

Efficient introduction of ionic compounds into LC-MS using the improved ESI unit; Application to dirty samples

Takanari Hattori, Miho Kawashima, Wataru Fukui, Kazuo Mukaibatake

Shimadzu Corporation. 1, Nishinokyo-Kuwabaracho Nakagyo-ku, Kyoto 604-8511, Japan

1. Overview

A Newly-developed ionization unit, IonFocus Unit introduces ions into the mass spectrometer more efficiently, while expelling contaminants. To evaluate this unit, we conducted two analyses of samples with complicated matrices.

2. Introduction

In the ESI (Electrospray Ionization), which is widely used as an ionization method for LC/MS, the sample in a mobile phase is sprayed by applying a high voltage and then it is dried and ionized. At this time, if not only ions but also contaminants enter the MS, they will contaminate the ion guides and lead to a decrease in sensitivity. They also may be one of the causes of the matrix effect. To prevent these issues, if we set the position of the ion spray far from the inlet of the MS, the sampling efficiency of the target ions is also decrease. This means that there is a trade-off between sensitivity and robustness. In this poster, we show one of the solution for this trade-off.

3. Materials and Methods

3-1. IonFocus Unit

To improve the trade-off between sensitivity and robustness, we have newly developed an IonFocus Unit (Figure 1). In this ionization unit, two electrodes (focus electrodes) were attached to create a potential difference. The focus electrodes introduce only ions into the MS with greater efficiency while expelling contaminants. Therefore, the IonFocus Unit keeps the sensitivity even if the ion spray away from the inlet of the MS to decrease the matrix effect and increase the robustness.

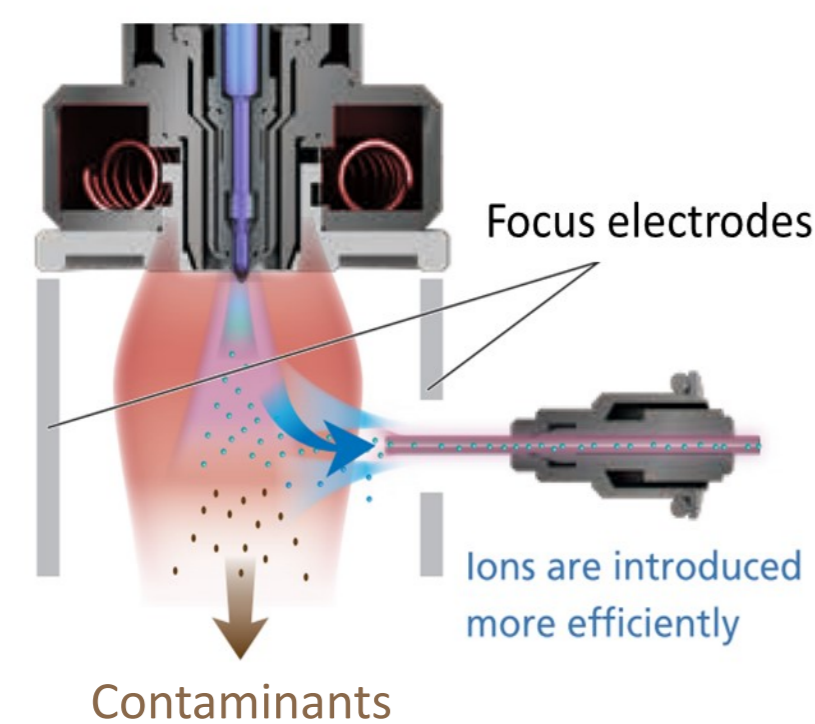


Figure 1 The concept of the IonFocus Unit

3-2. Sample Preparation

Tea Leaf

As sample for the evaluation of sensitivity and recovery, the extract of tea leaf was used. Figure 2 shows the process flow of sample preparation.

1. Add 20 mL water to 5 g tea leaf powder in 100 mL bottle (A)
2. Shake the bottle (A) by hands and wait for 30 min
3. Add 50 mL acetonitrile to the bottle (A)
4. Mix the bottle (A) by shaker at 260 rpm for 30 min
5. Transfer acetonitrile layer in the bottle (A) to 100mL volumetric flask (B)
6. Add 20 mL acetonitrile to the bottle (A)
7. Mix the bottle (A) by shaker at 260 rpm for 30 min
8. Filter all the solution and the sample residue in the bottle (A) into volumetric flask (B) and add extra 10mL acetonitrile via the filter
9. Add acetonitrile to the volumetric flask (B) (to 100 mL)
10. Take 5 mL solution in the volumetric flask (B)
11. Dry the 5 mL solution by vacuum concentrator
12. Reconstitute with 2.5 mL methanol
13. Filter (0.2 μm membrane filter)

Figure 2 Process flow of sample preparation

Urine

As sample for the evaluation of robustness, the urine provided by a healthy man was used. The urine was centrifuged at 14,000 g for 15 minutes. The supernatant was diluted 2-fold with water.

3-3. Analytical Conditions

Analysis of Pesticides in Tea Leaf Extract

UHPLC (Nexera X2™ system)

Column: Shim-pack Velox Biphenyl (100 mmL. × 2.1 mmI.D., 2.7 μm)
 Mobile phase A: 2 mmol/L Ammonium formate, 0.002% Formate/water
 B: 2 mmol/L Ammonium formate, 0.002% Formate/methanol
 Flow rate: 0.40 mL/min
 Time program: B conc. 3% (0.00 min) → 10% (1.00 min) → 55% (3.00 min) → 100% (10.50-12.00 min) → 3% (12.01-15.00 min)

Injection vol.: 1 μL
 Column temp.: 40°C

MS (LCMS-8060NX)

Ionization: IonFocus (ESI, Positive/Negative, MRM mode)
 DL temp.: 150°C HB temp.: 300°C
 Interface temp.: 350°C Nebulizing gas: 3.0 L/min
 Drying gas: 10 L/min Focus voltage: ±4 kV
 Heating gas: 10 L/min

Analysis of Metabolites in Urine

UHPLC (Nexera X2™ system)

Mobile phase A: Water
 B: Acetonitrile * B conc. 70%
 Flow rate: 0.40 mL/min
 Injection vol.: 10 μL

MS (LCMS-8060NX)

Ionization: IonFocus (ESI, Positive)
 DL temp.: 250°C HB temp.: 400°C
 Interface temp.: 400°C Nebulizing gas: 3.0 L/min
 Drying gas: 3 L/min Focus voltage: 4.5 kV
 Heating gas: 15 L/min MRM: m/z 348.00 > 136.05



Figure 3 LCMS-8060NX with the IonFocus Unit

4. Result

4-1. Evaluation of the Sensitivity and Recovery

Before the evaluation of the IonFocus Unit, the relationship among sensitivity, recovery, and ion spray position (the distance between ESI capillary and MS inlet) was investigated using normal ESI unit. The tea leaf extract spiked 100 pesticides (0.02 ppm) was used as a sample. Figure 4 shows the relationship among sensitivity, recovery rate, and ion spray position. As results of the investigations, the average peak intensity of pesticides was highest at 0 mm of ion spray position. Compared to 0 mm of ion spray position, the average peak intensity decreased by 17.4%, 42.3%, and 86.5% at +1 mm, +3 mm, and +5 mm, respectively.

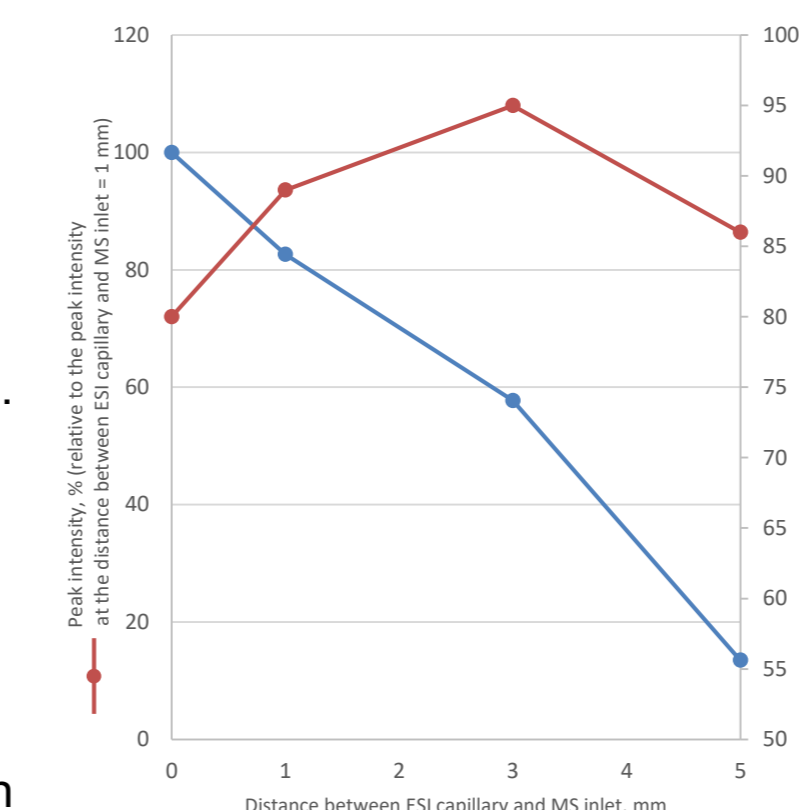


Figure 4 The relationship of sensitivity and recovery for position of the ion spray

It is considered that the amount of ions taken into MS decreases as the position of ion spray is far from the MS inlet. When ion spray position was +3 mm, 95% of pesticides showed good recovery (recovery rates: 70-120%). However, the closer the ion spray position was, the worse the recovery rate was. 89% and 80% of pesticides showed recovery rates between 70-120% at +1 mm and 0 mm of ion spray position, respectively. It is considered that the closer the ion spray distance is to the MS inlet, the more the sample matrix is affected.

Next, the evaluation of IonFocus Unit was performed. When the ion spray position was +3 mm, the average peak intensity was improved 1.5 times (max 3.9 times improvement). Figure 5 shows some of MS chromatogram. It is considered that the IonFocus Unit introduced ions into MS more efficiently, improving the signal intensity. Compared to the best condition for sensitivity (0 mm of ion spray position using normal ESI unit), the average peak height was decreased by only 13.4%.

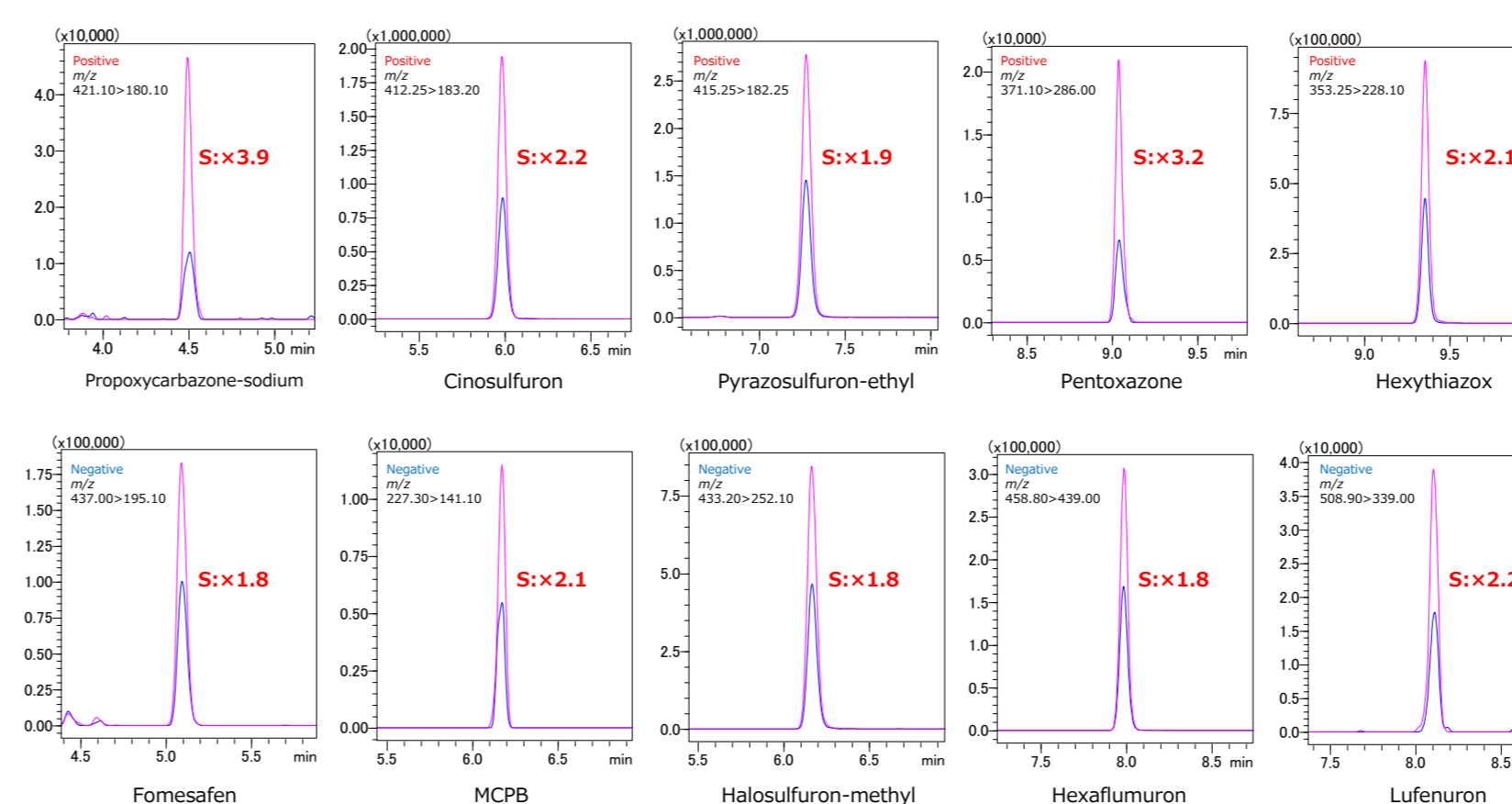


Figure 5 MS chromatograms of pesticides

Figure 6 shows distribution of recovery rates with IonFocus Unit. 96% of pesticides were recovered properly within a range of 70-120%. Therefore, it was suggested that both sensitivity and recovery could be compatible using IonFocus Unit.

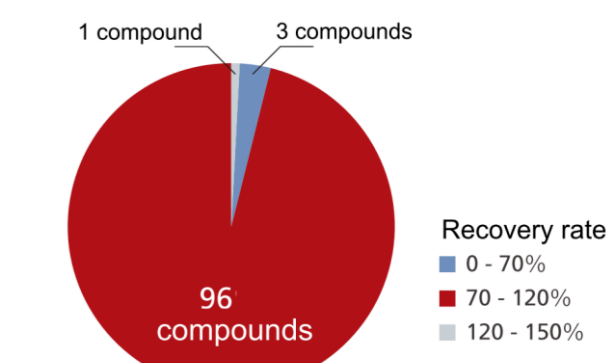


Figure 6 Distribution of recovery rates

4-2. Evaluation of Robustness

As explained before, the IonFocus Unit enables us to set the probe away from the MS inlet to improve robustness, while maintaining enough sensitivity. For the evaluation of robustness, urine sample diluted only with water was analyzed 1,000 times continuously (10 μL/injection). The results of stability study is shown in Figure 7. Even the continuous analysis of samples with complicated matrices was performed, the LCMS-8060NX with the IonFocus Unit showed superior data stability (%RSD 8.4%) without any loss of sensitivity.

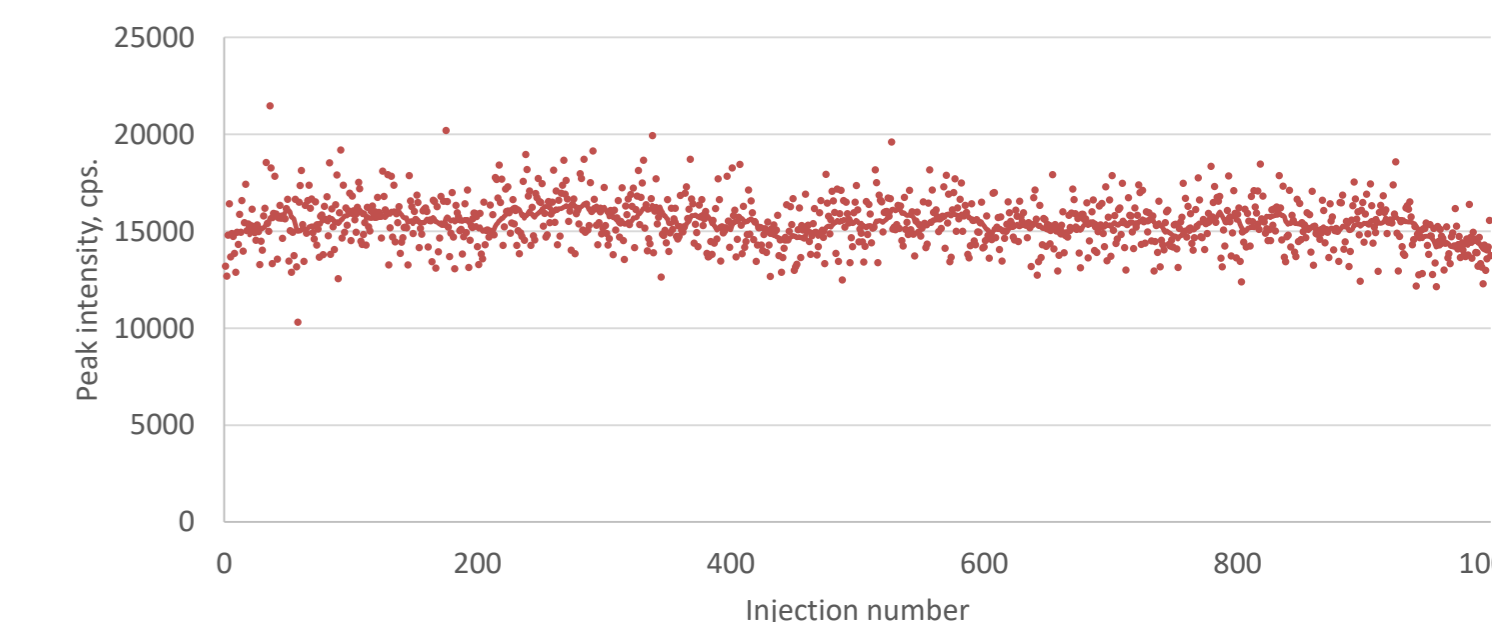


Figure 7 Peak intensity of endogenous compound in continuous analysis of urine

The pictures of the IonFocus Unit after the continuous analysis are shown in Figure 8. Although the inside of its chamber including the focus electrodes looked quite dirty, the intensity maintained and no severe accumulation of sample matrix residue at the MS inlet was observed.

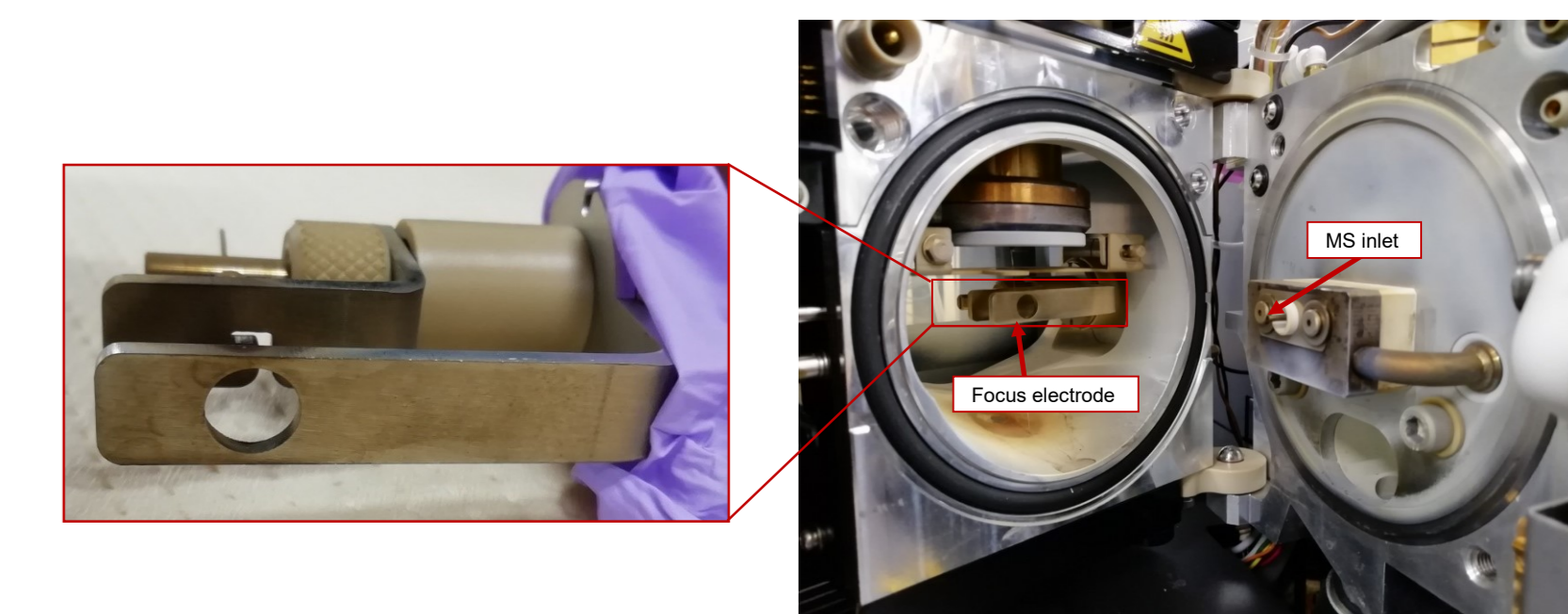


Figure 8 Ion source after 1,000 injections performed

5. Conclusions

- A Newly-developed ionization unit, IonFocus Unit enabled highly sensitive analysis with good recovery.
- It is possible to extend the use of the instrument over prolonged duration due to reduction of accumulation of material on the MS inlet even on the analysis of the sample with complicated matrices such as urine and crops.