Technical Overview

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Performance Characteristics of the Agilent 1260 Infinity II Online Sample Manager

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Introduction

In the pharmaceutical and biopharmaceutical industry, it is of crucial importance to monitor chemical or biological reaction processes to control the educts, products, and possible raising impurities in a reaction vessel. For that purpose, different monitoring techniques have been developed (Figure 1).

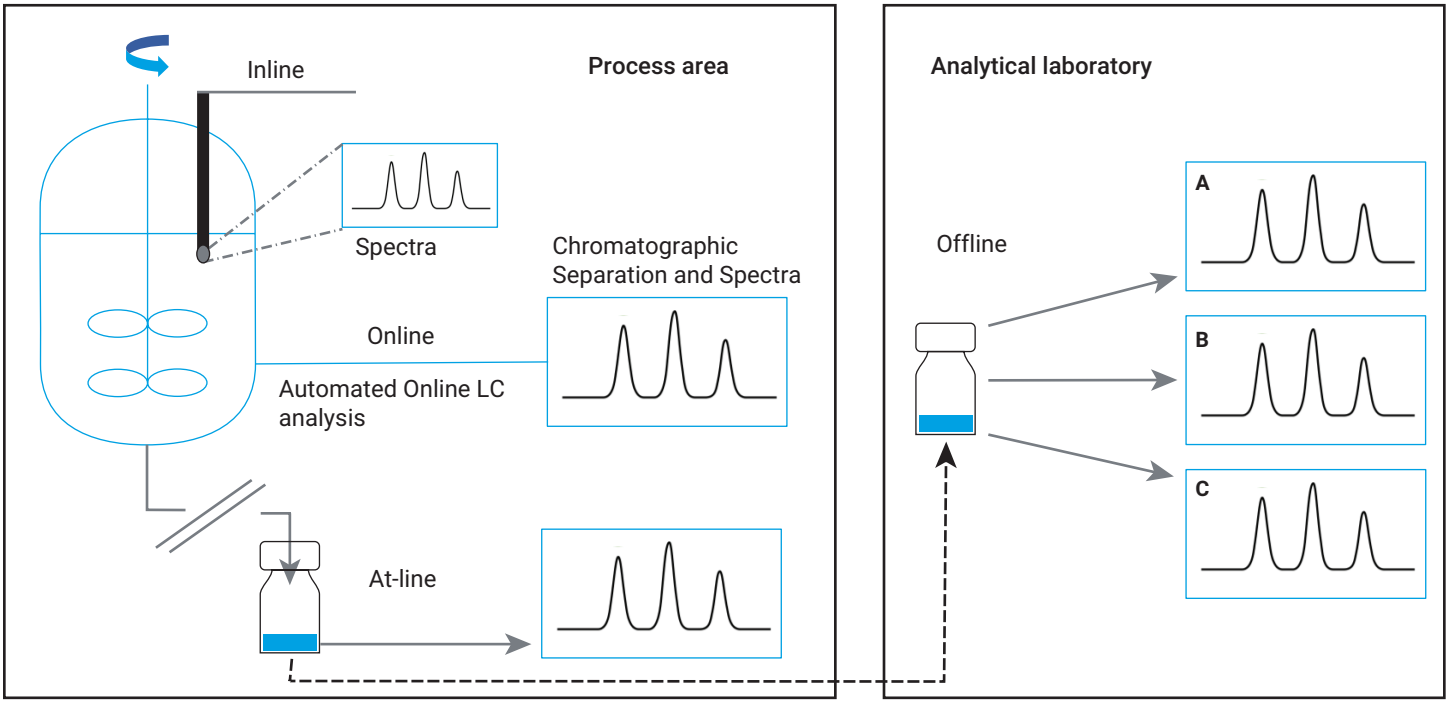

Figure 1. Established technologies for reaction monitoring.

The fastest response is given by inline monitoring, where a probe is directly located in the reaction medium (e.g., providing real-time infrared spectroscopy (IR) signals). However, such approaches are typically characterized by the inability to provide separation of inherent compounds. On the other side of the scale of response speed is the offline approach, where the sample is drawn, often manually, from the batch reactor and brought to an analytical laboratory, where it can be analyzed with more advanced equipment. The at-line approach is very similar, with an analytical instrument in the production area. The online approach offers the excellent opportunity to draw samples directly from the reaction vessel and transfer them automatically to an advanced analytical instrument located in the process development or production area, giving information about the status of the content inside the vessel within a short response time.

This technical overview demonstrates the performance of the Agilent 1260 Infinity II Online Sample Manager by the most important parameters like area precision, injection linearity, retention time precision, and carryover. The 1260 Infinity II Online Sample Manager can draw samples directly from the external reactor sampling interface with immediate injection or followed by dilution/quenching with subsequent injection. The capability to store diluted/quenched or even neat samples is a unique feature of the Online Sample Manager. The performance of the dilution and mixing capabilities of the Online Sample Manager is shown in another technical overview.[1] This provides a reliable picture of the reaction status nearly in time or at later analysis. This gives the user optimized reaction monitoring and handling with maximization of valuable products at fastest response times.

Experimental

Instrument

- Agilent 1290 Infinity II High-Speed Pump (G7120A)
- Agilent 1260 Infinity II Online Sample Manager Set (G3167AA): Agilent 1260 Infinity II Online Sample Manager (G3167A) clustered with external valve (5067-6680) located at the Agilent 1290 Infinity Valve Drive (G1170A) and Agilent Online LC Monitoring Software
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
- Agilent 1290 Infinity II Diode Array Detector (G7117B)

The new Online Sample Manager offers the capability to inject samples in two different modes: The classic flow-through mode and the novel Agilent Feed Injection mode. In the latter, the sample is merged online with the analytical flow at a settable flow rate, while the total flow rate towards the column is regulated to remain constant. Feed Injection could be especially used to infuse higher volumes of polar solvents to focus the analytes on the column head at less polar gradient starting conditions. This avoids the breakthrough of polar compounds or other effects, compromising peak shapes. Switching between the two modes can be done by selection in the method user interface of the Online Sample Manager in OpenLab CDS 2 (Figure 2). For the Feed Injection parameters, an automatic setting can be used for Feed Speed and Flush Out. They are optimized towards peak focusing and dispersion, as well as recovery, accounting for the complex hydrodynamic behavior. The Agilent Online LC Monitoring Software and its capabilities are introduced in more detail in another application note.[2]

Methods

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

Software

- Agilent OpenLab CDS revision 2.6
- Agilent Online LC Monitoring Software, revision 1.0

Columns

- Agilent ZORBAX SB-C18, RRHD, 3.0 × 100 mm, 1.8 µm (part number 858700-302)
- Agilent Pursuit XRs C18, 2.0 × 50 mm (part number A6001050X020)
- Agilent ZORBAX SB-C18, 3.0 × 100 mm, 3.5 µm (part number 861954-302)

Chemicals

- Caffeine
- Chlorhexidine

Samples

- Agilent isocratic checkout sample (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 system equipped with LC-Pak polisher and a 0.22 µm membrane point of use cartridge (Millipak).

Additional materials

- **Vials:** 2 mL glass vials, screw (part number 5182-0714)
- **Caps:** Screw caps with PTFE-S-PTFE septa (part number 5185-5862)/PTFE-red silicone septa (part number 5190-7024)

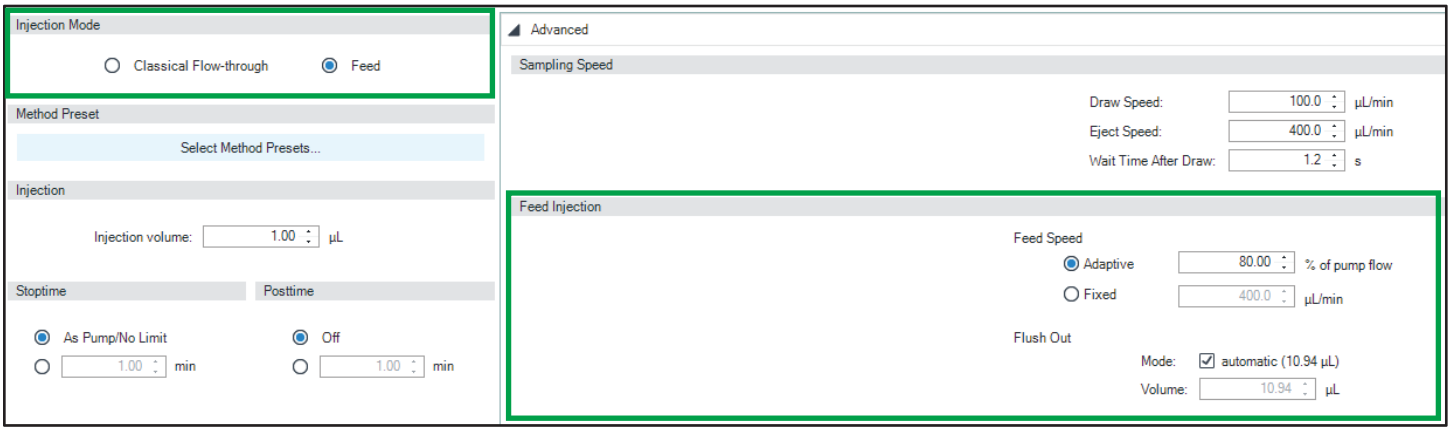

Figure 2. Agilent 1260 Infinity II Online Sample Manager user interface.

Results and discussion

Results for peak area RSDs

For the determination of peak area precision, three differently concentrated solutions of caffeine were applied to be in the linear range of the detector for all used injection volumes ranging from 0.1 to 40 µL. Each injection was repeated 10 times for the calculation of the standard deviation (SD) and relative standard deviation (RSD) of the peak area. The complete experiment was performed for both injection modes, Feed Injection and flow-through injection, from a vial and from the connection to the reactor on the external sampling interface of the Online Sample Manager.

As an example, Figure 3 displays overlaid chromatograms obtained for the injection volumes of 0.1, 1.0, 10, and 40 µL in Feed Injection mode drawn from the reactor. In the case of the Feed Injection from the external sampling interface, the peak area RSD of a 100 nL injection was only 1.40% (SD: 1.40 nL). For a 1.0 µL injection RSD 0.59% (SD: 5.86 nL), for a 10 µL injection RSD 0.07% (SD: 7.27 nL), and for a 40 µL injection RSD 0.04% (SD: 16.25 nL) were obtained. With increasing injection volume, the relative standard deviation of the peak area steadily declined and reached a level typically below 0.15% RSD for injections ≥5 µL. Figure 4 displays a comprehensive graphical overview of the RSD values obtained under Feed Injection and flow-through conditions from a vial and from the reactor interface.

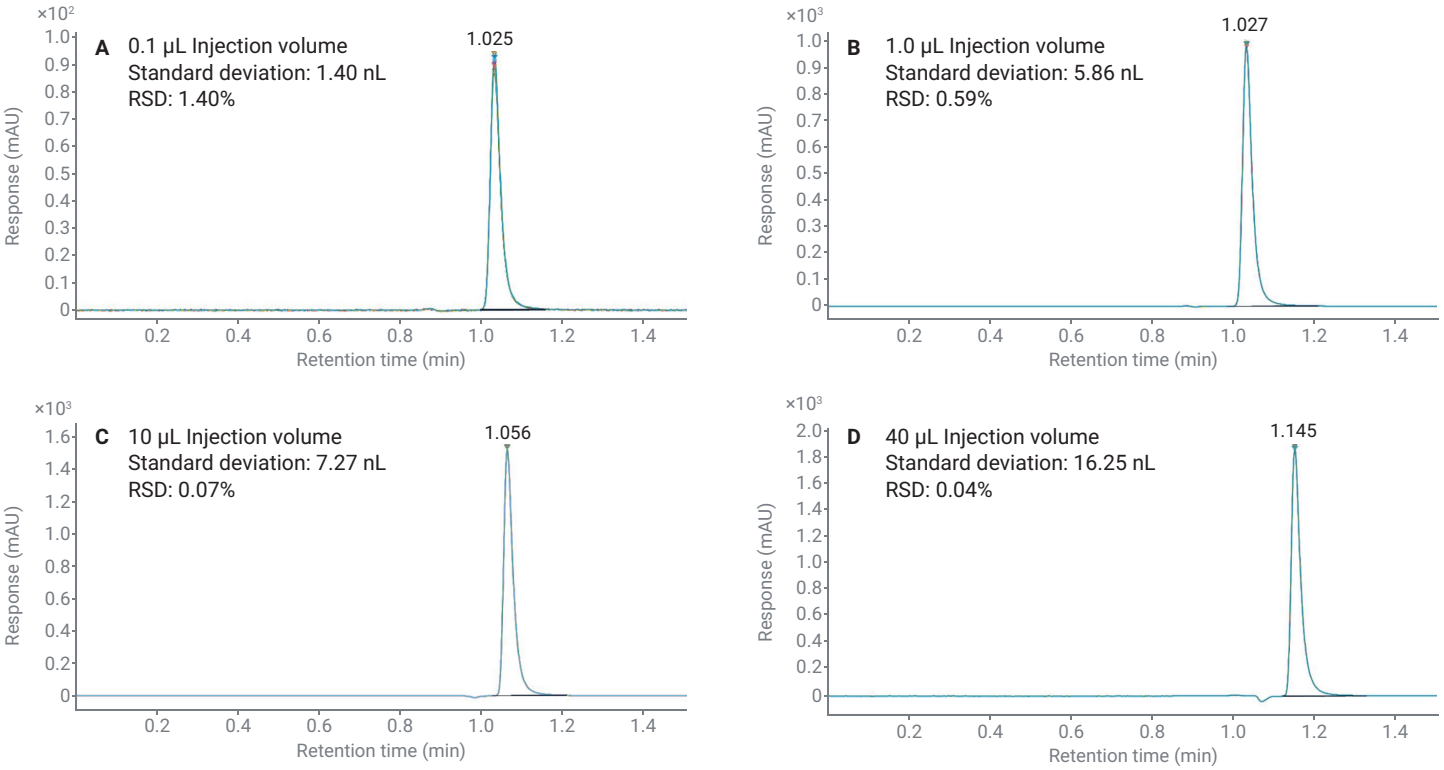

Figure 3. Overlay of chromatograms obtained for caffeine dilutions, injected under Agilent Feed Injection conditions from the reactor interface at different injection volumes (n = 10).

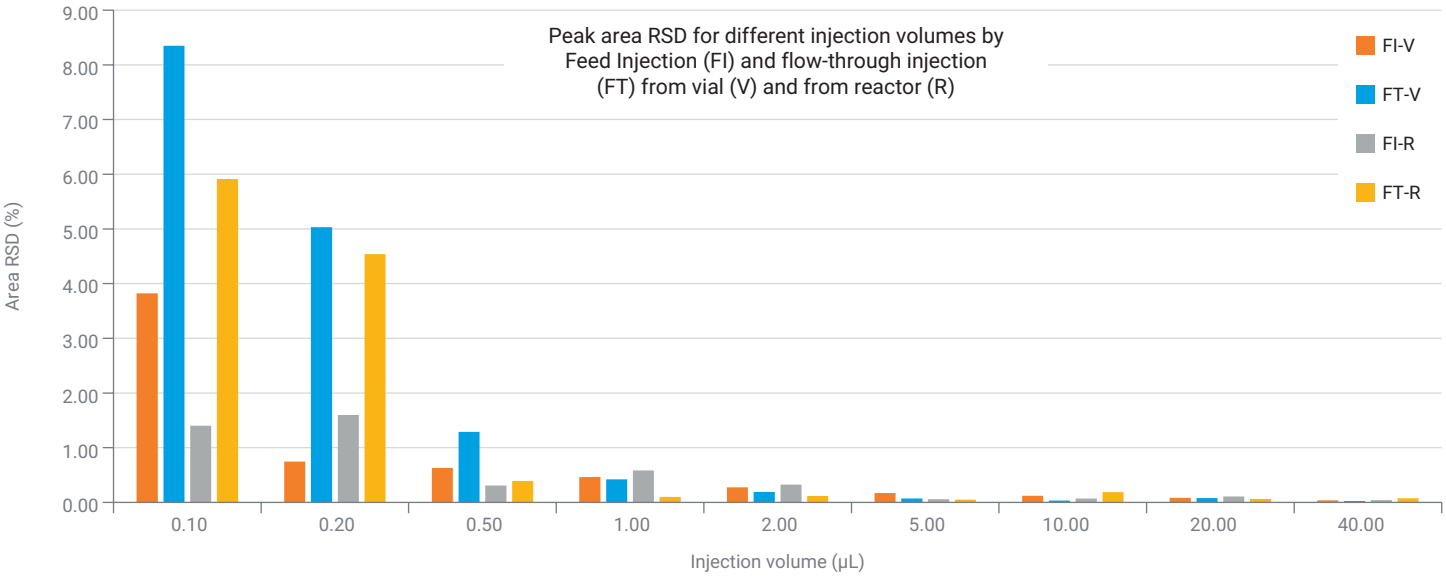

Figure 4. Peak area RSD values obtained for Agilent Feed Injection and flow-through injections from vials and from the reactor in the injection volume range of 0.1 to 40 µL.

The injection accuracy of a Feed Injection from the reactor is demonstrated by a normalization of the obtained peak areas for a given injection volume (n = 10) on its mean peak area obtained from vial injections (Figure 5). The peak area obtained by Feed Injection from vials was considered to be accurate, which was confirmed gravimetrically.

This demonstrated that the typical accuracy for Feed Injections from the reactor line was within ±1.5%.

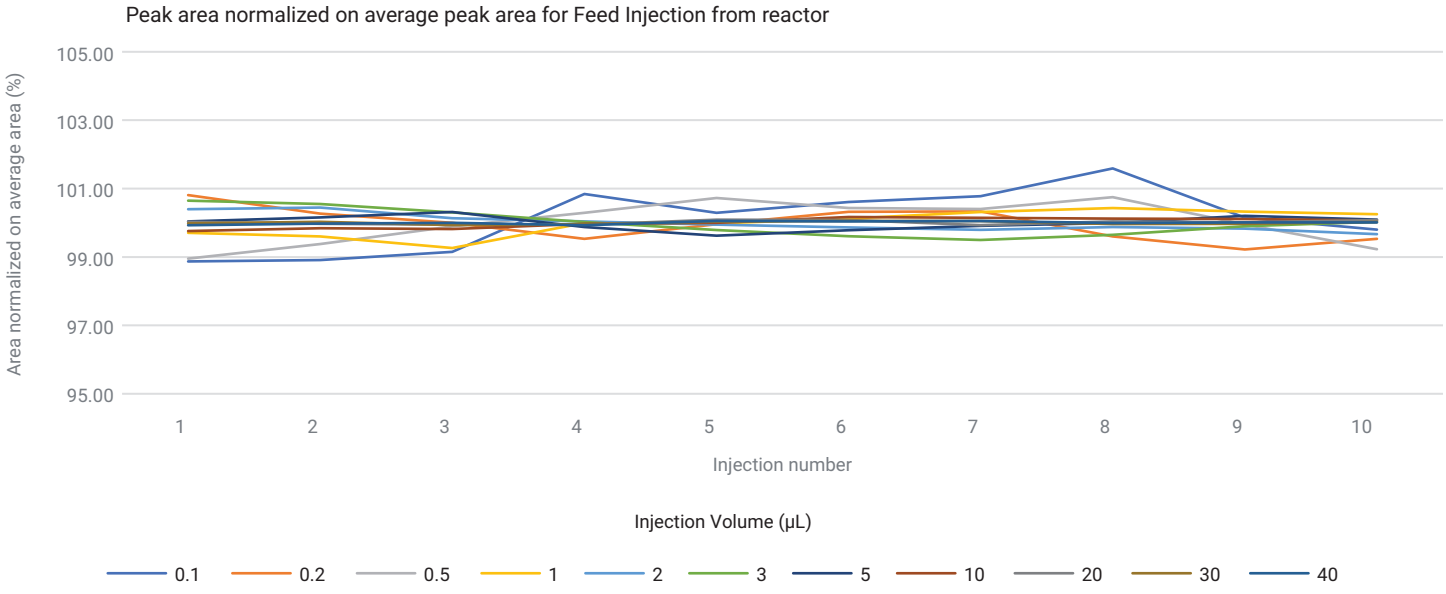

Figure 5. Peak area accuracy obtained by normalization of the peak area mean value within a series of injection volumes (n = 10).

The content of a typical reaction vessel is often more viscous than the aqueous solution used for the measurement of the area precision, as shown above. Therefore, it is of crucial interest to repeat a subset of these experiments to demonstrate the ability of the Online Sample Manager to handle samples of high viscosity. For these experiments, a solution of caffeine in glycerin (60%, w/w), which has a viscosity of 10 cP, was used to measure the area precision at different injection volumes (Figure 6). The obtained RSD values were typically below 0.15% for injection volumes ≥5 µL. This demonstrates that the area precision for injections of highly viscous solutions is in the typical range, as demonstrated above. This is true for injection from vials and from the reactor line in Feed Injection mode as well as in classic flow-through mode.

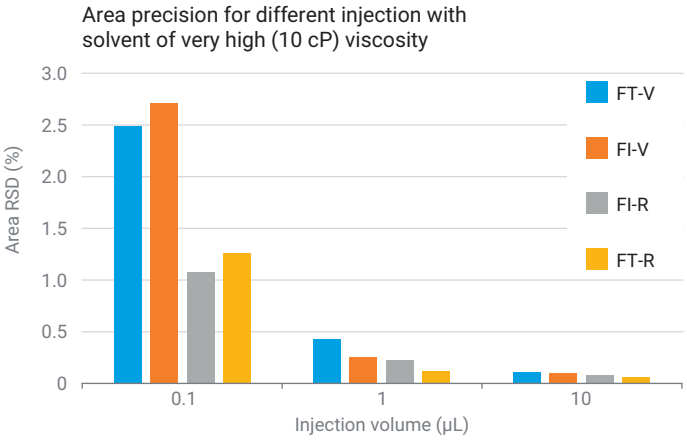

Figure 6. Area precision (RSD) for injections at different injection volumes of 0.1, 1.0, and 10 µL by means of Feed Injection mode (FI) and flow-through injection mode (FT) from vials (V) and from the reactor (R).

The results obtained for injection linearity of the Online Sample Manger are shown in Figure 7. Two levels of dilutions were used to cover the range of injection volume from 0.1 to 10 µL and from 5 to 40 µL. Both dilution levels were measured in Agilent Feed Injection and flow-through injection mode for injections from vials and from the reactor line. For all experiments, an excellent linearity at R^2 >0.9999 was obtained.

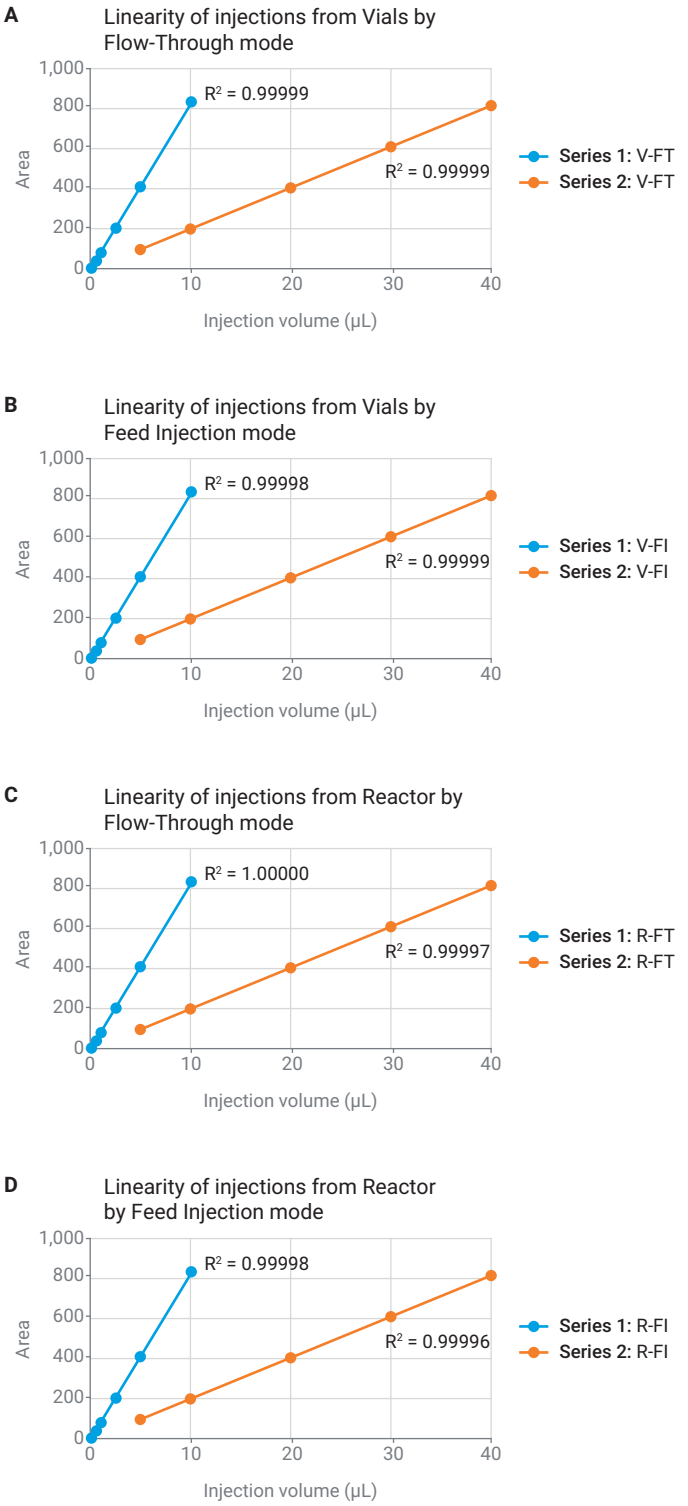

Figure 7. Determination of injection linearity achievable by the Agilent 1260 Infinity II Online Sample Manager for the injection range from 0.1 to 40 µL. From vials in flow-through mode (A), from vials in Agilent Feed Injection mode (B), from the reactor line in flow-through mode (C) and from the reactor line in Feed Injection mode (D).

Method for determination of peak area RSDs and linearity

Sample Preparation For Area Precision Measurement	
Diluent	2% acetonitrile in water
Stock Solution	1,520 mg/L caffeine
Dilutions	**Level 1:** 20 mL stock/100 mL **Level 2:** 3 mL stock/100 mL **Level 3:** 2 mL/250 mL
Sample Preparation For Linearity Measurement	
Diluent	2% acetonitrile in water
Stock Solution	3,000 mg/L caffeine/100 mL
Dilutions	**Level 1:** 0.5 mL stock/100 mL **Level 2:** 0.125 mL stock/100 mL

Instrument Settings	
Agilent 1290 Infinity II High-Speed Pump	
Mobile Phase	30% acetonitrile in water, premixed
Flow Rate	0.5 mL/min
Flow-Ramp Up/Down	1,000 mL/min^2
Stop Time	1.5 min
Agilent 1290 Infinity II Multicolumn Thermostat	
Temperature	30 °C
Column	**For RSD Measurement:** Agilent ZORBAX SB-C18, RRHD, 3.0 × 100 mm, 1.8 µm **For Linearity Measurement:** Agilent ZORBAX SB-C18, 3.0 × 100 mm, 3.5 µm
Agilent 1290 Infinity II Diode Array Detector	
Wavelength	273 nm, bandwidth 4 nm
Reference	360 nm, bandwidth 100 nm
Frequency	40 Hz
Slit	4 nm

Instrument Settings	
Agilent 1260 Infinity II Online Sample Manager	
Operation Mode	Feed Injection/flow through according to experiment
Injection Volumes	**For Area Precision Measurement:** **Level 1:** 0.1, 0.2, 0.5, 1.0 µL **Level 2:** 2.0, 5.0, 10 µL **Level 3:** 20, 40 µL **For Linearity Measurement:** **Level 1:** 0.1, 0.5, 1.0, 2.5, 5.0, 10.0 µL **Level 2:** 5, 10, 20, 30, 40 µL
Needle Wash Options	**Inner wash:** off **Outer wash:** standard (flush port, 3 sec, 30% ACN)
Draw Speed	50 µL/min
Eject Speed	100 µL/min
Sampling Speed	130 µL/min (reactor only)
Wait Time After Draw	1.2 sec
Feed Speed	400 µL/min (N/A for flow-through injections)
Flush Out Solvent	30 % acetonitrile in water
Flush Out Volume	Automatic (N/A for flow-through injections)

Results for retention time precision

Although this is typically a test for analytical pumps, it was also carried out for the Online Sample Manager due to the fact that it can operate in Feed Injection mode. When the sample is fed into the analytical flow, the pump flow is reduced, and the metering device of the Online Sample Manger delivers part of the analytical flow. Any potential issues arising during this process will have an impact on the analytical flow rate, and hence on retention time.

The isocratic checkout sample containing four compounds was injected multiple times into the analytical system (Figure 8), and the RSD of the peak retention times were calculated. The experiment was run with the sample drawn from the reactor and from a vial.

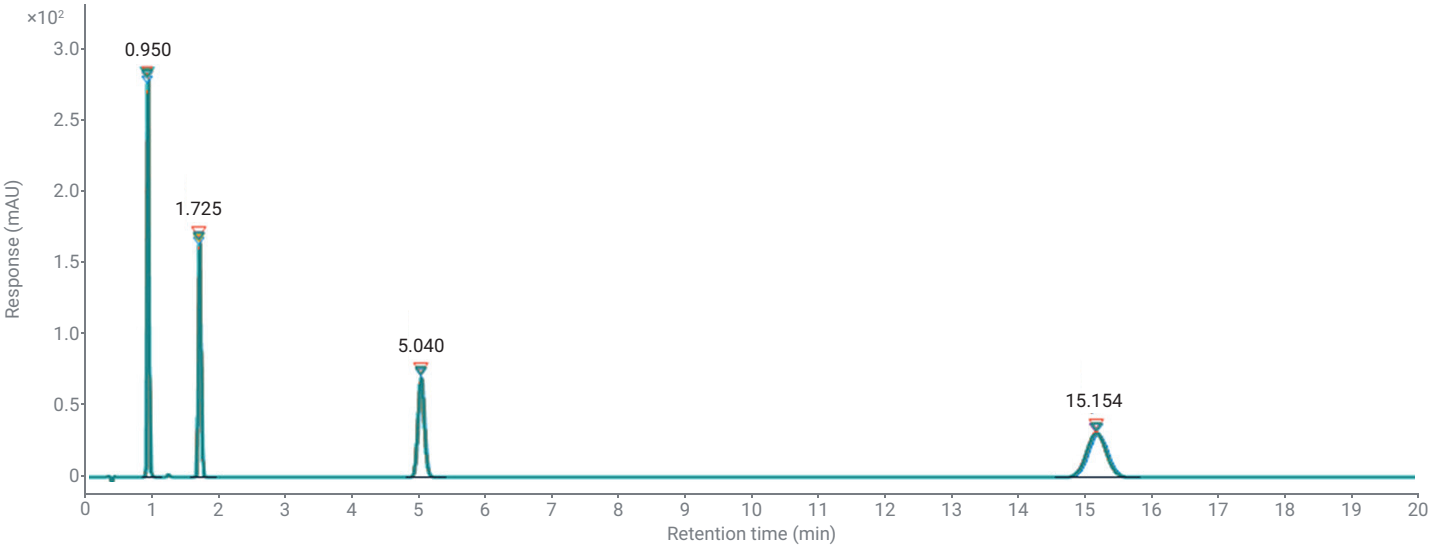

Figure 8. Separation of the isocratic checkout sample to determine retention time precision for Agilent Feed Injection from the reactor via the external sampling interface (overlay, n = 10).

The test sample was injected 10 times either from a vial or from the reactor interface as described. To see potential impacts of the change in pump flow rate on retention time, the Feed Injection mode was used at two different feed speed settings, namely 20% and 80% of the current flow rate. The calculated retention time RSDs were typically below 0.03% (Table 1).

Table 1. Determination of retention time precision (%RSD) for Agilent Feed Injections from a vial and from the reactor interface at different feed speed.

	From Reactor		From Vial	
	Feed Speed, % of Flow Rate		Feed Speed, % of Flow Rate	
Peak No.	20	80	20	80
1	0.03	0.02	0.04	0.01
2	0.02	0.02	0.02	0.01
3	0.01	0.03	0.01	0.02
4	0.01	0.04	0.01	0.02

Method for the determination of retention time precision

Sample preparation:

The isocratic checkout sample (p/n 01080-68704, comprising dimethyl phthalate: 0.15% (w/w), diethyl phthalate: 0.15% (w/w), o-terphenyl: 0.03% (w/w), and biphenyl: 0.01% (w/w)) was filled into a vial and into the reactor groove without any further sample preparation.

Instrument Settings	
Agilent 1290 Infinity II High-Speed Pump	
Mobile Phase	50% acetonitrile in water + 0.1% TFA, premixed
Flow Rate	1 mL/min
Flow-Ramp Up/Down	1,000 mL/min^2
Stop Time	20 min
Agilent 1290 Infinity II Multicolumn Thermostat	
Temperature	30 °C
Column	Agilent ZORBAX SB-C18, RRHD, 3.0 × 100 mm, 1.8 µm
Agilent 1290 Infinity II Diode Array Detector	
Wavelength	254 nm, bandwidth 4 nm
Reference	360 nm, bandwidth 100 nm
Frequency	40 Hz
Slit	4 nm
Agilent 1260 Infinity II Online Sample Manager	
Operation Mode	Feed Injection mode
Injection Volume	1 µL
Needle Wash Options	**Inner wash:** off **Outer wash:** standard (flush port, 3 sec, 30% ACN)
Draw Speed	100 µL/min
Eject Speed	100 µL/min
Wait Time After Draw	1.2 sec
Feed Speed	20%/80% of analytical pump flow rate
Overfeed Solvent	Automatic
Sample Settings in Online LC Monitoring Software	
Sampling Speed	40 µL/min (draw from reactor)

Results for carryover

The determination of carryover from one injection to the following one was examined for both injection modes, Feed Injection and flow-through injection, under three different scenarios:

- In the vial-to-vial scenario, a concentrated solution of chlorhexidine was injected from a vial followed by an injection of a blank solution from another vial.
- In the vial-to-reactor scenario, the concentrated chlorhexidine solution was injected from a vial followed by a blank solvent injection drawn from the reactor line.
- In the reactor-to-vial scenario, the concentrated chlorhexidine solution was drawn from the reactor line followed by a blank solvent injection from a vial.

These scenarios should reflect typical workflows of analysis and sampling sequences.

Figure 9 displays the result of carryover obtained in a vial-to-vial scenario for the flow-through mode. After injecting the extremely concentrated chlorhexidine sample (a compound that is considered very sticky and difficult to wash off), there was only a very small residual amount of carryover from chlorhexidine of less than 30 ppm detectable in the flowing blank injection. Table 2 lists the measured carryover values for the three different scenarios in Feed Injection and flow-through mode.

Table 2. Carryover determined in flow-through and Feed Injection mode for the vial-to-vial, vial-to-reactor, and reactor-to-vial scenarios.

	Flow-Through	Feed Injection
Vial-to-Vial	<30 ppm	<30 ppm
Vial-to-Reactor	<30 ppm	<30 ppm
Reactor-to-Vial	<30 ppm	<30 ppm

Method for the determination of carryover

References/Blanks	
Reference 1	1.2 mg/mL chlorhexidine in water/0.1% TFA
Blank Solution	Water/0.1% TFA
Instrument Settings	
Agilent 1290 Infinity II High-Speed Pump	
Mobile Phase	31% acetonitrile in water/0.1% TFA, premixed
Flow Rate	0.5 mL/min
Run Time	2.5 min for blanks, 12 min for references
Flow-Ramp Up/Down	1,000 mL/min
Agilent 1290 Infinity II Multicolumn Thermostat	
Temperature	30 °C
Column	Agilent Pursuit XRs C18, 2.0 × 50 mm
Agilent 1290 Infinity II Diode Array Detector	
Wavelength	257 nm, bandwidth 4 nm
Reference	360 nm, bandwidth 16 nm
Frequency	10 Hz
Slit	4 nm
Agilent 1260 Infinity II Online Sample Manager	
Operation Mode	Feed Injection/flow through according to experiment
Injection Volume	1 µL
Needle Wash	Flush port, 3 sec, 50% acetonitrile in water + 0.1% TFA
Inner Wash	**FT mode:** OFF **Feed mode:** 250 µL water/0.1% TFA, 250 µL mobile phase
Seat Wash	**FT mode:** OFF **Feed mode:** 250 µL water/0.1% TFA, 250 µL mobile phase
Draw Speed	100 µL/min
Eject Speed	100 µL/min
Wait Time After Draw	1.2 sec
Flush Out Solvent	Mobile phase
Flush Out Mode	Automatic (N/A for flow-through injections)
Feed Speed	400 µL/min (N/A for flow-through injections)
Sampling Speed	Set 1

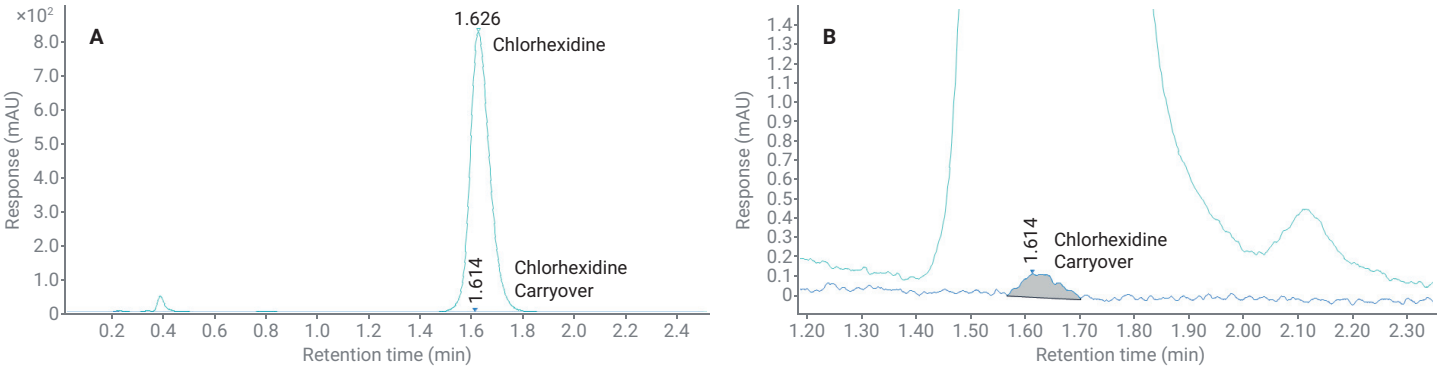

Figure 9. (A) Overlay of injection of highly concentrated chlorhexidine in flow-through mode followed by a solvent injection. (B) Zoom of A to visualize the carryover peak, which was 14 ppm in this example.

Conclusion

This technical overview provides an introduction to the new Agilent 1260 Infinity II Online Sample Manager for the control of chemical and biological reaction processes. The Online Sample Manager can be easily interfaced with the process via the inherent interface, and it supports both Feed Injections and classic flow-through injections for sample introduction. The sample can be injected directly from the reactor stream or subsequent to dilution/quenching. The performance of the module was demonstrated by the measurement of critical performance criteria like peak area precision and injection linearity, retention time precision, and carryover for different injection scenarios, all with excellent results. This allows optimized reaction monitoring and handling of samples to maximize valuable chemical and biological products.

References

1. Reactor Sample Dilution and Mixing Performance of the Agilent 1260 Infinity II Online Sample Manager. *Agilent Technologies technical overview*, publication number 5994-3679EN, **2021**.
2. Online Reaction monitoring by the Agilent InfinityLab Online LC Solutions. *Agilent Technologies application note*, publication number 5994-3528EN, **2021**.

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