mL/min
Gradient	0.0 minutes – 2% B 0.5 minutes – 2% B 5.3 minutes – 98% B 6.4 minutes – 98% B 6.5 minutes – 2% B	0.0 minutes – 10% B 0.5 minutes – 10% B 4.0 minutes – 95% B 6.0 minutes – 95% B 6.1 minutes – 10% B
Stop Time	7.5 minutes	7.0 minutes
Injection Volume	10 µL	300 µL
Injection Parameters	**Method Preset 2** Postsample plug: 180 µL, 25% methanol in water Sample homogenization enabled: 15 cycles (default) Sample dilution enabled: dilution factor 20	**Method Preset 3** Pre- and postsample sandwich plug: 30 µL each, 100% methanol postsample plug: 180 µL, 25% methanol in water
Temperature	Ambient	Ambient
UV Detection	A: 254 nm Peak width >0.0063 minutes (0.13 seconds response time) (40 Hz)	A: 254 nm Peak width >0.025 minutes (0.5 seconds response time) (20 Hz)
Fraction Collection	Not applicable	Peak-based, using signal A Trigger mode: threshold Threshold: 10 mAU

Results and discussion

The preparative sample was separated in preparative scale, with fraction collection set to peak-based mode with a trigger threshold of 10 mAU for UV signal A. Figure 1 shows that the four compounds were collected into two separate fractions. In fraction 1, butylparaben (A) and Sudan Orange G (B) were collected. Fraction 2 contains valerophenone (C) and amcinonide (D). Compounds B and D represent impurities of the main peaks A and C that are unintentionally collected for lack of method optimization. Both impurities were collected, as peaks A/B and C/D are not baseline separated. The signal between the peak pairs does not fall below the threshold of 10 mAU, causing the fraction to end only after the impurity.

After fraction collection, the reinjection sequence for both fractions was conveniently set up in the data analysis view of OpenLab CDS ChemStation edition. The vessels holding the fractions were selected as the samples, and manual interaction was not necessary.

In the first step of the reanalysis, the fractions were mixed using the automated homogenization feature, which enables a simplified reanalysis workflow. This feature is available in the analytical operation mode of the instrument (see Figure 2). Homogenization is performed cycle-wise, and air is used for mixing. This technical overview used a default value of 15 cycles. In general, the number of cycles necessary for thorough mixing depends on the fill height and tube volume as well as the gradient range during fraction collection. It is suggested to increase the cycles by increments of 5, if necessary. After mixing, the fractions were automatically diluted by a factor of 20 to prevent saturation of the detector signal.

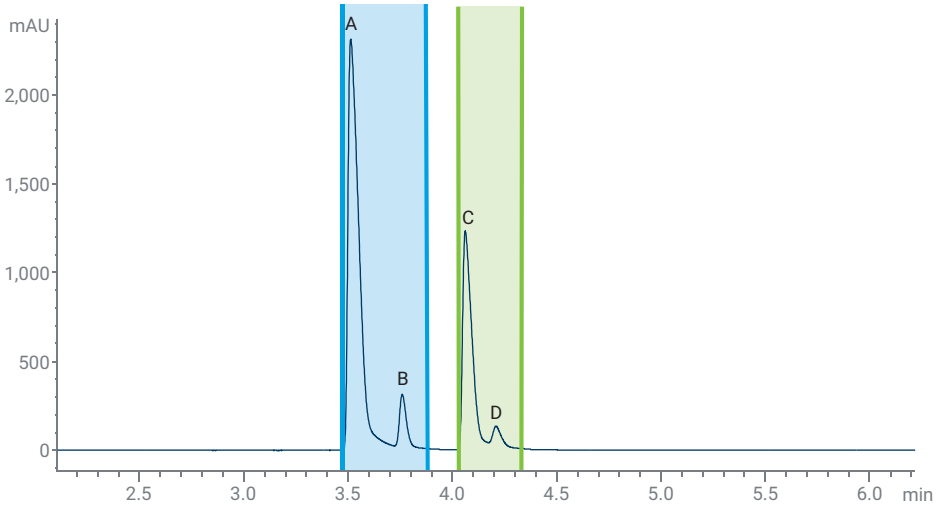

Figure 1. Chromatogram of the preparative sample with collection of compounds A and B in fraction 1 (blue) and compounds C and D in fraction 2 (green).

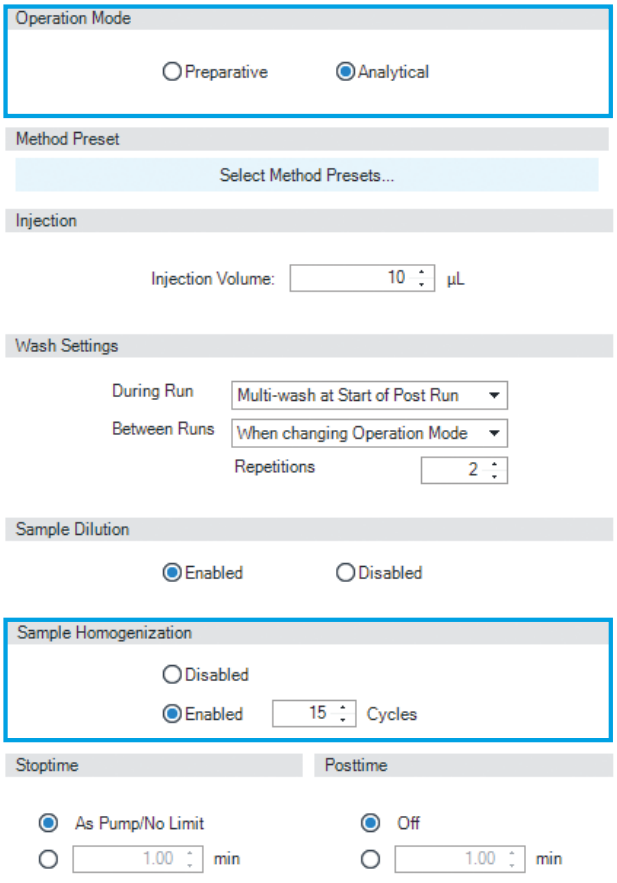

Figure 2. Method settings for the Agilent 1290 Infinity II Preparative Open-Bed Sampler/Collector with OpenLab CDS ChemStation edition. The Sample Homogenization feature is selectable in operation mode "Analytical", and was enabled using the default of 15 cycles.

Figure 3 shows a chromatogram overlay of the reanalyses of fraction 1 with and without mixing. The importance of homogenization is clearly visible when comparing the two traces. Before homogenization, only the main peak A is detected in the reanalysis run, suggesting a fraction purity of ≥99%. After homogenization, the concentration of the main peak A decreases, and the second compound B is detected. The homogeneous fraction shows that the actual sample purity is 92%.

In the purification run (Figure 1), compounds C and D are not baseline separated and are collected in fraction 2. Fraction reanalysis without mixing suggests a fraction purity of ≥99% (see Figure 4). Similar to fraction 1, homogenization of fraction 2 enabled the detection of the impurity peak D and reveals that the purity is in fact only 93%. In comparison to the purification run, the separation between main peak and impurity was increased in the reanalysis run. The comparison proves the importance of homogenization for spotting fraction impurities.

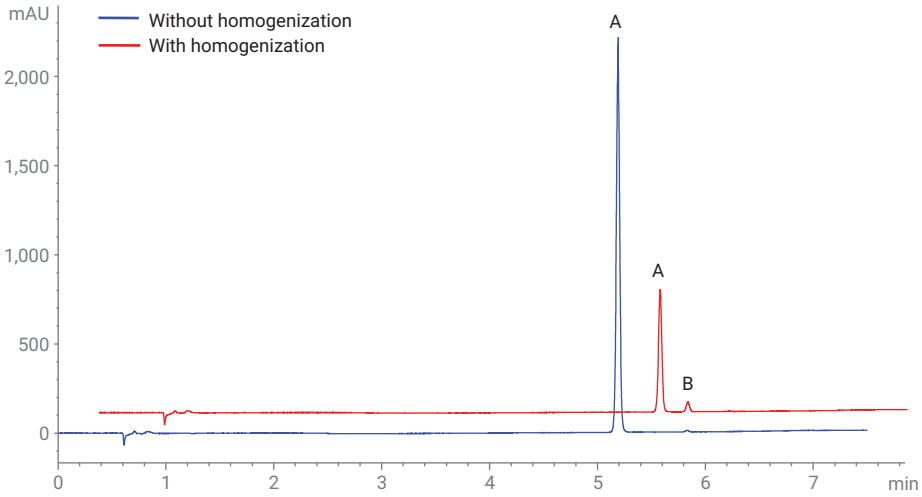

Figure 3. Reanalysis of fraction 1 before (blue) and after homogenization (red).

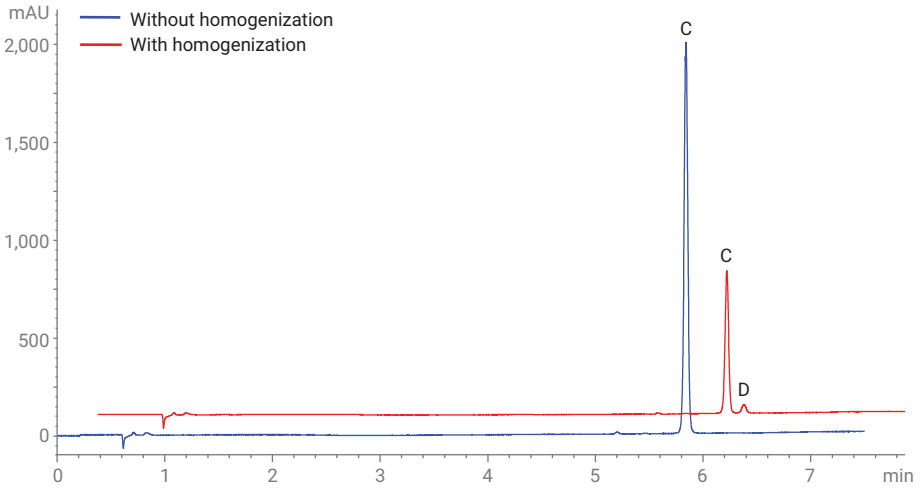

Figure 4. Reanalysis of fraction 2 before (blue) and after homogenization (red).

Figure 5 shows the fraction collection vessels after successful collection of fraction 1 before and after homogenization. The sample is drawn from the bottom of the vessel where compound A is located directly after fraction collection. Therefore, without homogenization, a high absorbance of compound A is detected (see Figure 3). After homogenization, compounds A and B are equally distributed in the vessel, enabling representative reanalysis results.

Conclusion

The Agilent 1290 Infinity II Preparative Open-Bed Sampler/Fraction Collector combines analytical and preparative tasks in one instrument, reducing manual sample interaction to a minimum. With the capability to sample from any position of the fraction bed, purification and fraction reanalysis can easily be combined, including automated homogenization of the fractions before reinjection. The reinjection sequences are conveniently set up in the data analysis view of Agilent OpenLab CDS ChemStation edition, enabling a workflow without manual handling of the fractions. This technical overview proves that the homogenization of fractions is crucial for spotting impurities: without homogenization, a fraction purity of ≥99% was determined, while the reanalysis of homogenized fractions showed an actual purity of 92 to 93%. The 1290 Infinity II Preparative Open-Bed Sampler/Fraction Collector with automated homogenization provides more confidence in fraction reanalysis, and obsoletes manual interaction with the fractions, saving time and eliminating sources of error.

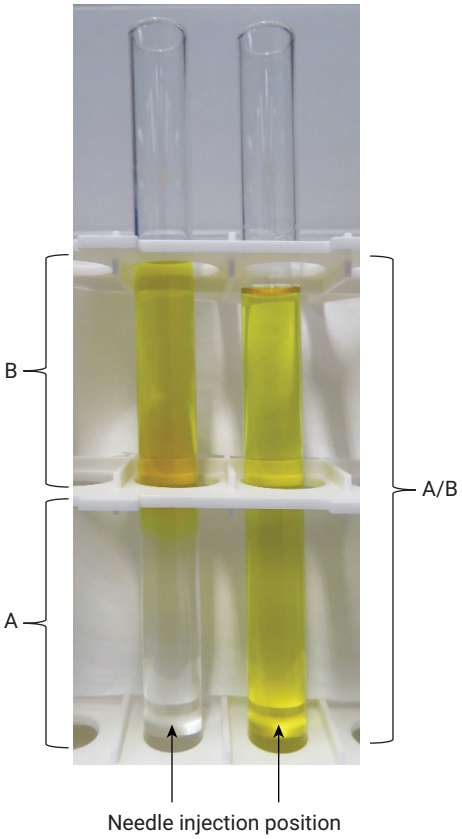

Figure 5. Fraction 1 containing compounds A and B directly after fraction collection and before homogenization (left). Homogenized fraction after reanalysis (right). The sample is drawn from the bottom of the fraction vessel. Fill height decreases due to duplicated sample draw for dilution.

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