Supercritical Fluid Chromatography with Flexible Injection Volumes at Highest Precision

Performance Evaluation of the Agilent 1260 Infinity II SFC Multisampler in the Agilent 1260 Infinity II SFC System

Technical Overview

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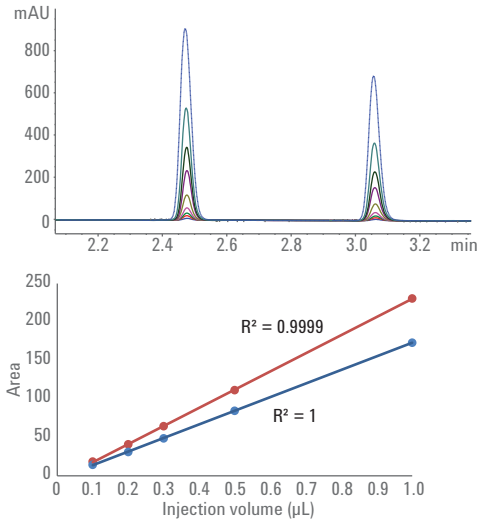
Abstract

This Technical Overview demonstrates the injection principle used in the Agilent 1260 Infinity II SFC Multisampler. In the Agilent 1260 Infinity II SFC Multisampler, the sample volume is drawn under atmospheric pressure conditions, pressurized to system pressure, and injected by an ultrafast syringing process. Data are presented that the 1260 Infinity II SFC Multisampler enables the injection of flexible sample volumes with highest precision, and excellent linearity over a broad volume range.



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Introduction

In contrast to the variable sample introduction of classical HPLC instruments, where the mobile phase filled sampling path can harmlessly experience atmospheric pressure, SFC instruments must avoid using mobile phase at ambient pressure in sampling paths to prevent evaporation of the dense CO_2. Evaporation of the CO_2 in the sampling path could lead to either a complete loss of the sample or an incomplete injection. Therefore, the fixed loop approach, where a previously filled loop was switched into the pressurized CO_2 stream, has been the method of choice for SFC. While this approach yields good peak area precision for full loop injections, it requires loop overfilling and hence a waste of sample. When used for partial loop filling, it requires complicated implementations, and compromises precision performance.

This Technical Overview demonstrates the injection principle used in the Agilent 1260 Infinity II SFC Multisampler. The 1260 Infinity II SFC Multisampler enables the injection of flexible sample volumes with the highest precision, in contrast to the widely used fixed-loop approach, which wastes sample by loop overfilling or suffers poor injection precision for partial loop injections. In the 1260 Infinity II SFC Multisampler, the sample volume is drawn under atmospheric pressure conditions, and pressurized to system pressure before it is injected into the analytical flow path by an ultrafast syringing process.

Experimental

Instrumentation

Agilent 1260 Infinity II SFC System comprised:

- Agilent 1260 Infinity II SFC Control Module (G4301A)
- Agilent 1260 Infinity II SFC Binary Pump (G4782A)
- Agilent 1260 Infinity II SFC Multisampler (G4767A)
- Agilent 1260 Infinity II DAD (G7115A) with high-pressure SFC flow cell
- Agilent 1260 Infinity II Multicolumn Thermostat (G7116A)

Instrumental setup

The 1260 Infinity II SFC Multisampler is connected directly to the SFC pump and downstream to the column. All necessary flushing and washing steps are done through the factory-installed plumbing. It is only necessary to connect two solvents: one for the flushing and feeding process, and one for the needle wash.

Column

Agilent ZORBAX Rx-Sil, 4.6 × 150 mm, 5 µm

Software

Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS Systems, revision C.01.07 SR3

Samples

Solutions of caffeine and theobromine (250 mg/L each in methanol), caffeine (0.5 g/100 mL in methanol) and theobromine (250 mg/L in methanol).

Chemicals

All solvents were purchased from Merck, Germany. Chemicals were purchased from Sigma-Aldrich (Germany).

Results and Discussion

The fixed-loop approach is used for sample injection in SFC instruments as a state-of-the-art technique. This approach enables the injection of fixed volumes with high precision, but volumes that are injected by a partial loop fill suffer from compromised precision. The variable-loop concept that is widely used for sample injection in HPLC instruments cannot be used because liquid CO_2 cannot be subjected to atmospheric pressure. This would lead to partial or complete loss of the sample due to evaporation. To overcome this drawback, a flexible injection principle was introduced with the 1260 Infinity II SFC Multisampler for the Agilent 1260 Infinity II SFC System.

The Agilent feed-injection technology provides a pressurized sample, which is injected into the CO_2 stream prior to the column by a syringing process. Before the sample is drawn, the connected loop, needle, and seat are cleaned by purging with feed solvent, while the SFC pump is connected to the column. After drawing the flexible sample volume, it is pressurized in the loop to the pressure of the system. The pressurized loop containing the sample is connected to the CO_2 stream coming from the analytical SFC pump and leading to the column. In this position, the sample can be injected by a syringing process with variable injection speed (feed speed). To flush the complete sample out of the loop, an overfill volume (over-feed volume) can be defined.

SFC method

Parameter	Value
Solvent A	CO_2
Modifier B	Methanol
SFC flow	2.5 mL/min
Isocratic elution	12 %B
Stop time	6 minutes
Gradient elution	5 to 35 %B in 4 minutes
Stop time	6 minutes
Post time	2 minutes
Gradient for large volume injection	0 to 1 minute – 1 %B 1.1 minutes – 5 %B 4 minutes – 35 %B
Stop time	6 minutes
Post time	2 minutes
Back pressure regulator (BPR) temperature	60 °C
BPR pressure	130 bar
Column temperature	40 °C
Injection volume	0.1, 0.2, 0.3, 0.5, 1.0, 2.0, 3.0, 5.0, and 10.0 µL
Large volume injection	10, 20, 30, 40, 50, 60, 70, and 80 µL
Feed solvent	Methanol
Over-feed volume	4 µL
Feed speed	400 µL/min (up to 10 µL injection) and 100 µL/min for large volume injection (>10 µL)
Needle wash	3 seconds in methanol
Detection	272 nm/bandwidth 4 nm; reference 360 nm/bandwidth 100 nm; standard high-pressure SFC flow cell; data rate 10 Hz

There are two instrument parameters controlling the injection: the feed speed and the over-feed volume. For standard injections (0.1 to 10 µL), the feed speed should typically be higher than 100 µL/min (default 400 µL/min, up to 1,000 µL/min) to avoid peak broadening. A lower feed speed could be used for trapping injections into an initial isocratic step. The over-feed volume should not be below 2 µL (default 4 µL) due to possible sample loss. Higher values could be used to flush out sticky compounds or heavy matrix loaded samples. The influence of these parameters on chromatographic performance is discussed in more detail in an Agilent Technical Overview[1].

To determine the performance of the 1260 Infinity II SFC Multisampler, the injection linearity and peak area precision were determined under isocratic and gradient elution conditions for the injection volume range from 0.1 µL to 10.0 µL (Figures 1, 2, and 3). For both sets of experiments, the default conditions for over-feed volume and feed speed (see method) were applied. This ensured that the sample was fed into the CO_2 stream quickly, and made certain that the sample was flushed out completely from the sampling loop. Under isocratic conditions, both compounds were well separated, and peak shapes were excellent for all injection volumes from 0.1 to 10.0 µL (Figure 1A). The injection linearity was calculated for the different ranges of injection volumes: 0.1 to 10.0 µL, 0.1 to 1.0 µL, and 1.0 to 10.0 µL. In all cases, the injection linearity was excellent, with $R^2 > 0.9995$ (Figures 1B to 1D) for both compounds. The results for the gradient separation also showed excellent peak shapes for all tested injection volumes from 0.1 µL up to 10.0 µL (Figure 2A). The injection linearity for both compounds showed excellent values of $R^2 > 0.9999$ for all tested injection volume ranges at 0.1 to 10.0 µL, 0.1 to 1.0, and 1.0 to 10.0 µL (Figures 2B to 2D).

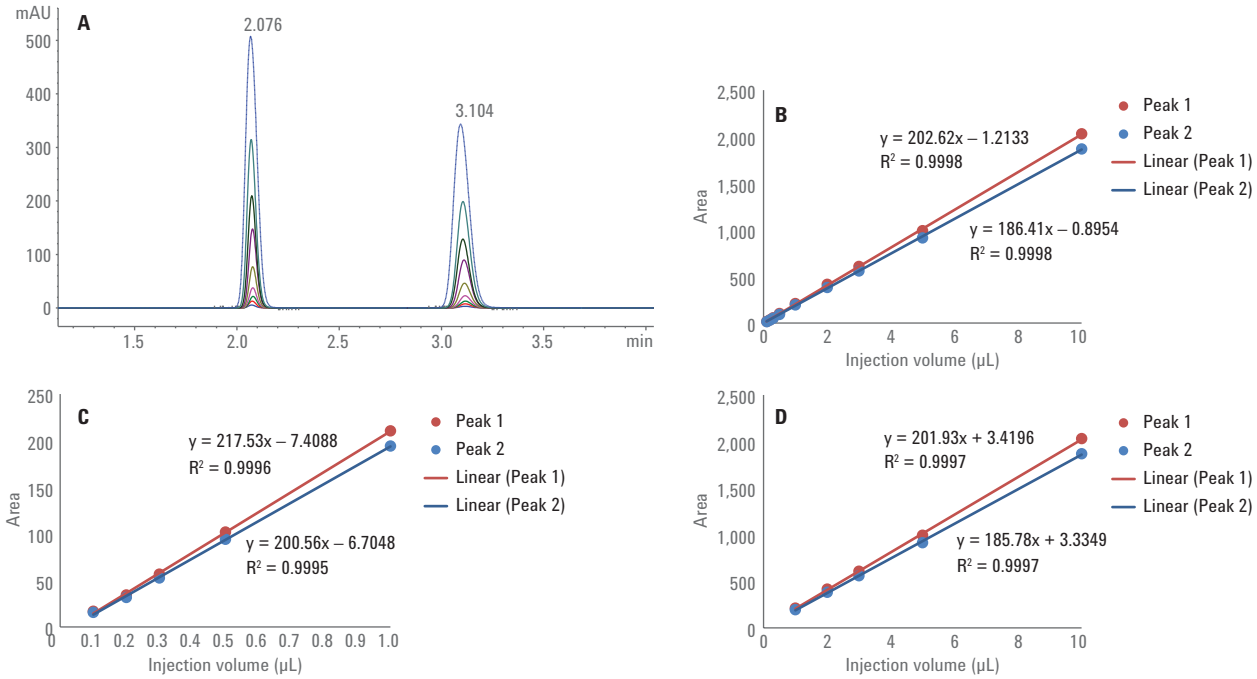


Figure 1. Injection linearity for peak 1 and peak 2 under isocratic elution conditions. A) Chromatogram of the isocratic separation. Peak 1: Caffeine, 2.076 minutes. Peak 2: Theobromine, 3.104 minutes. Injection volume: 0.1 to 10.0 µL. B) Linearity for 0.1 to 10 µL injection volume. C) Linearity for 0.1 to 1.0 µL injection volume. D) Linearity for 1.0 to 10 µL injection volume. R^2 is typically >0.9995.

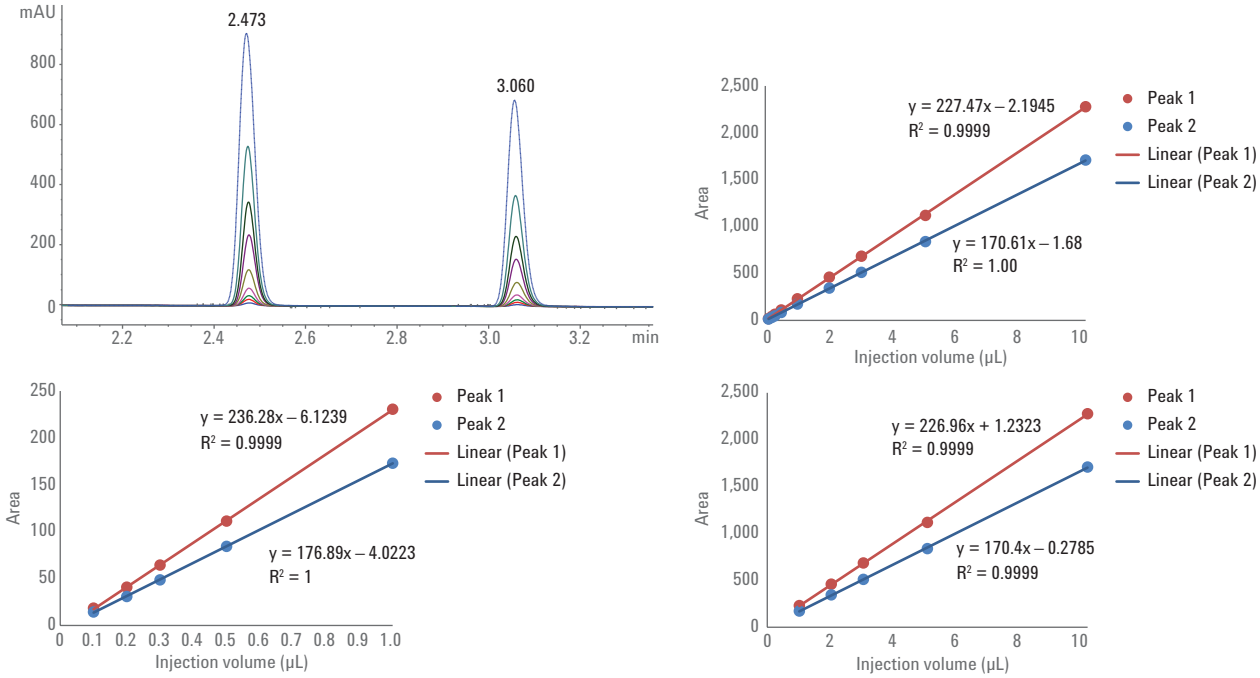


Figure 2. Injection linearity for peak 1 and peak 2 under gradient elution conditions. A) Chromatogram of the gradient separation. Peak 1: Caffeine, 2.473 minutes. Peak 2: Theobromine, 3.060 minutes. Injection volume 0.1 to 10.0 µL. B) Linearity for 0.1 to 10 µL injection volume. C) Linearity for 0.1 to 1.0 µL injection volume. D) Linearity for 1.0 to 10 µL injection volume. R^2 is typically >0.9999.

The area RSD values for all injection volumes were calculated from 10 replicates for the isocratic and gradient experiments (Figure 3). For both compounds, the area RSDs start for the lowest volume injection (0.1 µL) at 3.0 to 3.5 % for the isocratic separation (Figure 3A), and at 2.0–2.5 % for the gradient separation (Figure 3B). For both cases, the RSD values decline to 0.3 % and below for increasing injection volume above 0.5 µL. For all higher injection volumes, up to 10.0 µL, the RSD values remain below 0.3 %.

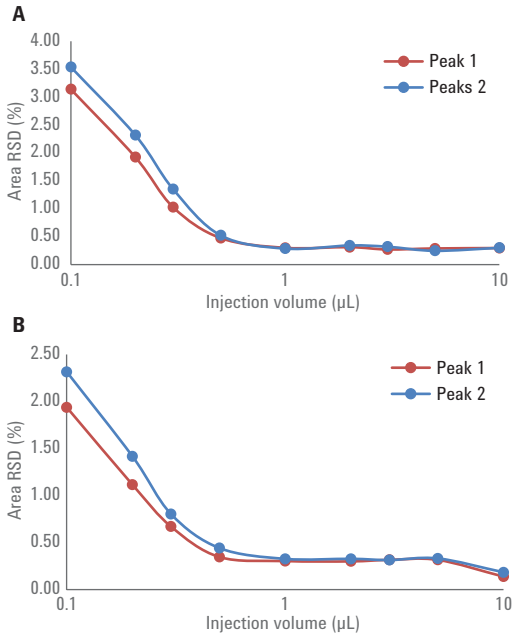
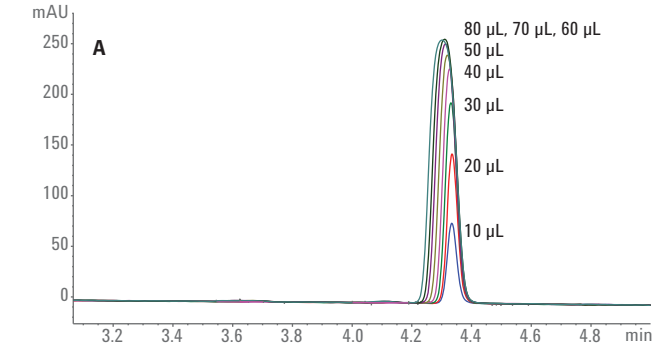


Figure 3. Area RSDs for injection volumes of 0.1 to 10 µL for peak 1 and 2 under A) isocratic and B) gradient separation conditions.

Beyond the range of up to 10 µL injections, the SFC multisampler is capable of injecting even larger volumes. For that purpose, it has a 100 µL sample loop installed. The injectable sample volume can be calculated by subtraction of the used over-feed volume from 100 µL loop volume. To demonstrate this capability, increasing volumes from 10 to 80 µL of a solution of theobromine were injected (250 mg/L in methanol). The large sample volume of methanol solution, which is a strong eluting solvent, was slowly fed into the CO_2 stream (100 µL/min). After the feeding process, the modifier concentration was increased from 1 % to 5 % in a fast step, and the eluting gradient was started. The theobromine peaks increased with the injection volume in peak height and peak width (Figure 4). For the higher injection volumes, the peak height does not increase much, but with the increase of the peak width, the peak area increases linearly (see the table in Figure 4). The correlation of the injection volume versus the peak area shows excellent linearity, with a correlation of 0.9999 (Figure 4B). The peak width at half height increased from 0.04 minutes at 10 µL injection volume to very acceptable 0.1 minutes for 80 µL injection volume, showing symmetrical peaks for all injection volumes (see table in Figure 4). From the peak areas of all injection volumes, the relative standard deviations were calculated, and showed excellent values typically at or below 0.3 % area RSD (Figure 4C).



Inj. volume (µL)	RT (min)	Area	Height	Width (min)	Symm.
10	4.337	187.27	79.89	0.036	0.875
20	4.336	375.48	148.32	0.039	0.876
30	4.331	566.40	197.70	0.045	0.872
40	4.326	760.59	231.65	0.053	0.881
50	4.320	938.66	246.14	0.062	0.891
60	4.314	1,127.29	255.96	0.073	0.905
70	4.309	1,315.80	261.58	0.084	0.933
80	4.302	1,494.58	261.62	0.096	0.947

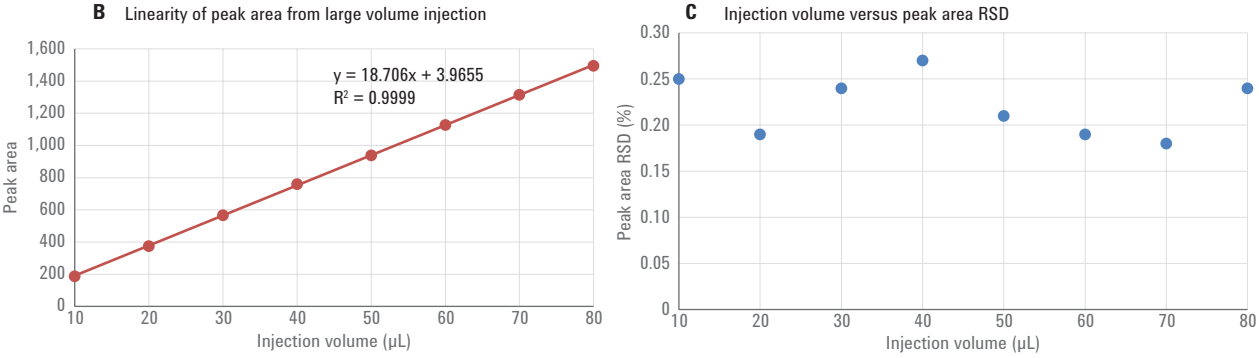


Figure 4. Results of large volume injections.
A) Overlay of the chromatograms obtained for injection volumes from 10 to 80 µL sample.
B) Area linearity obtained from large volume injection with R^2 0.9999.
C) Peak Area RSDs (%) calculated from 10 replicate injections of each large volume injection.
Table) Summary of all parameters measured for large volume injections.

Finally, the carryover behavior of the 1260 Infinity II SFC Multisampler caused by an injection of a highly concentrated caffeine sample (5 g/L in MeOH, injection volume 5 µL) was examined. The carryover was determined from the first blank injection after the injection of the high caffeine concentration. The carryover was calculated as area percentage in comparison to the peak of the high caffeine concentration to be 0.0014 % (14 ppm). No carryover was detected in the second blank injection after the injection of the high caffeine concentration (Figure 5).

Conclusion

This Technical Overview discusses the performance results obtained for the Agilent 1260 Infinity II SFC Multisampler, which enables variable injection volumes at highest precision. The area RSDs for injection volumes between 0.5 and 10 µL are typically below 0.3 %. Even lower injection volumes, down to 0.1 µL, showed area RSDs typically below 2.5 %. The demonstrated injection linearity is typically better than 0.9995. Even for injection volumes of up to 80 µL, excellent area RSD values below 0.3 % could be achieved. The linearity of the peak area for large injection values is also extremely good. The flexible sample introduction showed a negligible carryover of only 14 ppm. This offers performance that is comparable to the typically used fixed-loop autosamplers in full-loop mode, but with the high flexibility of a variable-loop autosampler.

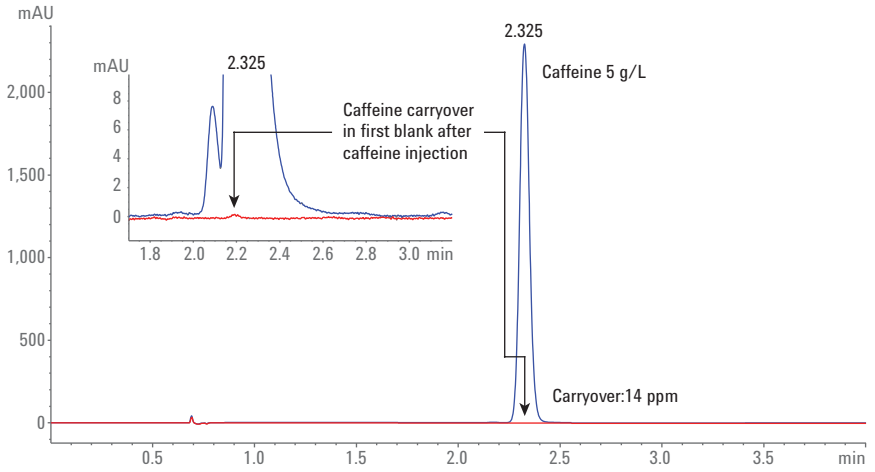


Figure 5. Determination of carryover of the Agilent 1260 Infinity II SFC Multisampler by an injection of a high concentrated caffeine sample (5 g/L in MeOH, injection volume 5 µL). Carryover was determined from the first blank injection after the injection of the high caffeine concentration. No carryover was detected in the second blank injection after the injection of the high caffeine concentration. Due to the fact that the injected amount of caffeine reaches the nonlinear range of the detector, the correct area for the 5 µL injection was calculated by extrapolation from lower volume injections.

Reference

1. Naegele, E. Feed Speed and Over-Feed Volume – New Parameters for SFC Injection, *Agilent Technologies Technical Overview*, publication number 5991-7626EN, **2017**.

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Published in the USA, October 15, 2017
5991-7623EN

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