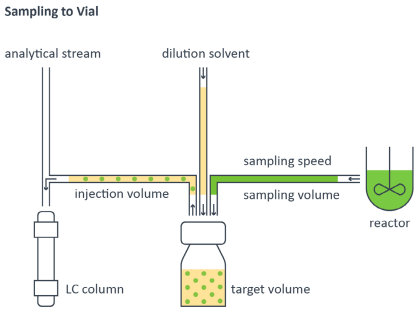
Technical Overview

Reactor Sample Dilution and Mixing Performance of the Agilent 1260 Infinity II Online Sample Manager



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Introduction

In modern pharmaceutical production, process analytical technology (PAT) plays an important role in controlling and optimizing chemical and biological reactions to generate valuable products. For online analysis, typically, the samples of a chemical or biological reaction are drawn from a reactor containing a highly concentrated solution. This solution has to be diluted to come within a concentration range that is acceptable for analysis by, for example, HPLC. The final concentration must be in a range where column and detector overloading are avoided. Therefore, the drawn reaction sample has to be placed in a vial or well plate with dilution and mixing prior to injection. To achieve reliable analytical results and even proper quantification of the content of the reaction vessel, it is of crucial importance to dilute and mix the sample with high precision and repeatability.

The Agilent InfinityLab Online LC Solutions comprising the Agilent 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 1260 Online Sample Manager. This provides a reliable picture of the reaction status nearly in real time. This offers the user optimized reaction monitoring and handling with maximization of valuable products at fastest response times.

This technical overview will demonstrate the dilution and mixing performance of the 1260 Infinity II Online Sample Manager for samples drawn from a reactor via the reactor interface of the Online Sample Manager. The results will highlight the RSDs of dilutions with and without the influence of internal mixing and a comparison of linearity of samples diluted and mixed by the Online Sample Manager to additional externally mixed samples. Other important performance parameters are shown in another application note.[1]

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)

Instrument configuration

The Online LC system is designed as a modular LC instrument equipped with a newly developed module, the Online Sample Manager. The Online Sample Manager contains two unique valves, one inside and an additional one outside (Figure 1). The developed valve concept, with its specialized switching modes, enables Agilent Feed Injection and classic flow-through mode. The design of ports and grooves and the specific plumbing allows direct drawing and injection from a reaction vessel (Figure 1A) as well as sample handling like dilution/quenching prior to injection (Figure 1B). The Online Sample Manager is typically connected to the reaction vessel by a sampling unit providing a stream of solvent from the reactor, either neat or prepared (e.g., filtrated). The complete sampling and injection process is controlled by the specialized Agilent Online LC Monitoring Software, working seamlessly with the Agilent OpenLab CDS 2 software. The Online LC Monitoring Software and its capabilities are introduced in more detail in another application note.[2]

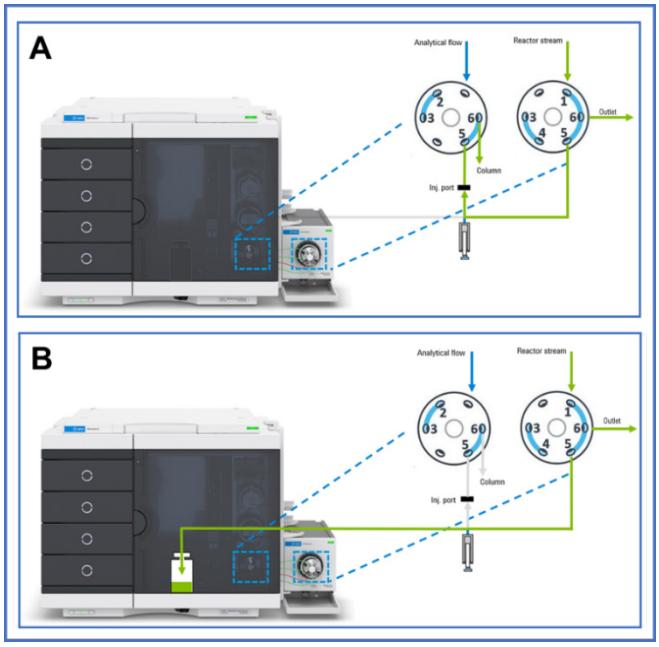

Figure 1. Schematic of the Agilent 1260 Infinity II Online Sample Manager and respective valve configuration enabling: (A) Direct injections from the external reactor sampling interface. (B) Sample handling for dilution/quenching prior to injection.

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)

Chemicals

Caffeine

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).

Method for the determination of dilution and mixing precision

Sample Preparation	
Diluent	2 % acetonitrile in water
Stock Solution	152 mg caffeine/100 mL
Dilution Factor 10	5 mL stock solution/50 mL
Dilution Factor 50	1 mL stock solution/50 mL
Dilution Factor 500	1 mL stock solution/500 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	Agilent ZORBAX SB-C18, RRHD, 3.0 × 100 mm, 1.8 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
Agilent 1260 Infinity II Online Sample Manager	
Operation Mode	Classic flow-through
Injection Volume for Dilution Experiments	1 µL at dilution factor 10, 5 µL at dilution factor 50, 40 µL at dilution factor 500
Injection Volume for Mixing Experiments	1 µL at dilution factor 10, 2 µL at dilution factor 50, 3 µL at dilution factor 500
Needle Wash Options	Inner wash: Off Outer wash: Standard (flush port, 3 s, 30% ACN)
Draw Speed	50 µL/min
Eject Speed	100 µL/min
Wait Time After Draw	1.2 s
Sample settings in Online LC Monitoring Software	
Dilution Speed	10,000 µL/min
Sampling Speed	Set 2 (draw speed: 100 µL/min, wait time: 3.6 s, dispense speed: 130 µL/min)
Dilution Solvent	2% acetonitrile in water
Dilution Factor	10, 50, 500
Target Volume	600 µL

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)

Results and discussion

Explanation of sampling possibilities

The sampling from the reaction vessel and subsequent sample handling is set up in the Online LC Monitoring Software. In its simplest form, it is a direct injection from the reaction vessel. Injections from a vial are also supported. This is schematically shown in Figure 2. The use of the Feed Injection mode offers a direct injection of the drawn sample in the flow to the column with minimized delay volume, which is especially useful in fast gradient applications. For the injection of the sample from the reactor, it is not even necessary to lift and move the needle: the sample will be handled within the valves.

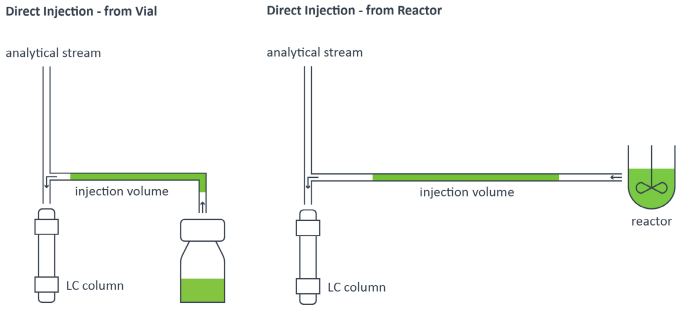

Figure 2. Schematic of the possible direct injections controlled from the Agilent Online LC Monitoring Software.

A more sophisticated sample handling method includes transfer of the drawn reactor sample to a vial with dilution/quenching. This is done by dispensing the sample into the vial followed by infusing the dilution/quenching solvent with very high flow rate into the vial (Figure 3). The diluted sample can either be analyzed immediately or kept for later analysis.

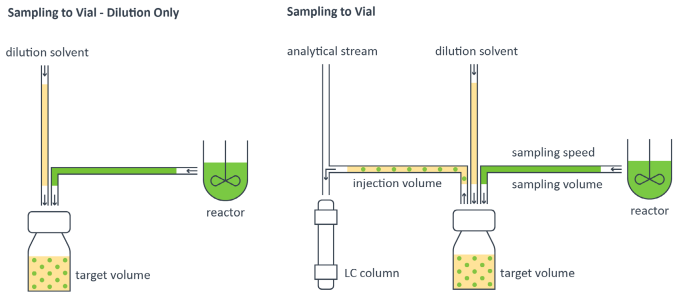

Figure 3. Schematic of the possible dilution/quenching of the reactor sample into a vial with subsequent analysis controlled by the Agilent Online LC Monitoring Software.

Where the sample needs to be undiluted, the Online LC Monitoring Software offers the possibility to create an undiluted retained sample. If necessary, it is also possible to perform a subsequent injection of the undiluted retained sample (Figure 4).

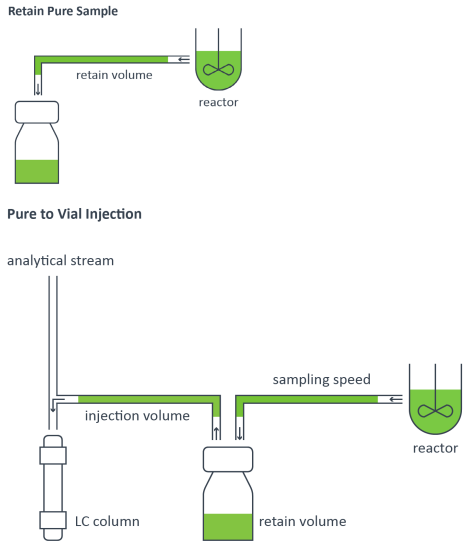

Figure 4. Schematic of the possibility to create an undiluted retain sample with or without subsequent injection of the undiluted sample.

Results for dilution and mixing accuracy and precision

For reliable determination of the educt and product concentration in the reactor, it is of crucial importance that the dilution/quenching and mixing of the drawn sample with the chosen solvents takes place with highest possible accuracy and precision. To determine the range of highest accuracy and precision for dilution/quenching and mixing, three different levels of dilution were chosen (Figures 5 and 6). For the determination of dilution accuracy, the dilutions at 1:10, 1:50, and 1:500 were prepared automatically by the sampler and by manual dilution. Both series of dilution were mixed by a thorough vortexing step. To get an impression of the quality of dilution accuracy, the peak area obtained for the dilutions prepared with the Online Sample Manager were normalized on the handmade dilution, which shows that they are all in a range of ±3% (Figure 5A). In general, there is a trend to lower accuracy for lower dilution ratios. The calculated area RSDs of the dilutions on the Online Sample Manager were typically below 1% in the chosen dilution range (Figure 5B). In general, there is a trend to lower precision (higher RSD) values for higher dilution ratios.

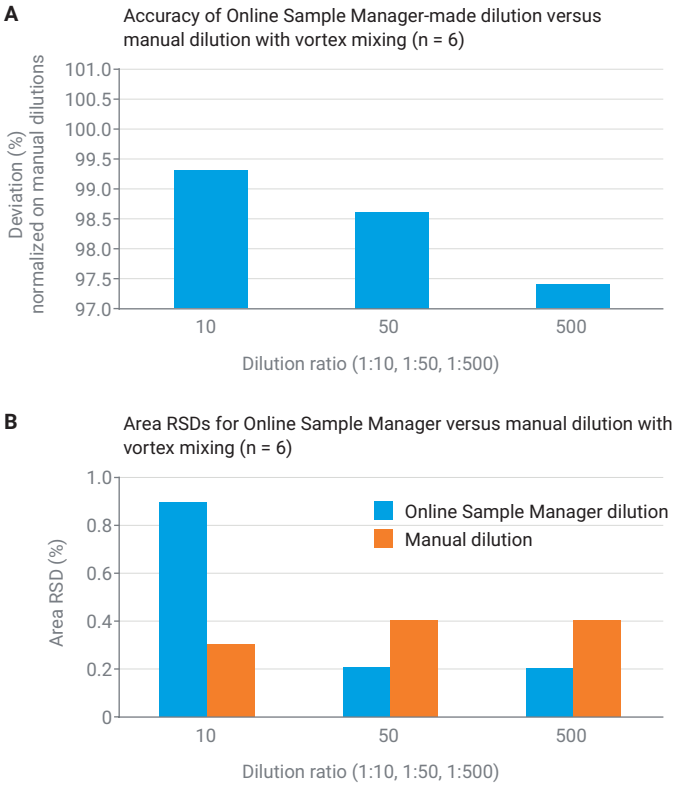

Figure 5. A comparison of areas from three dilution series prepared automatically by the Agilent 1260 Online Sample Manager and manually. (A) Accuracy of the automated dilutions with the Online Sample Manager compared with handmade dilution. (B) Area RSD values of the Online Sample Manager and manually prepared dilutions.

In addition to the pure ratios of sample to solvents used for the dilution, the mixing step also has a critical impact on the achievable accuracy and precision of the combined dilution-mixing process. The mixing step is responsible for the homogeneous distribution of the sample in the dilution solvent. The mixing is performed by the Online Sample Manager by means of flushing the diluent solvent after the sample at very high speed into the vial, typically at 10,000 µL/min. To determine the influence of the mixing itself, the three dilution ratios were automatically prepared by the Online Sample Manager and injected. This set of samples was then thoroughly vortexed and injected again. After normalization on the peak area of the vortexed sample, it turns out that the area's accuracies were in a window of ±5% (Figure 6A). The area precision (RSD) of the sampler mixed dilutions were below 2.5% (Figure 6B).

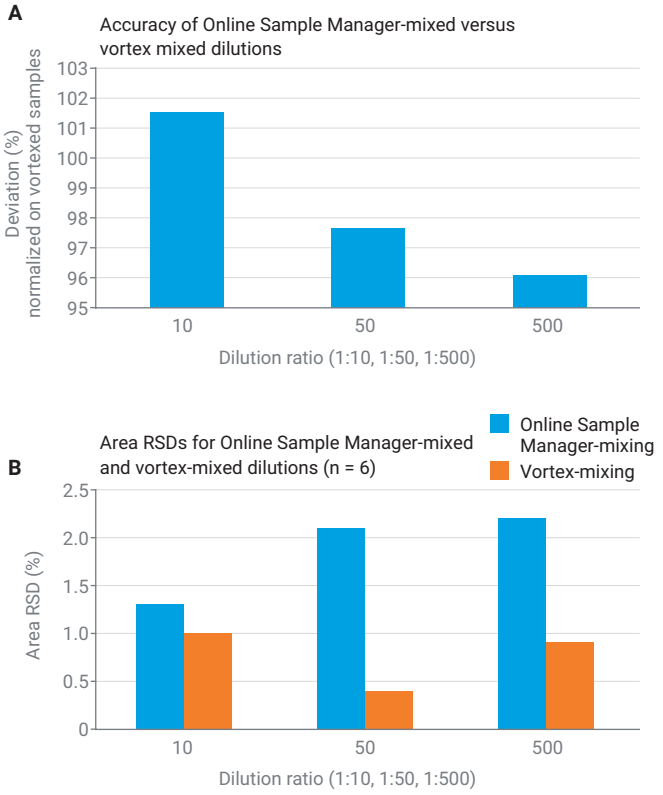

Figure 6. Performance of the mixing process. A) Accuracy of peak areas of sampler mixed dilutions to additionally vortexed dilutions. B) Area RSDs of the dilutions at 1:10, 1:50, and 1:500.

Dilution and mixing linearity

Besides the obtained peak area RSD values of dilution and mixing, the linearity, which can be obtained in a series of dilutions, is also very important. For that purpose, two series of dilutions (1:10 to 1:100 and 1:100 to 1:500) were prepared by the Online Sample Manager with mixing of the drawn caffeine sample with the diluent. After measurement of these dilutions, they were thoroughly vortexed and measured again. The obtained calibration curves are compared in Figure 7. Both series of dilutions showed excellent linearity coefficients and a complete overlap.

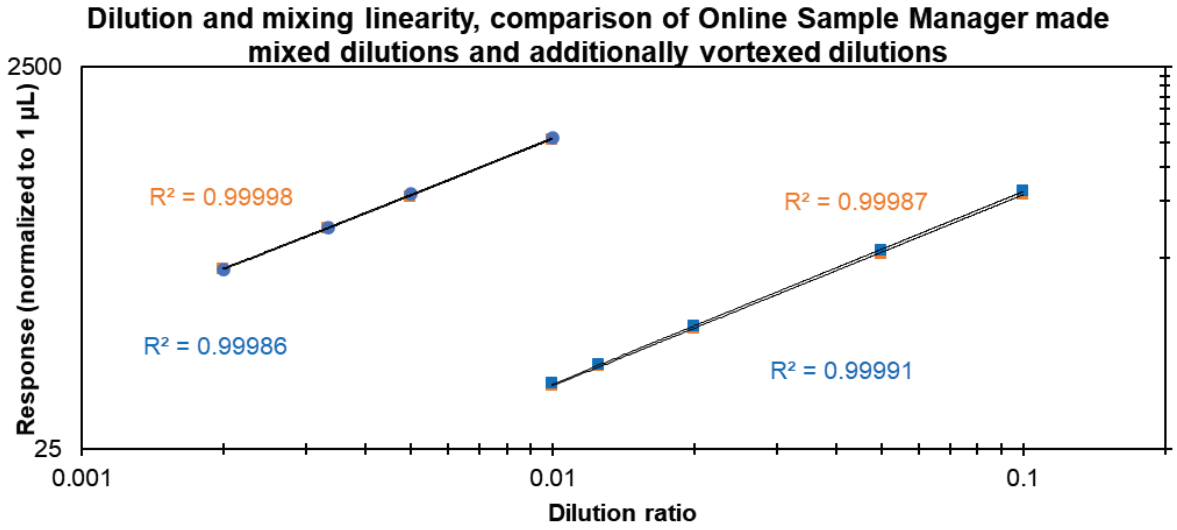

Figure 7. Determination of dilution and mixing linearity for dilutions made by the Agilent 1260 Online Sample Manager including mixing (orange series) and after an additional vortex step (blue series).

Conclusion

The Agilent 1260 Infinity II Online Sample Manager enables the drawing of a flexible amount of sample from the reactor and dilution/mixing for retain samples and injection.

The dilution and mixing process is performed with high precision over a broad range, providing a precise picture of the reaction status. This enables optimized reaction monitoring and handling to gain a maximum yield of valuable products.

References

1. Performance Characteristics of the Agilent 1260 Infinity II Online Sample Manager. *Agilent Technologies technical overview*, publication number 5994-3529, **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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