

Direct Coupling of Semi-micro Liquid Chromatography and Sonic Spray Ionization Mass Spectrometry for Pesticide Analysis

Yukiko Hirabayashi*, Yasuaki Takada, Atsumu Hirabayashi, Minoru Sakairi, and Hideaki Koizumi
 Central Research Laboratory, Hitachi, Ltd., 1-280 Higashi-koigakubo, Kokubunji-shi, Tokyo 185, Japan.

Direct coupling of semi-micro liquid chromatography and sonic spray ionization mass spectrometry is demonstrated and shown to be a very powerful technique for pesticide analysis. Direct coupling is achieved by using a high flow rate of nitrogen gas in sonic spray ionization and a high operating temperature in the sampling orifice of the atmospheric ionization mass spectrometer. The detection limit for simazine is about 300 fmol based on quantitative analysis in the range between 1 pmol and 1 nmol. A mixture of three pesticides was successfully analyzed using semi-micro liquid chromatography/mass spectrometry.

Analysis of environmental-polluting compounds in solution is becoming more important in the field of analytical science. For example, the concentrations of pesticides in drinking water have recently come under regulation in Japan. With the rapidly growing numbers of items that must be monitored, tools that can analyze mixtures with high sensitivity are becoming more important. Liquid chromatography/mass spectrometry (LC/MS) is one such tool for analyzing mixtures in a solution. In the combination of liquid-phase separation techniques with mass spectrometry, the interface between LC and MS is very important; in this respect, atmospheric pressure ionization (API)^{1–7} has become a standard LC/MS interface.

We previously developed a sonic spray ionization (SSI) interface for LC/MS.^{8,9} In this method, compounds are ionized by nebulizing sample solutions using only a high-speed gas flow at room temperature. However, the solution flow rate ($\sim 30 \mu\text{L}/\text{min}$) was too low for use with semi-micro LC. One way to achieve compatibility is to use a splitter to control the flow rate into the ion source. Direct coupling is a better approach because the system is easier to operate and maintains higher separation efficiency.

In this paper, we describe new operating conditions for SSI that enable direct coupling of semi-micro LC with SSI-MS and then present the results for pesticide mixture analysis using semi-micro LC/SSI-MS.

EXPERIMENTAL

Figure 1 shows a cross-sectional view of the sonic spray ion source. The details have been described elsewhere.^{8,9} For the measurement of spectra, solution is pumped through the

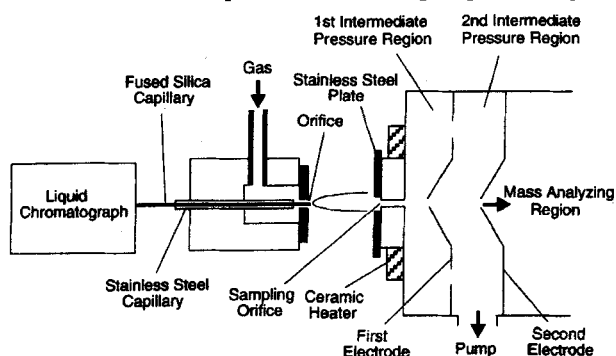


Figure 1. Cross-sectional view of sonic spray interface.

* Author for correspondence.

fused-silica capillary (0.1 mm i.d., 0.2 mm o.d.) at a flow rate ranging from 100 to 200 $\mu\text{L}/\text{min}$. The end of the capillary extends 0.2 mm beyond the duralumin orifice (0.4 mm i.d.). The center axes of the fused-silica capillary and the orifice are aligned. The potential of the ion source is set to ground. Nitrogen gas flows through the orifice into the atmosphere. The flow rate of the nitrogen gas in the standard state, as determined with a mass-flow controller (5850E, Brooks Instrument, Tokyo), is 6 L/min. A spray is thereby generated in which droplets and free ions are produced.

For the LC/MS measurements, the sample mixture was separated with a semi-micro LC column (TSK-GEL PTH PAK, TOSOH, Tokyo, Japan). The mobile phase was pumped by a Hitachi L-6200 LC pump into the fused-silica capillary at a flow rate of 200 $\mu\text{L}/\text{min}$. The mobile phase was water+methanol (60/40) for the first ten minutes, then a linear gradient from 60% water+40% methanol to 100% methanol over ten minutes was used for separation.

The distance between the fused-silica capillary tip of the ion source and the sampling orifice of the mass spectrometer is 3 mm. The sampling orifice (0.25 mm i.d., 25 mm long) of the mass spectrometer was heated with a ceramic heater (50 W) to 120–130 °C and covered with a stainless steel plate with a 2 mm aperture to avoid cooling of the sampling orifice due to gas flow and droplet evaporation. As shown in Fig. 1, ions produced at atmospheric pressure pass into the first intermediate-pressure region through the sampling orifice. The ions then pass into the second intermediate-pressure region through a 0.5 mm aperture in the first electrode. Finally, the ions pass into the mass analyzing region through a 0.2 mm aperture in the second electrode. A drift voltage of 10 V was applied between the sampling orifice and the first electrode, and another drift voltage of 30 V was applied between the first and second electrodes.

RESULTS AND DISCUSSION

Under our original operating conditions,⁹ intense analyte ions were obtained from the mass spectrometer at a sample solution flow rate of approximately 30 $\mu\text{L}/\text{min}$. At flow rates ranging from 100 to 200 $\mu\text{L}/\text{min}$, however, intense ions could not be obtained. Therefore direct coupling of semi-micro LC and SSI-MS could not be achieved. Apparently, the charged droplets produced by the SSI at high solution flow rates could not be efficiently evaporated⁹ by the use of a nitrogen gas flow rate of about 3 L/min and

a sampling orifice which was held at a relatively low temperature.¹⁰ Therefore, we have investigated settings of the nitrogen gas-flow rate and the sampling orifice temperature that will enable direct coupling of semi-micro LC with SSI-MS.

The amount of heat transferred to a droplet in the sampling orifice, ΔQ , is described by the following equation:

$$\Delta Q = \alpha \cdot S \cdot \Delta T \cdot \Delta \tau \quad (1)$$

In Eqn (1), α , S , ΔT , and $\Delta \tau$ are the coefficient of heat transfer, the surface area of the droplet, the temperature difference between the atmosphere and the droplet, and the time for the droplet to pass through the sampling orifice, respectively. Since we use a long sampling orifice (25 mm), $\Delta \tau$ is considered to be sufficiently long for evaporation of the droplet. Therefore, we assumed that the parameters ΔT and α are most important for efficient evaporation.

Empirically, the temperature in the 25 mm long sampling orifice needed to evaporate the droplets is about 120 °C.⁹ However, the sampling orifice is quickly cooled in the SSI method due to evaporation of droplets from the solution and the high-speed gas flow. We therefore measured the temperature change in the sampling orifice at a 200 $\mu\text{L}/\text{min}$ solution flow rate. As shown in Fig. 2, 40 min. after starting the gas flow, the temperature of the sampling orifice, heated with 30 and 50 W ceramic heaters, was below 80 °C and about 120 °C, respectively. It is therefore necessary to heat the sampling orifice with a 50 W ceramic heater to maintain the required temperature of 120 °C for semi-micro LC.

We estimated a suitable gas flow rate for nebulization of a solution of 50% water+50% methanol at a flow rate of 200 $\mu\text{L}/\text{min}$ as follows. The saturated vapor pressure of

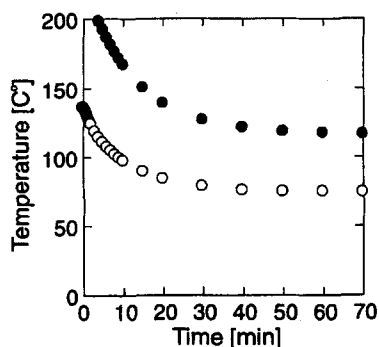


Figure 2. Temperature changes in sampling orifice heated with 30 W (○) and 50 W (●) ceramic heaters at 200 $\mu\text{L}/\text{min}$ solution flow rate and 3 L/min gas flow rate.

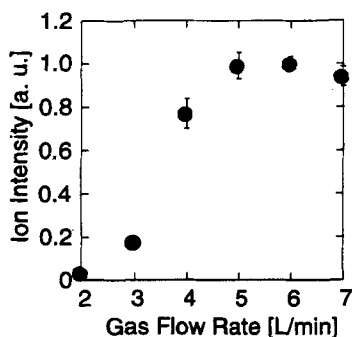


Figure 3. Relation between ion intensity of the protonated simazine molecule (m/z 202) (introduced at 200 $\mu\text{L}/\text{min}$ solution flow rate) and the nitrogen gas flow rate. Simazine concentration was 1 μM .

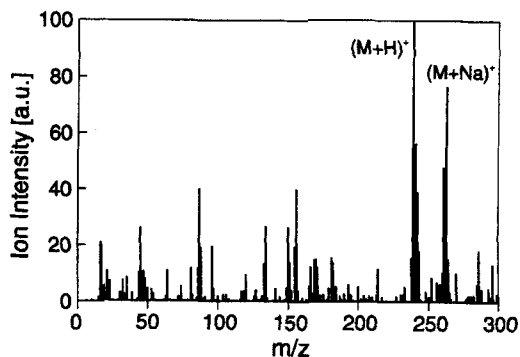


Figure 4. Mass spectrum obtained from a 50:50 methanol+water solution of thiuram by the sonic spray ionization method. The solution concentration was 10 μM .

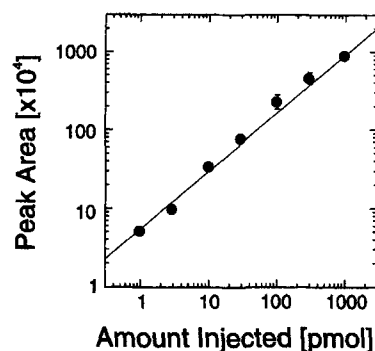


Figure 5. Calibration curve for the protonated simazine molecule (m/z 202).

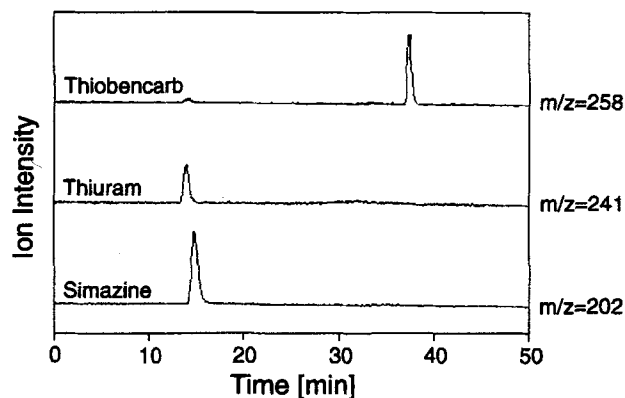


Figure 6. On-line LC/MS analysis of a three-pesticide mixture (simazine, thiuram and thiobencarb). Each component represents 50 pmol.

water at 20 °C is approximately 180 torr, which is 2.3% of atmospheric pressure. When water flowing at 100 $\mu\text{L}/\text{min}$ is completely evaporated, the resultant flow of water vapour is about 0.12 L/min. To keep the partial pressure of the water vapour below the value of the saturated vapor pressure, the nebulizing gas volume must then be more than 5.2 L/min. On the other hand, the saturated vapor pressure of methanol at 20 °C is about 100 torr, so the required nebulizing gas flow rate for methanol is less than 0.5 L/min, derived in the same manner as for water. This SSI ion source thus requires a total flow of nebulizing gas in excess of 5.7 L/min for water+methanol at a 200 $\mu\text{L}/\text{min}$ solution flow rate.

Figure 3 shows the relation between the ion intensity of the protonated simazine molecule (m/z 202), a regulated pesticide, and the nebulizing gas flow rate with a 200 $\mu\text{L}/\text{min}$ solution flow rate. In this experiment, in which the solvent was 50% water+50% methanol and the sampling

orifice was heated with a 50 W ceramic heater, the ion intensity reached a maximum at a gas flow rate of 5–6 L/min. This agrees with our simple approximation. Therefore, a gas flow rate of about 6 L/min is suitable for performing LC/MS at the liquid flow rate needed for semi-micro LC/SSI-MS.

We choose thiuram as a model compound because it is a typical thermolabile compound and it is difficult to analyze using a gas chromatograph (GC) or by GC/MS techniques. A typical mass spectrum of thiuram as measured using the SSI-MS interface is shown in Fig. 4. The thiuram solution was made up 50:50 methanol+water at a concentration of 10 μM , the gas flow rate was 6 L/min, and the solution flow rate was 100 $\mu\text{L}/\text{min}$. Protonated and sodiated molecules of thiuram were detected at $m/z=241$ and 263, respectively. The random noise in the spectrum was caused by the detection of charged droplets produced at atmospheric pressure whose m/z values were too high to be affected by the electric field of the quadrupole mass spectrometer.

We measured the dynamic range for quantitative analyses using SSI-MS under these new operating conditions. The calibration curve for simazine is shown in Fig. 5. We repeatedly injected 10 μL sample solutions with different concentrations without a column; the protonated molecules ($m/z=202$) were detected in the selected-ion monitoring (SIM) mode. The flow rate of the mobile phase (water+methanol, 50%:50%) was set at 100 $\mu\text{L}/\text{min}$. Good linearity was obtained in the range between 1 pmol and 1 nmol. Based on these data, we estimate the detection limit for simazine, at which the S/N ratio is 2, to be about 300 fmol. Thus, this SSI method is sensitive enough for the quantitative determination of simazine.

Figure 6 shows the analysis result from a mixture of pesticides using semi-micro LC/SSI-MS with multiple-ion detection (MID). Simazine, thiuram, and thiobencarb were used in this measurement and each injected component was 50 pmol. To separate these compounds, the mobile phase

was 60% water+40% methanol for the first ten minutes, followed by a linear gradient from 60% water+40% methanol to 100% methanol over ten minutes. The flow rate of the mobile phase was set at 200 $\mu\text{L}/\text{min}$. Since intense protonated molecules can be obtained for these compounds, we monitored these ions at m/z values of 202, 241, and 258 for simazine, thiuram, and thiobencarb, respectively. Peaks at each m/z value are clearly observed at retention times of 15, 14, and 37 min, respectively. A smaller peak corresponding to protonated molecules of thiuram combined with water molecules ($m/z=258$) is also observed at 14 min.

These results show that semi-micro LC/SSI-MS is a very powerful tool for pesticide analysis.

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