Technical Overview

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Online LC Reaction Monitoring with Fraction Collection

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Abstract

The objective of this technical overview is to demonstrate the use of the Agilent 1290 Infinity III Online LC System in combination with Agilent Fraction Collectors to collect large scale fractions of reaction mixtures during the course of the reaction in parallel to Online LC reaction monitoring. This enables access to further analytical techniques with the collected reaction fractions in case a larger amount of sample is required. This avoids having to draw larger amounts of reaction solvent directly and manually from the reactor.

Introduction

The Agilent 1290 Infinity III Online LC System is a versatile tool for online monitoring of chemical reactions with a multiplicity of functions for sampling from reaction vessels. The Agilent Online LC Monitoring Software[1,2] controls the Online LC, offering direct injection of the sampled reaction solution, and injection after dilution and quenching or derivatization. An additional sampling functionality that generates retained samples without dilution is also provided. Using this option, reaction solution samples up to 240 µL can be collected. For some analytical techniques, it is possible that this function will not provide enough material for further analysis (like NMR). In such cases, an Agilent Fraction Collector can be connected to the Online LC integrated in the Agilent OpenLab CDS acquisition method.

This technical overview will describe the easiest way to connect an Agilent Fraction Collector to the Agilent 1290 Infinity III Online LC and will demonstrate the functionality by simulating a reaction monitoring experiment.

Experimental

Instrumentation

- Agilent 1290 Infinity III High-Speed Pump (G7132A)
- Agilent 1290 Infinity III Online Sample Manager (G3167B)
- Agilent 1290 Infinity III MCT (7116B)
- Agilent 1290 Infinity III DAD (G7117B) with Agilent InfinityLab Max-Light Cartridge Cell (10 mm, p/n G4212-60008)
- Agilent 1290 Infinity III Quaternary Pump (G7111B)
- Agilent 1290 Infinity III Fraction Collector (G5664B)

Method

Table 1. Method parameters.

Parameter	Value
Binary Pump	
Solvent	A) Water B) ACN
Flow Rate	0.6 mL/min
Gradient	Time (min) %B 0 15 4 98 6 98 Stop time: 6 min Post time: 2.5 min
Online Sample Manager	
Injection Volume	10 µL
Needle Wash	3 s, Solvent 1 (ACN:Water 1:1)
Feed Injection	Feed speed: 80% of flow rate Flush out volume: Automatic Flush out solvent (S2): Water
Column Compartment	
Column Temperature	30 °C
DAD	
Data Rate	10 Hz
Wavelength	310 nm ±4
Fraction Collector	
Time 0.01 min	Change Fraction Mode, time based, collecting volume slices, 1 mL
Time 1.01 min	Change Fraction Mode, Off
Quaternary Pump	
Flow Rate	1 mL/min
Channel A	Reactor
Channels B, C, and D	Other solvents for e.g. dilution, quenching, and flushing

Online LC Monitoring Software

In the experimental setup of Agilent Online LC Monitoring Software, the chosen OpenLab acquisition and data analysis methods were combined to create a method set.[1,2] For this method set, "Direct Injection" was selected as a sampling method. Five injections with 10-minute intervals were scheduled for the experiment.

Instrumental setup

The flow path for the fraction collection was set up by connecting the outlet of the reactor to channel A of the quaternary pump (Figure 1). This pump is constantly running and delivering reactor content to the valve interface of the Online LC System. The outlet of the Online LC valve interface was connected to the diverter valve in the analytical fraction collector and from the diverter valve back to the reactor (or to waste, depending on the setup). With the Online LC valve interface, a sample can be drawn directly out of the stream and the Fraction Collector diverter valve can be switched to sample reactor solvent. In addition, three more solvents can be connected to channels B, C, and D of the quaternary pump (e.g., for dilution and quenching). For a more complex setup comprising more than one reactor with additional flushing options, the use of an Agilent 1290 Infinity Flexible Cube (G4227A) equipped with one or two selection valves (G4235A) is possible (described in a previous application note[3]). Electronically, all modules were connected by CAN and can be controlled by Agilent OpenLab Software.

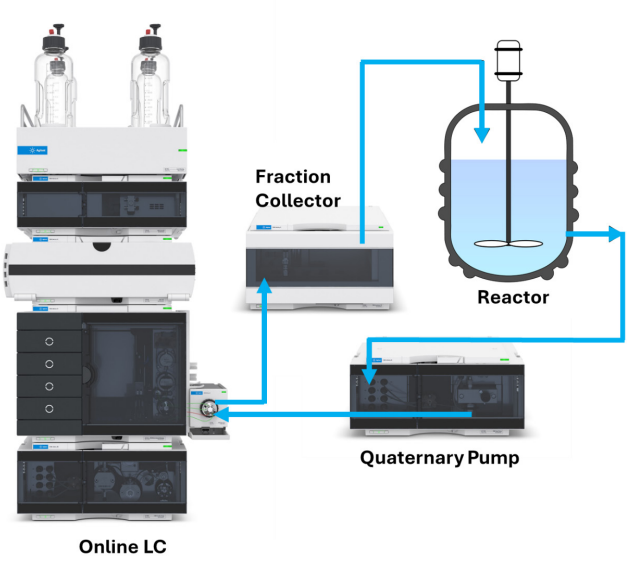

Figure 1. Setup of the Agilent 1290 Infinity III Online LC with fraction collection.

Column

Agilent InfinityLab Poroshell 120 SB-C18, 3.0 × 100 mm, 2.7 µm (p/n 685975-302)

Software

- Agilent OpenLab Software, version 2.8
- Agilent Online LC Monitoring Software, version 1.3

Reactor sample model

Mixture of Dye Mix (Agilent Delay and Checkout Calibrant), 5 mL in 1 L acetonitrile (p/n 5190-8223).

Chemicals

Agilent Delay and Checkout Calibrant (p/n 5190-8223), Patent Blue VF sodium salt, Sunset Yellow FCF, Sudan Orange G, dissolved in DMSO, 2 mg/mL

Solvents

- InfinityLab Acetonitrile for LC/MS (p/n 5191-5100-002), HPLC Gradient Grade, 1 × 2.5 liters
- InfinityLab Water for LC/MS (p/n 5191-5120-002), HPLC Gradient Grade, 1 × 2.5 liters

Results and discussion

For the simulation of the reactor solution, a dilution of a three-dye mixture was prepared. The flask was connected to the A channel of a quaternary pump as described in the experimental section. Online LC Monitoring Software was programmed for direct injection and on a schedule to draw five samples, 10 minutes apart. The outlet of the Online LC reactor interface valve was connected to the diverter valve of the Fraction Collector (FC). The FC was programmed for time-based fractionation in OpenLab Software. Therefore, fractions of one minute for each sample analysis were introduced in the timetable for time-based fractionation (see Experimental section). The result of the experiment comprising all five sampling points was displayed in the Online LC Monitoring Software (Figure 2).

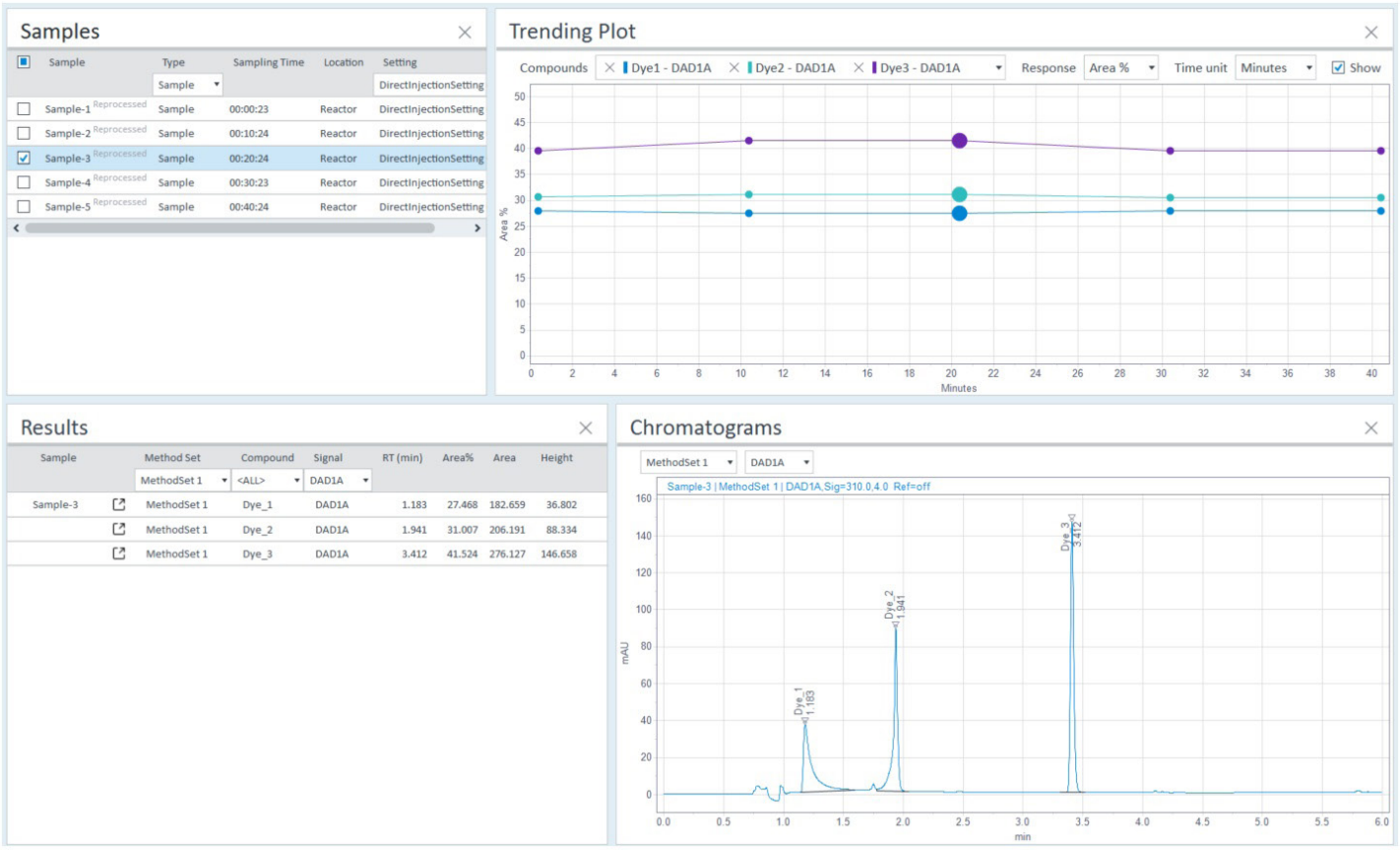

Figure 2. Agilent Online LC Monitoring Software, displaying the result of the five samples drawn from the reactor simulation flask. Samples: Sampling time of each sample from the reactor for direct injection, and selection of Sample 3 for result display. Trending plot: Measured peak area percentage of the three dyes in the mixture for all five sampling points. Results: Retention time, area, area percentage, and height for the three dyes in Sample 3. Chromatograms: Chromatogram of Sample 3.

To display the result of the fractionation, OpenLab Software was used (Figure 3). This software can display the area of sampling in each chromatogram, the fraction results, and an image showing the locations of the fractions in the used container.

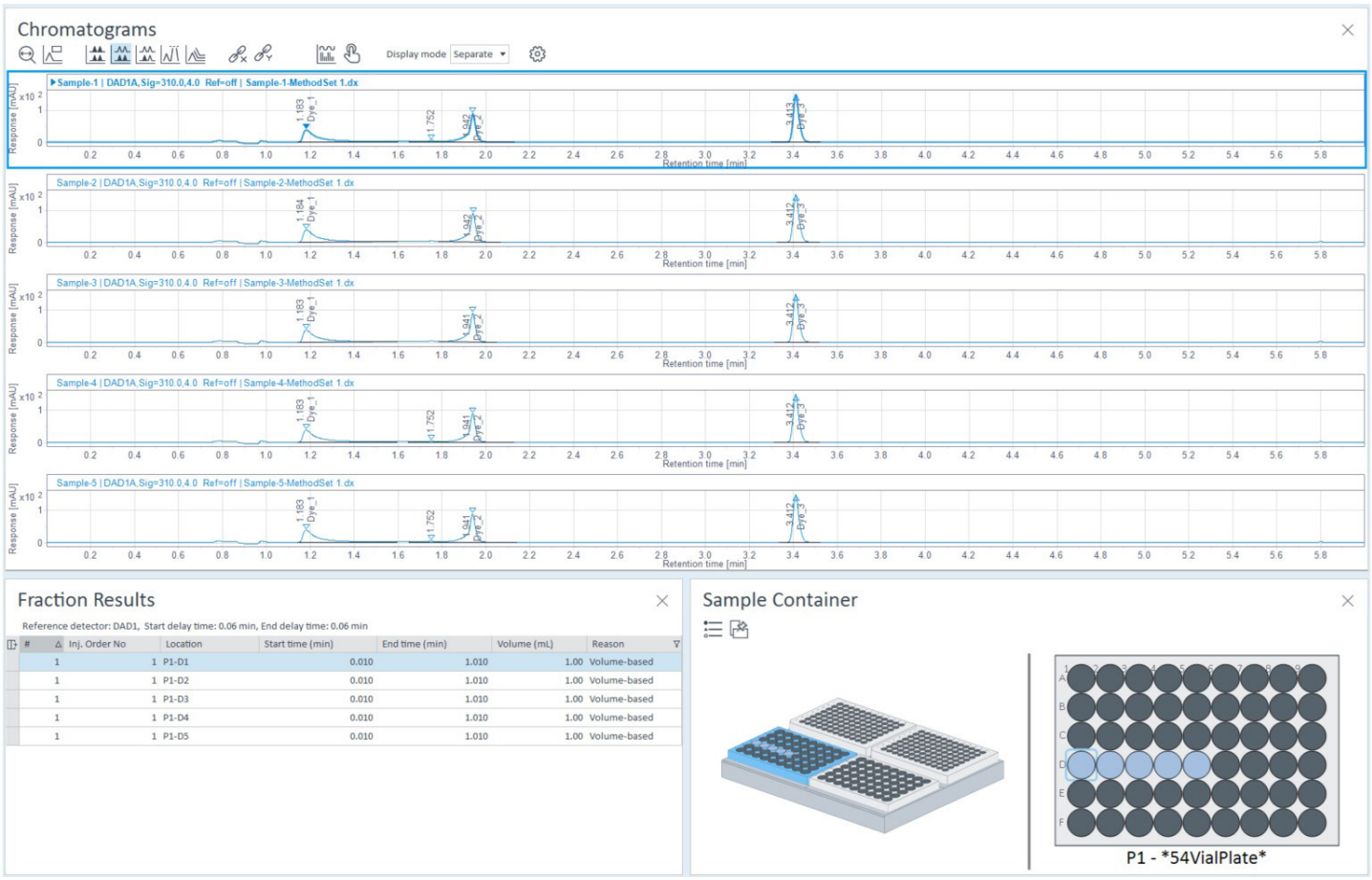

Figure 3. Fraction collection of the five reactor sampling runs (five injections with 10-minute intervals). In the upper section, the five chromatograms for Samples 1 to 5 are shown. From each run, the time range of one minute was drawn directly after the injection of the sample taken from the reactor solvent. In this time range, the complete solution is collected, comprising all compounds of the reactor solution. The bottom section shows the fractionation results (left side) with the corresponding location of each fraction sample run in the container (right side).

Conclusion

This technical overview demonstrates the pairing of the Agilent 1290 Infinity III Online LC System with an Agilent Fraction Collector. This combination enables the drawing of large volume retained samples of 1 mL from each reactor sampling point (as shown in this study) or even more with higher-capacity Agilent Fraction Collectors. Additionally, it is also possible to draw more than one fraction during the run time of the Online LC. The fractionation was done in parallel to the near real-time analysis on the Online LC, saving time to gain samples for further analytical techniques.

References

1. Performance Characteristics of the Agilent 1260 Infinity II Online Sample Manager. *Agilent Technologies technical overview*, publication number 5994-3529EN, **2024**.
2. Online Reaction Monitoring by the Agilent InfinityLab Online LC Solutions. *Agilent Technologies application note*, publication number 5994-3528EN, **2024**.
3. Dual-Reactor Sampling with Agilent InfinityLab Online LC Solutions. *Agilent Technologies application note*, publication number 5994-5811EN, **2024**.

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