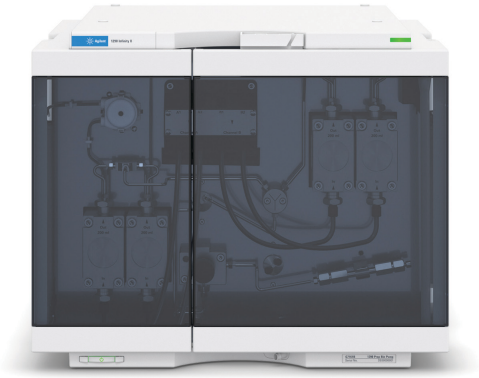
Technical Overview

Fast and Easy Injection of Large Sample Volumes in Preparative HPLC

Agilent 1290 Infinity II Preparative Binary Pump with Solvent Selection Valve



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Abstract

The Agilent 1290 Infinity II Preparative Binary Pump is the most flexible and high-performance pump of the Agilent InfinityLab LC Purification Solutions. Two sets of exchangeable pump heads enable operation from 1 to 200 mL/min. A built-in solvent selection valve allows users to switch between different eluents, or to use the pump as a high-flow, high-pressure injection pump. This Technical Overview demonstrates the use of the 1290 Infinity II Preparative Binary Pump as an injection pump, and presents performance characteristics of this application.

Introduction

The 1290 Infinity II Preparative Binary Pump is the powerhouse of the InfinityLab Preparative-Scale LC Purification Systems. Its high-performance and flexible design features exchangeable pump heads that enable operation from 1 to 50 and 4 to 200 mL/min, respectively. This feature facilitates fast and easy scale-up from semipreparative to wide-bore preparative columns, when methods are being developed and sample loads increase. A built-in solvent selection valve allows the connection of two solvents to each of the two channels, further increasing flexibility and reducing manual interaction when switching between methods.

Another application of the solvent selection valve is to use one of the channels to introduce large volumes of diluted sample directly onto the column. This application takes advantage of the wide flow range and high-pressure stability in comparison to common injection or metering pumps. Being able to inject at 150 mL/min with a pressure limit of 420 bar (or even at 200 mL/min with a limit of 300 bar) speeds up injections and increases sample throughput. As no separate injection pump or autosampler is needed, this feature also saves bench space and a considerable amount of investment.

This Technical Overview describes the use of a 1290 Infinity II Preparative Binary Pump as an injection pump using the built-in solvent selection valve to introduce large sample volumes directly onto the column. Loading accuracy and precision are evaluated and demonstrated by a representative sample and fraction collection.

Experimental

Instrumentation

The system used for this Technical Overview consisted of the following modules:

- Agilent 1290 Infinity II Preparative Binary Pump (G7161B) with preparative pump heads (option #206)
- Agilent 1260 Infinity II Diode Array Detector WR (G7115A) with 0.3 mm preparative flow cell (option #084)
- Agilent 1260 Infinity II Preparative Valve-Based Fraction Collector (G7166A)

Column

Agilent Prep-C18, 30 × 150 mm, 10 µm (p/n 413910-302)

Software

Agilent OpenLab CDS ChemStation edition for LC and LC/MS Systems, version C.01.09 [144]

Sample

The Agilent Prep LC Standard 1 (p/n 5190-6886) was diluted 1:200 (v:v) with water in a volumetric flask. The mix contains caffeine and four parabens. Fraction collection was focused on the closely eluting parabens.

Solvents

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).

Method parameters

The integrated solvent selection valve of the 1290 Infinity II Preparative Binary Pump cannot be switched during a run. Therefore, separate methods need to be set up for sample loading and elution. The loading method is described in detail in the results section. Table 1 lists parameters of the elution method.

Table 1. Parameters of the elution method.

Parameter	Value
Mobile Phase	A) Water B) Acetonitrile
Flow Rate	50 mL/min
Gradient	0.0 minutes, 5 %B 0.5 minutes, 5 %B 5.0 minutes, 70 %B
Stop Time	7.0 minutes
Post Time	2.0 minutes
Injection Volume	100 mL
Temperature	Ambient
UV Detection	254 ±2 nm, peak width >0.025 minutes (0.5 seconds response time), 10 Hz data rate
Fraction Collection	Peak-based, threshold 10 mAU, starting at 4.00 minutes

Results and discussion

Loading precision and accuracy

Whether samples are injected by an autosampler, a manual injector, or a loading pump—precise and accurate metering of the sample volume is key to obtaining reproducible results from day to day on a single instrument or when transferring methods between instruments. Loading performance of the 1290 Infinity II Preparative Binary Pump was determined by sampling 100 mL of water while applying different loading speeds. Precision and accuracy were calculated gravimetrically using the average sample mass and relative standard deviation (RSD) over a series of 10 injections. For these calculations, the density of pure water at 22 °C was used. The 1290 Infinity II Preparative Binary Pump delivered highly precise and accurate results over a wide range of loading speeds, as shown in Table 2.

Sample loading and elution

The 1290 Infinity II Preparative Binary Pump features an integrated solvent selection valve, which allows users to connect two different solvents to each of the two channels. In this experiment, channels A1 and B1 were connected to the eluents, whereas A2 was connected to a bottle of aqueous sample solution. Switching between A1 and A2 was facilitated by a method setting. Unlike the flow rate or eluent composition, this setting cannot be controlled by a timetable. Therefore, a separate method is needed to load the sample onto the column. Separate methods for loading and elution have two advantages:

- First, the flow rate of loading and elution can be different, enabling precise loading at low flow, and fast elution with high flow.
- Second, a common elution method can be used to separate different amounts of sample injected by different loading methods.

Table 3 shows the pump timetable used to load the sample onto the column in this experiment. Table 1 lists the elution method.

Methods for loading and eluting the sample were conveniently entered in the sequence table in alternating order (Figure 1).

Table 2. Loading precision and accuracy over a series of 10 consecutive injections of 100 mL of sample.

Loading speed (mL/min)	25	50	100
Average sample mass (g)	99.79	99.74	99.57
RSD (%)	0.097	0.024	0.030
Sample volume (mL)	100.01	99.96	99.79

Table 3. Pump timetable used to load 100 mL of sample through channel A2.

Time (min)	B (%)	Flow (mL/min)
0	0	0
0.01	0	50
2.00	0	50
2.01	0	0

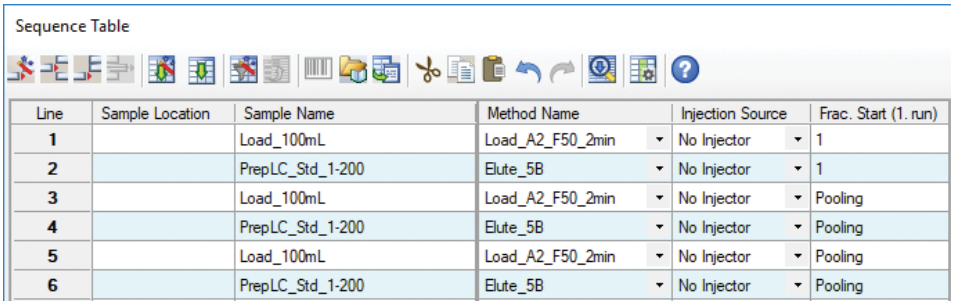

Figure 1. Excerpt of the sequence table used to load and elute multiple samples of 100 mL each. Separate methods for loading and elution allow using the same elution method for different sample loads.

Note that the Sample Location field is left blank, and **No Injector** is selected as the Injection Source (Figure 2). Fraction collection was set to pooling for all runs except the first one, enabling collection of the four target compounds in four vessels throughout the complete sequence. Figure 3 depicts an overlay of six consecutive 100 mL injections of diluted sample. No sample breakthrough could be observed. Retention times (RTs) and peak areas were highly reproducible, allowing precise collection and pooling of the four target compounds. With the exception of one compound, injection precision was comparable with the specifications of the 1260 Infinity II Preparative Autosampler[1] (<0.25 %). Table 4 shows the detailed peak properties.

When an injection pump is used instead of an autosampler, the sample and sample solvent flow through the pump head and the inlet/outlet valves of the pump channel that is used for injection. To avoid sample sticking to and damaging these parts, it is crucial to flush the pump channel immediately after injection. The integrated solvent selection valve of the 1290 Infinity II Preparative Binary Pump facilitates this task: After injecting from solvent A2, the elution method was programmed to switch the solvent selection valve to A1 (aqueous eluent). Eluting the sample with highly aqueous starting conditions flushed all channel A parts of the pump downstream of the solvent selection valve. Thus, only the tubing from the sample bottle to the solvent selection valve is contaminated with the sample. Since solvent A usually has low elution strength, it is not recommended to use concentrated sample solutions for injections through the pump.

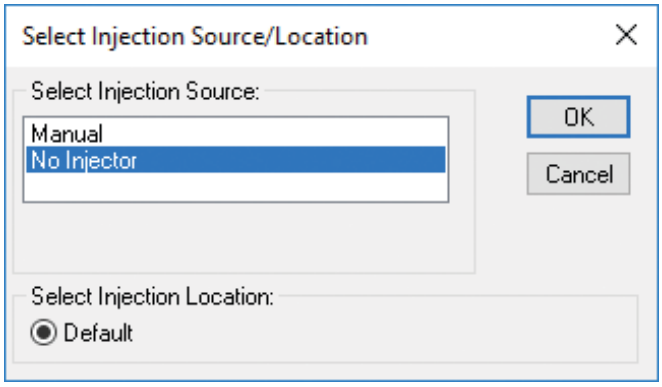

Figure 2. When an injection pump is used, the injection source (Instrument → Select Injection Source…) needs to be set to **No Injector**.

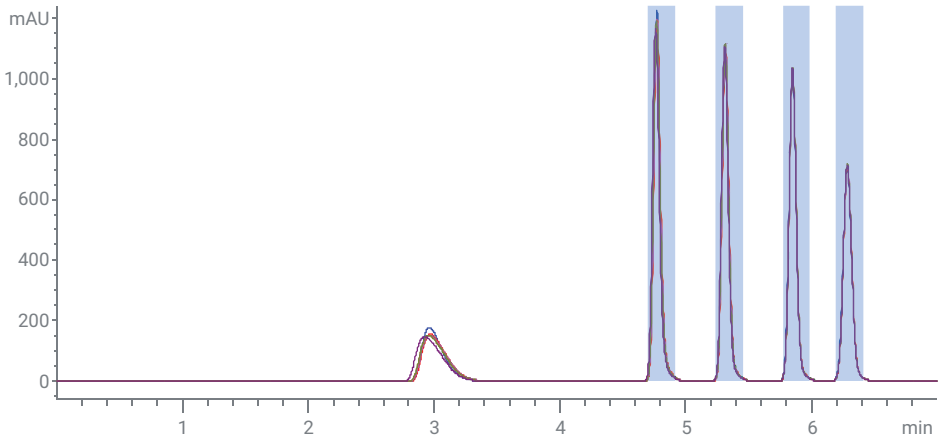

Figure 3. Chromatogram overlay (UV 254 nm) of six consecutive runs using a loading pump. Blue bars represent periods of fraction collection.

Table 4. Peak properties of a series of six consecutive runs injected using the 1290 Infinity II Preparative Binary Pump as the loading pump.

	RT (min)	RT RSD (%)	Area (mAU × s)	Area RSD (%)
Caffeine	2.957	0.534	2123	0.134
Methylparaben	4.769	0.082	4735	0.185
Ethylparaben	5.314	0.038	4376	0.212
Propylparaben	5.850	0.009	4263	0.267
Benzylparaben	6.286	0.010	3467	0.216

Conclusion

The 1290 Infinity II Preparative Binary Pump offers outstanding performance and flexibility. The built-in solvent selection valve was successfully used to inject large sample volumes. Taking advantage of the wide flow range and high-pressure stability enabled automatic high-speed injections, saving a considerate amount of time and effort. Over a wide range of flow rates, injection accuracy and precision were at a high level. RTs and peak areas were found to be highly accurate and precise; peak area RSDs were 0.26 % or lower and comparable to preparative autosamplers. These data demonstrate that the 1290 Infinity II Preparative Binary Pump can be used to inject high volumes of diluted samples, eliminating the need and costs of purchasing an autosampler or a separate injection pump.

Reference

1. Agilent InfinityLab LC Series 1260 Infinity II Preparative Autosampler, *Agilent Technologies User Manual*, **2017**.

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