Technical Overview

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Performance Characteristics of the Agilent 1260 Infinity II Hybrid Multisampler

Introduction

A typical sample introduction principle used for HPLC autosampler modules is the flow-through injection (Figure 1A). With this injection mode, the metering device, the loop, and the needle are switched to bypass while drawing the sample. During the injection step, the complete hydraulic path is switched into the flow path and the sample is injected towards the column. In contrast, for Agilent Feed Injection, the sampling part of the autosampler is independent and the pump flow is always connected to the column without any change in the internal volume (Figure 1B). The pressurized sample is directly injected into the flow stream towards the column by switching the injection valve. This mode enables the mediation of sample diluent effects and offers lowest delay volumes for fast gradients. With the Agilent 1260 Infinity II Hybrid Multisampler, it is possible to use both injection modes due to the built-in specialized valve.

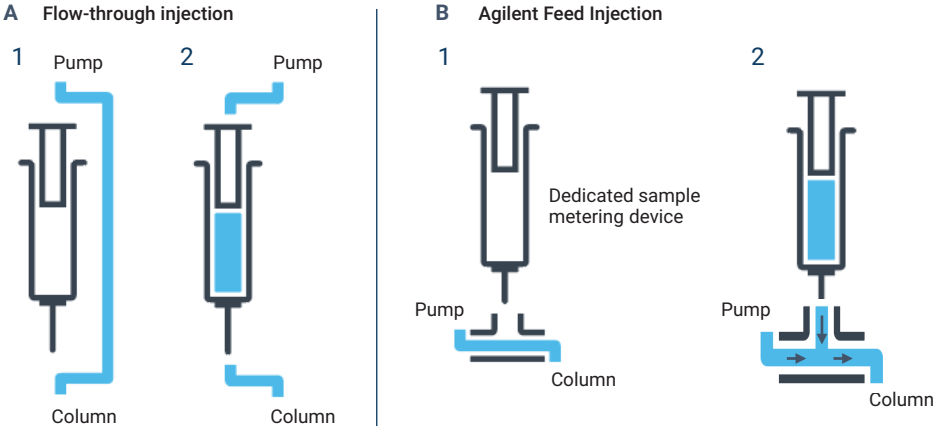

Figure 1. Schematic principle of flow-through injection and Agilent Feed Injection modes.

This technical overview demonstrates the performance of the different LC injection modes, flow-through injection (FT) and Agilent Feed Injection (FI), which are possible with the new Agilent 1260 Infinity II Hybrid Multisampler. High precision of peak area and retention time, excellent linearity, and lowest carryover provide high confidence in the generated data.

Experimental

Instrumentation

- Agilent 1260 Infinity II Hybrid Multisampler (G7167C)
- Agilent 1290 Infinity II High-Speed Pump (G7120A)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
- Agilent 1290 Infinity II Diode Array Detector (G7117B) with Agilent InfinityLab Max-Light Cartridge Cell, 10 mm (part number G4212-60008) or, for determination of carryover, Agilent Max-Light Cartridge Cell, 60 mm (part number G4212-60007)

Instrument setup

The 1260 Infinity II Hybrid Multisampler is built as a modular LC unit like a standard multisampler. It can be easily switched between the two injection modes, flow-through injection and Feed Injection, in the software user interface (Figure 2). The 1260 Infinity II Hybrid Multisampler allows a modulation of the injected flow by an adaptive injection speed in relation to the pump flow. That means, the flow rate approaching the column is always constant due to the respective down regulation of the HPLC pump. The flush out volume is calculated automatically or can be set manually. The combined injection and flush out volume must not exceed 71 µL.

Methods

All methods are outlined in the Results and discussion section, together with the corresponding results.

Software

- Agilent OpenLab CDS, version 2.7

Columns

- Agilent ZORBAX RR StableBond C18, 3.0 × 100 mm, 3.5 µm (part number 861954-302)
- Agilent Pursuit XRs C18, 2.0 × 50 mm, 3 µm (part number A6001050X020)

Chemicals

- Caffeine
- Chlorhexidine

Samples

- Agilent isocratic standard (part number 01080-68704)

Solvents and chemicals

All solvents were purchased from Merck, Germany. Chemicals were purchased from VWR, Germany. Fresh ultrapure water was obtained from a Milli-Q Integral Water Purification System equipped with LC-Pak polisher and a 0.22 µm membrane point-of-use cartridge (Millipak).

Additional materials

- Vial, screw top, clear, certified, 2 mL (part number 5182-0714)
- Screw cap, blue, certified, preslit PTFE/silicone septa (part number 5185-5865)
- Screw cap, bonded, blue, PFTE/red silicone septa (part number 5190-7024)

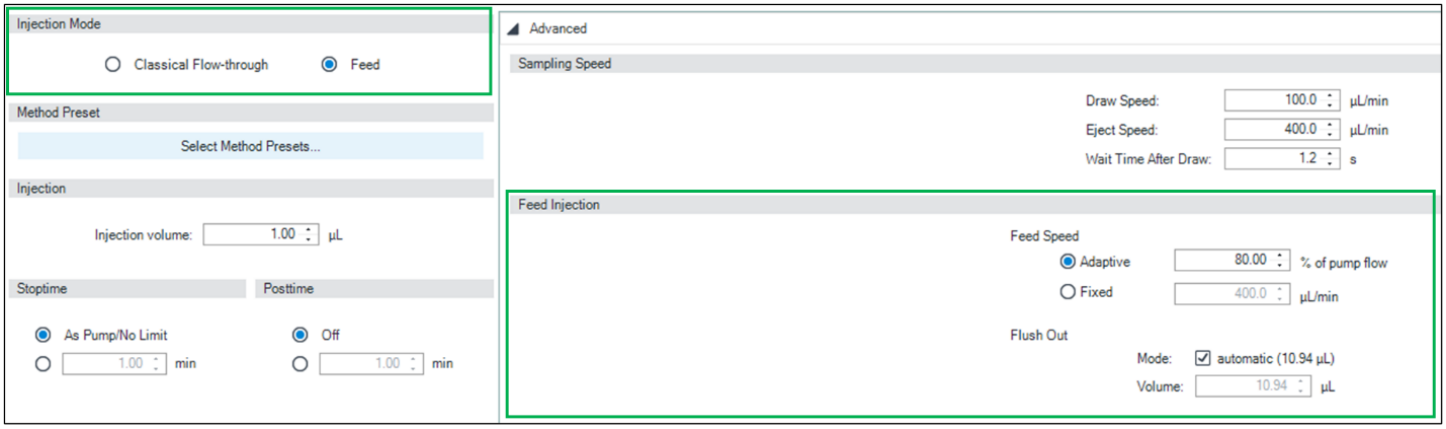

Figure 2. Software user interface of the Agilent 1260 Infinity II Hybrid Multisampler.

Results and discussion

Peak area precision

Table 1. Method settings for measurement of area precision.

Module	Parameter	Information
Pump	Mobile phase	Water:acetonitrile (85:15), premixed (v:v)
	Gradient	Isocratic, premixed (v:v), channel A:B, 50%
	Flow rate	1 mL/min
	Flow ramp up/down	100 mL/min²
	Stop time	2.0 min
Column Compartment	Temperature	30 °C
	Column	Agilent ZORBAX RR StableBond C18, 3.0 × 100 mm, 3.5 µm (p/n 861954-302)
Sampler	Temperature	Ambient
	Draw/Eject speed	100/100 µL/min
	Wait time after draw	1.2 s
	Outer needle wash: Time/Solvent	3 s/water
	Inner needle wash: Time/Solvent	Off
	Feed speed	80% of analytical pump flow rate (NA for flow-through injections)
	Flush out: Volume/Solvent	Automatic/15% acetonitrile in water (NA for flow-through injections)
	Operation mode	Agilent Feed Injection/ Flow-through injection
	Injection volume	See Table 2
UV Detector	Detection	Sig = 273, 4 nm; Ref = 360, 100 nm
	Peak width	>0.025 min (0.5 s response time), 10 Hz

Table 2. Dilutions used for measurement of area precision. Sample preparation: Caffeine stock solution: 300 mg caffeine + 100 mL diluent. Diluent: 2% acetonitrile in water.

Dilution Factor	Preparation	Concentration (mg/mL)	Injection Volume (µL)
NA	stock solution, neat	3	0.1
2	5 mL stock solution + 10 mL diluent	1.5	0.2
5	5 mL stock solution + 25 mL diluent	0.6	0.5
10	5 mL stock solution + 50 mL diluent	0.3	1
20	2.5 mL stock solution + 50 mL diluent	0.15	2
50	1 mL stock solution + 50 mL diluent	0.06	5
100	0.5 mL stock solution + 50 mL diluent	0.03	10
200	0.25 mL stock solution + 50 mL diluent	0.015	20
400	0.25 mL stock solution + 100 mL diluent	0.0075	40
1,000	0.1 mL stock solution + 100 mL diluent	0.003	100

For the determination of peak area precision in Feed Injection mode and flow-through injection mode, caffeine was used as a model compound and measured with an isocratic chromatography method (Table 1). For the Feed Injection, a feed speed of 80% of the pump flow rate was applied, and the flush out volume was set to automatic. For flow-through injection, the typical default conditions for draw speed and wait time were applied. The applied injection volumes, 0.1 to 40 µL for Feed Injection and 0.1 to 100 µL for flow-through injection, were kept inverse to the dilution ratio to achieve comparable peak areas (Table 2).

In Feed Injection mode, the peak area relative standard deviation (RSD) is between 3.7 and 2.3% for the lowest injection volumes of 100 and 200 nL, respectively. For 0.5 and 1.0 µL, the RSDs are 0.67 and 0.55%. The standard deviations are 4.0, 5.0, 3.0, and 5.0 nL, respectively. For higher injection volumes, the peak area RSD is typically below 0.15% (Figure 3). In comparison, the peak area RSDs obtained from the measurements in flow-through mode are comparable.

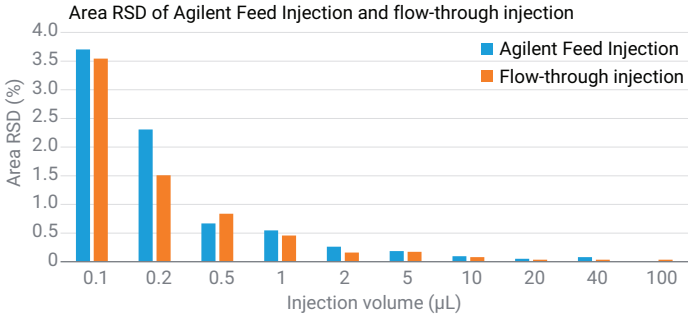

Figure 3. Peak area RSD for Agilent Feed Injection from 0.1 to 40 µL and flow-through injection from 0.1 to 100 µL.

Injection linearity

For the determination of the injection linearity of the 1260 Infinity II Hybrid Multisampler, two dilutions of the described caffeine stock solution were prepared with a dilution ratio of 1:100 and 1:1,000 (Table 3). These solutions were injected with the Feed Injection and flow-through injection. The more concentrated solution was used for injections with lower injection volumes up to 20 µL (level 1), and the less concentrated solution was used for the larger injection volumes up to 40 µL in Feed Injection mode and 100 µL in flow-through injection mode (level 2).

The obtained injection linearities for both levels in the Feed Injection mode and in the flow-through injection mode show linearity correlation of 0.9999 or better (Figure 4).

Table 3. Dilutions for measurement of injection linearity in Agilent Feed Injection and flow-through injection modes. Sample preparation: Diluent: 2% acetonitrile in water. Caffeine stock solution: 300 mg caffeine + 100 mL diluent. Injection volumes: Flow-through: Level 1: 0.1, 0.5, 1.0, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, and 20.0 µL. Level 2: 10, 20, 50, 70, and 100 µL. Feed Injection: Level 1: 0.1, 0.5, 1.0, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, and 20.0 µL. Level 2: 5, 10, 15, 20, and 40 µL.

Dilution Factor	Preparation	Concentration (mg/mL)	Injection Volume (µL)
100	0.5 mL stock solution + 50 mL diluent	0.03	Level 1
1,000	0.1 mL stock solution + 100 mL diluent	0.003	Level 2

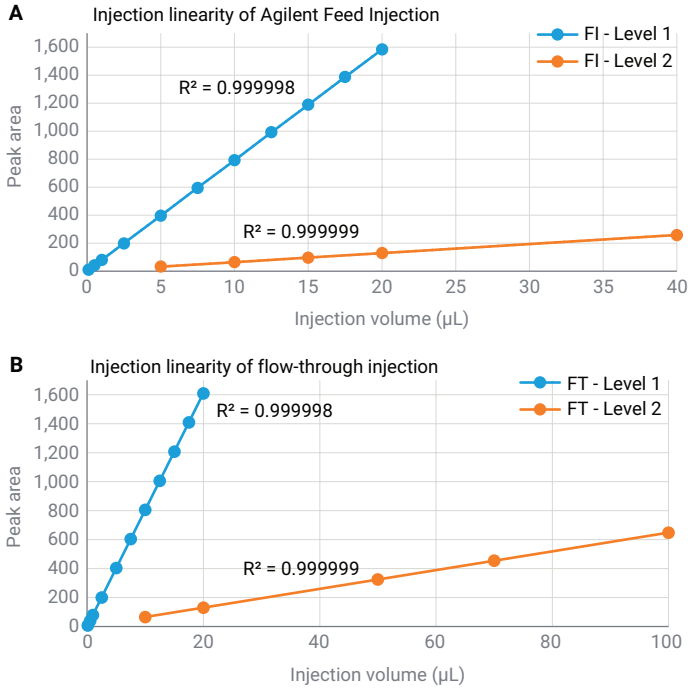

Figure 4. Injection linearities in Agilent Feed Injection mode (A) and flow-through injection mode (B) over the complete injection volume range.

Retention time precision

For the determination of the retention time RSD, the Agilent isocratic standard (part number 01080-68704) comprising four compounds was used (Figure 5). For the separation of the compounds in about 17 minutes, an isocratic method was applied (Table 4). The retention time RSD values were determined for feed speeds of 20 and 80% of the pump flow rate to prove the retention time stability over the range of feed speeds due to the changes in pump flow during the Feed Injection process (see Experimental – Instrument setup). All retention time RSD values obtained at Feed Injection with a feed speed of 20 and 80% of pump flow were below 0.01% (Table 5).

Table 4. Method for the determination of retention time precision. Sample preparation: Agilent isocratic standard (p/n 01080-68704) was used without further dilution and transferred directly into a sample vial.

Module	Parameter	Information
Pump	Mobile phase	Water:acetonitrile (50:50) + 0.1% TFA, premixed (v:v)
	Gradient	Isocratic, premixed (v:v), channel A:B, 50%
	Flow rate	1 mL/min
	Stop time	20 min
Column Compartment	Temperature	30 °C
	Column	Agilent ZORBAX RR StableBond C18, 3.0 × 100 mm, 3.5 µm (p/n 861954-302)
Sampler	Temperature	Ambient
	Draw/Eject speed	100/100 µL/min
	Wait time after draw	1.2 s
	Outer needle wash: Time/Solvent	3 s/mobile phase
	Inner needle wash: Time/Solvent	Off
	Feed speed	20 and 80% of pump flow
	Flush out: Volume/Solvent	Automatic/mobile phase
	Operation mode	Agilent Feed Injection
	Injection volume	1 µL
UV Detector	Detection	Sig = 254, 4 nm; Ref = 360, 100 nm
	Peak width	>0.025 min (0.5 s response time), 10 Hz

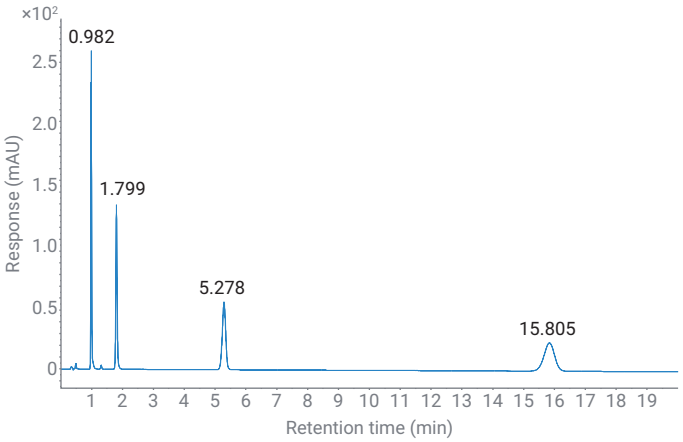

Figure 5. Separation of the four compounds in the Agilent isocratic standard (p/n 01080-68704) separated with the method given in Table 4 at 80% feed speed.

Table 5. Retention time precision obtained for four compounds at two different feed speeds (n = 10).

Feed speed: 80% of pump flow rate			
Peak	**RT (min)**	**SD**	**RSD (%)**
1	0.982	0.00044	<0.01
2	1.799	0.00134	<0.01
3	5.278	0.00507	<0.01
4	15.805	0.02136	<0.01
Feed speed: 20% of pump flow rate			
Peak	**RT (min)**	**SD**	**RSD (%)**
1	0.991	0.00058	<0.01
2	1.806	0.00078	<0.01
3	5.276	0.00128	<0.01
4	15.763	0.00448	<0.01

Carryover

From one sample injection to the next, it is possible that an amount of sample is transferred by the injection system between the samples. To determine a typical value for this instrument-related carryover, a high concentration of the sticky model compound chlorhexidine was injected, followed by a blank solvent injection. The carryover was calculated in relation to a more diluted reference solution (Figure 6). The measurement of chlorhexidine was done by an isocratic method in Feed Injection and flow-through injection modes (Table 6).

As a test setup for Feed Injection and flow-through injection, eight solvent blanks were injected to ensure that the instrument was clean, followed by three injections of chlorhexidine reference solution 2. The following injection contained the high concentrated chlorhexidine reference solution 1. Finally, blank solution was injected three times to determine the amount of carryover (Table 7).

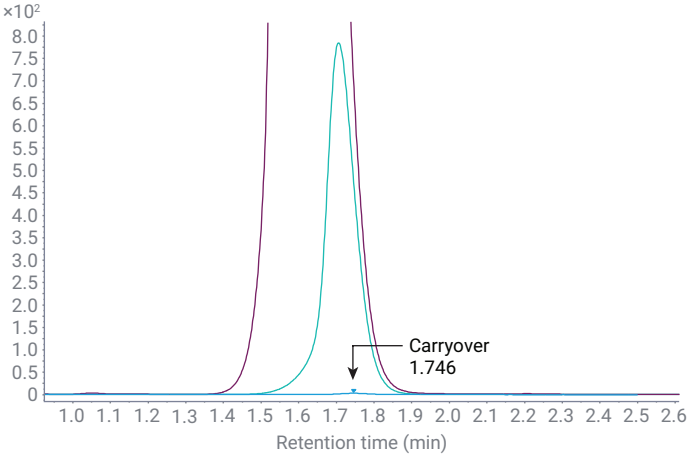

Figure 6. Determination of carryover. Overlay of injection of highly concentrated reference solution 1 (purple), reference solution 2 (green), and carryover obtained in the blank solution injection after the high concentration injection (blue) at 1.746 minutes.

Table 6. Isocratic method for the determination of carryover in Agilent Feed Injection mode and flow-through injection mode.

Module	Parameter	Information
Pump	Mobile phase	Water:acetonitrile (69:31) + 0.1% TFA, premixed (v:v)
	Gradient	Isocratic, premixed (v:v), channel A:B, 50%
	Flow rate	0.5 mL/min
	Stop time	2.5 min for blanks, 12 min for references
Column Compartment	Temperature	30 °C
	Column	Agilent Pursuit XRs C18, 2.0 × 50 mm, 3 µm (p/n A6001050X020)
Sampler	Temperature	Ambient
	Draw/Eject speed	100/100 µL/min
	Wait time after draw	1.2 s
	Outer needle wash: Time/Solvent	3 s, 50% acetonitrile in water + 0.1% TFA
	Inner needle wash: Volume/Solvent	1) 250 µL/50% acetonitrile in water + 0.1% TFA 2) 250 µL/mobile phase
	Seat wash: Volume/Solvent	1) 250 µL/50% acetonitrile in water + 0.1% TFA 2) 250 µL/mobile phase
	Feed speed	80% of analytical pump flow rate (NA for flow-through injections)
	Flush out: Volume/Solvent	Automatic/mobile phase (NA for flow-through injections)
	Operation mode	Agilent Feed Injection/Flow-through injection, see Table 7
	Injection volume	1 µL
UV Detector	Detection	Sig = 257, 4 nm; Ref = 360, 16 nm
	Peak width	>0.025 min (0.5 s response time), 10 Hz

Table 7. Experimental workflow for determination of carryover in Agilent Feed Injection mode and flow-through injection mode. Reference 1: 1.2 mg/mL chlorhexidine in water + 0.1% TFA. Reference 2: Reference 1 diluted 1:10 with blank solution. Blank solution: water + 0.1% TFA.

Step	Test Step Description	Injection Sequence
1	Preparation	Blank: 8 injections Reference 2: 3 injections
2	Agilent Feed Injection mode and flow-through injection mode	Reference 1: 1 injection Blank: 3 injections

Carryover is assessed using the peak area ratio of residual chlorhexidine in blank injections and reference injections expressed in parts per million (ppm):

Carryover [ppm] = Peak area chlorhexidine in blank/Ref. 2 average × 100.000.

For the determination of carryover in both injection modes, the described experiment was repeated 10 times. The carryover obtained from the flow-through injection mode was typically between 1 and 3 ppm. The carryover typically obtained from Feed Injection mode was between 4 and 8 ppm (Figure 7). Both results are in an excellent range, ensuring lowest carryover for problematic compounds.

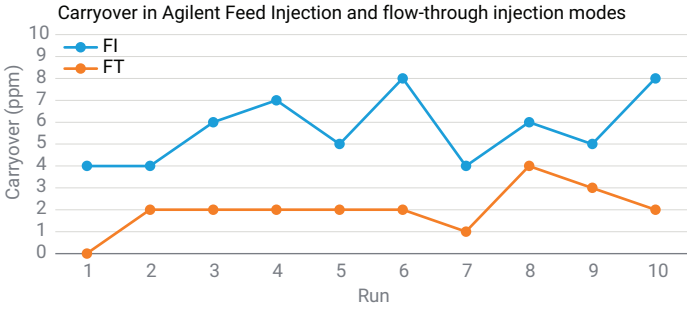

Figure 7. Results of the measurement of carryover in Agilent Feed Injection mode and flow-through injection mode from 10 repeated experiments.

Conclusion

This technical overview describes the performance characteristics of the Agilent 1260 Infinity II Hybrid Multisampler in Feed Injection mode and classical flow-through injection mode. In Agilent Feed Injection mode, the obtained results of injection area precision were typically below 0.15% RSD for high-volume injections and below a standard deviation of 20 nL for the lower volume injections. Injection linearities were always higher than 0.99990. Retention time precision for Feed Injection at 20 and 80% of pump flow rate were less than 0.01% RSD. Carryover was measured to be between 4 and 8 ppm. The obtained performance ensures the usability of the 1260 Infinity II Hybrid Multisampler for all quantitative applications in different application areas.

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