

Real-Time Monitoring of Chemical Reactions Using a Single Quadrupole Mass Spectrometer

Takanari Hattori and Yasuko Shibayama

User Benefits

- ◆ The progress of chemical reactions can be accurately determined by real-time tracking of the mass information of components related to the reactions.
- ◆ Direct injection is performed by flow injection, so the chemical reactions can be continuously monitored at 1-minute intervals.

Introduction

In the chemical field, tracking the reaction process or the reaction speed of synthesis reactions is important for accurately determining what chemical reactions have occurred.

This Application News describes an example of real-time monitoring of chemical reactions using flow injection and a single quadrupole LC-MS. By injecting samples using flow injection, chemical reactions can be continuously monitored at 1-minute intervals. When the chemical reactions are fast and it is necessary to monitor at intervals shorter than 1 minute, the combination of probe electrospray ionization (PESI) and the mass spectrometer introduced in Application News No. eC159 is useful. By combining both methods, the chemical reactions can be monitored in more detail.

Derivatization Reaction with 3-NPH

In some cases when analyzing short-chain fatty acids or organic acids by LC-MS, 3-nitrophenylhydrazine (3-NPH) is used to derivatize the short-chain fatty acids or organic acids in order to increase retention in the ODS column and the sensitivity of MS detection. As shown in Fig. 1, 2-ethylbutyric acid (2-EBA) was derivatized with 3-NPH. With an aqueous solution of 75 % methanol, solutions were prepared with 100 ppm 2-EBA, 1, 5, and 20 mmol/L 3-NPH, 5 mmol/L 1-Ethyl-3-(3-dimethylamino-propyl)carbodiimide (EDC, a condensing agent), and 0.75 % pyridine (a catalyst). To provide the samples for analyses, 50 μ L of these solutions were mixed with 50 μ L of the 75 % methanol aqueous.

Instruments and Analytical Conditions

The analyses were performed using a system combining the Nexera™ series and the LCMS-2050, as shown in Fig. 2. The LCMS-2050 single quadrupole mass spectrometer is compact, but it affords excellent ease of use and performance. Normally, direct injection of samples by flow injection without a column can contaminate the mass spectrometer easily. However, the LCMS-2050 is highly robust, and even if it is contaminated, it is easily maintained, so it is suitable for flow injection analysis.

The analytical conditions are shown in Table 1. 2-EBA, 3-NPH, and products (2-EBA + 3-NPH) were analyzed by SIM mode at m/z 115 (-), 154 (+), and 252 (+), respectively. Other peaks were analyzed by Scan mode (m/z 50-2000).



Fig. 2 Nexera™ and LCMS-2050 System

Table 1 Analytical Conditions

[Flow Injection Conditions] (Nexera XR)	
Flowrate:	0.2 mL/min
Mobile Phase:	Water / Methanol = 50:50
Injection Volume:	1 μ L
[MS Conditions] (LCMS-2050)	
Ionization:	ESI/APCI (DUIS), Positive and Negative mode
Mode:	SIM (m/z 154 (+), 252 (+), 115(-)) or Scan (m/z 50-2000)
Interface Voltage:	+3.0 kV / -2.0 kV
Nebulizing Gas Flow:	2.0 L/min
Drying Gas Flow:	5.0 L/min
Heating Gas Flow:	7.0 L/min
Desolvation Temp.:	450 °C
DL Temp.:	200 °C

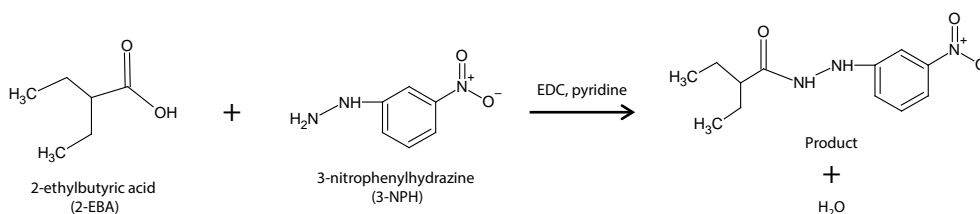


Fig. 1 Derivatization Reaction of 3-nitrophenylhydrazine

Effect of Derivatization Reagent Concentration

The difference in the amount of product and 2-EBA when the concentration of the derivatization reagent 3-NPH was 1, 5, and 20 mmol/L was investigated. Each solution for derivatization and sample was mixed and waited 3 hours before the mixed solutions were analyzed. As shown in Fig. 3, the higher the concentration of 3-NPH the greater the peak area of the product, and conversely the smaller the peak area of 2-EBA. It can be seen that the higher the concentration of the derivatization reagent the more the reaction occurred.

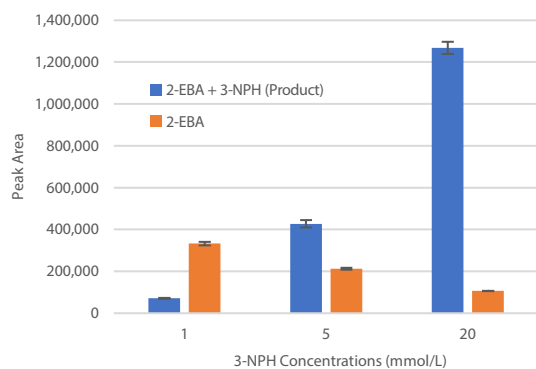


Fig. 3 Effect of 3-NPH Concentrations

Real-Time Monitoring of Reaction Processes

In order to perform real-time monitoring of the derivatization reactions, immediately after the samples were mixed, they were injected into the LC-MS every minute by flow injection, and analysis was performed continuously for 1 hour. The concentration of 3-NPH was 5 mmol/L. As shown in Fig. 4 (A), the peak area of the product increased with the elapsed time. Next, the samples were injected every 1 hour. The monitoring results for 15 hours are shown in Fig. 4 (B). The peak area of the products increased continuously, while conversely the peak area of the 2-EBA decreased continuously, from which it can be seen that the reaction continued for 15 hours.

Fig. 5 shows the mass spectra (positive mode) immediately after mixing the solutions and 20 hours later. Apart from the peak originating from the product of m/z 252, the peaks of m/z 188, 272, and 309 also changed greatly.

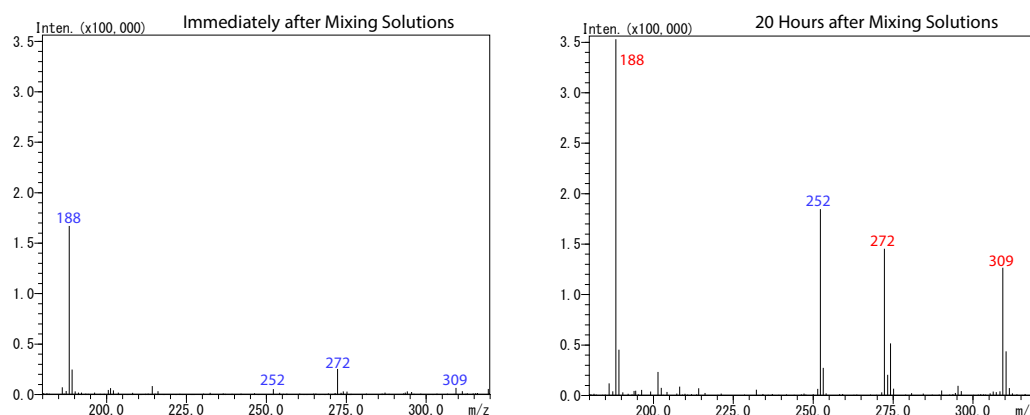


Fig. 5 Comparison of Mass Spectra (Positive Mode) Immediately after Mixing Solutions and 20 Hours after Mixing Solutions

<Related Application News>

1. Real-Time Monitoring of Chemical Reactions [Application News No. C159](#)

Nexera is a trademark of Shimadzu Corporation or its affiliated companies in Japan and/or other countries.

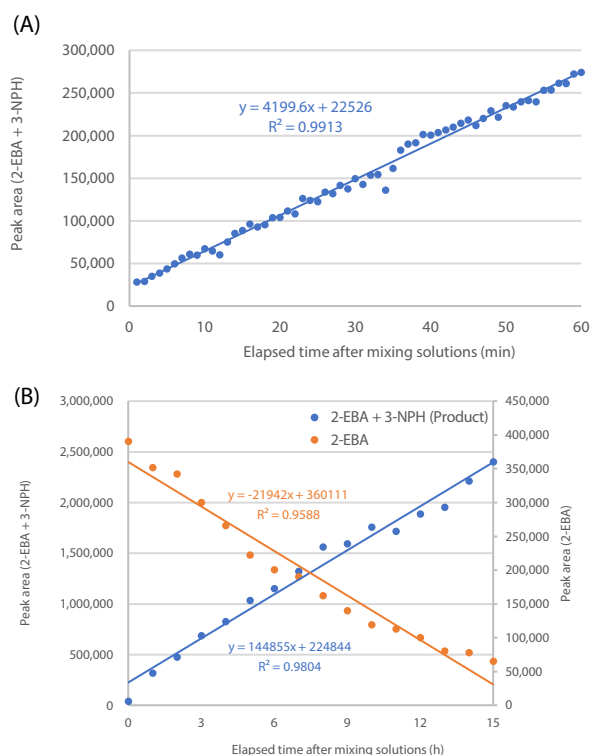


Fig. 4 Change of Derivatization Reaction with Time

Conclusion

This Application News describes an example of real-time monitoring of chemical reactions using a single quadrupole LC-MS. By tracking in real-time the peak areas of components related to the chemical reactions, the progress of the reactions was accurately determined. This method used flow injection analysis with an autosampler, so it can be fully automated for continuous monitoring at 1-minute intervals. In the future, it is expected that the spread of this method will lead to the development of technology and product development in fields such as synthetic chemistry.