Application Note

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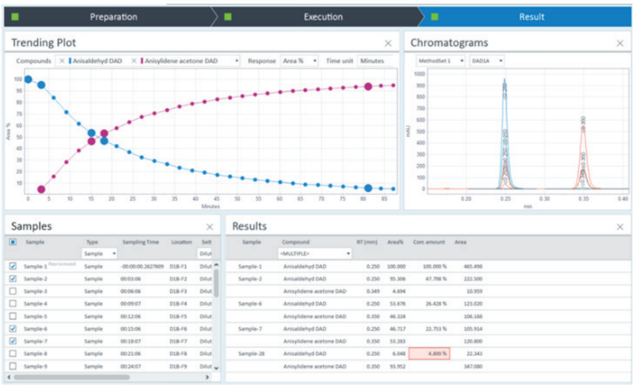
1260 Infinity III LC

Automated Reaction Monitoring by the Agilent 1260 Infinity II Prime Online LC

Sampling from the reactor, dilution, quenching, and analysis



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Abstract

This application note demonstrates the online monitoring of a small molecule reaction with the Agilent 1260 Infinity II Prime Online LC. The experiment described in this application note demonstrates the capability of the Agilent 1260 Infinity II Online Sample Manager to sample directly from the reaction vessel. The sampled amount was diluted or quenched as needed, preparing the sample for immediate or subsequent analysis. Sampling was done in a completely automated fashion with relative quantification of the educt and fast display of the results during the reaction. The experimental setup, control, and display of the results was performed entirely within the Agilent Online LC Monitoring Software.

Introduction

The modern small molecule pharmaceutical industry often produces compounds by chemical reactions, using valuable reactants or catalysts to generate highly potent active pharmaceutical ingredients (APIs). Therefore, it is crucial to closely monitor the contents of a reaction vessel in a timely manner as the reaction progresses. This real-time data allows the operator to stop the reaction at an optimum point to maximize the amount of valuable API or to prevent degradation of the product.

This application note will demonstrate the use of the 1260 Infinity II Prime Online LC with the Online LC Monitoring Software. An aldol condensation will be used as the example reaction (Figure 1).[1] The online experiment will be completely orchestrated by the seamlessly integrated Online LC Monitoring Software, which schedules sample drawing from the reactor, runs the analytical method, and displays the progressing results during the reaction.

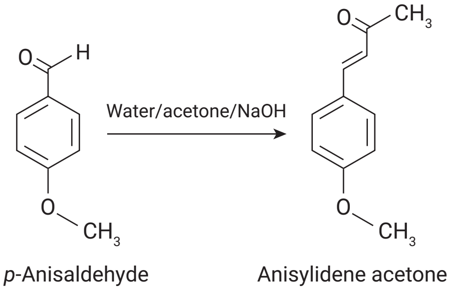


Figure 1. Aldol condensation reaction of *p*-anisaldehyde and acetone.

Experimental

Instrument

- Agilent 1260 Infinity II Flexible Pump (G7104C)
- Agilent 1260 Infinity II Online Sample Manager Set (G3167AA): Agilent 1260 Infinity II Online Sample Manager (G3167A) clustered with external valve (part number 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)

Column

Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 30 mm, 1.9 µm (part number 695775-302)

Software

- Agilent OpenLab CDS, version 2.6 or later
- Agilent Online LC Monitoring Software, version 1.0

Chemicals

- *p*-Anisaldehyde
- Acetone
- NaOH
- Formic acid

Additional material

- 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 educt of the aldol reaction, *p*-anisaldehyde, was dissolved in acetone/water and pumped circularly from the reaction vessel to the Online Sample Manager interface and back to the reaction vessel. The sampling, dilution, and analysis were started without adding the sodium hydroxide to get the first data point of pure educt. The first data point was used for setting up a calibration curve for relative quantification in OpenLab Data Analysis and enabled on-demand reprocessing in the Online Monitoring Software. The reaction was started by adding the sodium hydroxide solution immediately after the first sample was drawn (10 seconds). The first sample that shows conversion of the educt is sample number 2, drawn after 3 minutes, where the reaction product was first detected. The compounds of interest were separated with high resolution using a fast chromatographic method on an 800 bar UHPLC. The UHPLC system combined feed injection with a steep gradient, which allowed short cycle times to monitor the reaction closely and continuously. The trending plot obtained for area percent of educt and product displays the course of the reaction (Figure 2). The trending plot shows that the peak area of product and educt were equal after about 17 minutes. After a reaction time of approximately 82 minutes, the area percent of the educt was approximately 6%, and the product reached approximately 94 area percent.

Instrumental conditions

Analytical Method	
Solvents	A) Water + 0.1% formic acid (FA) B) Acetonitrile (ACN) + 0.1% FA
Analytical Flow Rate	1.3 mL/min
Gradient	40% B to 90% B in 0.85 min Stop time: 1.0 min
Column Temperature	45 °C
Agilent Feed Injection (Automatic)	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:ACN 1:1 + 0.1% FA (S1)
Sampling	See sampling methods for sampling to vial
Diode Array Detector	290 ±4 nm, 40 Hz data rate
Sampling to Vial (Dilutions)	
	Sampling from reactor to deep well plate sealed with silicon mats.
Target Volume	600 µL
Dilution Factor	100
Sample Volume	6 µL
Draw Speed	Setting 2 (draw speed: 100 µL/min, wait time: 3.6 s, dispense speed: 130 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: 3 min, run time: 90 min
Sample Delivery Pump	
Pump Used	Agilent 1260 Infinity II Isocratic Pump (G7110B)
Flow Rate	5 mL/min
	Solvent stream from reaction vessel to Online Sample Manager reactor interface and back to reaction vessel.
Reaction Conditions	
Educt	*p*-Anisaldehyde, 1 mL
Solvent	100 mL acetone:water 2:1 (v/v)
	Stirring at room temperature
Reaction Start	Add 100 µL of NaOH 50% in water (w/w)

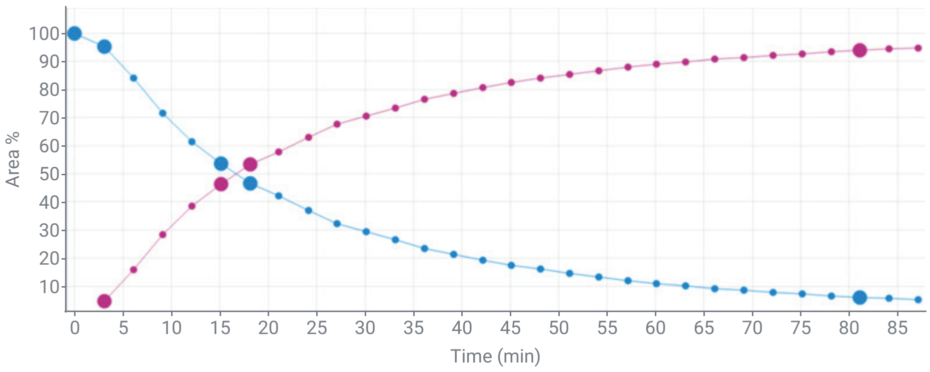


Figure 2. Trending plot for the area percent of educt *p*-anisaldehyde (blue) and product anisylidene acetone (purple) in the reaction vessel.

An overlay of the chromatograms obtained from samples 1, 2, 6, 7, and 28 is shown in Figure 3. This overlay shows a declining signal of the educt *p*-anisaldehyde at a retention time of 0.250 minutes and an increasing peak for the product anisylidene acetone at a retention time of 0.350 minutes.

For a proper relative quantification of the educt, the peak obtained from the first sample, in which the reaction was not yet started, was taken as 100%. The quantification was set up in OpenLab Data Analysis with reanalysis of already processed samples on demand in the Online LC Software. The results show that the reaction initially proceeds quickly, sample 1 to sample 2, and slows down for later samples (Figure 4). As a control for the reaction, a lower limit of 5% educt residue was introduced. When this limit was passed, a triangle with exclamation mark was displayed in the plot, as shown from sample 28 to the end.

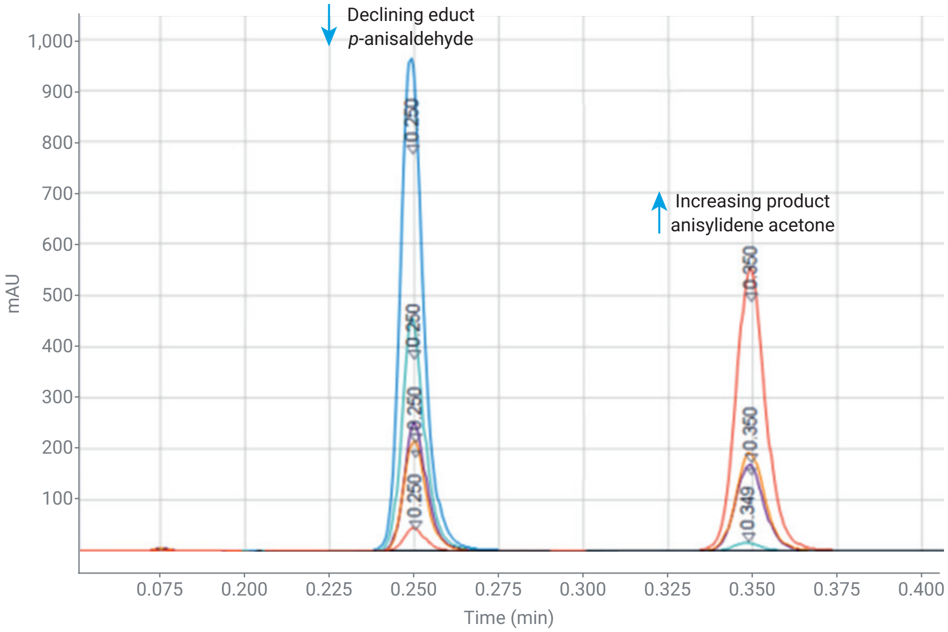


Figure 3. Overlay of chromatograms from samples 1, 2, 6, 7, and 28 showing declining educt and increasing product in the reaction vessel throughout the reaction.

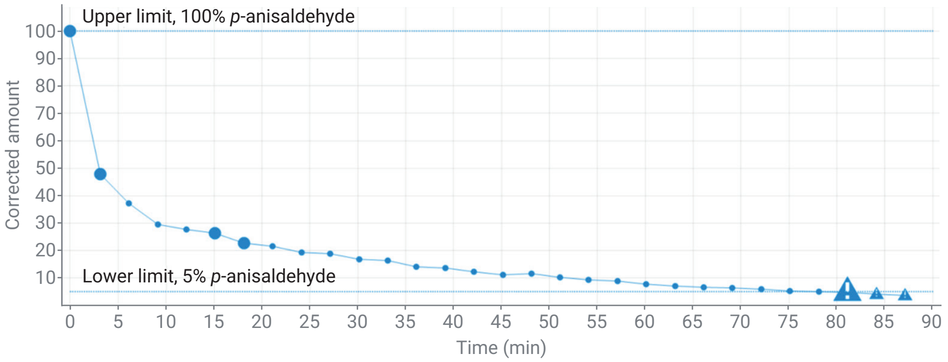
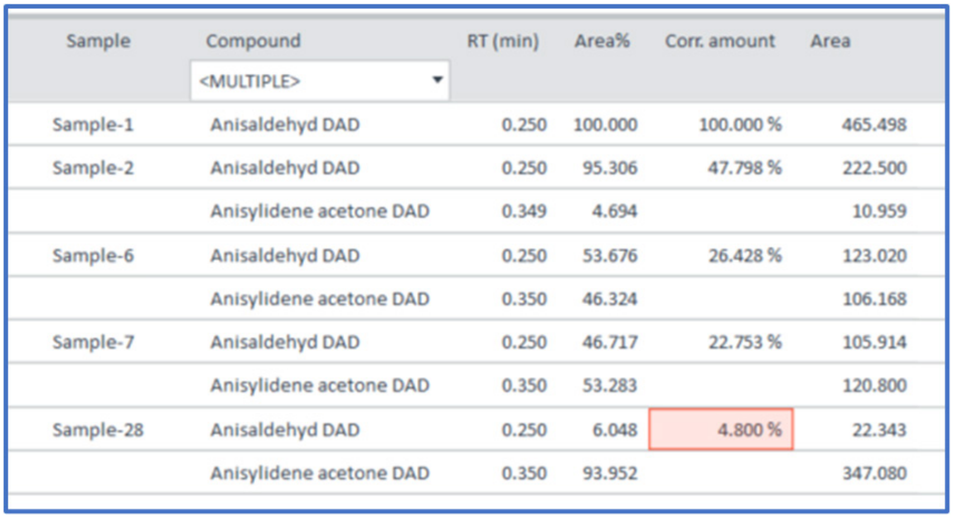


Figure 4. Relative quantification of *p*-anisaldehyde. The quantified amount is corrected by the dilution and initially set as 100%. The lower limit was set to 5% of the educt as the notification level. The triangles with exclamation mark indicate exceeding this lower limit.

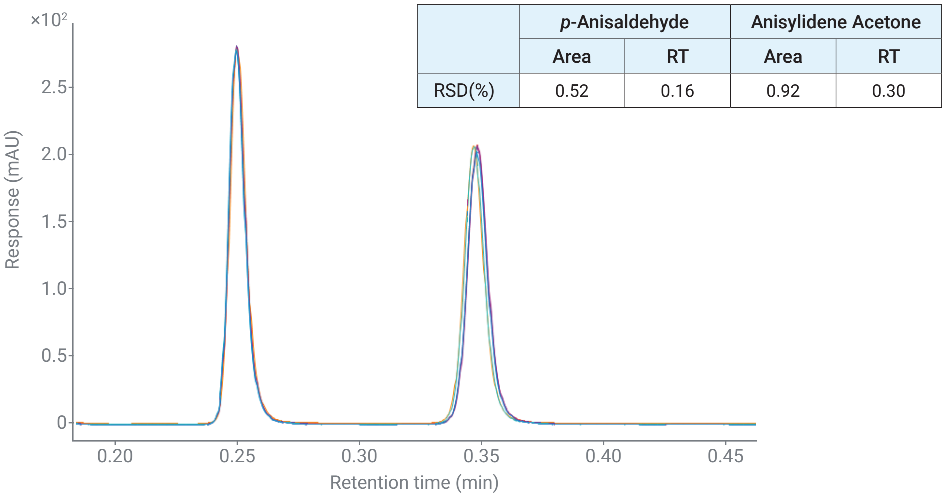
The results for samples 1, 2, 6, 7, and 28 are summarized in Table 1. Sample number 2 showed an amount of 47.8% educt, corrected for dilution, which is 95.3 peak area percent. Sample 6 showed an educt residue of 26.4%. The peak area percent, however, was about 54 and 46% for the educt and product respectively. The difference between the percent educt or product and the area percent was due to the different relative UV absorbance coefficients for both compounds. Therefore, the relative quantification of the educt is a more valid number to monitor the progress of the reaction. The set limit was undershot in sample 28 with the highlighted value of 4.8%.

The capability of the 1260 Infinity II Online Sample Manager to dilute or quench reaction samples offers the possibility to retain them for later analysis.[2] In this case, sample number 6 was taken for a reanalysis after 24 hours to determine major performance parameters of this fast separation method—the relative standard deviation (RSD) values for retention time and peak area. An overlay of six injections of sample number 6 is shown in Figure 5. The RSD values of the retention times were 0.16 and 0.30% for *p*-anisaldehyde and anisylidene acetone respectively (shown in the table in Figure 5). Peak area RSDs were at 0.52 and 0.92% for *p*-anisaldehyde and anisylidene acetone respectively. This experiment also demonstrates that the reaction was fully quenched during the 1:100 dilution performed during the sampling campaign. A more detailed overview about the performance of the Online Sample Manager is shown in other Agilent technical overviews.[3,4] In addition, more sensitive and selective detection could be achieved by adding a mass spectrometer such as the Agilent InfinityLab LC/MSD iQ to the Infinity II Prime Online LC for the detection of low-level impurities.[5]

Table 1. Summary of samples 1, 2, 6, 7, and 28 with area percent and relative quantification.



Sample	Compound	RT (min)	Area%	Corr. amount	Area
Sample-1	Anisaldehyd DAD	0.250	100.000	100.000 %	465.498
Sample-2	Anisaldehyd DAD	0.250	95.306	47.798 %	222.500
	Anisylidene acetone DAD	0.349	4.694		10.959
Sample-6	Anisaldehyd DAD	0.250	53.676	26.428 %	123.020
	Anisylidene acetone DAD	0.350	46.324		106.168
Sample-7	Anisaldehyd DAD	0.250	46.717	22.753 %	105.914
	Anisylidene acetone DAD	0.350	53.283		120.800
Sample-28	Anisaldehyd DAD	0.250	6.048	4.800 %	22.343
	Anisylidene acetone DAD	0.350	93.952		347.080



	p-Anisaldehyde		Anisylidene Acetone	
	Area	RT	Area	RT
RSD(%)	0.52	0.16	0.92	0.30

Figure 5. Overlay (n = 6) of sample 6 analyzed after 24 hours. The table displays the calculated RSD performance for retention time and peak area.

Conclusion

This application note demonstrates the use of the Agilent 1260 Infinity II Prime Online LC with the Agilent 1260 Infinity II Online Sample Manager for the monitoring of a small molecule reaction. For reaction control, a relative quantification method was used with lower limit alarm for the given educt. The complete experiment is controlled by the Agilent Online LC Monitoring Software.

The process of sampling from the reactor is a fully automated procedure, including dilution or quenching and analysis. The obtained sample could be retained and used for later confirmation even with other analytical techniques. Confident data and fast results are provided during the reaction run time.

Acknowledgment

The results shown in this application note are derived from a beta-test evaluation. We would like to thank Dr. Christian Lautz and his colleagues for providing valuable feedback while working with the prototype.

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