Technical Overview

Overlapping Reaction Sampling and Analysis for Online LC Reaction Monitoring of Fast Processes

Automated sampling and analysis scheduling by Agilent Online LC Monitoring Software

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Introduction

In the arena of small-molecule reactions as well as large-biomolecule processes, there are transformations that are fast in comparison to the time required for their analysis. This challenges existing process analytical technologies by providing an adequately fast sampling time, and even intensifies the challenge when initial analytical data is required to control the reaction.

A possible example of such a fast reaction is the formation of derivatized urea by the reaction of 1,1-carbonyl-di-1,2,4-triazole (CDT) with benzylamine (Figure 1). The reaction of CDT with the first equivalent of benzylamine occurs immediately and leads to the production of an intermediate, benzyl triazole urea (BTU), by loss of one triazole moiety. This intermediate has enough stability to be detected while the reaction with the second equivalent of benzylamine continues. The formation of both the intermediate and the final product, 1,3-dibenzylurea (DBU), can be monitored simultaneously.

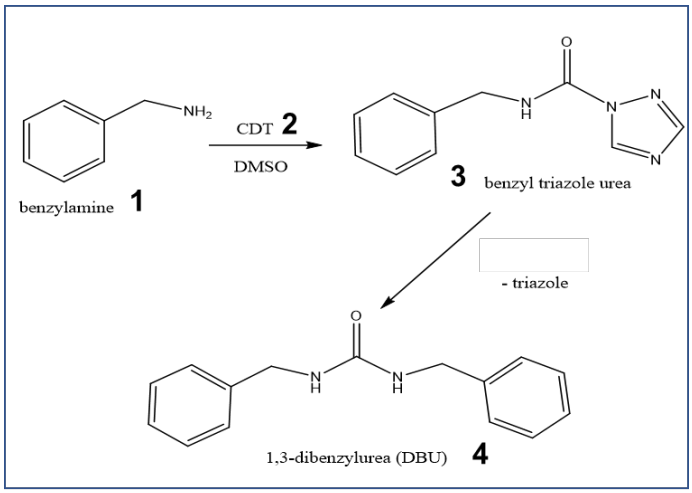

Figure 1. Reaction of benzylamine (1) with CDT (2) to the intermediate, BTU (3), and the final product, DBU (4).

This discrepancy between fast sampling and slower analysis requires the capability of the Agilent InfinityLab Online LC Solutions to draw samples while the analysis of another sample is currently running, and, after completion of the sampling, to analyze the remaining samples (Figure 2, A to C).

After completion of the first sampling, the analysis of this sample was started. While the analysis of sample 1 was running, samples 2 and 3 were drawn. In Figure 2A, the analysis of sample 2 and the sampling of 4 are currently running. Also, the analysis of sample 3 is already waiting and the sampling of 5 is scheduled. At a later stage in Figure 2B, the drawing of samples has greatly surpassed the number of samples that have been analyzed. In Figure 2C, the sampling is finished, and only the samples waiting for analysis will be completed to finalize the result set.

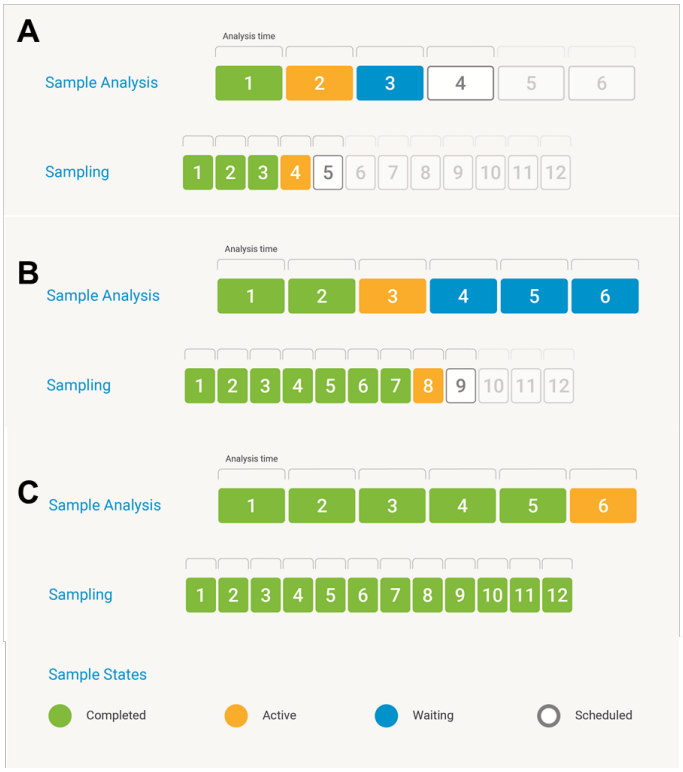

Figure 2. Schematic workflow of fast sampling with slower sample analysis.

This technical overview demonstrates the capability of the Agilent InfinityLab Online LC Solutions to sample from a fast chemical reaction while a slower gradient is used for the analysis of the samples. This is even possible with quenching and dilution to store the chemical reaction samples for later reanalysis of retained samples. It will be shown that no sampling point will be skipped, and that the Agilent Online LC Monitoring Software is able to schedule sampling and analysis in a parallel manner. The software-aided, automated scheduling of sampling and analysis loses no information about the reaction, and works in an unattended, time-and-money-saving manner, gaining all information in one experiment.

Experimental

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

Table 1. Instrumentation.

Product Type	Agilent Product Description
Instrument	– 1290 Infinity II High-Speed Pump (G7120A) – 1260 Infinity II Online Sample Manager Set (G3167AA): – 1260 Infinity II Online Sample Manager (G3167A), clustered with external valve (p/n 5067-6680) located at the 1290 Infinity Valve Drive (G1170A), and Online LC Monitoring Software – 1290 Infinity II Multicolumn Thermostat (G7116B) – 1290 Infinity II Diode Array Detector (G7117B) with InfinityLab Max-Light Cartridge Cell, 10 mm (G4212-60008)
Column	InfinityLab Poroshell 120 EC-C18, 2.1 x 50 mm, 1.9 µm (p/n 699675-902)
Software	– OpenLab CDS, version 2.6 or later – Online LC Monitoring Software, version 1.0

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	0.5 mL/min
Generic Gradient	3% B to 95% B in 5 min, stop time: 5 min, post time: 2 min
Column Temperature	50 °C
Agilent Feed Injection (Automatic)	80% of analytical flow rate
Flush-Out Solvent	Water (S2)
Flush-Out Volume	Automatic
Injection Volume	3 µL
Needle Wash	3 s, 1:1 water:ACN + 0.1% FA (S1)
Sampling	See sampling methods for sampling to vial
Diode Array Detector	210 ± 16 nm, reference: 310 ± 40 nm, 20 Hz data range
Sampling to Vial (Dilutions)	
Sampling	Sampling from reactor to deep-well plate sealed with silicon mat
Target Volume	250 µL
Dilution Factor	5
Sample Volume	50 µL
Draw Speed	**Setting 1** – **Draw speed:** 130 µL/min – **Wait time:** 1.2 s – **Dispense speed:** 155 µL/min (Ejection of sample into well before dilution)
Dilution Solvent	S2
Dilution Eject Speed	10,000 µL/min (after sample ejection for mixing)
Schedule	– **Interval:** 1.5 min – **Calculated time for sampling of 14 samples:** 19.5 min
Sample Delivery Pump	
Pump	Agilent 1260 Infinity II Isocratic Pump (G7110B)
Flow Rate	5 mL/min
Solvent Stream	Solvent stream from reaction vessel to Online Sample Manager reactor interface and back to reaction vessel
Reaction Conditions	
Reactant	82 mg CDT in 45 mL DMSO (final concentration 10 mmol)
Solvent	Anhydrous DMSO
Stirring	At 20 °C
Reaction Start	Reaction started by adding educt: 120 µL benzylamine in 5 mL DMSO (2.2 mol equivalent)

Chemicals

- Benzylamine
- CDT
- Anhydrous DMSO
- Formic acid

Other materials

- Agilent 96-deep-well plates, 1 mL, polypropylene (part number 5043-9305)
- Agilent sealing mat, 96 wells, round, preslitted, silicone (part number 5043-9317)

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

The fast chemical reaction applied as an example was started by adding benzylamine to the CDT in the reaction vessel (Figure 1). At the same time, the experiment was started by the Online LC Monitoring Software and an execution list was created (Figure 3A). This list initially shows a schedule of all expected sampling times, the status of the scheduled samples, general information, and empty fields that will be populated during the experiment. In Figure 3A, two samples are already sampled at 9 seconds and at 1 minute 31 seconds, respectively. In the Info field, a status can be found explaining that the analysis of the first sample is already running, and the sampling for the second sample has already been completed, while the third sample has started to be drawn from the reactor line. This means that the sampling was continued at the expected time—while the analysis of the first sample was running.

Figure 3B describes the situation after an experiment run time of 12 minutes. It can now be seen that the first sample was injected at 3 minutes 20 seconds. The Online LC Monitoring Software automatically arranged this injection with the sampling of sample 3, with an actual sampling time of 3 minutes 42 seconds. During the analytical run of sample 2, the sampling from the reactor continued with only a minor delay. For instance, sample 4, scheduled at 4 minutes 20 seconds, was sampled at 4 minutes 55 seconds. Sample 5, scheduled at 6 minutes, was drawn in at 6 minutes 9 seconds. This software-guided scheduling enables the sampling of all required reaction samples with an automated scheduling if necessary due to the analysis running in parallel. Most importantly, no sampling point will be lost, and in the worst case, sampling will be delayed for a few seconds due to other actions performed by the 1260 Infinity II Online Sample Manager at the same time.

Figure 3C describes the situation towards the end of the experiment: the sampling of the last scheduled sample from the reactor was completed at 19 minutes 30 seconds and the samples stored in the Online Sample Manager will be analyzed in a sequential manner. For instance, sample 10, which was drawn at 13 minutes 56 seconds, was injected for analysis at 1 hour 18 minutes. The currently running sample, sample 11, was sampled at 15 minutes 10 seconds.

This special feature of parallel sampling and analysis can be utilized due to the Feed Injection mode where the sample loop does not need to be kept in the flow path, in contrast to the classical flowthrough injection mode.

A

State	Type	Name	Expected Time	Start Time	Info	Sample	Location	Sampling Time	Injection Time	Analytical Method Set
Completed	Action	Start	00:00:00	00:00:00						
Analysis	Diluted to vial	DilutedToVialSetting 01	00:00:00	00:00:00	Running - Sample-1 - MethodSet...	Sample-1	D1B-D1	00:00:09		
Analysis	Diluted to vial	DilutedToVialSetting 01	00:01:30	00:01:30	Sampling finished	Sample-2	D1B-D2	00:01:31		
Sampling	Diluted to vial	DilutedToVialSetting 01	00:03:00	00:03:00	Start sampling	Sample-3	D1B-D3			
Scheduled	Diluted to vial	DilutedToVialSetting 01	00:04:30							
Scheduled	Diluted to vial	DilutedToVialSetting 01	00:06:00							
Scheduled	Diluted to vial	DilutedToVialSetting 01	00:07:30							
Scheduled	Diluted to vial	DilutedToVialSetting 01	00:09:00							
Scheduled	Diluted to vial	DilutedToVialSetting 01	00:10:30							
Scheduled	Diluted to vial	DilutedToVialSetting 01	00:12:00							
Scheduled	Diluted to vial	DilutedToVialSetting 01	00:13:30							
Scheduled	Diluted to vial	DilutedToVialSetting 01	00:15:00							
Scheduled	Diluted to vial	DilutedToVialSetting 01	00:16:30							
Scheduled	Diluted to vial	DilutedToVialSetting 01	00:18:00							
Scheduled	Diluted to vial	DilutedToVialSetting 01	00:19:30							

Figure 3A. Agilent Online LC Monitoring Software execution list showing the situation at the beginning of the experiment with the first sample already running, the second sampled, and the third has started sampling in parallel to the analytical run of the first sample.

B

State	Type	Name	Expected Time	Start Time	Info	Sample	Location	Sampling Time	Injection Time	Analytical Method Set
Completed	Action	Start	00:00:00	00:00:00						
Completed	Diluted to vial	DilutedToVialSetting 01	00:00:00	00:00:00		Sample-1	D1B-D1	00:00:09	00:03:20	MethodSet 1
Analysis	Diluted to vial	DilutedToVialSetting 01	00:01:30	00:01:30	Running - Sample-2 - MethodSet...	Sample-2	D1B-D2	00:01:31		
Analysis	Diluted to vial	DilutedToVialSetting 01	00:03:00	00:03:00	Sampling finished	Sample-3	D1B-D3	00:03:42		
Analysis	Diluted to vial	DilutedToVialSetting 01	00:04:30	00:04:53	Sampling finished	Sample-4	D1B-D4	00:04:55		
Analysis	Diluted to vial	DilutedToVialSetting 01	00:06:00	00:06:08	Sampling finished	Sample-5	D1B-D5	00:06:09		
Analysis	Diluted to vial	DilutedToVialSetting 01	00:07:30	00:07:30	Sampling finished	Sample-6	D1B-D6	00:07:31		
Analysis	Diluted to vial	DilutedToVialSetting 01	00:09:00	00:09:00	Sampling finished	Sample-7	D1B-D7	00:09:01		
Analysis	Diluted to vial	DilutedToVialSetting 01	00:10:30	00:10:30	Sampling finished	Sample-8	D1B-D8	00:10:31		
Sampling	Diluted to vial	DilutedToVialSetting 01	00:12:00	00:12:00	Start sampling	Sample-9	D1B-D9			
Scheduled	Diluted to vial	DilutedToVialSetting 01	00:13:30							
Scheduled	Diluted to vial	DilutedToVialSetting 01	00:15:00							
Scheduled	Diluted to vial	DilutedToVialSetting 01	00:16:30							
Scheduled	Diluted to vial	DilutedToVialSetting 01	00:18:00							
Scheduled	Diluted to vial	DilutedToVialSetting 01	00:19:30							

C

State	Type	Name	Expected Time	Start Time	Info	Sample	Location	Sampling Time	Injection Time	Analytical Method Set
Completed	Action	Start	00:00:00	00:00:00						
Completed	Diluted to vial	DilutedToVialSetting 01	00:00:00	00:00:00		Sample-1	D1B-D1	00:00:09	00:03:20	MethodSet 1
Completed	Diluted to vial	DilutedToVialSetting 01	00:01:30	00:01:30		Sample-2	D1B-D2	00:01:31	00:12:20	MethodSet 1
Completed	Diluted to vial	DilutedToVialSetting 01	00:03:00	00:03:00		Sample-3	D1B-D3	00:03:42	00:21:19	MethodSet 1
Completed	Diluted to vial	DilutedToVialSetting 01	00:04:30	00:04:53		Sample-4	D1B-D4	00:04:55	00:30:20	MethodSet 1
Completed	Diluted to vial	DilutedToVialSetting 01	00:06:00	00:06:08		Sample-5	D1B-D5	00:06:09	00:38:43	MethodSet 1
Completed	Diluted to vial	DilutedToVialSetting 01	00:07:30	00:07:30		Sample-6	D1B-D6	00:07:31	00:46:40	MethodSet 1
Completed	Diluted to vial	DilutedToVialSetting 01	00:09:00	00:09:00		Sample-7	D1B-D7	00:09:01	00:54:39	MethodSet 1
Completed	Diluted to vial	DilutedToVialSetting 01	00:10:30	00:10:30		Sample-8	D1B-D8	00:10:31	01:02:35	MethodSet 1
Completed	Diluted to vial	DilutedToVialSetting 01	00:12:00	00:12:00		Sample-9	D1B-D9	00:12:42	01:10:33	MethodSet 1
Completed	Diluted to vial	DilutedToVialSetting 01	00:13:30	00:13:55		Sample-10	D1B-D10	00:13:56	01:18:31	MethodSet 1
Analysis	Diluted to vial	DilutedToVialSetting 01	00:15:00	00:15:09	Running - Sample-11 - MethodSe...	Sample-11	D1B-D11	00:15:10		
Analysis	Diluted to vial	DilutedToVialSetting 01	00:16:30	00:16:30	Sampling finished	Sample-12	D1B-D12	00:16:31		
Analysis	Diluted to vial	DilutedToVialSetting 01	00:18:00	00:18:00	Sampling finished	Sample-13	D1B-E1	00:18:01		
Analysis	Diluted to vial	DilutedToVialSetting 01	00:19:30	00:19:30	Sampling finished	Sample-14	D1B-E2	00:19:31		

Figure 3B,C. (B) Situation of the experiment after 12 minutes. Sampling is continued automatically in parallel to the running sample 2. No sample will be lost, a sampling delay of a few seconds was accepted. (C) Situation towards the end of the experiment. All scheduled samples were drawn from the reactor and are stored in the Agilent 1260 Infinity II Online Sample Manager for their analytical run.

The trending plot of the reaction generated by the Online LC Monitoring Software shows the course of the complete experiment (Figure 4). The intermediate, BTU, is formed immediately and decreases, while the product, DBU, is generated gradually during the reaction. Therefore, the initial composition at sampling point 1 already is BTU/DBU 80/20. The reagents benzylamine and CDT could not be monitored due to coelution of benzylamine with DMSO in a large peak at the beginning of the chromatogram and a tiny peak for CDT at the used wavelength.

The advantage of parallel sampling and analysis in cases where a sampling is faster than the chromatographic separation is that the results of the initial sampling points can be seen early, and decisions can be made as soon as possible. The influence of the software-guided scheduling of sampling and injection for analysis can be seen between samples 2 and 3, and 8 and 9. No sampling point is lost due to the automated scheduling.

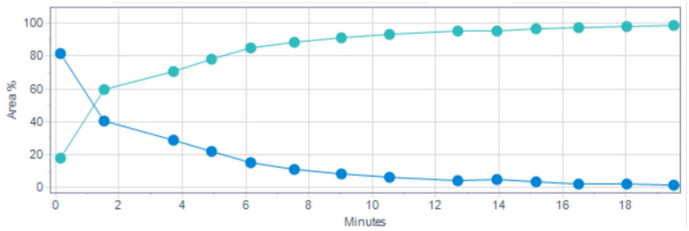

Figure 4. Trending plot of the reaction between CDT and benzylamine showing the sampling time and the corresponding area (%) of BTU (blue) and DBU (green).

An overlay chromatogram of samples 1, 2, and 10 is shown in Figure 5. The intermediate, BTU, eluting at 1.80 minutes, declines during the reaction from 81.715 to 4.659% area (Table 3). The main product, DBU, eluting at 2.32 minutes, increased from 18.285 to 95.341% area.

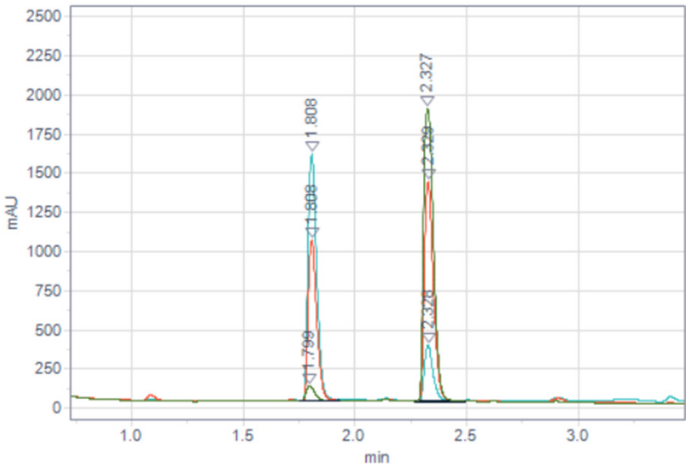

Figure 5. Chromatographic separation of the intermediate, BTU, and the main product, DBU. BTU elutes at 1.808 minutes and declines during the reaction. Increasing DBU elutes at 2.327 minutes.

Table 3. Values obtained for percent peak area, peak area, peak height, and retention time (RT) of BTU and DBU in samples 1, 2, and 10.

Sample	Compound (Multiple)	RT (min)	Area %	Area	Height
1	BTU	1.808	81.715	4,254.787	1,571.218
	DBU	2.328	18.285	952.047	352.121
2	BTU	1.808	40.670	2,700.315	1,028.197
	DBU	2.329	59.330	3,939.274	1,398.267
10	BTU	1.799	4.659	284.761	99.714
	DBU	2.327	95.341	5,827.224	1,870.620

Conclusion

This technical overview describes the fast sampling from a fast, small-molecule reaction using the Agilent InfinityLab Online LC Solutions and the parallel starting of the analysis of collected samples. The complete scheduling of sampling and analysis was performed by the Agilent Online LC Monitoring Software. This provides early, near-real-time information of fast reactions with high sampling frequency and without loss of sampling points. This enables sampling and analysis in a single experimental setup in an economic, time-saving way.

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DE26049924

Printed in the USA, December 6, 2022
5994-5583EN

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