Application Note

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Online Monitoring of a Heterogenic Liquid/Liquid Reaction

Combination of the Agilent 1260 Infinity II Online LC System with the Mettler Toledo EasySampler 1210 by the Snapdragon Chemistry SRS module

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Abstract

This application note will describe the combination of the Agilent 1260 Infinity II Online LC with the Mettler Toledo EasySampler 1210, using the Snapdragon Chemistry Sample Relay System (SRS) module, for the monitoring of heterogenic reactions. This combination allows automated sampling and dilution from a chemical reactor with a heterogenic liquid/liquid reaction, enabling unattended use of a chemical reactor with continuous monitoring of chemical reactions.

Introduction

Small molecule chemical reactions often take place in heterogenic mixtures like liquid/solid and liquid/liquid media. Their analysis, especially by online LC, can be challenging due to inhomogeneities in the sampling process and clogging of LC capillaries and tubing by solid matter. In addition, it is necessary to stop the reaction in the sample by quenching and diluting to a concentration appropriate for LC analysis. This process is typically done manually, with the drawback of at-line analysis and the associated delay relative to the reaction progress in the vessel.

This application note describes the online LC analysis of a heterogenous liquid/liquid phase reaction. The sampling is done by a probe especially designed for heterogenic sampling. The Mettler Toledo EasySampler, which is connected to the probe, moves the sample to the Snapdragon Chemistry SRS interface for quenching, dilution, mixing, and finally transferring of the sample to the 1260 Infinity II Online LC. This enables determination of the reaction vessel content in near real time, allowing for unattended supervision of the reaction.

The reaction chosen as an example for a heterogenic liquid/liquid reaction is a hydrolytic ester cleavage of benzyl benzoate (Figure 1). The educt, benzyl benzoate, is soluble in methyl-THF, and the products, potassium benzoic acid and benzyl alcohol, are soluble in the aqueous phase. The transfer of the educt to the aqueous phase is accelerated by strong stirring to enlarge the contact surface of the phases and the addition of a phase transfer catalyst, tetrabutylphosphonium hydroxide. The increased reaction temperature of 60 °C additionally increases the speed of the reaction. After quenching with aqueous acid, benzoic acid will be formed.

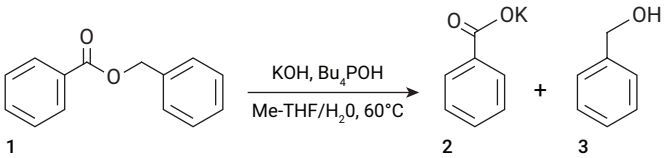

Figure 1. Hydrolytic cleavage of benzyl benzoate (1) to potassium benzoate (2) and benzylic alcohol (3).

Experimental

The instrumentation used in this study is detailed in Table 1, and the method parameters are outlined in Tables 2 and 3. The instrumental setup and the software setup are described in detail in a separate technical overview.[1]

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	– Snapdragon Chemistry SRS – Mettler Toledo EasySampler 1210, including 33 cm probe – Mettler Toledo EasyMax 102 Reactor – Julabo Cooler
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 – 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
Gradient	0 min 5% B, 2 min 95% B, stop time: 2 min
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	1 µL
Needle Wash	3 s, water:MeOH 80:30 + 0.1% FA (S1)
Diode Array Detector	215 ± 4 nm, reference: off, 40 Hz data rate
Agilent 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 the SRS software

Table 3. Snapdragon Chemistry SRS software and hardware settings; solvents used with the Mettler Toledo EasySampler.

Sample Relay System Software Settings	
Dilution	1:200
Mixing Time	5 s
Cleaning Volume	1.6 mL, two wash repeats
Easy Sampler Single Solvent	No
Sampling Interval	15 min
Sample Relay System Hardware Settings	
Pressure	– 30 psi on inlet of instrument – 15 psi on regulator on front of instrument – 0.1 L/min on flow meter
Mettler Toledo EasySampler	
Solvents	Reactor solvent: water Dilution: MeOH Quenching: MeOH:Water 80:20 + 1% FA

Reaction conditions

Benzyl benzoate 25 mM (4.8 mL) was dissolved in 60 mL methyl-THF and transferred to the 100 mL reaction vessel of the Mettler Toledo EasyMax 102 (equipped with an internal temperature sensor and the EasySampler probe). KOH 1.5 equivalent (2.1 g) was dissolved in 40 mL water and added to the reactor. The reaction mixture was stirred rapidly (500 rpm) and was brought to 60 °C (25 °C at the external Julabo cooler for reactor temperature regulation). The reaction was started by the addition of tetrabutylphosphonium hydroxide, 0.1 equivalent (1.8 mL, 40% in water) acting as a phase transfer catalyst.

Chemicals

- Benzyl benzoate
- Potassium hydroxide
- Tetrabutylphosphonium hydroxide (40% in water)
- Methyl-THF
- Formic acid

Solvents and chemicals

- Agilent InfinityLab acetonitrile (ACN) for HPLC (part number 5191-5100) was used as mobile phase.
- 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 trending plot of the described reaction is shown in Figure 2. The first sampling point only shows the educt benzyl benzoate (blue trace) with 100 area%. The products benzoic acid (violet trace) and benzylic alcohol (green trace) start to appear in the second sampling point after 15 minutes. The third sampling point showed a decline of the educt to 73.7 area% and an increase of the products benzoic acid and benzylic alcohol to 14.7 area% and 11.4 area%, respectively. The reaction was monitored for 800 minutes (13.3 hours) with a sampling point every 15 minutes. By the last sampling point, the educt had decreased to 8.5 area% and the products had increased to 55.8 area% and 35.5 area% (Table 1).

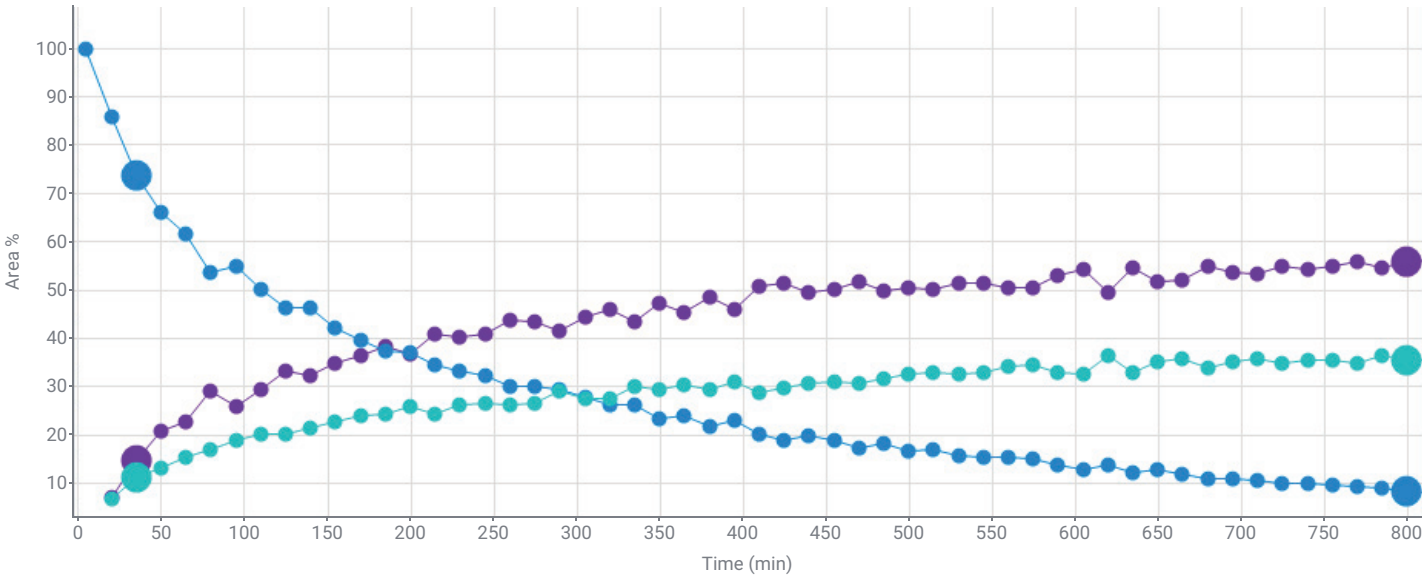

Figure 2. Trending plot of the heterogenic liquid/liquid reaction of benzyl benzoate (blue) to benzoic acid (violet) and benzylic alcohol (green).

The chromatographic separation of the educt and product compounds was achieved using a short gradient where benzyl benzoate elutes at 1.489 minutes, benzoic acid at 0.849 minutes, and benzylic alcohol at 0.774 minutes (Figure 3).

Figure 3 also shows an overlay of sampling point 3 (blue) and the last sampling point (green), both highlighted in the trending plot (Figure 2). These chromatograms display the declining peak area of benzoyl benzoate and the increasing peak areas of benzoic acid and benzylic alcohol for the selected sampling points.

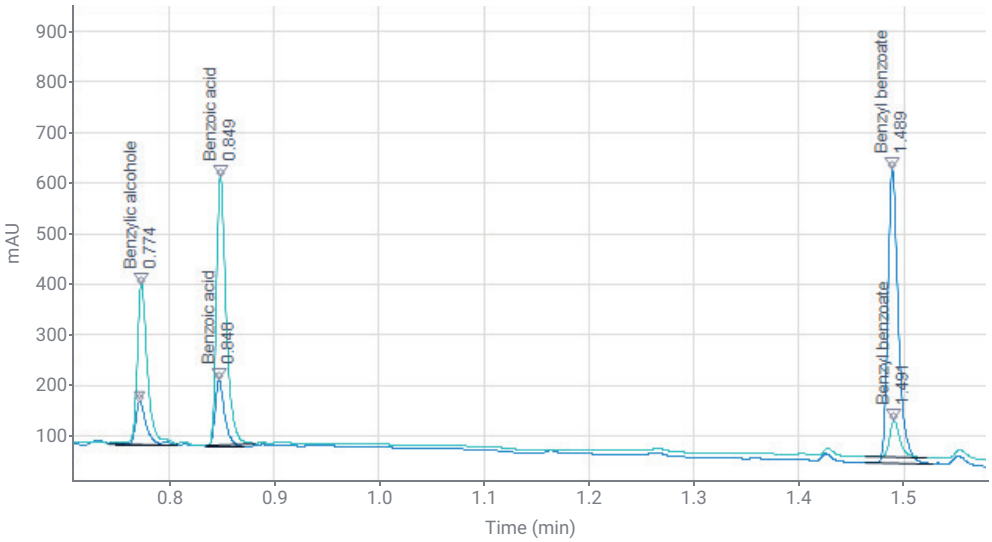

Figure 3. Chromatographic separation of benzyl benzoate (1.489 min), benzoic acid (0.849 min), and benzylic alcohol (0.774 min). Sampling point 3 (blue) and sampling point at 800 minutes (green).

Table 4. Details of sampling point 3 (upper three rows) and the last sampling point (lower three rows) at 800 minutes.

Compound	RT (min)	Area %	Area	Height
Benzylic alcohol	0.772	11.44	63.36	87.98
Benzoic acid	0.848	14.97	81.95	129.87
Benzyl benzoate	1.489	73.77	408.70	578.31
Benzylic alcohol	0.774	35.56	217.37	312.53
Benzoic acid	0.849	55.85	341.42	525.72
Benzyl benzoate	1.491	8.59	52.49	71.82

Conclusion

This application note demonstrates the use of the Agilent 1260 Infinity II Online LC with the Mettler Toledo EasySampler and probe using the Snapdragon Chemistry SRS for monitoring of heterogenic chemical reactions. The heterogenic liquid/liquid reaction, which was used as an example, was monitored over more than 13 hours with a sampling point every 15 minutes. The obtained data and trending plot demonstrate the proper sample handling including transport, quenching, dilution, and mixing, clearly showing the expected trending curves for such a reaction.

Reference

1. Performance Evaluation of a Reactor Sampling Device for Online LC. *Agilent Technologies technical overview*, publication number 5994-6983EN, **2024**.

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