

Higher Capacity, Lower Complexity

InfinityLab LC fraction collector clusters



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Abstract

Preparative high-performance liquid chromatography (HPLC) is a widely adapted technique to purify chemical compounds. With growing demands of synthetic and medicinal chemists, larger fraction capacities are required. Agilent InfinityLab LC Purification Solutions offer unique scalability to meet changing demands: multiple fraction collectors can be clustered to increase the capacity of existing systems. This technical overview demonstrates how Agilent hardware and software facilitate fraction and recovery collector clusters without increasing the complexity of the system.

Introduction

Whether it is a plant extract, a synthetic peptide, or a new small molecule drug candidate, preparative HPLC is an established method of choice for routine purification tasks. As synthesis methods and extraction throughput evolve, the demand for fraction capacity increases. Small fraction beds mean short administration intervals in which fractions need to be processed and the fraction collector cleared. Many labs are not able or willing to provide this level of administration, and seek solutions that offer fraction capacity for at least an entire working day without intermittent maintenance.

Agilent InfinityLab LC Purification Solutions offer scalability and modularity for the complete purification system. Up to three fraction collectors plus another three recovery collectors can be clustered to meet the growing demands of system users (the Agilent 1290 Infinity II Preparative Open-Bed Sampler/Fraction Collector (G7158B) can be clustered with up to two additional 1290 Infinity II Open-Bed Fraction Collectors (G7159B)). With sophisticated hardware solutions such as the 1290 Infinity II Preparative Open-Bed Sampler/Fraction Collector, the footprint of the system stays reasonably small. Smart software keeps the complexity of the system low, e.g. by enabling users to choose sampling or fraction locations in a graphical way.

This technical overview demonstrates the ease-of-use of a fraction/recovery collector cluster. Fifteen samples are purified, each in a dedicated fraction tray, without pausing for intermittent cleanup of the fraction bed. Smart method settings and graphical reports help to keep a high level of order with a low level of user interaction. To assess the performance of the extended system, fraction purity and recovery are determined.

Experimental

Instrumentation

The Agilent 1290 Infinity II Preparative LC/MSD system used in this experiment comprised the following modules:

- Agilent 1290 Infinity II Preparative Binary Pump (G7161B) with 200 mL pump heads (option 206)
- Agilent 1260 Infinity II Quaternary Pump (G7111B) with active seal wash (option 30) and active inlet valves (option 032)
- Agilent 1290 Infinity II Preparative Open-Bed Sampler/Collector (G7158B) with 5 mL sample loop (option 241)
- Agilent 1290 Infinity II Preparative Open-Bed Fraction Collector (G7159B)
- Two Agilent 1260 Infinity II Preparative Valve-Based Fraction Collectors (G7166A)
- Agilent 1260 Infinity II Variable Wavelength Detector (G7114A) with 0.3 mm preparative flow cell (option 024)
- Agilent 1290 Infinity II MS Flow Modulator (G7170B)
- Agilent 1290 Infinity II Preparative Column Compartment (G7163B)
- Agilent 1260 Infinity II Delay Coil Organizer (G9324A)
- Two Agilent 1260 Infinity II Cluster Valves (G9322A)
- Agilent LC/MSD XT (G6135B)

During instrument setup, the software recognizes the configuration and offers different topologies that can be configured using the connected modules. The system was configured as a cluster of two fraction collectors (one of which was a combined autosampler/fraction collector) and two recovery collectors, each connected by a cluster valve (see Figure 1). The software displays the cluster as a single fraction collector and automatically switches the cluster valves to provide the full capacity of the fraction and recovery collectors. Fraction and recovery start locations can also be chosen manually, which is facilitated by graphic selection.

Both fraction collectors were equipped with SBS containers (p/n G9321-60051) holding a total of 18 trays with a standard microplate footprint (as defined by ANSI/SLAS 1-2004). The three leftmost trays, located in the 1290 Infinity II Preparative Open-Bed Sampler/Collector, were reserved for injection purposes, leaving 15 trays for fraction collection. These were equipped with trays for 24 test tubes, 18 × 100 mm (p/n 5042-8544), or trays for 15 × 6 mL vials (p/n 5043-1826). The latter tray type was also used for samples, allowing to dedicate one fraction tray to each sample. Another 15 × 6 mL vial tray was used for postsample plugs, which were linked to the samples using relative positioning in the method (see Table 1).

FCC Post Auto Config

Communication
 Device name: Fraction Collector
 Type ID: FCC
 Connection settings...

Module List

Module Identifier	Is combined device	Name	Is used
▶ G7159B:PRBBN00090	<input checked="" type="checkbox"/>	AFC1	<input checked="" type="checkbox"/>
G7159B:PRBBE00058	<input type="checkbox"/>	AFC2	<input checked="" type="checkbox"/>
G7166A:DE00000119	<input type="checkbox"/>	AFC3	<input checked="" type="checkbox"/>
G7166A:DEAEF00135	<input type="checkbox"/>	AFC4	<input checked="" type="checkbox"/>
G9322A:PRAGT00052	<input type="checkbox"/>	Valve1	<input checked="" type="checkbox"/>
G9322A:PRAGT00054	<input type="checkbox"/>	Valve2	<input checked="" type="checkbox"/>

Add ... Configure ... Remove

Topology
 Selected Topology: V(F)n-V(R)n

Peak Detectors

Module Type	Serial Number
G7114A	DEACX00102

Add... Remove

Digital Triggers
 MSD Installed

Offline Configuration
 These options are for information only or for configuring an offline system. Please see help for instructions on how to change the configuration.
 Linked Pump: G7161B:DEBBK00124

Figure 1. Auto configuration dialog during instrument setup. A topology of two fraction collectors and two recovery collectors, each clustered by a valve, was chosen.

Method settings

Table 1. Chromatographic conditions of analytical and preparative runs.

Parameter	Value
Mobile Phase	A) 0.1% formic acid in water B) 0.1% formic acid in acetonitrile
Flow Rate	20 mL/min
Gradient 1	Time (min) %B
	0 10
	0.35 10
	0.36 25
	2.86 45
	3.30 98
Gradient 2	Time (min) %B
	0 10
	0.35 10
	0.36 40
	2.86 60
	3.30 98
Gradient 3	Time (min) %B
	0 10
	0.35 10
	3.30 98
	4.40 98
	4.5 10
Stop Time	5 min
Post Time	1 min
Injection Volume	200 µL
Sampler Method Preset	Preset 2: Nonpolar sample matrix Plug solvent: 25% methanol Relative position: Container, middle
Temperature	Ambient
UV Detection	254 nm 10 Hz data rate
MS Detection	Signal 1: positive scan <i>m/z</i> 100 to 600 Signal 2: negative scan <i>m/z</i> 100 to 600
Split Ratio to MSD	4,000:1 (mode M6) Turn ON after 1.50 min
Fraction Collection	1.50 min: Peak-based, UV AND MSD UV Threshold: 10 mAU UV Upslope: 5 mAU/s UV Downslope: 10 mAU/s MSD Threshold: 10,000 cps Fraction start location: next container

Table 2. MSD spray chamber and fraction collection settings.

Parameter	Value
Make Up Solvent	0.1% formic acid in methanol:water (70:30)
Make Up Flow	1.5 mL/min
Ionization Source	Agilent Jet Stream Electrospray
Nebulizer Pressure	35 psig
Drying Gas Temperature	300 °C
Drying Gas Flow	12.0 L/min
Sheath Gas Temperature	350 °C
Sheath Gas Flow	11.0 L/min
Capillary Voltage	± 4,000 V
Nozzle Voltage	± 600 V
Target Mass (<i>m/z</i>)	166.1
Ion Species	[M+H] ⁺ , [M-H] ⁻

Column

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

Software

Agilent OpenLab CDS ChemStation
edition for LC and LC/MS Systems,
version C.01.10 [239]

Solvents

LC-grade acetonitrile was purchased from Merck (Darmstadt, Germany). Analytical-grade dimethyl sulfoxide (DMSO) was obtained from VWR (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).

Samples

Three different samples were prepared in DMSO, each containing two arbitrary compounds and the target compound, which was weighed in a volumetric flask to exactly 50 mg/mL. Each sample was separated by a dedicated solvent gradient, see Table 1. Aliquots of each sample were diluted 250 × with acetonitrile/water (1:9 by volume) in volumetric flasks for recovery calculations.

Results and discussion

A preparative LC/MSD system with a fraction and recovery collector cluster was used to separate and purify target compounds out of 15 samples. To simulate different instrument operators in a multi-user environment, three different samples were injected five times each in random order. While the tray at the front position of the first drawer was used for samples, postsample plug solvents were provided on a separate sample tray. The position of the postsample plug solvent was mirrored from the sample location to the middle tray on the first drawer (e.g., sample 1-D1F-A1 uses the plug solvent from position 1-D1M-A1). This setting enables the instrument administrator to prepare fresh solvent for each sample, minimizing cross-contamination of samples.

Each sample was separated with a dedicated gradient, and the collection of the target compound was triggered by a target mass. Figure 2 depicts representative chromatograms for the three samples, and shows that the target peak was always separated with high resolution. With the fraction start location set to next container, each run's collection started on an empty tray. This setting has several advantages, for example:

- Better tracking of the fractions belonging to a sample
- Reduced risk of mixing fractions of different users and/or samples
- Further processing of completed containers while the instrument collects the next run's fractions into another container

To benefit from the last mentioned advantage, it is recommended to select the column-by-column collection order (see Figure 3), which uses the space of the fraction bed from left to right, and thus moves to another fraction drawer

for the next sample. To further facilitate the assignment of fractions to a sample, the software features intelligent reports with graphics highlighting the respective positions of fractions and recovery locations (see Figure 4).

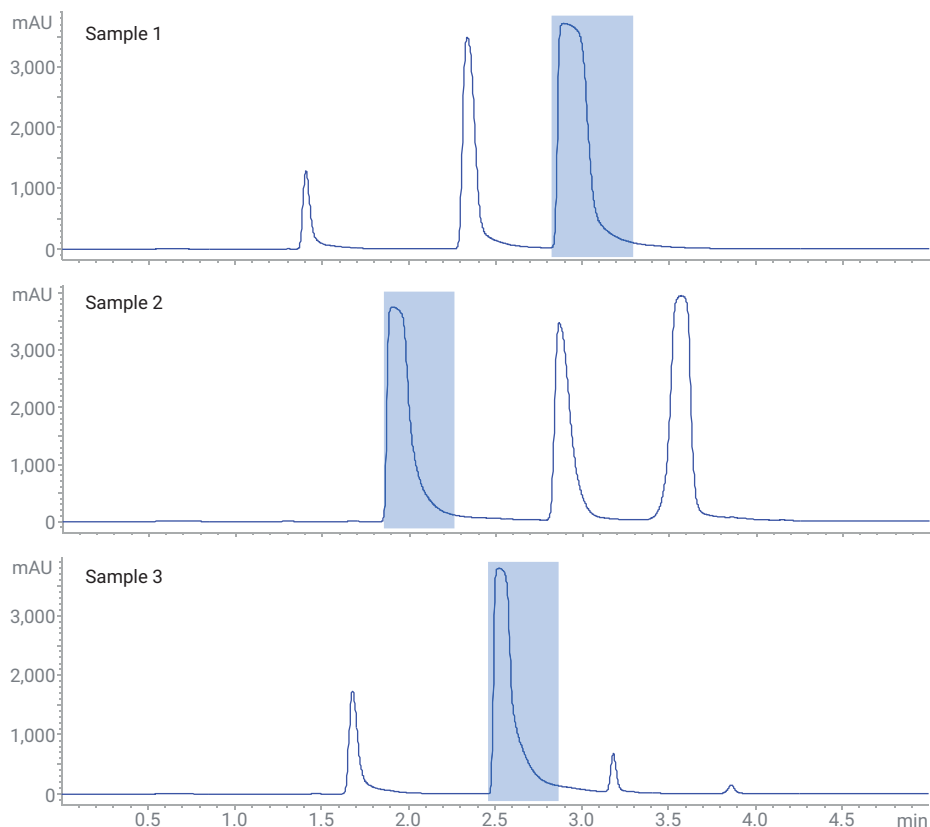


Figure 2. Chromatograms recorded at 254 nm show the separation of the three samples by different gradients. The target compound was collected (blue bars) using the UV and MSD signals.



Figure 3. Fraction collection orders available with the open-bed fraction collector. The column by column mode proceeds from left-to-right and front-to-back.

#	Location	Start Time	End Time	Volume	Reason
1	2-D2F-A1	2.811	3.111	6003	VWD1
1	2-D2F-A2	3.117	3.417	6000	Overflow
1	2-D2F-A3	3.422	3.489	1327	Overflow

Reference Detector = VWD1 (Delay Time = 0.1382)

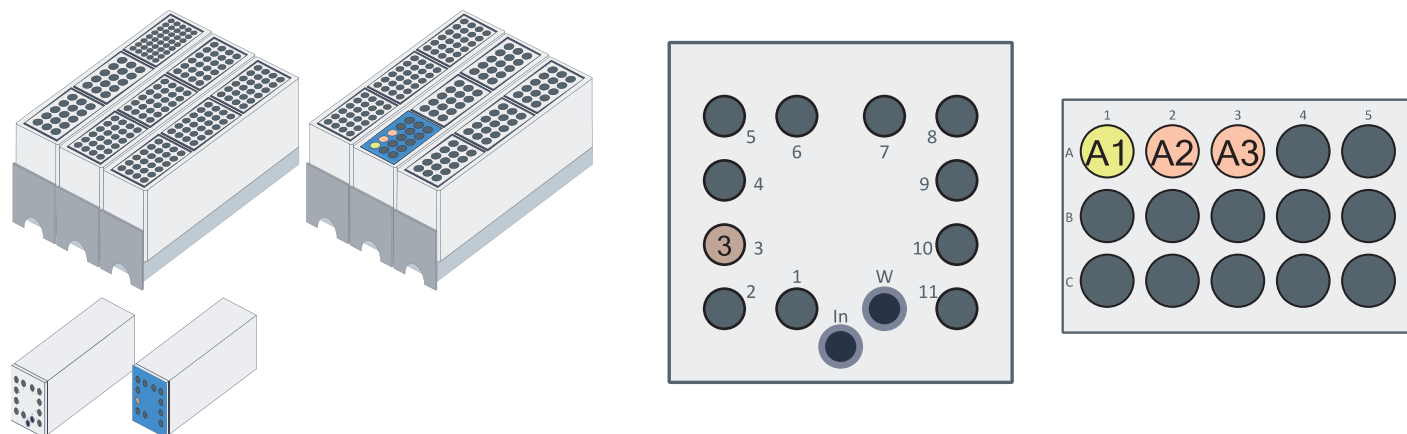


Figure 4. Cut-out from a sample report created with the Preparative LC W MS Spectra template. All fractions and the recovery location are listed and displayed in a bird's eye and detailed view of the fraction and recovery collectors, respectively.

Fractions were diluted with acetonitrile/water (1:9 by volume) in volumetric flasks to a final dilution factor of 250 × with respect to the injection sample. Analyzing these diluted fractions and the diluted target compound solution, peak areas of the target compound were compared. The recovery was then calculated as the peak area of the diluted fraction divided by the peak area of the diluted sample. Purity was assessed by the peak area of the target compound compared to the total peak area at 254 nm.

Figure 5 depicts a representative chromatogram of a re-analyzed fraction and the diluted injection sample. Recovery varied slightly among the three samples and ranged between 93 and 96%. Purity was always very high, with 97 to 99%.

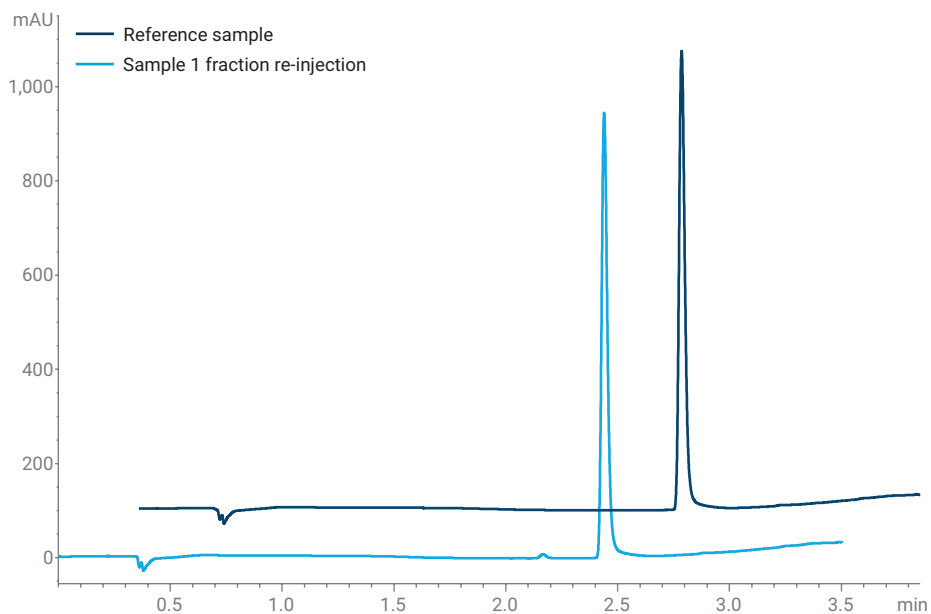


Figure 5. Chromatogram overlay (UV 254 nm) of a fraction re-analysis (here: target collected from sample 1) and the reference sample. Chromatograms are stacked for better view. This fraction has a recovery of 96% and is 99% pure.

Conclusion

This technical overview demonstrates the scalability of InfinityLab LC Purification Systems. Fraction capacity can be increased easily by adding another fraction collector and a cluster valve. Multiple injections and collections across the expanded bed show that recovery of fractions can be 93% or greater. Smart method settings facilitate the use in a large lab with multiple users: injection solvents can automatically be referenced to the samples, and each sample can be collected into a dedicated container, which reduces the risk of losing track of fractions or mixing those of different users. Comprehensive reports indicate the exact fraction and sample location on the fraction bed graphically, adding further clarity and traceability.

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