

Sensitive Detection of Low-Level Byproducts in a Small Molecule Reaction by the Agilent 1260 Infinity II Prime Online LC/MS System



Author

Edgar Naegele
Agilent Technologies, Inc.

Abstract

This application note demonstrates the online monitoring of a chemical reaction with an occurring byproduct by means of the Agilent 1260 Infinity II Prime Online LC/MS System, including a mass spectrometer, the Agilent LC/MSD iQ single quadrupole. The inclusion of the MSD iQ offers additional sensitivity and selectivity for the identification of low-level impurities for more reliable control of chemical reactions. With the experiment described in this application note, the capability of the Agilent 1260 Infinity II Online Sample Manager to sample directly from the reaction vessel and dilute/quench automatically is demonstrated. Relative quantification by the MSD iQ in SIM mode of the educt and fast display of the results during the reaction is shown. The experimental setup, control, and display of the results was completely performed in the Agilent Online LC Monitoring Software.

Introduction

In the production process of modern small-molecule pharmaceuticals, it is of crucial importance not only to control the educts and products through the course of the reaction, but also the possibly occurring impurities. Such impurities can pollute the valuable active pharmaceutical ingredient (API). This would make it necessary to introduce additional costly purification steps into the production. An aldol condensation producing a main product and a byproduct is used as a reaction example (Figure 1).¹

This application note demonstrates the use of the 1260 Infinity II Prime Online LC/MS System, including the LC/MSD iQ, in combination with Agilent Online LC Monitoring Software. The online experiment was completely orchestrated by the seamlessly integrated Online LC Monitoring Software, which schedules sample drawing from the reactor, the analytical runs, and display of the progressing results during the reaction.

Experimental

Instruments

- 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 (5067-6680) located at the Agilent 1290 Infinity Valve Drive (G1170A) and Agilent Online LC Monitoring Software
- Agilent 1290 Infinity II MCT (G7116B)
- Agilent 1290 Infinity II DAD (G7117B)

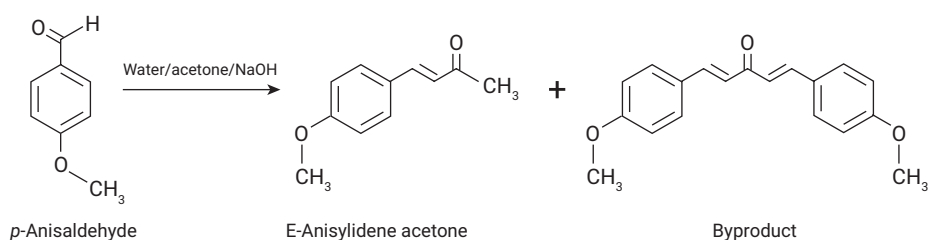


Figure 1. Aldol condensation reaction of *p*-Anisaldehyde and Acetone to the main product E-Anisylidene acetone, and a byproduct form a second aldol condensation.

Column

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

Software

- Agilent OpenLab CDS rev. 2.6 or later versions
- Agilent Online LC Monitoring Software, v. 1.0

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
Feed Injection (Automatic)	80% of analytical flow rate
Flush-Out Solvent (S2)	Water/ACN 10% + 0.1% FA
Flush-Out Volume	Automatic
Injection Volume	1 μL
Needle Wash	3 Seconds, water/ACN 50% + 0.1% FA (S1)
Sampling	See sampling methods
DAD	290 ±4 nm, 40 Hz data rate

MSD iQ method

Automated Method Setting	ESI source and fragmentor voltage
Polarity	Positive
Scan Mode	100 to 500 <i>m/z</i>
SIM Mode	137, 177, and 295 <i>m/z</i>

Sampling (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 sec, dispense speed: 130 μL/min (ejection of sample into well prior to 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	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	<i>p</i> -Anisaldehyde, 1 mL
Solvent	100 mL Acetone:water 2:1 (v:v)
Stirring at Room Temperature	
Reaction Start	Add 100 μL NaOH 50% in water (w:w)

Chemicals

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

Additional material

- Agilent InfinityLab deep-well plates for sampling, 31 mm, 1 mL (part number 5042-6454)
- Agilent InfinityLab silicone sealing mats for well plates (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

Compared to UV-based reaction monitoring², a more specific and sensitive control of the individual compounds during the reaction is possible by adding a single quadrupole mass spectrometer to the 1260 Infinity II Prime Online LC. Best suited for that purpose is the Agilent MSD iQ, which is designed for easiest use in an LC instrument. The educt and product of the reaction were controlled by monitoring of their SIM traces at 133 m/z and at 177 m/z for *p*-anisaldehyde and E-Anisylidene acetone, respectively (Figure 2). The declining compound *p*-anisaldehyde is shown in Figure 2A, and the increasing E-anisylidene acetone in Figure 2B as a trending plot of their peak areas. Since the SIM traces are acquired as separate signals, and the peak areas were highly different due to different ionization behavior, relative peak area percentages cannot be displayed.

The different ionization behavior and the resulting difference in peak area makes it unreliable to use the relative peak area percentage as an indicator for the progress of the reaction. To overcome this obstacle, a relative quantification of the educt SIM trace was done by a one-point calibration using the initial

concentration of *p*-anisaldehyde at 100% value. This calibration was done in OpenLab Data Analysis with the first sample, which was drawn before the starting sodium hydroxide was added. The calibration was applied with the data analysis method on the fly in the

Agilent Online LC Monitoring Software. The lower limit for the relative amount in the reaction vessel was set to 5% *p*-anisaldehyde with the requisition of a warning when this value will be undershot (Figure 3).

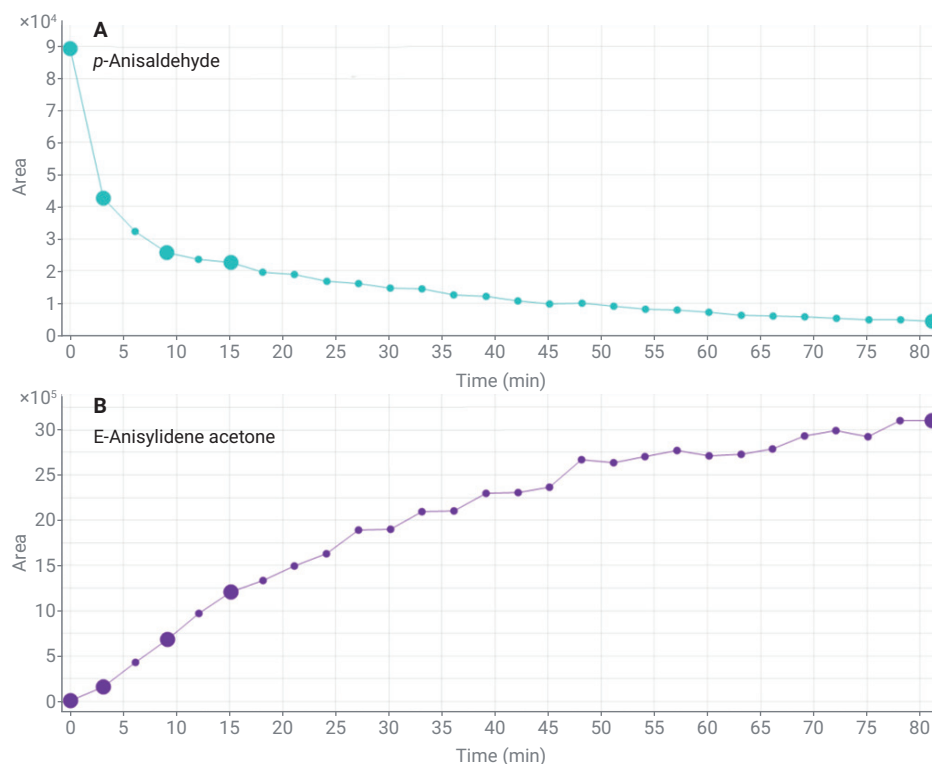


Figure 2. Peak area trending plots of reaction educt *p*-anisaldehyde and reaction product E-anisylidene acetone measured in SIM mode. (A) Declining area of reaction educt. (B) Increasing peak area of reaction product (each dot represents a sample drawn from the reactor).

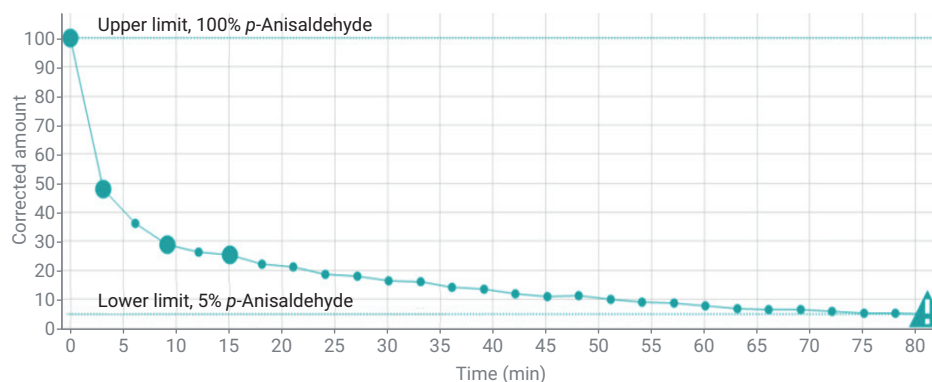


Figure 3. Relative quantification of the reaction educt *p*-anisaldehyde by means of its SIM trace (each dot represents a sample drawn from the reactor).

At the start of the reaction, in the first drawn sample, there is only educt detectable, without product (green peak in Figure 4A, red base line in Figure 4B). The second sample, drawn at three minutes reaction time, shows a decline of educt of approximately half of the amount at reaction start, and the product came up (blue peak in Figure 4A, green peak in Figure 4B). Due to the fact that the product showed a much better ionization behavior, the response is approximately a factor of five higher than the one for the educt. The second sample, drawn after six minutes reaction time, showed a response of approximately 1.8×10^4 in the SIM trace of the educt, and approximately 0.1×10^6 in the one of the products. The reaction was considered complete when the lower limit of 5% residual educt was undershot in sample 28.

In addition, it is possible to monitor likely occurring impurities. Here, a late-eluting compound at $295\ m/z$ could be detected, which is a byproduct of a double aldol condensation. This trace was also monitored in SIM mode. The measured peak area was shown in a trending plot, and unravels the first detection of the byproduct after nine minutes reaction time in sample 4 (Figure 5). The SIM traces for the byproduct in the samples highlighted in the trending plot are shown in Figure 6. The byproduct of a double aldol condensation eluted at 0.831 minutes in the gradient. A summary of the results from the mass spectrometric detection is shown in Table 2. The measured peak areas and heights are declining for the educt, while the main product and a small amount of byproduct contamination was rising.

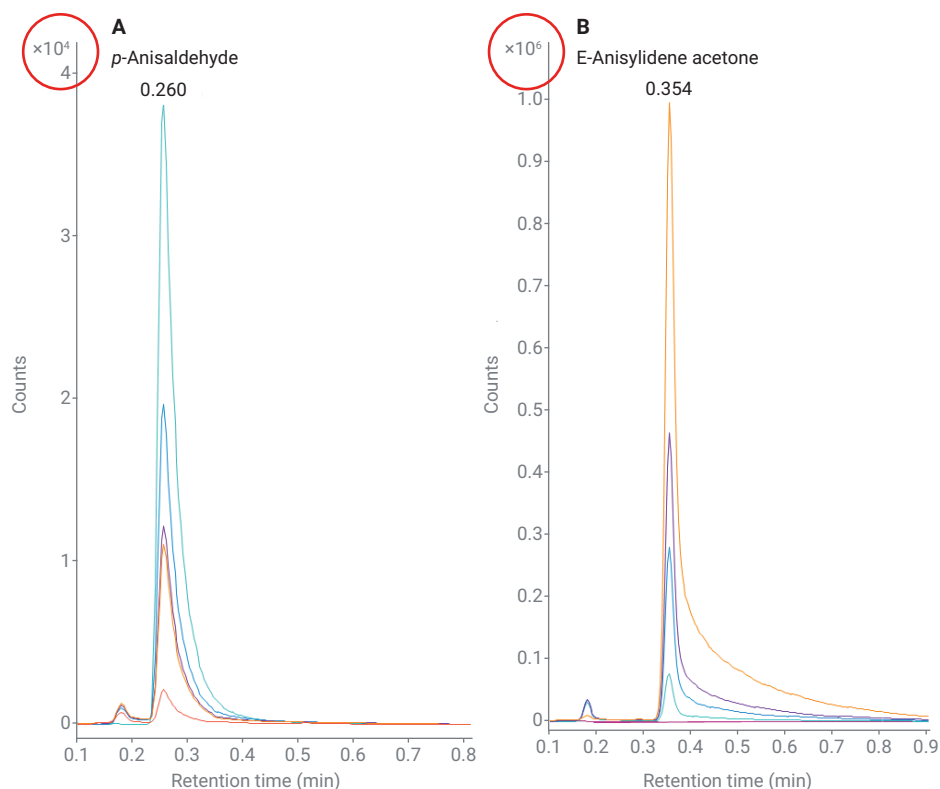


Figure 4. SIM traces during the course of the reaction. (A) Educt *p*-anisaldehyde (Sample 1: green, Sample 2: blue, Sample 4: purple, Sample 6: red, Sample 28: orange). (B) Product E-anisylidene acetone (Sample 1: red baseline, Sample 2: green, Sample 4: blue, Sample 6: purple, Sample 28: orange).

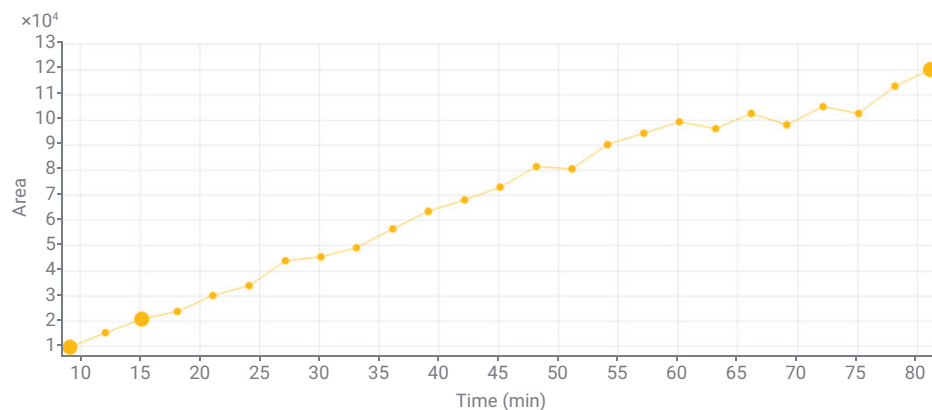


Figure 5. Trending plot of the SIM peak area ($295\ m/z$) for the minor impurity appearing in the course of the aldol reaction (Samples 4, 6, and 28 are highlighted).

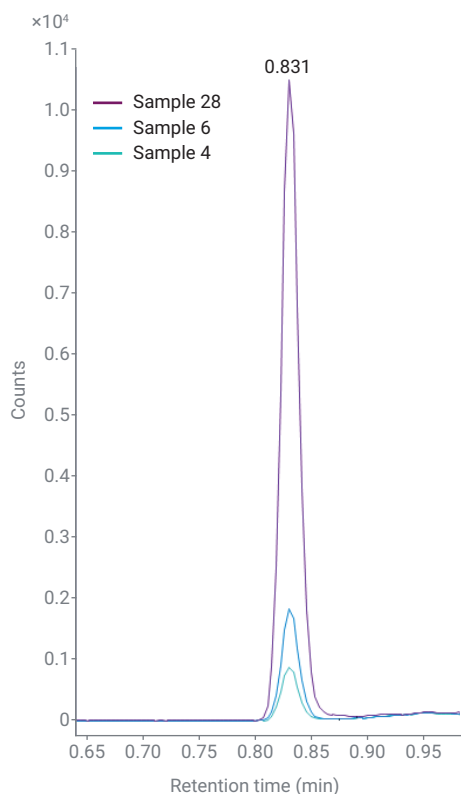


Figure 6. SIM trace of the byproduct appearing from a double aldol condensation (295 m/z).

Conclusion

This application note demonstrates the use of the Agilent 1260 Infinity II Prime Online LC/MS, comprising the Agilent 1260 Infinity II Online Sample Manager and the Agilent MSD iQ for the monitoring of a small molecule reaction. For reaction control, a relative quantification method was used for the SIM trace, with lower limit alarm for the given educt. The complete experiment was controlled by the Agilent Online LC Monitoring Software. The identity of the compounds and appearing byproducts were confirmed by the connected Agilent MSD iQ. The process of sampling from the reactor is a completely automated procedure, comprising dilution/quenching and analysis. The obtained retention sample could be used for later confirmation, even with other analytical techniques. Confident data and rapid results are provided during reaction run time.

References

1. Viviano M. *et al.* A Scalable Two-Step Continuous Flow Synthesis of Nabumetone and Related 4-Aryl-2-butanones. *Org. Process Res. Dev.* **2011**, 15, 858–870.
2. Automated Reaction Monitoring by the Agilent 1260 Infinity II Prime Online LC system. *Agilent Technologies application note*, publication number 5994-3980EN.

Table 2. Results of the mass spectrometric monitoring on the anisaldehyde aldol condensation of selected samples at the beginning, at approximately 50% conversion and at the end of the reaction.

Sample	Compound	RT (min)	Corr. Amount (%)	Area	Height
1	<i>p</i> -Anisaldehyde	0.260	100.00	89,301	34,892
2	<i>p</i> -Anisaldehyde	0.261	47.83	42,710	17,742
	<i>E</i> -Anisylidene acetone	0.354		160,588	73,606
4	<i>p</i> -Anisaldehyde	0.260	28.86	25,789	11,002
	<i>E</i> -Anisylidene acetone	0.355		680,244	269,326
	Aldol dimer	0.831		972	832
6	<i>p</i> -Anisaldehyde	0.261	25.25	22,549	9,951
	<i>E</i> -Anisylidene acetone	0.355		1,211,467	443,855
	Aldol Dimer	0.831		2,095	1,756
28	<i>p</i> -Anisaldehyde	0.261	4.76	4,256	1,842
	<i>E</i> -Anisylidene acetone	0.356		3,095,747	956,425
	Aldol dimer	0.831		12,015	12,015

www.agilent.com

DE44455.0872106481

This information is subject to change without notice.

© Agilent Technologies, Inc. 2021, 2022
Printed in the USA, March 16, 2022
5994-3981EN