Performance Characteristics of the Agilent 1290 Infinity II Preparative Open-Bed Fraction Collector

Purity and Recovery of Purified Compounds

Technical Overview

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Abstract

The Agilent 1290 Infinity II Preparative Open-Bed Fraction Collector was developed and optimized to enable efficient fraction collection with extended capacity and flexibility while providing the highest recovery and purity of the collected fractions. This Technical Overview demonstrates the performance of the 1290 Infinity II Preparative Open-Bed Fraction Collector when peak-based fraction collection is used. Using only the ultraviolet (UV) detection signal or a combination of UV and mass-selective detection (MSD) signals as a fraction trigger, purity and recovery of collected fractions are determined. Furthermore, the **peak-based, collecting time slices** fraction mode is discussed from a quantitative point of view.

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Introduction

Fraction collection in preparative-scale high-performance liquid chromatography (HPLC) can be challenging. Along with a suitable solvent gradient that separates the compounds of interest from unwanted impurities, the delay between the detector and the fraction collector plays a crucial role in the optimization process. Pure fractions with high recovery can only be collected with accurate setting of the time needed for a substance to migrate from the detector cell to the fraction nozzle. The Agilent 1290 Infinity II Preparative Open-Bed Fraction Collector[1] has been optimized in this respect to deliver efficient and flexible fraction collection with high purity and recovery. A built-in delay sensor allows precise calibration of the delay between each detector and the fraction valve. The calibration procedure does not require any hardware modifications, and needs to be done only once on UV-based systems; when the flow rate changes, the delay time is recalculated automatically, and adjusted accordingly. On systems including an MSD, the delay time is not only dependent on the flow rate, but also on the split ratio of the main flow and the make-up flow.

Exchangeable tubing kits are available with two different inside diameters to fit a wide range of flow rates. They can be cut in length to minimize peak dispersion between the detector and fraction collector. Fraction tubes, available in four different diameters (12, 16, 25, and 30 mm) and two lengths (100 and 150 mm), offer the right choice for any flow rate and fraction size. Carefully choosing fraction tubes is recommended to ensure short pathways of the fraction nozzle between two fractions, or to provide enough space for large fractions without the need to split them into multiple tubes.

Eight peak- and time-based modes, including two recovery modes, are available for fraction collection triggering. The different fraction modes, along with their typical applications, are described in detail in another Technical Overview[2]. This document focuses on the recovery and purity of fractions that were collected in peak-based mode. Fraction collection is triggered by the UV signal only, or by a logical AND combination of UV and MSD signals. The collected fractions are re-analyzed and quantified.

Experimental

Instrumentation

The Agilent 1260 Infinity Preparative-scale Purification System consisted of the following modules:

- Agilent 1260 Infinity Preparative Pump Cluster (G1361A and G1391A)
- Agilent 1260 Infinity Quaternary Pump (G1311B) for MSD makeup flow
- Agilent 1260 Infinity Dual-Loop Autosampler (G2258A) equipped with 50 µL (lower) and 5 mL (upper) injection loops
- Agilent 1260 Infinity Diode Array Detector (G1315C) equipped with a 3 mm preparative flow cell (Option #22)
- Agilent 1290 Infinity II Preparative Open-Bed Fraction Collector (G7159B) equipped with fraction containers for 16 × 150 mm (G9312-60129) and 12 × 150 mm (G9321-60045) tubes
- Agilent Active Flow Splitter (G1968F) connected through an Agilent 1200 Infinity Universal Interface Box II (G1390B)
- Agilent 6150 Single Quadrupole LC/MS with Agilent Jet Stream technology (G6150B)

For fraction re-analysis an Agilent 1260 Infinity II Binary LC was used in a configuration as follows:

- Agilent 1260 Infinity II Binary Pump (G7112B)
- Agilent 1260 Infinity II Vialsampler (G7129A)
- Agilent 1260 Infinity II Diode Array Detector (G7115A) equipped with a 10 mm standard cell (G1315-60022)

Columns

Preparative column

Agilent ZORBAX SB-C18 PrepHT,
21.2 × 100 mm, 5 µm (p/n 880966-122.01)

Analytical column

Agilent ZORBAX SB-C18
4.6 × 50 mm, 5 µm (p/n 846975-902)

Software

The analytical system was controlled by the Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS Systems, version C.01.07 SR1 [110]. The preparative system was controlled by the same software, version C.01.07 SR2 [257].

Solvents and samples

LC grade acetonitrile was purchased from Merck, Darmstadt, Germany. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak). Formic acid, dimethyl sulfoxide, and the eight components of the preparative sample were of analytical grade or higher, and purchased from Sigma-Aldrich, Taufkirchen, Germany.

A sample was prepared from a mixture of acetaminophen, sulfamerazine sodium salt, caffeine, methylparaben, sulfadimethoxine, ethylparaben, propylparaben, and benzyl-4-hydroxybenzoate, dissolved in dimethyl sulfoxide at a final concentration of 10 mg/mL.

Chromatographic conditions

Table 1. Preparative gradient, used with a 21.2 × 100 mm, 5 µm column.

Parameter	Description
Mobile phase	A) 0.1 % Formic acid in water B) 0.1 % Formic acid in acetonitrile
Flow rate	25 mL/min
Gradient	0.00 minutes – 10 %B 2.00 minutes – 10 %B 7.00 minutes – 50 %B 8.60 minutes – 98 %B 9.60 minutes – 98 %B 9.70 minutes – 10 %B
Stop time	11.00 minutes
Injection volume	500 µL, sandwich injection with 50 µL water plugs
Detection	UV 254 nm, no reference Peak width > 0.05 minutes (5 Hz)

Table 2. Analytical gradient for fraction re-analysis, used with a 4.6 × 50 mm, 5 µm column.

Parameter	Description
Mobile phase	A) 0.1 % Formic acid in water B) 0.1 % Formic acid in acetonitrile
Flow rate	1.5 mL/min
Gradient	0.00 minutes – 10 %B 0.30 minutes – 10 %B 3.00 minutes – 64 %B 3.10 minutes – 98 %B 4.10 minutes – 98 %B 4.11 minutes – 10 %B
Stop time	5.50 minutes
Injection volume	5 µL
Detection	UV 254 nm, no reference Peak width > 0.013 minutes (20 Hz)

Table 3. MSD spray chamber and signal settings.

Parameter	Description
Make-up flow	1.5 mL/min
Make-up solvent	Methanol:water 80:20 + 0.1 % formic acid
Spray chamber	Agilent Jet Stream Electro Spray
Signal 1	Positive scan 125–725 Fragmentor 125 V
Signal 2	Negative scan 125–725 Fragmentor 125 V
Nebulizer pressure	35 psig
Drying gas temperature	300 °C
Sheath gas temperature	250 °C
Sheath gas Flow	10.0 L/min
Capillary voltage	±1,300 V
Nozzle voltage	±2,000 V

Results and Discussion

Peak-based fraction collection

The preparative sample was purified using peak-based fraction collection. To collect three closely eluting compounds, the fraction collection was activated from 5.60 to 7.60 minutes. Trigger settings based on the UV signal at 254 nm were as follows: threshold 50 mAU, upslope 50 mAU/s, downslope 5 mAU/s.

Figure 1 shows a clipping of the chromatogram with overlaid tick marks of the fraction start and stop time. With the gradient used, compounds 4 and 5 were slightly overlapping, making a separate collection by threshold alone impossible with the chosen parameters. Instead, collection of the two peaks into different fractions was triggered by the slope change from negative to positive at the local minimum between the two peaks. Compounds 5 and 6 were separated to baseline and easily collected into two separate fractions.

All three fractions were diluted in volumetric flasks and quantified by duplicate analysis on an Agilent 1260 Infinity II LC. Recovery was calculated as the percentage of the quantified amount in the diluted fraction with reference to the total amount of sample injected to the purification system. The purity of the fractions was determined based on the ratio of the respective compound and the total peak area at 254 nm. Figure 2 presents a chromatogram overlay of the re-analysis of three compounds purified by peak-based fraction collection. Purity and recovery were excellent, and above 97 % for each compound.

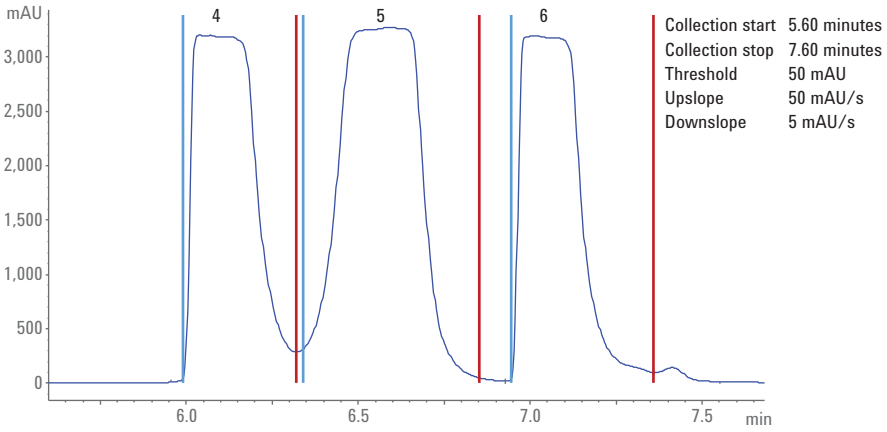
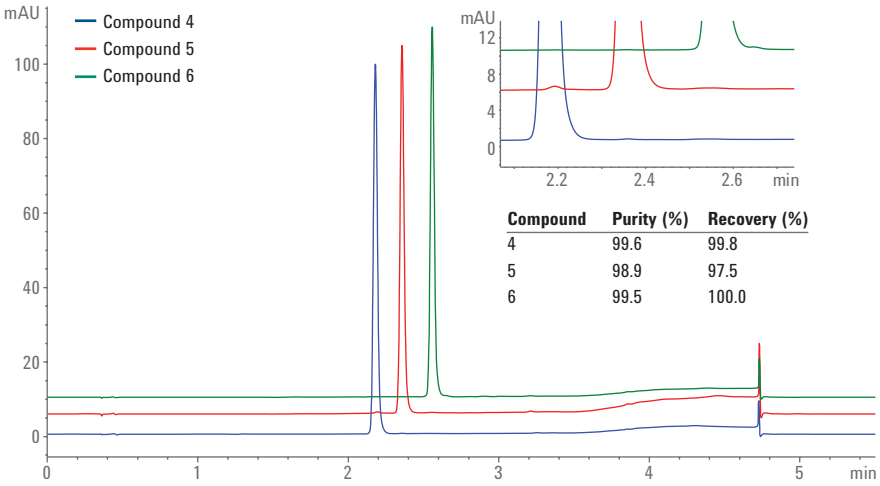


Figure 1. Chromatogram (clipping) of a purification run using peak-based fraction collection.



Compound	Purity (%)	Recovery (%)
4	99.6	99.8
5	98.9	97.5
6	99.5	100.0

Figure 2. Fraction re-analysis of three closely eluting compounds collected with peak-based fraction mode.

Peak-based fraction collection with MSD

Mass-based fraction collection is typically used to obtain highly specific peak triggering. With Agilent OpenLAB CDS ChemStation Edition, multiple target masses can be defined for each run to trigger fraction collection. Three compounds of the preparative sample were purified using peak-based fraction collection triggered by the UV and MSD signals connected by a logical AND combination. This combination triggers a fraction start as soon as both detector signals have exceeded the user-defined threshold/slope settings. Collection continues until one of the detector signals drops below the settings. Threshold and up-/downslope settings were the same as in the previous example. Target masses were entered for each of the three compounds of interest, with a threshold of 50,000 counts per second.

Figure 3 shows the chromatogram of the resulting purification run. Compounds 4 to 6 were collected in distinct fractions. In contrast to simple peak-based collection triggered by UV only, the combination with the MSD signal caused the fractions to be cut more restrictively. Figure 4 shows an overlay of the UV chromatogram with the MSD traces of the unique trigger ions.

All three fractions were quantified and analyzed with respect to purity. Figure 5 presents an overlay of the fraction re-analysis, along with purity and recovery data. As compared to peak-based fraction collection triggered by UV signals only, purity was slightly higher in all three fractions. Without any changes in the separation gradient, higher purity can only be obtained by a more restrictive cut of the fractions, which in turn causes a slight decrease in recovery. However, recovery was still above 96 % for all three compounds.

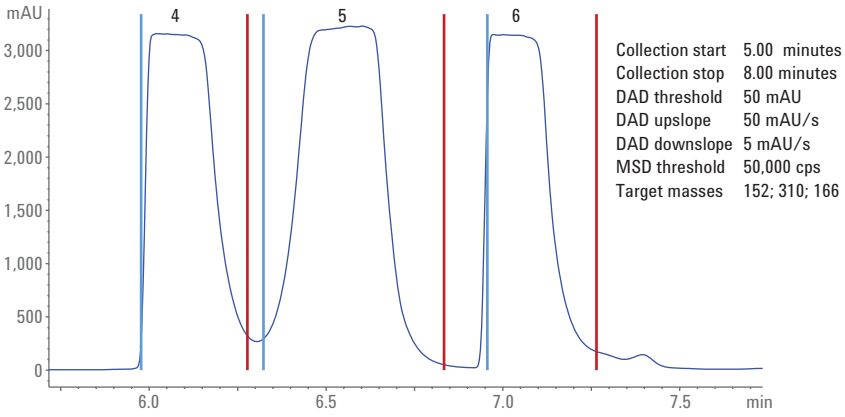


Figure 3. Chromatogram (clipping) of a purification run using peak-based fraction collection triggered by UV combined with MSD by a logical AND connection.

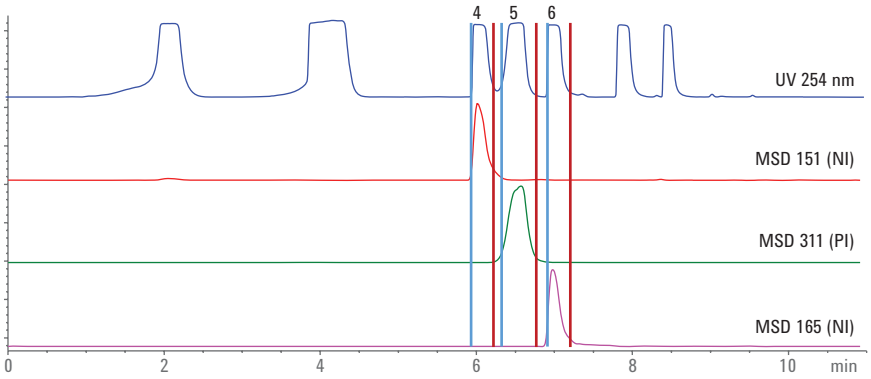


Figure 4. Chromatogram overlay of a purification run with peak-based fraction collection based on UV and MSD signals connected by logical AND combination. Unique trigger ions for each compound detected in negative (NI) or positive ionization (PI) mode are highlighted in red, green, and purple.

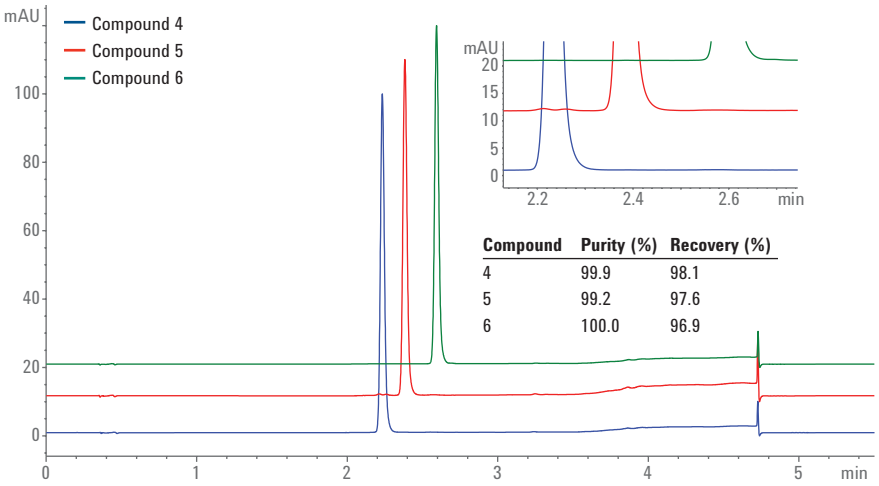


Figure 5. Fraction re-analysis of three closely eluting compounds purified with peak-based fraction collection triggered by UV and MSD signals.

Peak-based fraction collection with time slices

Typical applications for, and the benefits of peak-based fraction collection with time slices have been described in another Technical Overview[2]. If the workflow demands, the user can cut peaks into time or volume slices and process only those slices with the highest purity. Between two slices, however, a small amount of the peak is diverted to the waste while the robot arm of the fraction collector is moving to the next position. Since the 1290 Infinity II Preparative Open-Bed Fraction Collector has been designed for this fraction mode, the speed of the robot arm is optimized for highest performance. The time needed to move to the next position is only one third of a second with containers bearing 16 mm outside diameter (od) tubes, and even lower with 12 mm od tubes. Depending on the number of slices into which a peak is cut, there is a small decline in recovery.

Compounds 4 and 5 of the preparative sample were purified with the peak-based fraction mode, collecting time slices of 0.06 minutes width, equaling aliquots of 1.5 mL at the flow rate used. Collection was active from 5.50 to 7.10 minutes, with the following trigger settings: threshold 20 mAU, upslope 20 mAU/s, downslope 5 mAU/s. Compounds 4 and 5 were successfully collected and cut into six and ten slices, respectively (Figure 6).

All collected slices of each compound were pooled and diluted in a volumetric flask. Purity and recovery were determined as in the previous examples. Figure 7 presents a chromatogram of the fraction re-analysis, as well as recovery and purity data. Purity was about 99 % for both compounds, which is equivalent to the purity obtained with simple peak-based fraction collection (Figure 2). Compared with that fraction mode, the recovery of the compounds in the pooled fraction slices is lower, but still above 92 %. This demonstrates that even with a relatively narrow peak of 0.3 minutes cut into six slices, recovery is on a more than satisfactory level if the collection settings and fraction tubes are thoroughly selected. Therefore, a time/volume slice collection is a useful fraction mode to check for impurities at the front and tail of a peak, while the recovery is high enough to pool and use all slices, if no impurities are found.

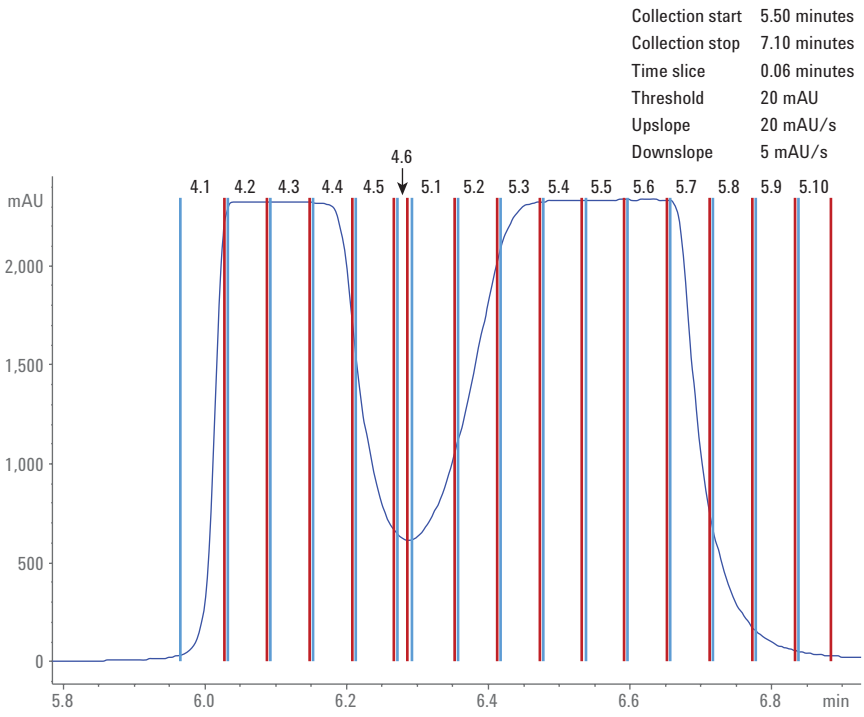


Figure 6. Chromatogram (clipping) of a purification run using the **peak-based, collecting time slices** fraction mode. Compounds 4 and 5 were cut into six and ten fractions, respectively.

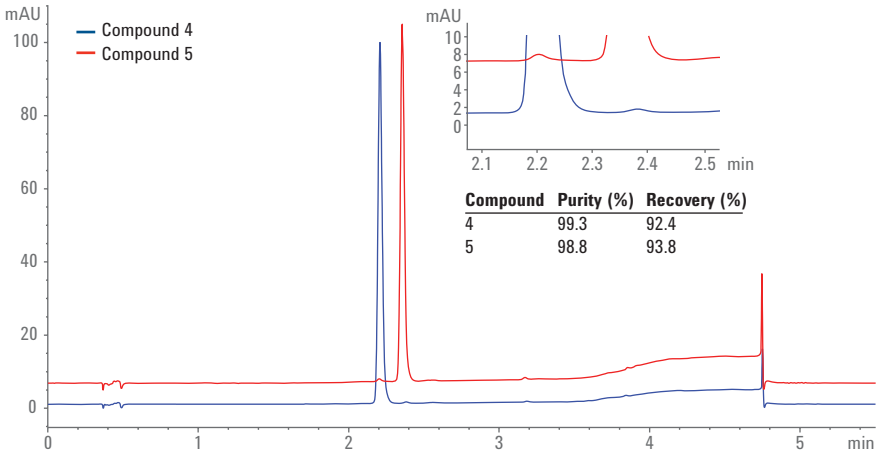


Figure 7. Fraction re-analysis of two closely eluting compounds purified with the **peak-based, collecting time slices** fraction mode. Chromatogram and data on purity and recovery refer to the pooled fractions of each compound.

Conclusion

The Agilent 1290 Infinity II Preparative Open-Bed Fraction Collector is a versatile instrument with enhanced fraction bed capacity and flexibility for diverse collection scenarios. Flow path and mechanics have been optimized for the highest purity and recovery of collected fractions. This Technical Overview demonstrates that fractions with high purity and recovery (above 98 % and 96 %, respectively) can easily be collected with the peak-based fraction mode triggered by UV signal only or a combination of the UV and MSD signals. When the **peak-based, collecting time slices** fraction mode is used to cut peaks into six or more slices, the purity of the pooled slices is on the same level as in peak-based collection. Sample loss caused by movement of the fraction valve between the slices is only marginal, yielding recoveries of the pooled fractions above 92 %.

References

1. Agilent 1290 Infinity II Preparative Open-Bed Fraction Collector – Collect Each and Every Drop, *Agilent Technologies Brochure*, publication number 5991-7225EN, **2016**.

2. Rieck, F. Time-, Peak-, and Mass-Based Fraction Collection with the 1290 Infinity II Preparative Open-Bed Fraction Collector. *Agilent Technologies Technical Overview*, publication number 5991-7654EN, **2016**.

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