Technical Overview

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Performance Evaluation of a Reactor Sampling Device for Online LC

Agilent 1260 Infinity II Prime Online LC System in combination with the Snapdragon Chemistry SRS and Mettler Toledo EasySampler

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Introduction

Small molecule chemical reactions often take place in heterogenous liquid/liquid, liquid/solid, or liquid/gas mixtures. Additionally, if the process is run above or below ambient temperature, it is often necessary to quench the reaction mixture at the process temperature to collect representative data. These factors make it difficult to sample automatically from such a reaction mixture for an online analysis by HPLC. To analyze heterogenic mixtures, it is essential to bring them into a homogenous state by quenching and dilution, applicable for an HPLC instrument. To execute such a task, the Agilent 1260 Infinity II Prime Online LC System can be connected to a Mettler Toledo EasySampler 1210. The interface between these two instruments can be established using the Snapdragon Chemistry Sample Relay System (SRS). This enables unattended operation of a chemical reactor with continuous monitoring of heterogenous reactions.

This technical overview describes the combination of the 1260 Infinity II Prime Online LC System with the Snapdragon Chemistry SRS and the Mettler Toledo EasySampler 1210 for reactor sampling. A description of the plumbing, electronic, and software setups is given, and critical performance data, such as peak area precision, linearity, recovery, and carryover are measured and discussed. The sampling and analysis of a typical heterogenic liquid/liquid reaction by means of the described setup is shown in another application note.[1]

Experimental

The instrumentation used in this study is detailed in Table 1, and the method parameters are outlined in Tables 2 and 3.

Table 1. Instrumentation.

Product Type	Agilent Product Description
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 Agilent 1290 Infinity Valve Drive (G1170A), featuring a reactor valve pod (part number 5067-6680) and Agilent Online LC Monitoring Software – Agilent 1290 Infinity II Multicolumn Thermostat (G7116B) – Agilent 1290 Infinity II Diode Array Detector (G7117B) with Agilent InfinityLab Max-Light Cartridge Cell (10 mm, G4212-60008)
Additional Hardware	– Mettler Toledo EasySampler 1210 – Snapdragon Chemistry SRS
Column	Agilent ZORBAX RRHD Eclipse Plus C18, 3.0 × 50 mm, 1.8 µm (p/n 959757-302)
Software	– Agilent OpenLab CDS, version 2.6 or later – Agilent Online LC Monitoring Software, version 1.2, and Remote Control License (G2956AA) – Snapdragon Chemistry SRS control software

Table 2. Method parameters.

Parameter	Value
Analytical Method Conditions	
Solvents	A) Water + 0.1% formic acid (FA) B) Acetonitrile (ACN) + 0.1% FA
Analytical Flow Rate	1.0 mL/min
Solvent Composition	Isocratic 15% B
Column Temperature	45 °C
Feed Speed	80% of analytical flow rate
Flush-Out Solvent	Water: ACN 9:1 + 0.1% FA (S2)
Flush-Out Volume	Automatic
Injection Volume	5 µL
Needle Wash	3 s, Water: ACN 1:1 + 0.1% FA (S1)
Diode Array Detector	273 ± 4 nm, reference: off, 20 Hz data rate
Online LC Monitoring Software	
Sampling	Direct injection only
Draw Speed	Setting 2 – Draw speed: 100 µL/min – Wait time: 3.6 s
Schedule	Not necessary, controlled by SRS software

Table 3. Snapdragon Chemistry SRS software and hardware settings, and MettlerToledo EasySampler 1210 solvents.

Sample Relay System Software Settings	
Dilution	1:150, 1:200, 1:400, 1:600, 1:700
Mixing Time	10 s
Cleaning Volume	1.6 mL, 2 Wash repeats
Easy Sampler Single Solvent	No
Pocket Out Time	NA (only used in single solvent mode)
Sampling Interval	10 min
Sample Relay System Hardware Settings	
Pressure	– 30 psi on inlet of instrument – 15 psi on regulator on front of instrument – 0.1 liter/min on flow meter*
Mettler Toledo EasySampler	
Solvents	Reactor probe cleaning, dilution, and quenching solvents: all water

* Parameter can only be set after selecting **set_sampling_phase**. Once done, select **set_holding_phase** to come back to initial conditions (Figure 2B).

Instrumental setup

For the combination of all instruments, a LAN connection was set up, connecting the Agilent Online LC, the Snapdragon Chemistry SRS, and the Mettler Toledo EasySampler to the controlling PC by means of a PC hub (Figure 1).

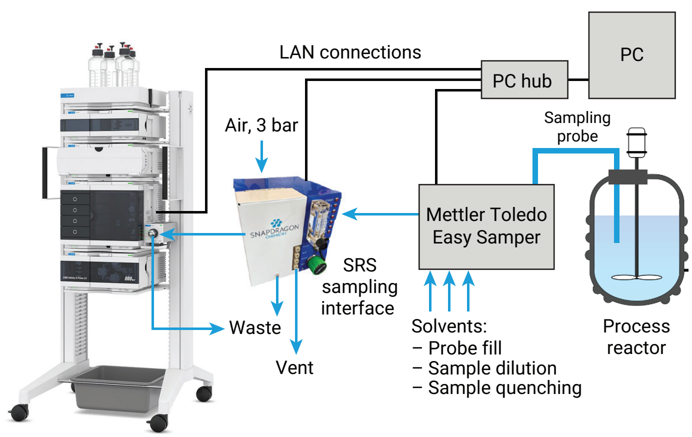

Figure 1. Flow path setup (blue arrows) and LAN connection setup (black lines) for the connection of the Agilent 1260 Infinity II Prime Online LC System with the Mettler Toledo EasySampler Probe via the Snapdragon Chemistry SRS.

For the setup of the flow path between the instruments, the tubing normally connected to the needle of the Mettler Toledo EasySampler was instead connected to the SRS. The EasySampler was connected to the required solvents for flushing the probe (identical to reactor solvent), the quenching solvent to stop the chemical reaction, and the sample dilution solvent. These solvents are connected to the EasySampler, allowing it to dispense the solvents into the probe. The tubing provided with the Snapdragon SRS is already in the appropriate lengths for the connection from the EasySampler to the SRS (0.6 mm id, 50 cm) and from the SRS to the Online LC (0.6 mm id, 50 cm). This length is critical for correct sample delivery and should not be modified. The SRS was connected to pressurized gas (air or nitrogen) for sample mixing and flushing of tubing.

Sampling process

Prior to the experiment, the EasySampler was cleaned and prepared with the internal routines accessible on the control display of the EasySampler. During this procedure, the probe can stay connected to the SRS, and solvents are guided to waste by the SRS.

The EasySampler has a cylindrical probe made of Alloy C-22 and PTFE wetted parts and an outer diameter of 9.5 mm. To draw the sample from the reactor, a small 20 µL cavity, the pocket, is moved out of the tip of the probe. This cavity fills up with the content of the reactor, and moves back into the probe. From the probe, the sample is moved to the SRS by the connected solvents and is deposited in a sample well. In this well, the sample is mixed with quenching and dilution solvent by mixing with a stream of air bubbles.

After proper mixing, the diluted reactor sample is guided directly to the Online LC interface for direct injection. The Online LC interface and SRS outlets for liquid and vented air were connected directly to waste. The sampling carousel of the EasySampler is not used in this process.

Software setup

Agilent OpenLab CDS: The acquisition and data analysis methods are run by OpenLab CDS. The CDS must be released for external control before the experiment is started.

Agilent Online LC Monitoring Software: A method for the experiment is set up by choosing the acquisition and data analysis methods previously created in OpenLab CDS. Direct injection must be chosen as the sampling setting, with the parameters listed in Table 2. The experiment setup must not contain more than one sampling setting. The schedule must not contain any time-based entries. Then, the experiment must be created in the Online LC Monitoring Software.

1. Select the **Require Manual Stop** check box during startup of the experiment.
2. Enable the communication of the Snapdragon Chemistry SRS control software with the Agilent Online LC Monitoring Software by starting the web application programming interface (API). This can be done in Microsoft Windows services by starting the appropriate service.
3. After opening the SRS control software, select and configure the AgilentSRS device class.

   After the configuration runs in the background, settable parameters will appear (Figure 2A, see also Appendix). After the initialization, a list of run commands will become available (Figure 2B, see also Appendix). For instance, the command **take_sample** will initiate one sampling event, followed by an analytical run.
4. To start a sequence of samplings and analyses, click **Set Repeated Manual Command**.
5. On the next screen (Figure 2C, see also Appendix), choose the command **take_sample** and input the time between manual commands.
6. Click **Create Routine** to start the sequence. The software will start to take samples in the defined interval until the user clicks **Stop**. After clicking stop, the current sample is still processed, but no more new samples are taken.

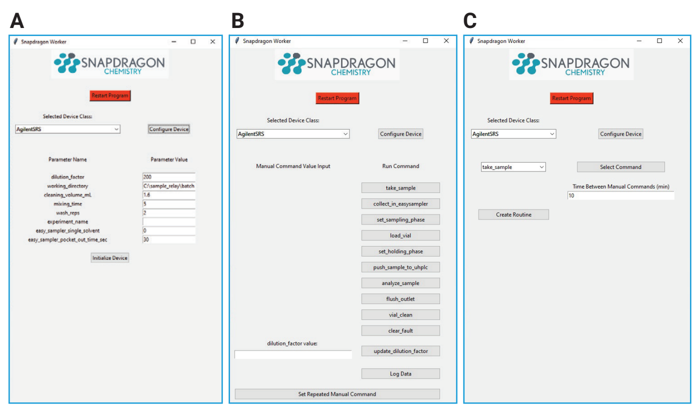

Figure 2. (A) Configuration and initialization of the AgilentSRS device class. (B) Manual commands and Set Repeated Manual Command access. (C) Select Command setting the for repetition and time between repeat for creation of the routine.

Sample

Caffeine, 15 g/L in water

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)

Results and discussion

For a multistep process which consists of drawing reactor sample by a probe, quenching, sending it to the SRS device for dilution and mixing, and sending the resulting sample solution to the online LC sampling interface valve, it is crucial to know the precision of different operating parameters, like dilution. To measure the dilution precision, the probe was placed in a highly concentrated aqueous caffeine solution (15 g/L). After drawing the sample, the SRS applied different dilution factors and the dilution was sent to the Online LC. Each experiment was repeated 10 times, and the relative standard deviation (RSD) of the peak areas was determined. The peak area RSD for dilution factors from 150 to 700 was typically below 0.3% (Figure 3).

Dilutions at 1:100 show increased peak area RSDs due to incomplete mixing and low sample volume. Dilutions higher than 1:750 suffer from loss of sample due to the volume of the mixing cup.

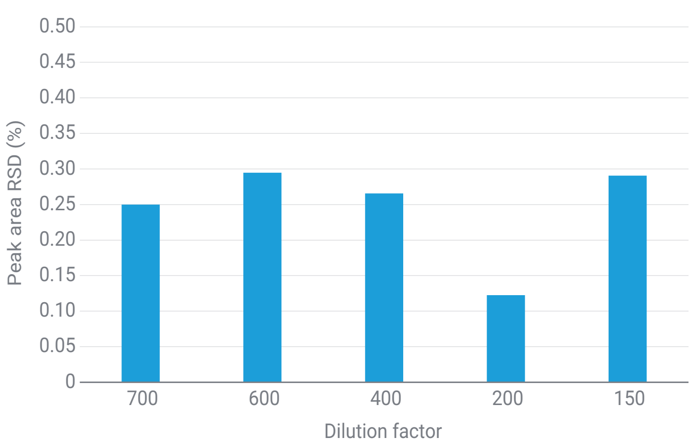

Figure 3. Dilution precision (n = 10) obtained for dilution factors in the range of 150 to 700.

The average peak areas obtained while measuring peak area precision were used for the determination of peak area linearity for the applied dilution factors, expressed as respective concentration (Figure 4). The linearity of the dilutions was excellent, with $R^2 > 0.9999$.

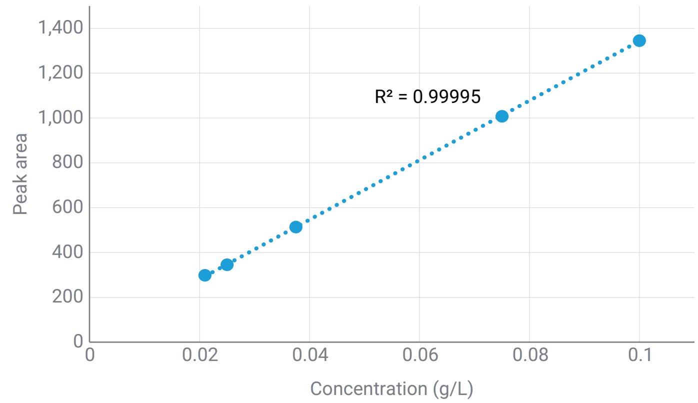

Figure 4. Linearity of average peak areas from the precision measurement experiment.

One critical value is the carryover between two sampling points, which expresses a value for the material remaining from sampling in the complete arrangement of probe, tubing, mixing cups, and valves. For the determination of carryover, the complete instrumental setup was cleaned by repeated samplings of pure water. Then, one sampling of the highly concentrated caffeine solution was taken, followed by an additional sampling of pure water. In the result obtained from the water sample, the remaining caffeine peak area was measured and put in relation to the caffeine peak from the highly concentrated sample. This was done for dilution factors from 150 to 600 (Figure 5). The carryover was typically < 1.0%.

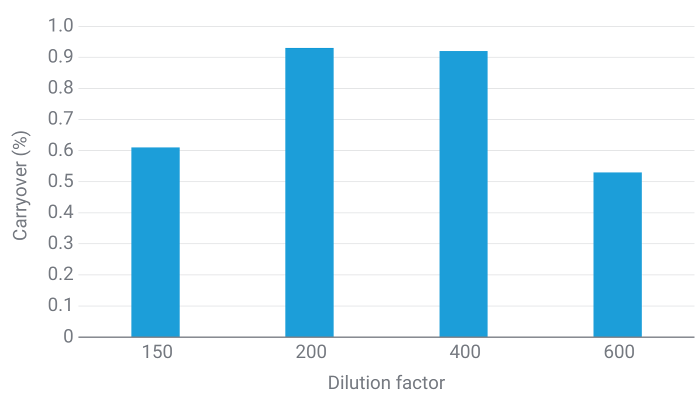

Figure 5. Sampling of a caffeine sample at 15 g/L, followed by water samples and determination of residual caffeine carryover.

For the determination of the loss of sample during the process, the recovery (accuracy) was determined by comparison of the peak areas obtained from the different dilution factors, compared to a handmade dilution. The recovery was typically better than 84% (Figure 6).

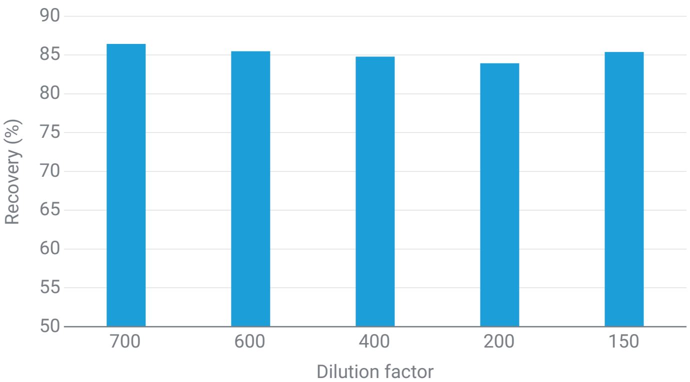

Figure 6. Recovery of sampling at different dilution factors.

In comparison, the Agilent 1260 Infinity II Online Sample Manager shows a peak area RSD performance of < 0.15% for injection volumes ≥ 5 µL done directly by the Sample Manager. Typical carryover is below 30 ppm.[2] The calculated area accuracies of the dilutions by means of the Online Sample Manager were typically below 1%.[3]

Conclusion

This technical overview describes the installation of a Mettler Toledo EasySampler with probe, in combination with the Snapdragon Chemistry SRS as a sampling device for the Agilent 1260 Infinity II Prime Online LC System. This includes software control of the sampling process and Online LC analysis by means of the Snapdragon Chemistry control software. The performance of the complete sampling process showed excellent values for peak area RSDs and linearity. Typical carryover between samplings was below 1% and obtained recoveries were typically higher than 84%.

References

1. Online Monitoring of a Heterogenic Liquid/Liquid Reaction, *Agilent Technologies application note*, publication number 5994-7000EN, **2024**.
2. Performance Characteristics of the Agilent 1260 Infinity II Online Sample Manager. *Agilent Technologies technical overview*, publication number 5994-3529EN, **2021**.
3. Reactor Sample Dilution and Mixing Performance of the Agilent 1260 Infinity II Online Sample Manager. *Agilent Technologies technical overview*, publication number 5994-3679EN, **2021**.

Appendix

SRS parameters (Figure 2A)

- **Dilution_factor**: Dilution factor, e.g. 200 implies a 200-fold dilution of 20 uL to 4 mL.
- **Working_directory**: Location in the file system where files related to the software are stored.
- **Cleaning_volume_ml**: Can be increased, if carryover is observed; wash-reps can also be increased in this case.
- **Mixing_time**: The time of mixing in the vessel by bubbling of nitrogen into the vessel from the bottom.
- **Wash_reps**: Number of cleaning cycles to run. Can be increased if carryover is observed.
- **Experiment_name**: Experiment name in Agilent Online Monitoring Software. Leave this blank if only a single experiment is running or to use the most recent experiment running. Must match experiment name exactly.
- **Easy_sampler_single_solvent**: 0 = NOT single solvent; 1 = single solvent. Single solvent mode means all three solvents for the EasySampler are identical for quenching, diluting, and cleaning. This mode allows for faster sampling because the lines do not need to be re-primed when changing from reaction solvent to quench, from quench to diluent, and from diluent back to reaction solvent.
- **Easy_sampler_pocket_out_time**: Only used in single solvent mode; gives the time the when sampling cavity is out of the tip of the probe.

SRS manual run commands (Figure 2B)

- **take_sample**: This will take a sample performing the entire chain of events from taking a sample to pushing it to the LC, and starting a chromatographic run.
- **Clear_faults**: Can be clicked if needed to reset a fault.
- **Set Repeated Manual Command**: Useful for a sequence of samples. Actions can be selected. The time interval before taking the next sample can be selected. Clicking **Create Routine** will initiate sampling in the defined interval until the user clicks stop. After clicking stop, the current sample is still processed, but no more new samples are taken.
- Additional commands are provided for testing and debugging. Details are provided in the user manual.

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