Technical Overview

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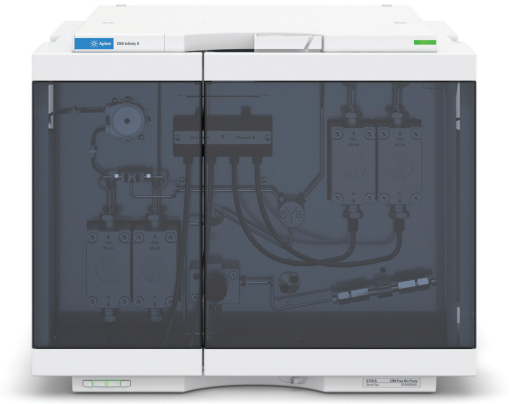
Performance Characteristics of the Agilent 1260 Infinity II Preparative Binary Pump



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Abstract

The Agilent 1260 Infinity II Preparative Binary Pump is the cornerstone of the 1260 Infinity II Preparative System, which offers outstanding flexibility and efficiency. With its high-performance binary design using two separate pump drives and parallel pistons within each channel, the 1260 Infinity II Preparative Pump delivers excellent results for a flow range of 1 to 50 mL/min at up to 420 bar. This Technical Overview presents superb accuracy and precision that push the boundaries of LC purification workflows.

Introduction

Agilent has developed a generation of preparative systems equipped with enhanced abilities to further unlock future potentials in purification workflows.

The 1260 Infinity II Preparative Binary Pump is an affordable high-pressure gradient pump, engineered for continuous use to provide robustness and reliable results through a dual-piston, rapid-refill design. It delivers flow rates from 1 to 50 mL/min, and is ideally suited for a broad range of semipreparative and preparative applications using columns up to 30 mm inside diameter (id). Figure 1 shows the hydraulic path of the pump.

Some of the features of the 1260 Infinity II Preparative Binary Pump are:

Purification efficiency

- Outstanding retention time stability for routine operation
- Automatic workflows using Agilent Automated Purification Software

Instrument efficiency

- Dynamic flow range starting at 1 mL/min, and up to 50 mL/min at 420 bar for semipreparative applications
- Outstanding binary gradient composition accuracy across a wide dynamic flow range

Laboratory efficiency

- Upper and lower pressure limits with automatic cutoff for increased safety in the event of column blockage or leakage
- Automated seal wash for extended pump seal lifetime

Another feature is flow reduction during column switching. If the pump is configured in a pressure cluster, this feature automatically detects a valve switch command issued by the user or method, ramps down the flow, and after the valve switch, automatically ramps the flow to the next setpoint defined by the user or method. This feature protects the system and column from pressure spikes, prolonging the lifetime of all parts.

This Technical Overview demonstrates the excellent flow precision, composition accuracy, and composition precision of the 1260 Infinity II Preparative Pump.

Experimental

Instrumentation

The Agilent 1260 Infinity II Preparative LC System comprised the following modules:

- Agilent 1260 Infinity II Preparative Binary Pump (G7161A)
- Agilent 1260 Infinity II Preparative Autosampler (G7157A)
- Agilent 1260 Infinity II Diode Array Detector WR (G7115A) with 3 mm preparative flow cell (option #022)
- Agilent 1260 Infinity II Preparative Valve-Based Fraction Collector (G7166A)

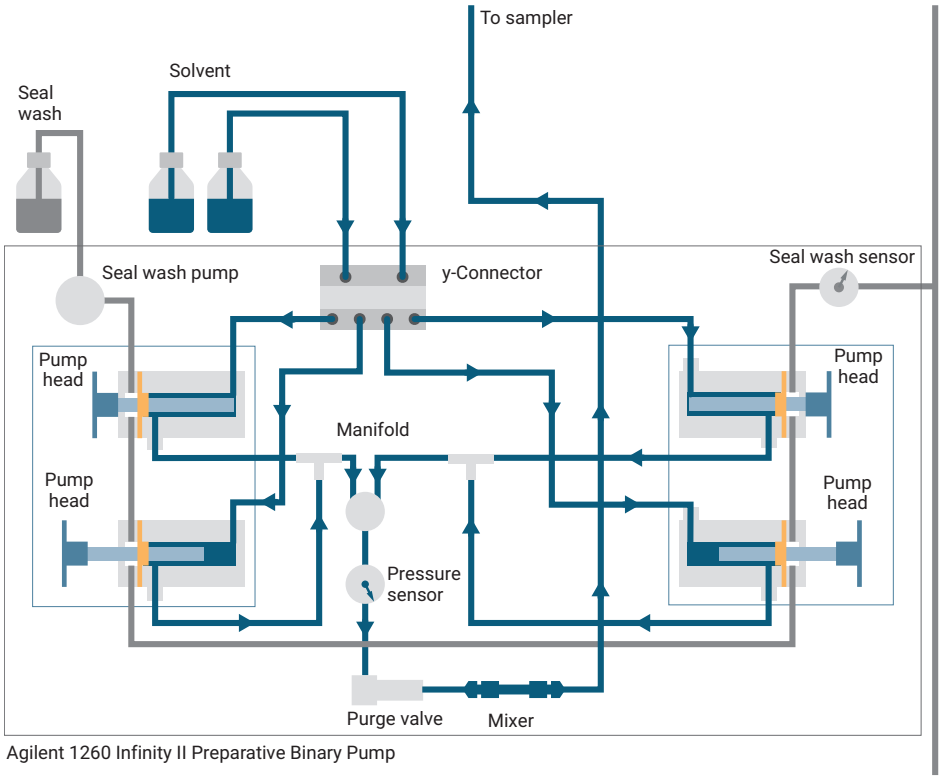

Figure 1. Hydraulic path of the Agilent 1260 Infinity II Preparative Binary Pump.

Columns

- Agilent ZORBAX SB-C18, 4.6 × 50 mm, 5 µm (part number 846975-902) for 1 mL/min
- Agilent ZORBAX SB-C18 PrepHT, 21.2 × 50 mm, 5 µm (part number 870050-902) with PrepHT end fittings (part number 820400-901) for 20 mL/min
- Agilent Prep-C18, 30 × 50 mm, 5 µm (part number 446905-302) for 42 and 50 mL/min
- InfinityLab Poroshell 120 SB-C18, 4.6 × 150 mm 2.7 µm (part number 683975-902) for a complex sample at 1 mL/min

Software

Agilent OpenLab CDS ChemStation edition for LC and LC/MS Systems, version C.01.08 [210]

Samples

- Agilent Prep LC Standard 1 (part number 5190-6886), diluted 1:40 (v:v) with dimethyl sulfoxide (DMSO)
- Caffeine, analytical grade (Sigma-Aldrich, Taufkirchen, Germany), dissolved in premixed water/acetonitrile 85:15 (v:v) to a final concentration of 2.5 mg/mL
- Reversed Phase Test Mix 2 (47641-U, Sigma-Aldrich, Munich, Germany), mixed with HPLC Gradient System Diagnostics Mix (48271, Sigma-Aldrich, Munich, Germany) and RRLC Checkout Sample (part number 5188-6529), v:v:v 1:1:2, 18 compounds.

Solvents

LC grade acetonitrile, DMSO, and methanol were 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). All solvents were freshly degassed either with helium or an inline degasser.

Results and discussion

Flow precision

To obtain reproducible results from run to run, high precision of the delivered flow is important, especially in isocratic methods. The precision of the flow was determined by running 10 replicates of an isocratic separation of caffeine samples with premixed solvents. Flow rates of 1, 20, and 42 mL/min were applied using isocratic elution (85% water, 15% acetonitrile) and appropriate chromatographic columns, as listed in the Experimental section. Peak retention times (RTs) of all runs were evaluated. The RT relative standard deviation (RSD) served as a measure of flow precision. Figure 2 and Table 1 summarize the results.

As shown in these tests, the pump delivered excellent flow stability and retention time reproducibility starting from 1 mL/min, which increases confidence in routine workflows.

Table 1. Peak properties and statistics of the flow precision experiments.

	Flow (mL/min)	RT (min)	RSD (%)
Channel A	1	1.461	0.137
	20	1.306	0.079
	42	1.087	0.048
Channel B	1	1.466	0.128
	20	1.315	0.042
	42	1.132	0.036

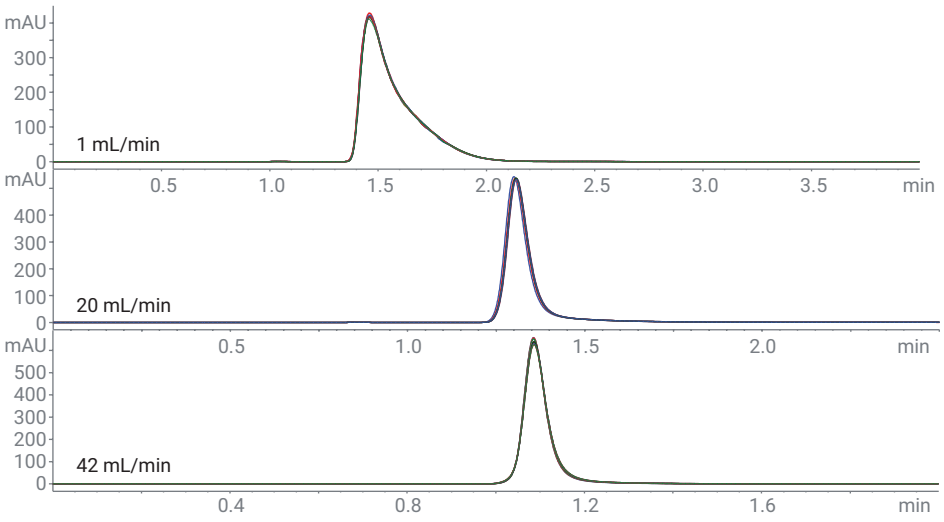

Figure 2. Flow precision: chromatogram overlay of 10 consecutive runs each, recorded at 1, 20, and 42 mL/min, respectively. Deployed columns for separation: 1 mL/min: Agilent ZORBAX SB-C18, 4.6 × 50 mm, 5 µm; 20 mL/min: Agilent ZORBAX SB-C18 PrepHT, 21.2 × 50 mm, 5 µm; and 42 mL/min: Agilent Prep-C18, 30 × 50 mm, 5 µm.

Composition accuracy

To maintain consistent results when applying or transferring gradient runs from different systems, the pump needs to accurately mix the two mobile phases. A step gradient using water in solvent A and water with 0.2% acetone as tracer in solvent B was applied to determine the composition accuracy of the pump. The gradient method was run without a column at 1, 20, and 50 mL/min.

A gradient program consisting of 17 steps was set and kept the same for all three tested flows. Figure 3 shows the program.

Composition accuracy was calculated with a three-point calibration, using two 0% B steps to assess the detector drift, and 100% B as the reference point. For each gradient step, the absorption was compared to a reference, and accuracy was evaluated. This Technical Overview reports excellent accuracy achieved by this binary pump, which is summarized in Table 2. The upper value stands for the highest numeric deviation of composition in percent among the gradient steps, whereas the lower value represents the lowest deviation.

Figure 4 shows the overlay of three runs at 1, 20, and 50 mL/min, and demonstrates comparable composition accuracy across the entire flow range. Note that the system volume remained constant for these experiments, making the delay at 1 mL/min more visible.

The results show that the binary high-pressure mixing principle ensures highly accurate gradient formation even at lower percentage difference (1%) of channel A or B. This feature is available for the entire flow range starting from 1 mL/min. Optimization steps such as deployment of an Agilent-designed capillary kit will exploit this performance feature further.

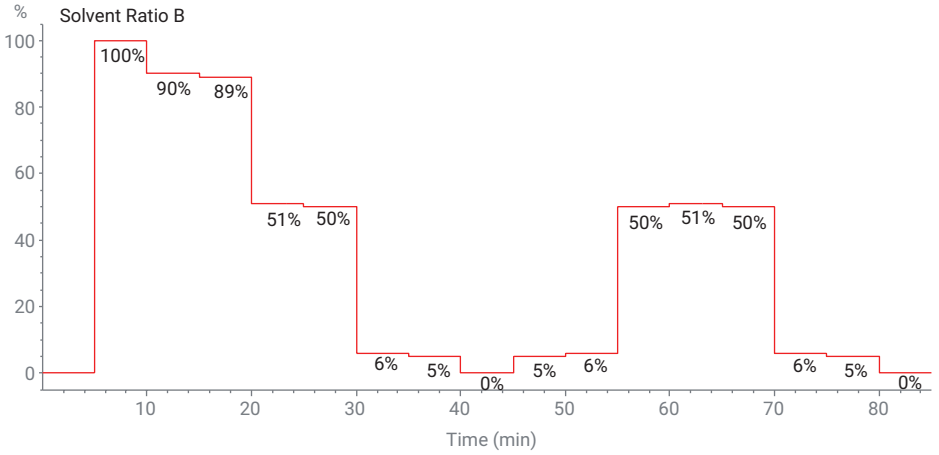

Figure 3. Programmed gradient consisting of 17 steps. This program was kept the same for flows of 1, 20, and 50 mL/min.

Table 2. Summary of composition accuracy runs.

Flow (mL/min)	Composition Accuracy in %	
	Lower Value	Upper Value
1	0.035	0.39
20	0.05	0.31
50	0.06	0.3

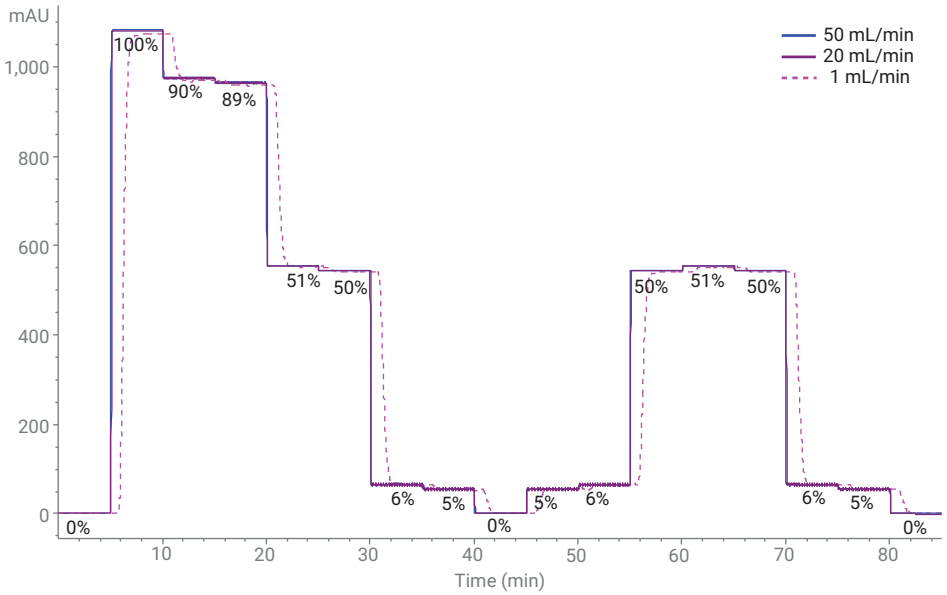

Figure 4. Composition accuracy: overlay of three consecutive runs at 1, 20, and 50 mL/min with 17 gradient steps. The system volume was kept constant.

Composition precision

Reproducible composition of the mixed mobile phase is essential to achieve consistent results from run to run when applying gradient runs. To determine the reproducibility of the mobile phase composition, a sample with five components was separated by a linear gradient from 2 to 98% acetonitrile versus water. Flow rates of 1, 20, and 42 mL/min were applied using appropriate chromatographic columns. RT RSDs were calculated for the five peaks for six consecutive runs to assess composition precision. Figure 5 and Table 3 show the results.

This pump demonstrated high degrees of run-to-run stability, which is highly favorable for everyday use.

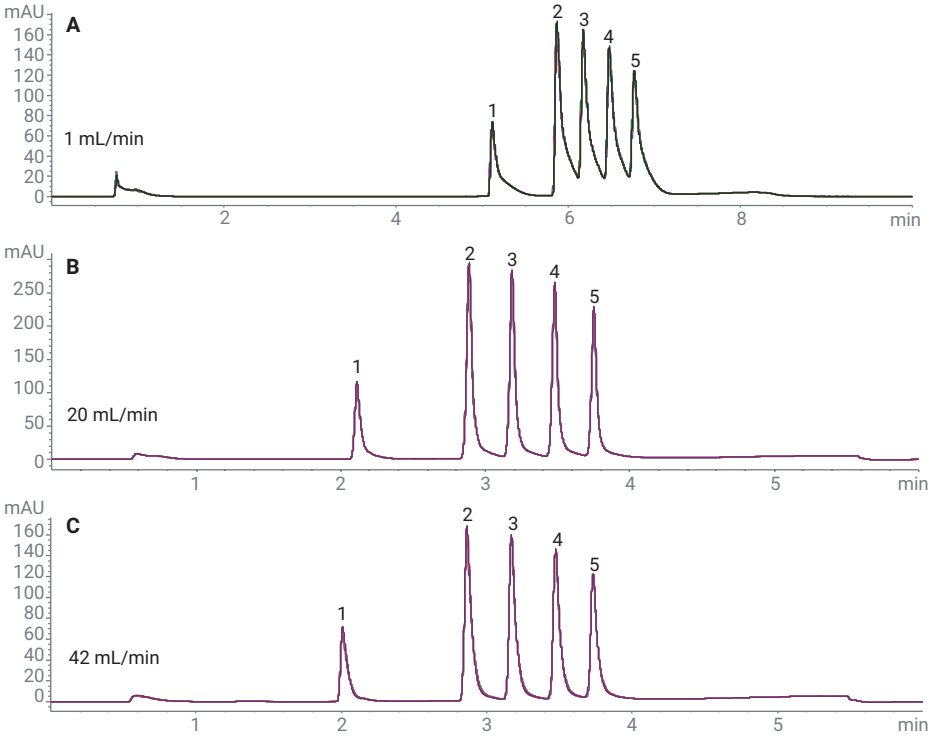

Figure 5. Composition precision: chromatogram overlay of six consecutive runs, recorded at 1, 20, and 42 mL/min, respectively. Deployed columns for separations: 1 mL/min: Agilent ZORBAX SB-C18, 4.6 × 50 mm, 5 µm; 20 mL/min: Agilent ZORBAX SB-C18 PrepHT, 21.2 × 50 mm, 5 µm; and 42 mL/min: Agilent Prep-C18, 30 × 50 mm, 5 µm. Eluents: water (A), acetonitrile (B). Gradient: 0–0.5 min: 2% B, 4.5 min: 98% B, 5 min: 2% B.

Table 3. Peak properties and statistics for the composition precision experiments.

	Flow (mL/min)					
	1		20		42	
Peak No.	RT (min)	RSD (%)	RT (min)	RSD (%)	RT (min)	RSD (%)
1	5.116	0.054	2.112	0.019	2.008	0.042
2	5.868	0.041	2.887	0.014	2.863	0.018
3	6.172	0.024	3.185	0.017	3.169	0.013
4	6.477	0.016	3.480	0.012	3.473	0.012
5	6.767	0.026	3.751	0.015	3.731	0.000

As an additional example for a challenging application, a more complex sample consisting of 18 compounds was separated by a shallower gradient starting at 2% acetonitrile over 30 minutes at 1 mL/min. Fourteen peaks were chosen and evaluated regarding RT RSDs. Figure 6 and Table 4 show the results.

As shown in the chromatograms and peak statistics in Figure 6 and Table 4, the 1260 Infinity II Preparative Pump achieved excellent results at 1 mL/min in terms of retention time stability and peak reproducibility.

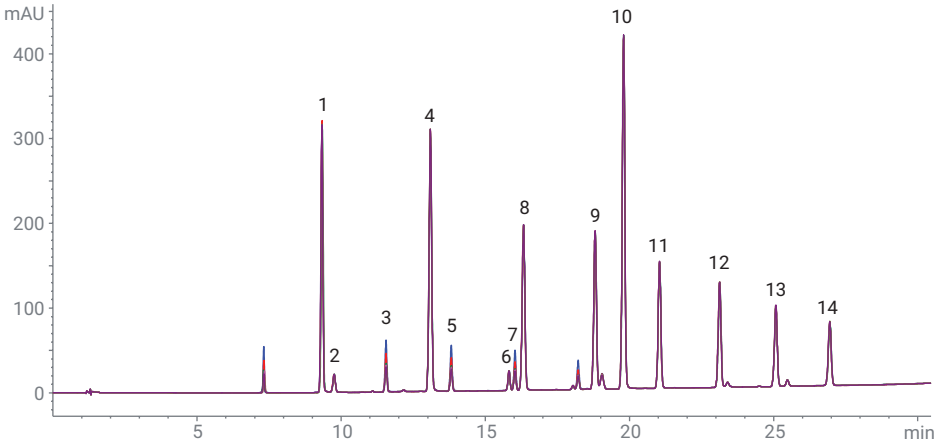

Figure 6. Composition precision: chromatogram overlay of six consecutive runs, recorded at 1 mL/min with a complex sample. Deployed column for this separation: Agilent InfinityLab Poroshell 120 SB-C18, 4.6 × 150 mm, 2.7 µm, thermostatted at 40 °C. Eluents: 0.1% formic acid in water (A), 0.1% formic acid in acetonitrile (B). Gradient: 0 minutes: 2% B, 30 minutes: 98% B, 31.1 minutes: 2% B.

Conclusion

As shown in the results, the Agilent 1260 Infinity II Preparative Binary Pump offers excellent accuracy and precision across a flow range of 1 to 50 mL/min. Isocratic elution experiments demonstrated highly precise solvent flows with RT RSDs of 0.14% or smaller. Composition deviations were assessed by step gradients with 1% steps, and were found to be smaller than 0.4% across the entire flow range. Gradient elution yielded highly reproducible results with RT RSDs of 0.06% or smaller. Equipped with such capabilities, the 1260 Infinity II Preparative Binary Pump will further unleash productivity potentials in your purification workflow.

Table 4. Peak properties and statistics for a complex sample.

Peak No.	RT (min)	RSD (%)
1	9.338	0.061
2	9.759	0.037
3	11.557	0.018
4	13.096	0.014
5	13.822	0.018
6	15.824	0.016
7	16.031	0.012
8	16.327	0.012
9	18.810	0.007
10	19.798	0.012
11	21.044	0.008
12	23.128	0.008
13	25.078	0.006
14	26.949	0.009

Reference

1. Agilent InfinityLab LC Series Preparative Binary Pump User Manual, *Agilent Technologies*, publication number G7161-90000 Rev.B.

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