Evaluation of improved ESI probe in pesticide analysis using triple quadrupole LC-MS

Takanari Hattori[1], Daiki Miyashiro[1], Yayoi Ichiki[1], Kazuaki Yamaguchi[1], Natsuyo Asano[1], Keisuke Iso[1]

(1) Shimadzu Corporation

1. Introduction

ESI using electrospray is the most widely used ionization technique in LC-MS. In conventional LC-MS analysis, the mobile phase for LC is delivered to MS in the order of several hundred µL/min. However, ESI is difficult to maintain stable electrospray due to the low charge supply efficiency to the liquid. So, a pneumatic nebulizer is widely used to achieve stable electrospray. We improved the pneumatic nebulizer to achieve more stable ionization in ESI. In this study, we report the performance of ESI probe with improved pneumatic nebulizer in pesticide analysis.

2. Sample and pretreatment

The mixed standard solutions of pesticides (PL2005 Pesticides LC/MS Mix I-III, 4-14) was obtained from Hayashi Pure Chemical Ind., Ltd. The black tea was purchased from the supermarket in Kyoto city and filtered by a membrane filter (Millex-GV, 0.22 µm). The mixed standard solutions of pesticides were added to the black tea and diluted 5-fold with MeOH (the final concentration of pesticides was 2 ppb).

3. Analytical conditions

The Nexera™ X3 and LCMS™-8060RX (Fig. 1) was used for pesticide analysis. Table 1 shows the analytivcal conditions of HPLC and MS. The analytical conditions that included in the LC/MS/MS Method Package for Residual Pesticides Ver. 3.01 was used.

Fig. 1 Nexera™ X3, LCMS™-8060RX

Table 1 Analytical conditions for HPLC and MS

[HPLC Conditions] (Nexera X3)	
Column:	Shim-pack Velox™ Biphenyl (2.1 mmI.D. x 100 mmL., 2.7 µm)
Mobile Phase A:	2 mmol/L Ammonium Formate, 0.002% Formic Acid in Water
	2 mmol/L Ammonium Formate, 0.002% Formic Acid in MeOH
Flow Rate:	0.4 mL/min
Injection Volume:	1 µL
Column Temp:	35°C
[MS Conditions] (LCMS-8060RX)	
Ionization:	ESI (Positive and Negative Mode)
Interface Voltage:	1 kV, -1 kV
Mode:	MRM
Nebulizing Gas Flow:	3.0 L/min
Drying Gas Flow:	10.0 L/min
Heating Gas Flow:	10.0 L/min
Interface Temp:	320°C
DL Temp:	150°C
Heat Block Temp:	300°C

4. Result

4-1. Improved ESI probe

The nozzle of the pneumatic nebulizer was improved to achieve more stable ionization. As a result of examining various nozzle shapes, the uniformity of the nebulizer flow was improved by using a coaxial nozzle that improved the concentricity of the mobile phase delivered from HPLC and the nebulizing gas (Fig. 2). Furthermore, the maximum flow rate of nebulizing gas increased from 3 L/min to 7 L/min.

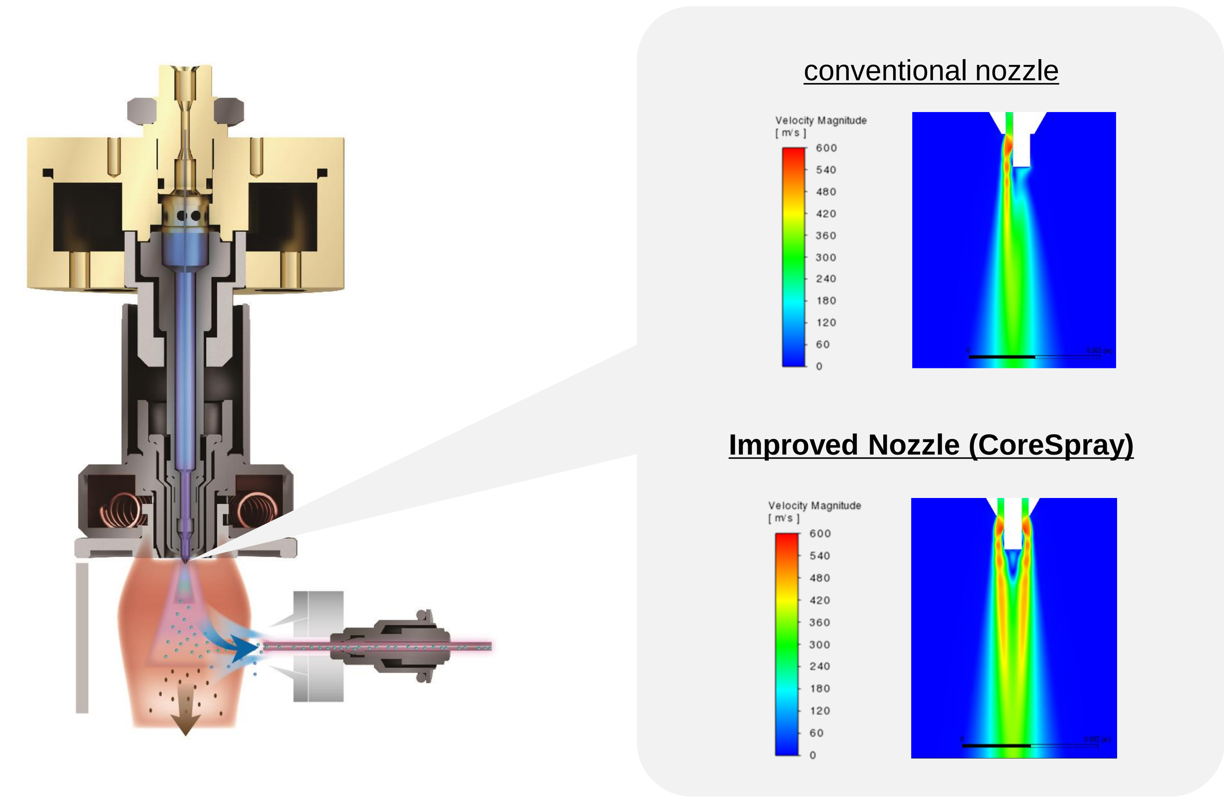

Fig. 2 Improved ESI probe

4-2. Variation due to ESI probes

Six improved ESI probes were prepared, and the variation due to ESI probes was evaluated. A standard solution of reserpine (1 ppb) was injected into the MS by flow injection. Fig. 3 shows the mass chromatograms obtained with different ESI probes. The relative standard deviation (%RSD) of the peak area was 4.7%.

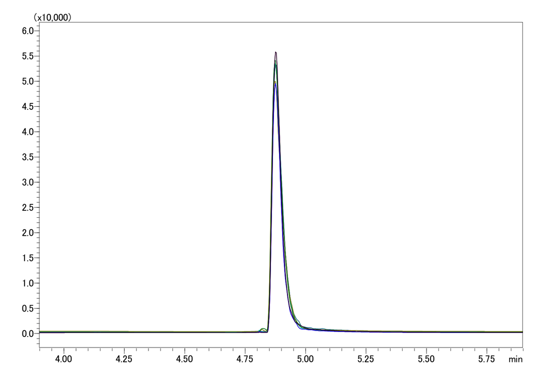

Fig. 3 Mass chromatograms obtained with different ESI probes (n=6)

4-3. Robustness in continuous analysis of high matrix sample

To evaluate the robustness in a short period of time, an analysis combining on-column and flow injection (repetition of on-column analysis and 99 injection by flow injection) was conducted as an accelerated degradation test. The black tea sample spiked with pesticides was injected 20,000 times, and the peak areas of the pesticides detected by on-column analysis were plotted (Fig. 4). The %RSDs of the peak areas of acetamiprid, spinetorum-L, and carbendazim were 2.52%, 2.92%, and 3.26%, respectively. Even in analysis of samples containing a large amount of matrix, such as black tea, there was almost no decrease in sensitivity, and stable analysis was possible.

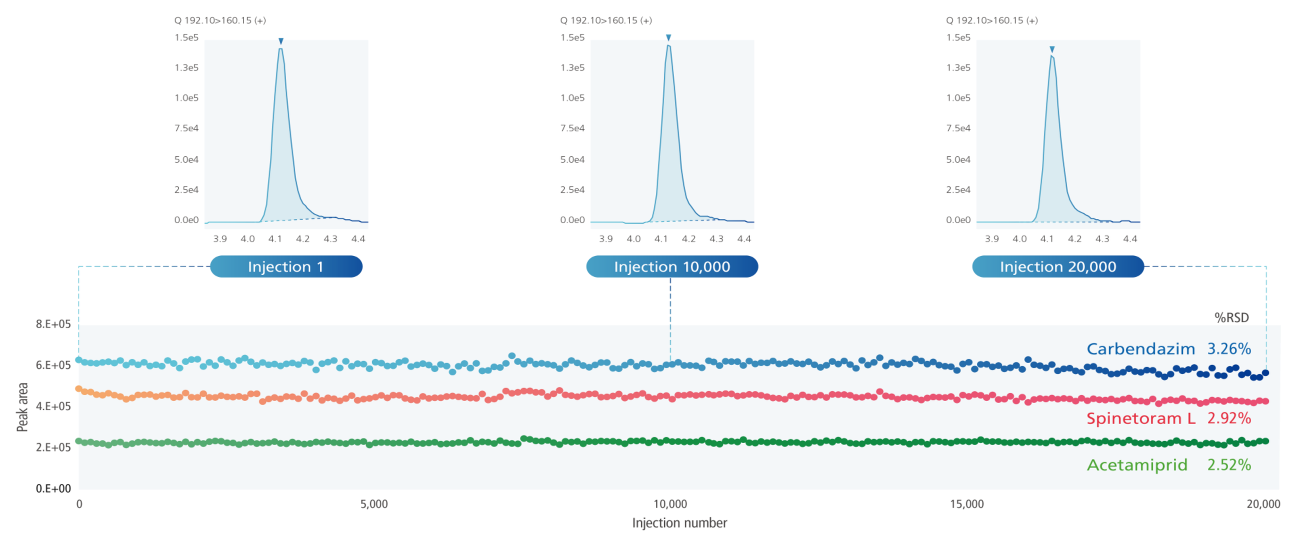

Fig. 4 Peak area of pesticides in continuous analysis

4-4. Flow rate of nebulizing gas

In the simultaneous analysis of pesticides using the conventional ESI probe, the optimal flow rate of nebulizing gas was 3 L/min. The improved ESI probe can flow nebulizing gas up to 7 L/min. So, the effect of increasing the nebulizing gas in pesticide analysis was evaluated. The flow rates of nebulizng gas were set to 3, 5, and 7 L/min. The peak heights of each pesticide were averaged and hierarchical clustering analysis was performed using a Multi-omics Analysis Package (Shimadzu). As shown in Fig. 5, the peak height of most pesticides was maximum at 3 L/min, but the peak height of some pesticides was maximum at 5 L/min or 7 L/min. Fig. 6 shows the mass chromatograms of pesticides whose sensitivity was improved by increasing the flow rate of nebulizing gas. The sensitivity improvement was about 4 times at the maximum.

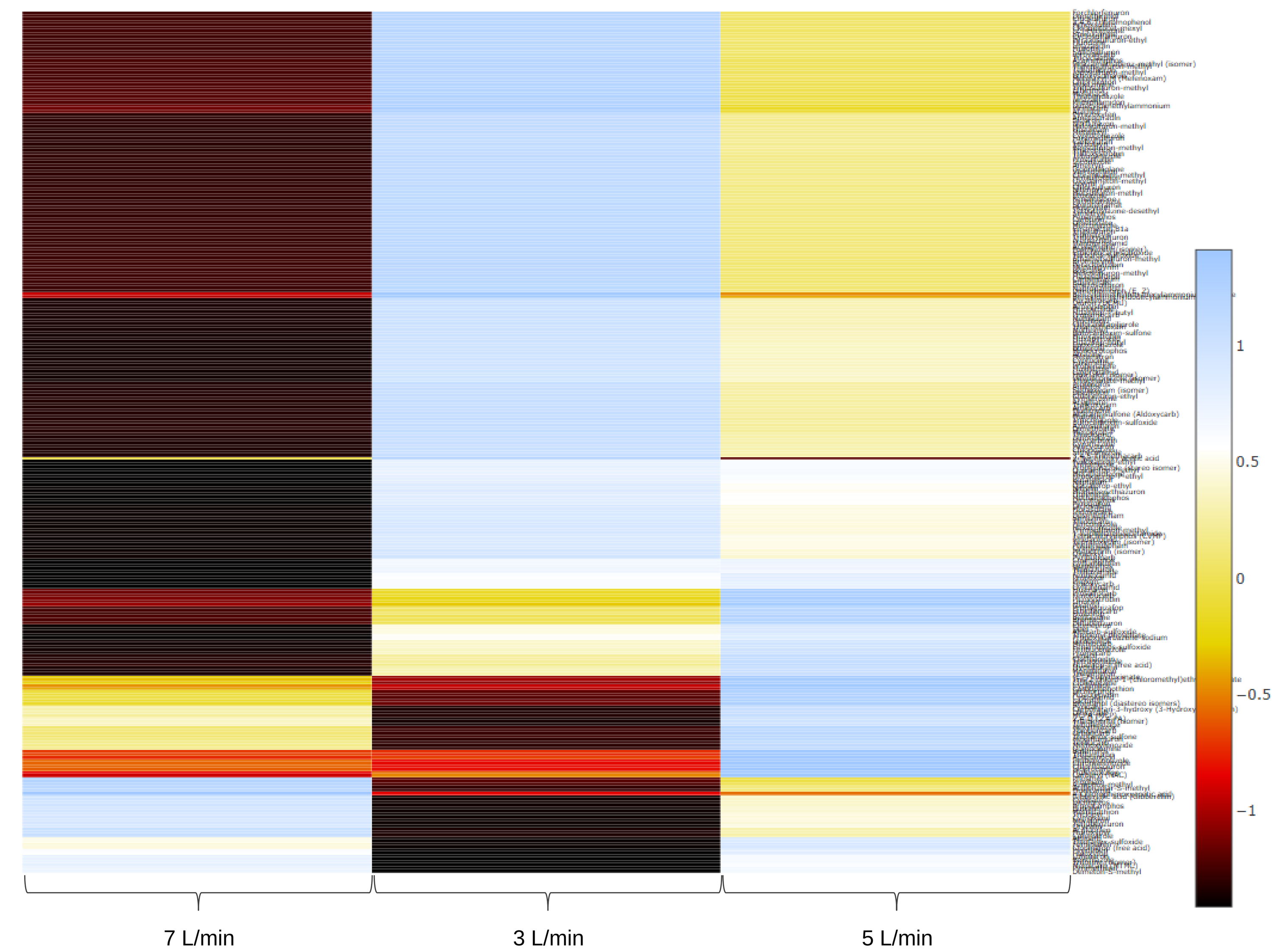

Fig. 5 Effect of nebulizing gas flow on sensitivity

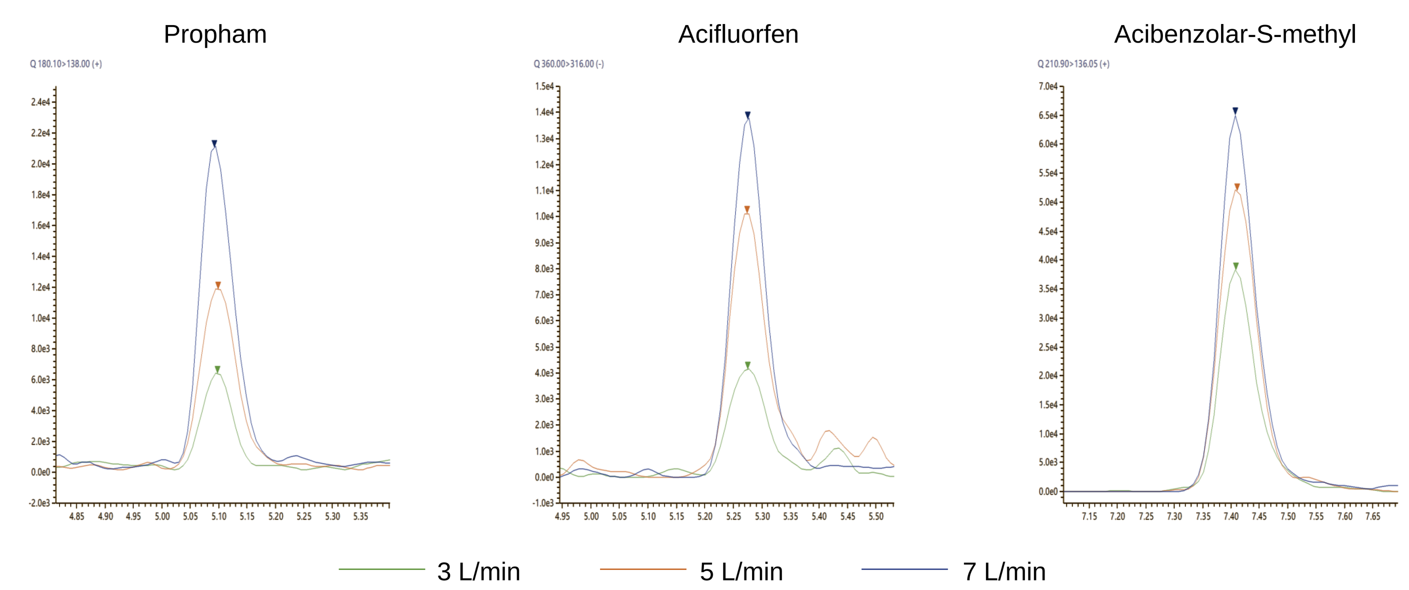

Fig. 6 Mass chromatograms of pesticides that sensitivity was improved

5. Conclusions

The performance of the improved ESI probe in pesticide analysis was evaluated. As a result, it was found that the improved ESI probe had little variation due to differences of ESI prove, and almost no sensitivity degradation was observed even for black tea samples containing a large amount of matrix, making stable analysis possible.The sensitivity of some pesticides was improved by increasing the flow rate of nebulizing gas. The improved ESI probe is expected to improve the sensitivity of compounds that are difficult to improve the sensitivity using the conventional ESI probe.