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Automated “Online“ LC-MS Small Molecule Reaction Monitoring with a Single Quadrupole MS

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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 2)[1].

This application 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.

Figure 1. 1260 Infinity II Prime Online LC/MS System

Experimental

Analytical Method

Solvents	A) Water + 0.1% FA B) 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

Sampling with Dilution

Sampling from reactor to sealed deep-well plate	
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

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 NaOH 50% in water (w:w)

Results and Discussion

Compared to UV-based reaction monitoring[2], 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 3). The declining compound p-anisaldehyde is shown in Figure 3A, and the increasing E-anisylidene acetone in Figure 3B 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.

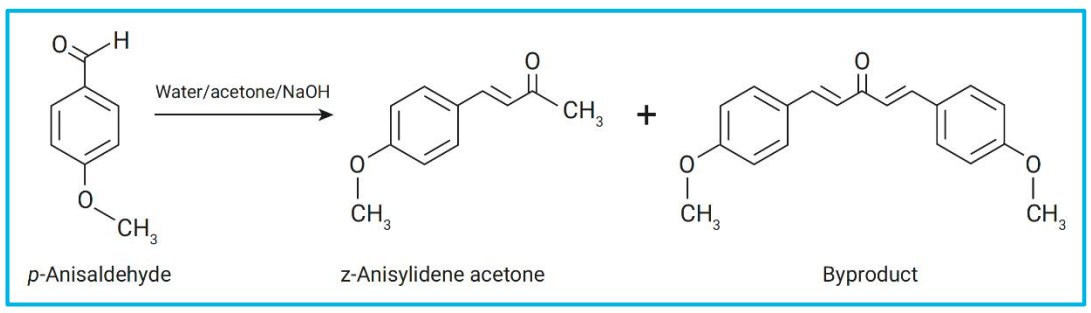

Figure 2. Aldol condensation reaction of p-Anisaldehyde and Acetone to the main product z-Anisylidene acetone, and a byproduct form a second aldol condensation.

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 by sodium hydroxide addition. 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 4).

MSD iQ Method

Automated Method Setting	ESI source and frag. vol.
Polarity	Positive
Scan Mode	100 to 500 m/z
SIM Mode	137, 177, and 295 m/z

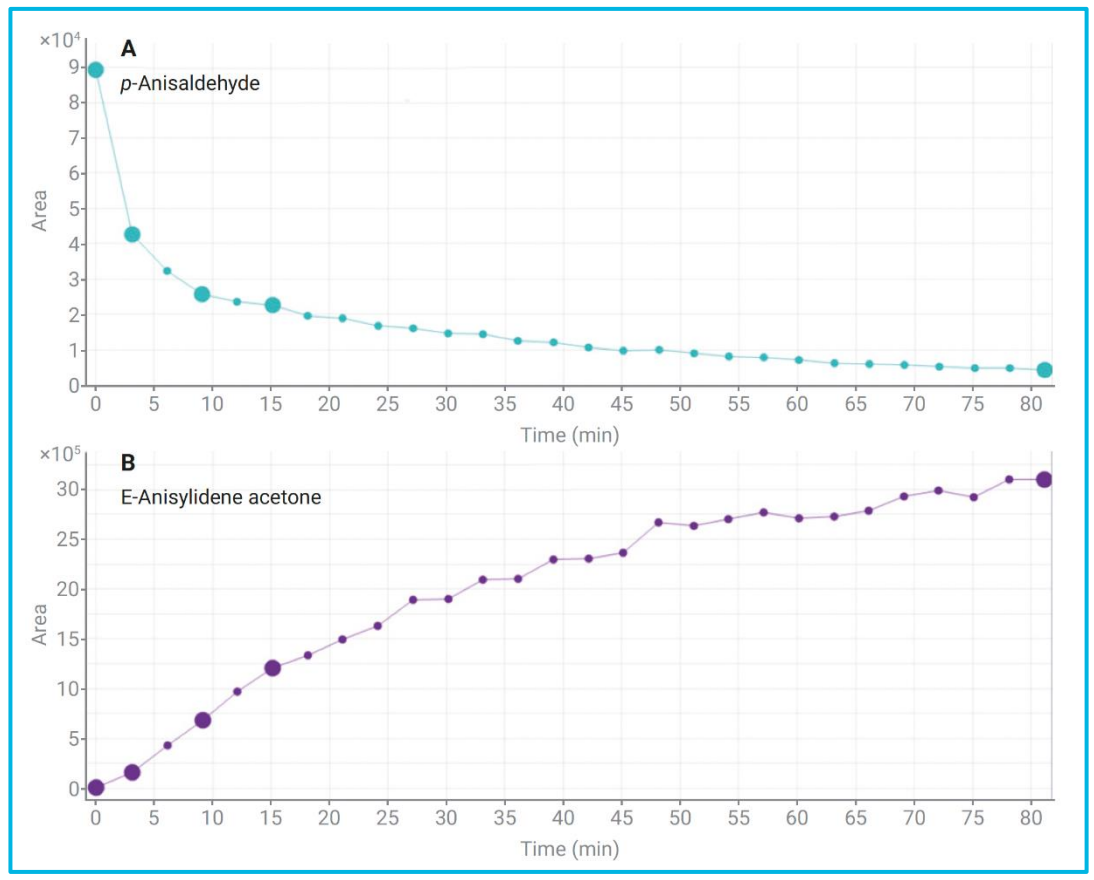

Figure 3. Peak area trending plots of reaction educt p-anisaldehyde and reaction product z-anisylidene acetone measured in SIM mode. (A) Declining area of reaction educt. (B) Increasing peak area of reaction product (each dot represents a sample drawn from the reactor).

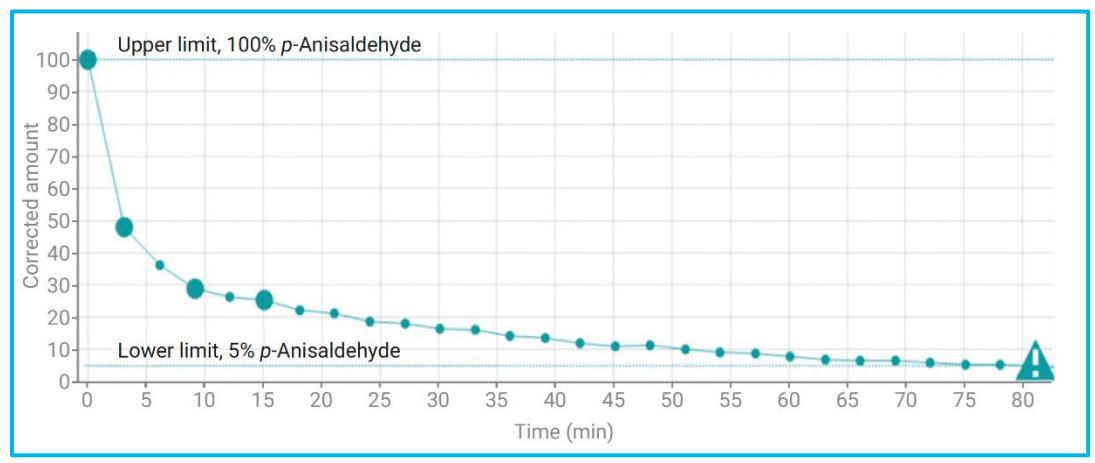

Figure 4. Relative quantification of the reaction educt p-anisaldehyde by means of its SIM trace (each dot represents a sample drawn from the reactor).

At the start of the reaction, in the first drawn sample, there is only educt detectable, without product (green peak in Figure 5A, red base line in Figure 5B). 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 5A, green peak in Figure 5B). 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 approx. 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.

Results and Discussion

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 6). The SIM traces for the byproduct in the samples highlighted in the trending plot are shown in Figure 7. The byproduct of a double aldol condensation eluted at 0.831 minutes in the gradient. The measured peak areas and heights are declining for the educt, while the main product and a small amount of byproduct contamination was rising.

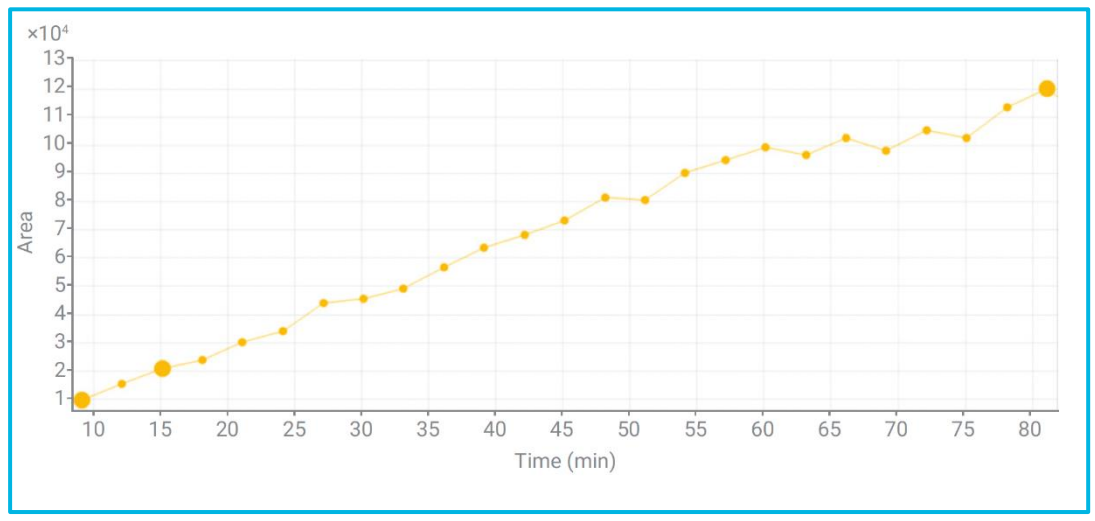

Figure 6. Trending plot of the SIM peak area (295 m/z) for the minor impurity appearing in the course of the aldol reaction (Samples 4, 6, and 28 are highlighted).

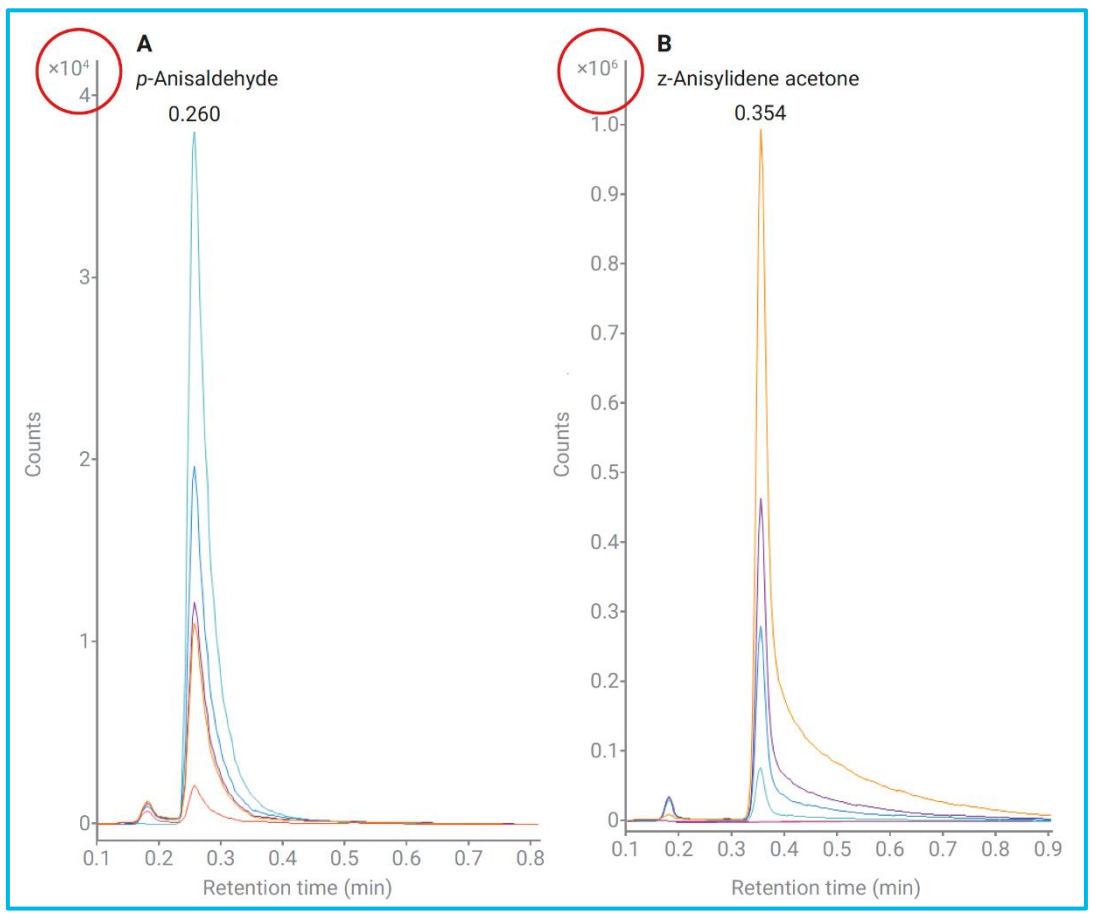

Figure 5. SIM traces during the course of the reaction. (A) Educt p-anisaldehyde (Sample 1: green, Sample 2: blue, Sample 4: purple, Sample 6: red, Sample 28: orange). (B) Product z-anisylidene acetone (Sample 1: red baseline, Sample 2: green, Sample 4: blue, Sample 6: purple, Sample 28: orange).

Conclusions

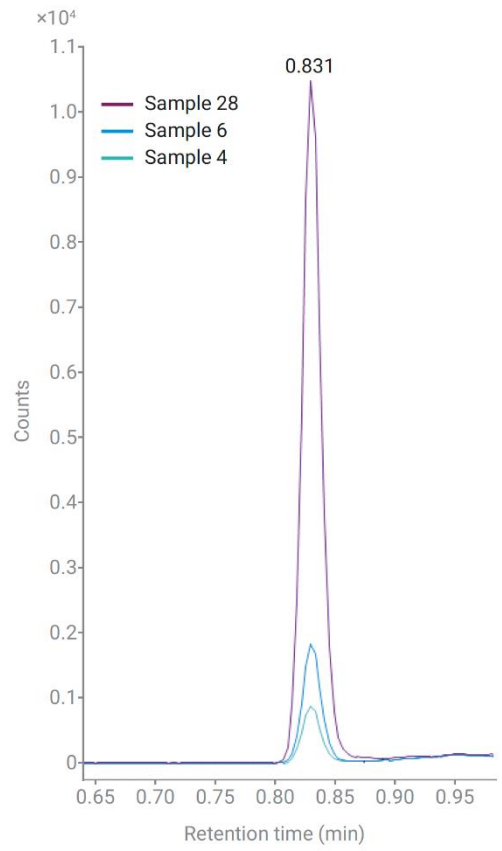

Figure 7. SIM trace of the byproduct appearing from a double aldol condensation (295 m/z).

This application 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.

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