Time-, Peak-, and Mass-Based Fraction Collection with the Agilent 1290 Infinity II Preparative Open-Bed Fraction Collector

Technical Overview

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Abstract

The Agilent 1290 Infinity II Preparative Open-Bed Fraction Collector sets new benchmarks in efficient and flexible fraction collection. The fraction bed can be equipped individually with six containers holding fraction tubes in eight different sizes. Three drawers provide easy access to collected fractions even during a run. Peak- and time-based fraction modes have been extended to eight combined modes, enabling the collection of volume or time slices, and including two recovery collection modes. Trigger combinations of ultraviolet (UV) with mass-selective detection (MSD) signals now include an AND/OR logic that gives extra flexibility for challenging purifications. This Technical Overview provides a deeper insight and typical application examples of the fraction modes, including collection results.

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Introduction

Fraction collection is the key feature that turns an analytical high-performance liquid chromatography (HPLC) system into a purification system. Simple as it may sound, fraction collection in general, and preparative-scale purification in particular, do have challenging facets. Flow rates in preparative-scale HPLC range from 5 up to 100 mL/min or above, generating fraction volumes of below 1 mL up to tens or even hundreds of mL. This demands collection vessels of different sizes, if the fraction collector capacity is to be used in an optimal way. Quick processing of collected fractions (re-analysis, pooling, and solvent evaporation) is desirable, creating the need for an easily accessible fraction bed.

The Agilent 1290 Infinity II Preparative Open-Bed Fraction Collector[1] contains new and improved features that address all of these topics. Fraction containers holding tubes of different diameters (12, 16, 25, 30 mm) and lengths (100 or 150 mm) are optimized to hold fractions of 8 to 78 mL. The fraction bed can be equipped individually with six containers, and holds up to 432 single fractions, or a maximum fraction volume of 5,940 mL. Three drawers separate the fraction bed, and enable the exchange of full drawers even during operation. Only the currently active drawer is locked to prevent spills and sample loss.

Tubing kits with different inside diameters are available for optimized setup according to the flow rate used. As with the Agilent 1260 Infinity fraction collectors, the 1290 Infinity II Preparative Open-Bed Fraction Collector features a built-in delay sensor, enabling precise calibration of the delay between the detector and the fraction valve without any hardware modifications.

Along with hardware improvements, the 1290 Infinity II Preparative Open-Bed Fraction Collector offers several enhanced fraction collection modes that combine peak- with time-based collection, and include recovery collections. Fraction collection triggered by a combination of ultraviolet (UV) with mass-selective detection (MSD) signals has also been extended by a third mode, combining the advantages of logical AND/OR connections. This Technical Overview describes the different fraction collection modes available in the 1290 Infinity II Preparative Open-Bed Fraction Collector, and presents typical applications of the feature enhancements.

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 make-up 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 × 100 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 to 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	Scouting gradient: 0.00 minutes – 10 %B 0.60 minutes – 10 %B 6.00 minutes – 90 %B 6.01 minutes – 98 %B 7.00 minutes – 98 %B 7.01 minutes – 10 %B	Optimized gradient: 0.00 minutes – 10 %B 0.60 minutes – 10 %B 7.00 minutes – 70 %B 7.01 minutes – 98 %B 8.00 minutes – 98 %B 8.01 minutes – 10 %B
Stop time	8.50 minutes	9.50 minutes
Injection volume	250 µL	
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

Time-based collection with volume slices

A perfect separation of a crude sample and purification of all desired compounds requires proper setting of fraction collection parameters and the solvent gradient. These parameters can only be determined by an experiment. In most cases, however, the available amount of sample will be too precious to be wasted for a simple scouting experiment without fraction collection. For situations such as this, the 1290 Infinity II Preparative Open-Bed Fraction Collector offers different time-based fraction modes, collecting either predefined volume or time intervals, or a fixed number of fractions within a specified time.

The preparative sample was separated by a scouting gradient, described in Table 1. Fraction collection was set to **time-based, collecting volume slices**, defining 21 mL volume slices, which is the maximum fill volume of 16 × 150 mm collection tubes. This fraction collection mode, being active from 0.00 to 7.00 minutes, enabled unspecific collection of all compounds into nine different fractions (Figure 1). In addition to this first crude fraction collection, the collected data can serve as a basis to determine optimum time, threshold, and slope parameters for a more sophisticated fraction collection. Based on this data file, the fraction preview functionality in the instrument control software simulates how peak parameter changes affect fraction collection in the following purification runs (Figure 2).

In addition to volume slice collection, time-based fraction collection can also be combined with a collection of time slices of definable duration. A third mode collects a user-specified number of fractions spread over a certain timespan, defined in the timetable.

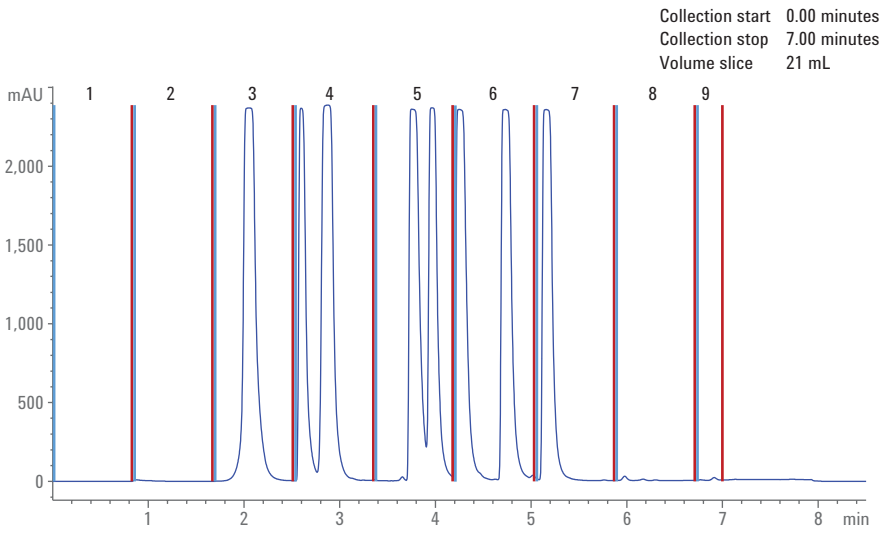

Figure 1. Purification of a preparative sample using a scouting gradient and the **time-based, collecting volume slices** fraction mode. Blue and red lines represent start and stop times of the nine collected fractions, respectively.

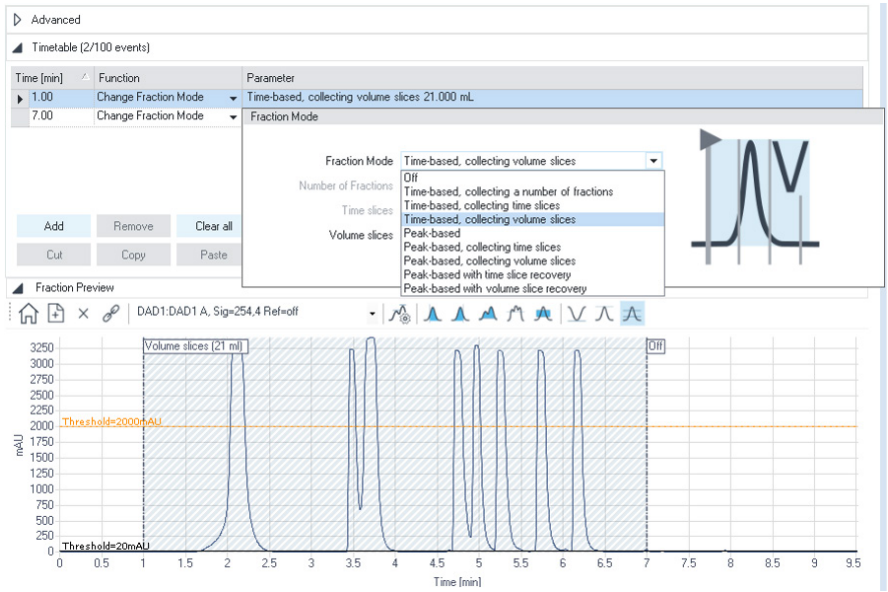

Figure 2. Fraction collector method settings in Agilent OpenLAB CDS ChemStation Edition, displaying collection timetable, available fraction modes, and fraction preview. The latter enables the user to review how changes of threshold, slope, and time settings affect the fraction collection.

Peak-based collection with volume slice recovery

Another approach for an unspecific fraction collection with a scouting gradient is possible using the **peak-based with volume slice recovery** fraction mode. Here, users can estimate and enter basic parameters for peak threshold and slope based on their experience and the expected sample concentration, as well as a recovery slice volume. Then, fraction collection will start with volume slice recovery at the time specified in the timetable. Whenever the signal rises above the entered threshold or slope parameters, the fraction mode switches to peak-based, and collects distinct fractions depending on the signal. This way, the compounds of interest are collected in single fractions, whereas the volume slice recovery collection prevents any sample loss in case threshold/slope parameters are improperly set.

Figure 3 shows the chromatogram and fractions collected with this fraction mode. Similar to the simple time-based fraction mode, the first and last two fractions are pure recovery, and do not contain any major peaks. Each of the eight sample compounds was collected in a distinct fraction. Note that peak-based collection is prioritized over volume slice recovery, causing the volume slices between the peaks to be smaller than the specified 21 mL. Collection start and stop times can also be defined in a timetable, enabling the collection of selected peaks only.

The second recovery collection mode combines peak-based fraction collection with time slices of user-definable duration. In addition to recovery collection, the 1290 Infinity II Preparative Open-Bed Fraction Collector features another combination of peak-based collection with time or volume slices, which is described in the following section.

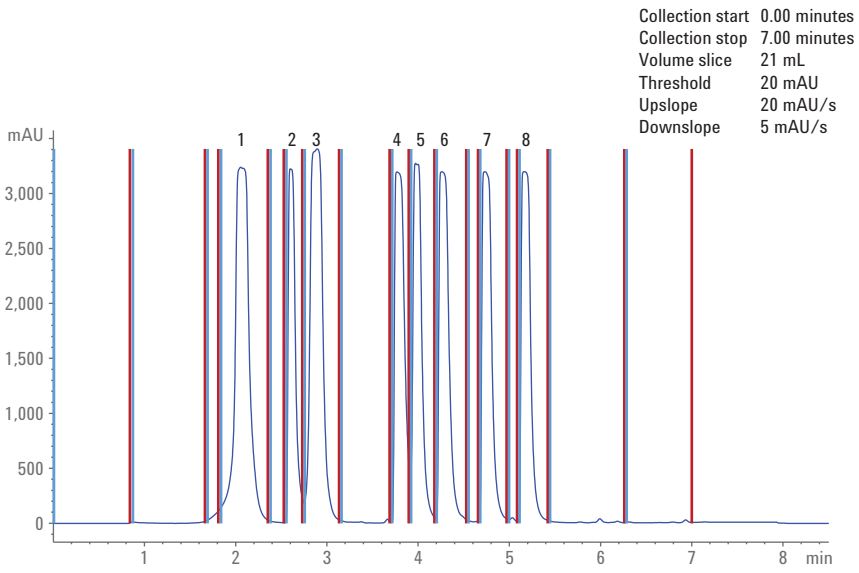

Figure 3. Purification of a preparative sample using a scouting gradient and the **peak-based with volume slice recovery** fraction mode. The eight sample components were collected in distinct fractions while the remaining chromatogram was cut into volume slices of ≤21 mL.

Peak-based collection with time slices

Finding the right peak threshold and slope parameters to collect peaks with highest purity without cutting too much of the front and tail of the peak can be difficult. This challenging task is addressed by the **peak-based, collecting time slices** and **peak-based, collecting volume slices** fraction modes. In either of these modes, parameters for peak threshold or slope have to be defined, along with a time or volume slice duration. As soon as the signal rises above the threshold/slope parameters, the fraction collector starts collecting, and cuts the peak into multiple fractions according to the user-defined slice duration/volume. This fraction mode enables the user to define conservative peak threshold/slope parameters, and decide after collection and reanalysis whether the outmost fractions of the peak front and tail shall be used or discarded due to insufficient purity.

Figure 4 shows a clipping of a chromatogram recorded with a gradient that barely separates compounds 4 and 5 intentionally. These two peaks were collected using peak-based fraction collection with 0.04 minute time slices. Both peaks were cut into nine slices each, triggered by a combination of threshold and upslope settings of 20 mAU and 20 mAU/s, respectively. In a separate experiment, the purity of each fraction was determined on an analytical system.

Figure 5 shows an overlay of the re-analysis chromatograms of the last three time slices of compound 4, and the first two time slices of compound 5. Fraction 4.7 contains the pure compound 4, whereas the last two slices (4.8 and 4.9) already contain traces of compound 5. Similarly, the first volume slice of compound 5 still contains traces of compound 4. To achieve the highest purity of both compound 4 and 5, the adjacent three fractions would have to be discarded. The topics of peak purity and recovery of the 1290 Infinity II Preparative Open-Bed Fraction Collector are addressed in more detail in another Technical Overview[2].

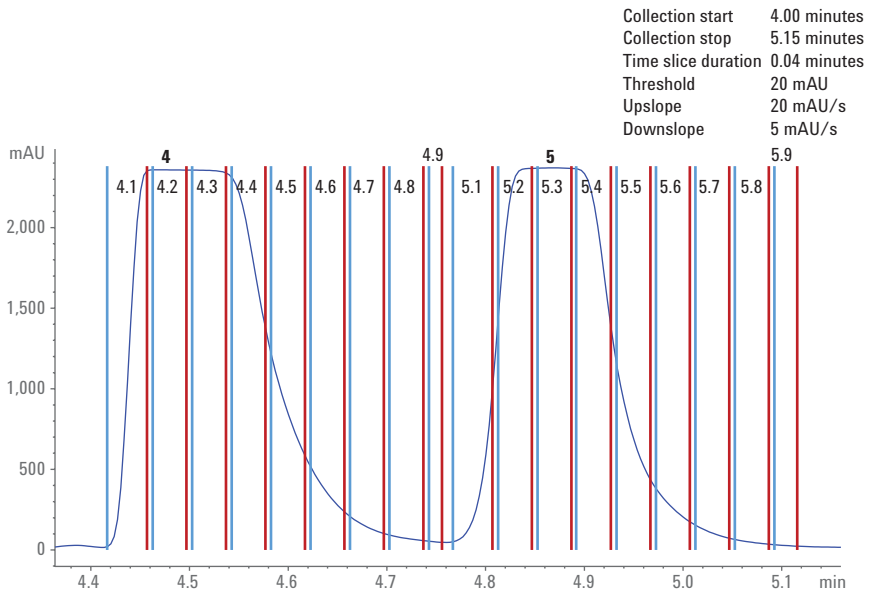

Figure 4. Purification of a preparative sample using an optimized gradient and the **peak-based, collecting time slices** fraction mode (clipping). Compounds 4 and 5 are barely separated and collected in nine time slices of 0.04 minutes each.

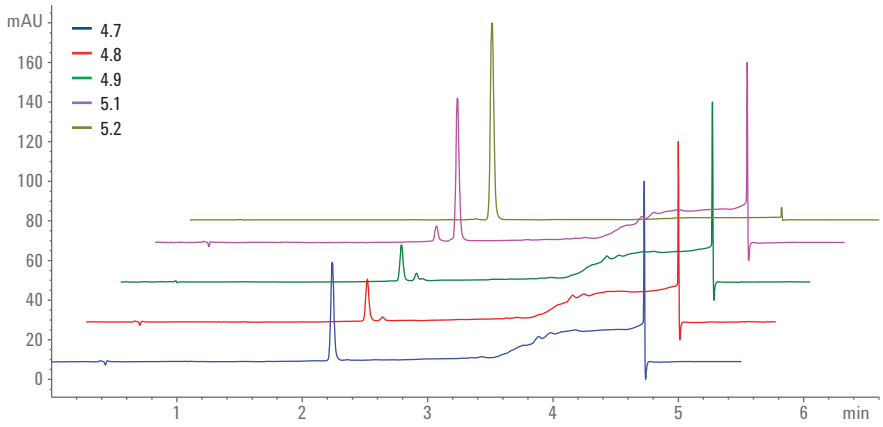

Figure 5. Overlay of fraction re-analysis: fractions 4.7 to 5.2 were collected using the **peak-based, collecting time slices** fraction mode (see Figure 4). Re-analysis revealed traces of compound 5 in the last fractions of compound 4, as well as traces of compound 4 in the first fraction of compound 5.

Peak-based collection

When the compounds of interest are well-separated and suitable peak threshold and slope parameters are known, peak-based fraction collection is the method of choice. This fraction mode can be activated for distinct time windows or for the complete runtime, and collects only peaks that meet the user-defined threshold or slope parameters.

The preparative sample was purified using an optimized gradient and peak-based fraction collection from 4.00 to 7.50 minutes, thus discarding the first three compounds. The signal threshold was set to 20 mAU, and upslope and downslope to 20 mAU/s and 5 mAU/s, respectively. The combination of threshold with slope settings allows a better separation of the fractions: if two peaks are not perfectly baseline-separated and the valley lies above the threshold, both peaks would be combined into one fraction if collection were triggered by threshold only. However, with downslope and upslope parameters, the falling and rising signal slope between two peaks triggers a separation into distinct fractions (Figure 6). As a plus, the upslope parameter prevents a false trigger; in case the baseline slowly rises above the threshold, fraction collection does not start until a peak causes the signal to rise with a steep slope.

Peak-based collection by UV and MSD signal

For highest specificity, fraction collection can be triggered by a combination of the UV signal with the MSD signal in each peak-based fraction mode. Multiple target masses can be specified for each run, either globally in the method, or specifically for each single sample in a sequence. In the MSD fraction collection parameters, ion species can be selected from a list of frequent adducts ($\pm H^+$, $+Na^+$, $+Cl^-$, and so forth) or entered arbitrarily. With this fraction mode, compounds can be purified with the highest specificity regardless of overlapping peaks or a complex sample matrix.

The preparative sample was separated using a logical AND combination of the UV and MSD signals to collect compound 5 with high specificity. Peak-based fraction collection was used, with the same UV trigger parameters as in the previous example. An additional MSD signal threshold was set to 50,000 cps, with a sample target mass of 310 Da. As ion species, only protonation/deprotonation was selected, monitoring both the negative and positive signal of the MSD.

Figure 7 shows the resulting chromatogram with the fraction marks displayed in both the UV and MSD signal. Contrary to the previous example, not every peak within the collection window from 4.00 to 7.50 minutes was collected, but only the one exceeding both UV and MSD threshold settings.

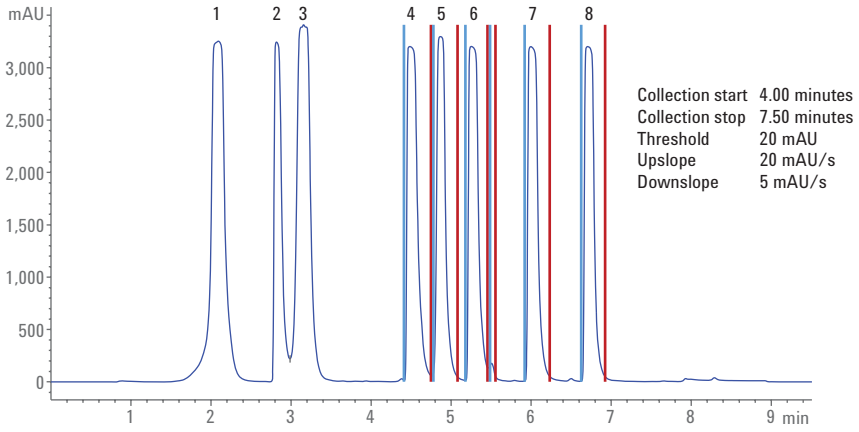

Figure 6. Purification of a preparative sample using an optimized gradient and the peak-based fraction mode, active from 4.00 to 7.50 minutes. Note that the impurity of compound 6 is separated from the main peak in a distinct fraction.

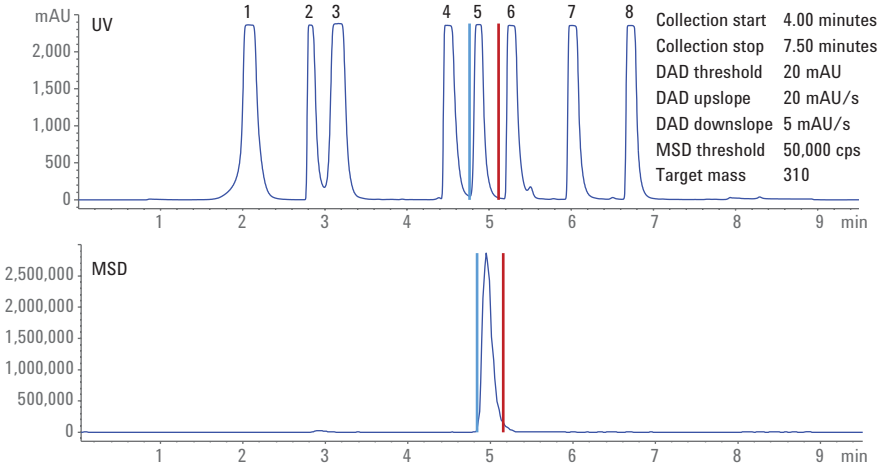

Figure 7. Purification of a preparative sample using an optimized gradient and the peak-based fraction mode with a logical AND combination of the UV and MSD signals.

The logical AND connection selected in this example is the most restrictive mode, triggering the fraction start only when both detector signals are above the threshold, but ending the fraction as soon as one of two detector signals drops below the settings. Alternatively, connecting UV and MSD signals with a logical OR condition causes the fraction collection to start as soon as one of the detector signals rises above the threshold. This mode can be unfavorable since it triggers the collection of a peak if only the UV threshold is exceeded, thereby overriding the specificity of the MSD.

The 1290 Infinity II Preparative Open-Bed Fraction Collector supports a third logical connection of UV and MSD signals, which is called AND/OR. This mode combines the advantages of both modes: fraction collection is triggered highly specifically by UV and MSD, starting only when both detector signals exceed the preset threshold/slope settings. Collection continues, however, until both detectors have sent a stop signal. Contrary to the AND connection, the AND/OR connection allows for a greater peak width in the MS signal, and does not cut off the fraction collection too soon. If the specificity of an MSD is desired, the choice between three logical connections of UV and MSD signals maintains maximum flexibility, and provides the optimum solution for any given scenario.

Conclusion

Besides a larger capacity, and a much more flexible configuration of the fraction bed, the Agilent 1290 Infinity II Preparative Open-Bed Fraction Collector offers new combined fraction modes that are tailored to the demands of different purification challenges. This Technical Overview provides application examples and collection outcomes of these fraction modes, including time-based collection combined with volume slices, peak-based collection combined with time slices, and two recovery collection modes. Peak-based fraction collection is demonstrated with a combination of ultraviolet (UV) and mass-selective detection (MSD) signals, connected by Boolean AND, as well as the novel AND/OR logic.

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. Performance Characteristics of the 1290 Infinity II Preparative Open-Bed Fraction Collector – Purity and Recovery of Collected Compounds, *Agilent Technologies Technical Overview*, publication number 5991-7655EN, **2016**.

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