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Determination of paraquat and diquat by liquid chromatography–thermospray mass spectrometry

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ABSTRACT

Thermospray liquid chromatography–mass spectrometry can be used for identifying and determining both paraquat ($C_{12}H_{14}N_2^{2+}$) and diquat ($C_{12}H_{12}N_2^{2+}$). Reversed-phase liquid chromatography was performed using a 15-cm Shim-pack CLC-ODS column, with methanol–water (80:20) + 0.1 *M* ammonium acetate (adjusted to pH 5 with trifluoroacetic acid) as buffers, at a flow-rate of 1.0 ml/min. The mass spectral sensitivity was best when the temperatures of the vaporizer, block and tip heater of the ion source block were set at 160, 310 and 320°C, respectively. When thermospray ionization was used, ions of *m/z* 186 and 183 were obtained as base peaks for paraquat and diquat, respectively. Detection limits by selected ion monitoring were of the order of 20 ng (S/N = 3.5). The mass spectra are influenced by temperature and therefore, precise temperature control is essential.

INTRODUCTION

Paraquat and diquat can be determined by methods such as spectrophotometry [1–5], gas chromatography [6–10], high-performance liquid chromatography [11–19], gas chromatograph–mass spectrometry (GC–MS) [20,21] and fast atom bombardment (FAB) MS [22]. However, these methods, with the exception of MS [5,6,22] and GC–MS, do not give a sufficiently complete identification of unknown compounds.

Recently, thermospray liquid chromatography–mass spectrometry (LC–TSP-MS) has been used in analysis for drugs, lipids, nucleotides, steroids and carbohydrates. In this work, the identification and determination of paraquat and diquat using this method was attempted. These herbicides could be detected without the need for derivatization.

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EXPERIMENTAL

A liquid chromatograph–tandem quadrupole mass spectrometer (Shimadzu LC-MS QP1000EX) equipped with a Vestec thermospray interface was used for recording mass spectra and selected ion monitoring. The column used was a 15 cm × 4.6 mm I.D. stainless-steel tube packed with a totally porous matrix, prepared by chemically binding octadecyl groups to the surface of spherical silica particles [Shim-pack CLC-ODS (Shimadzu), particle diameter 5 μm, pore diameter 100 Å]. The mobile phase was methanol–water (80:20, v/v) containing 0.1 *M* ammonium acetate and adjusted to pH 5 with trifluoroacetic acid (TFA). This was injected by a Shimadzu LC-9A pump at a flow-rate of 1.0 ml/min. Samples were injected using a Rheodyne Model 7125 injector fitted with a 100-μl loop or with a Shimadzu SIL-7A autoinjector.

The exit temperature of the vaporizer was 140–180°C . The block and tip heater temperature of the ion source block was 270–310°C. Positive-ion thermospray mass spectra were obtained using the thermospray ionization mode or the thermospray on

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filament ionization mode (filament-on ionization mode). Typical conditions for thermospray MS were a scan range of m/z 135–600 in 1 s and electron multiplier voltage 1400 V.

RESULTS AND DISCUSSION

Temperature of the ion source block and vaporizer

The areas of the fragment ions and the molecular ions change depending on the temperatures of the vaporizer and the ion source block. The temperature of the latter consists of the block and the tip heater temperatures, and these were examined by the use of selected ion monitoring. For this examination, paraquat was used owing to its lower detection sensitivity than diquat. In this context, the optimum operating conditions for paraquat were investigated.

Both the thermospray mode and filament-on mode methods of ionization were similar with respect to the vaporizer temperature change (Fig. 1). The areas of the fragment ions and the molecular ions were maximum when the vaporizer temperature was 161°C. The area of the ions measured using the thermospray ionization mode was greater than that with the filament-on ionization mode. Further, a high detection sensitivity was obtained when the vaporizer temperature was 161°C in the thermospray ionization mode.

The relationship between the temperature of the ion source block and the paraquat ion area is shown in Fig. 2. The ion area tended to increase with increase in temperature. The thermospray ionization mode was the best ionization method. However, the temperature there must be kept constant so that the ionization is controlled by the ion source block temperature, which means that for every temperature change the detection sensitivity was changed. Therefore, the temperature of the tip heater was set higher than that of the block in order to compensate for any temperature change during flow-rate changes of the mobile phase.

Based on the above, the temperatures giving maximum ion intensities were 161°C for the vaporizer, 310°C for the block and 320°C for the tip heater.

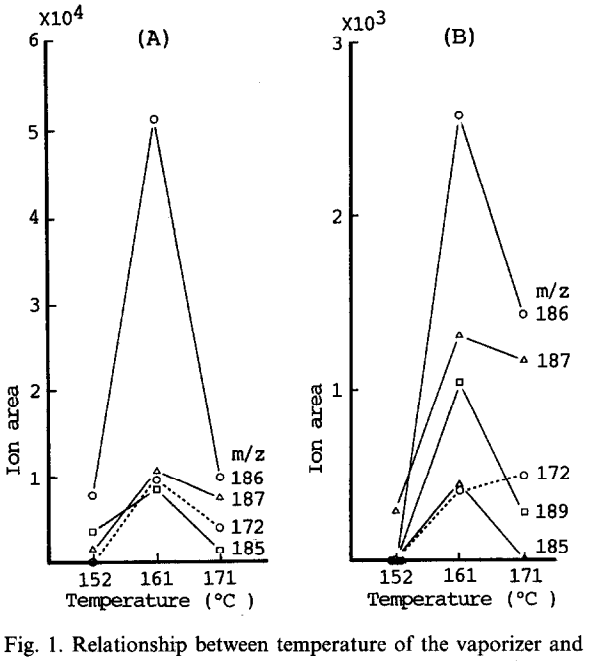

Fig. 1. Relationship between temperature of the vaporizer and ion area. A 200-ng paraquat sample was injected; single determination by selected ion monitoring. The temperatures of the block and tip heater were both 310°C. (A) Thermospray ionization mode; (B) filament-on ionization mode. Ion area represents peak area.

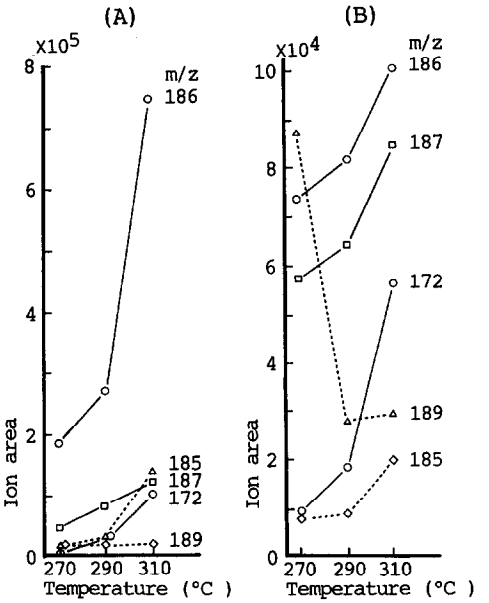

Fig. 2. Relationship between temperature of the ion source block and ion area. Temperature of the ion source block consists of the block and tip heater temperatures. A 200-ng paraquat sample was injected; single determination by selected ion monitoring. The temperature of the vaporizer was 161°C. (A) Thermospray ionization mode; (B) filament-on ionization mode. Ion area represents peak area.

Mass spectrum and mass chromatogram

Mass spectra in the thermospray ionization mode of paraquat and diquat are shown in Fig. 3. Ions of *m/z* 186 and 183 were obtained as base peaks for paraquat and diquat, respectively, under thermospray ionization conditions. The ions obtained in the mass spectrum of paraquat using the thermospray ionization mode were similar to those obtained by FAB-MS [22], whereas the ions for diquat were different from those obtained by FAB-MS. Mass chromatograms of paraquat and diquat obtained in the analysis of a standard mixture are illustrated in Fig. 4. Peaks representing paraquat were seen at *m/z* 186 and 171 at a retention time of 5.5 min and for diquat at *m/z* 183, 184 and 157 at a retention time of 4.8 min. The amounts injected to obtain the data in Figs. 3 and 4 were 200 ng each of paraquat and diquat. Hence, LC–TSP-MS analysis enabled paraquat and diquat to be identified.

However, the relative intensities of the fragment ions of paraquat in the mass spectra differed according to the scan number. This may be due to the peak of *m/z* 171 from the mass chromatogram, which was flat compared with that of *m/z* 186. Hence the mass spectrum of paraquat was obtained where the scan number indicated the peak top of the *m/z* 186 ion in the mass chromatogram.

In the filament-on ionization mode, the mass spectrum of paraquat was very similar to that obtained using the thermospray ionization mode. However, the intensity of the *m/z* 184 molecular ion of diquat was increased, so that the mass spectrum of diquat changed slightly. As filament-on ionization is similar to a chemical ionization process, the base peak of diquat in this mode was obtained at *m/z* 184, but the fragment ions were similar to those in the thermospray ionization mode.

We therefore decided to use both ionization modes because of their accuracy. Paraquat and diquat did not interfere with each other so that for quantification the fragment ions and the molecular ions of both were used.

Calibration

Typical calibration graphs for paraquat and diquat with selected ion monitoring, using the thermospray ionization mode, are shown in Fig. 5. The graphs are linear over the range 30–500 ng. When

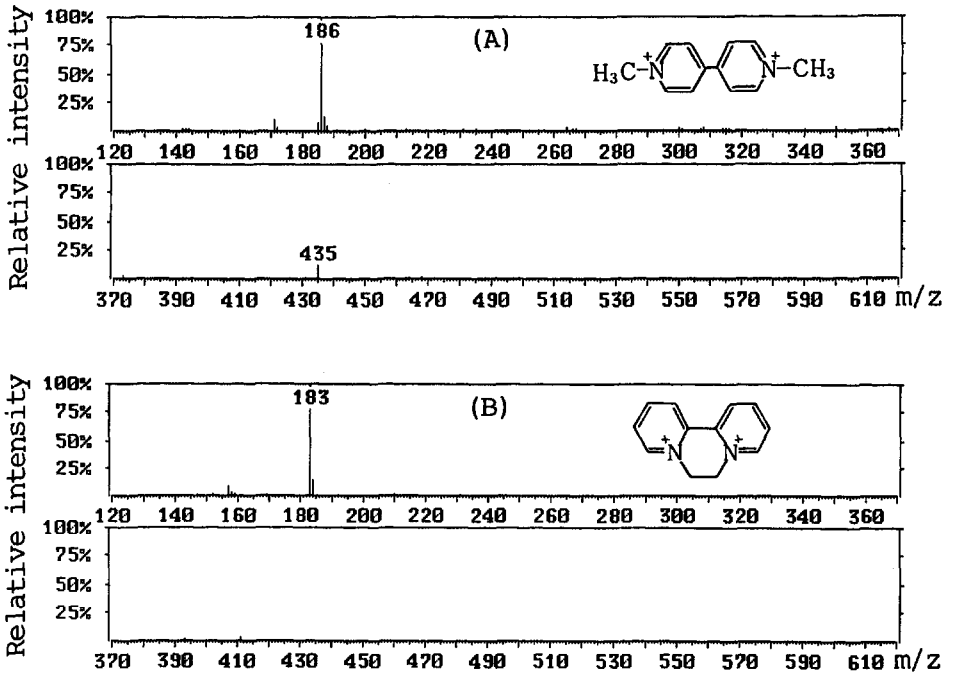

Fig. 3. Mass spectra of (A) paraquat and (B) diquat. The temperatures of the vaporizer, block and tip heater were 161, 310 and 320°C, respectively. Thermospray ionization mode; 200 ng of each sample injected.

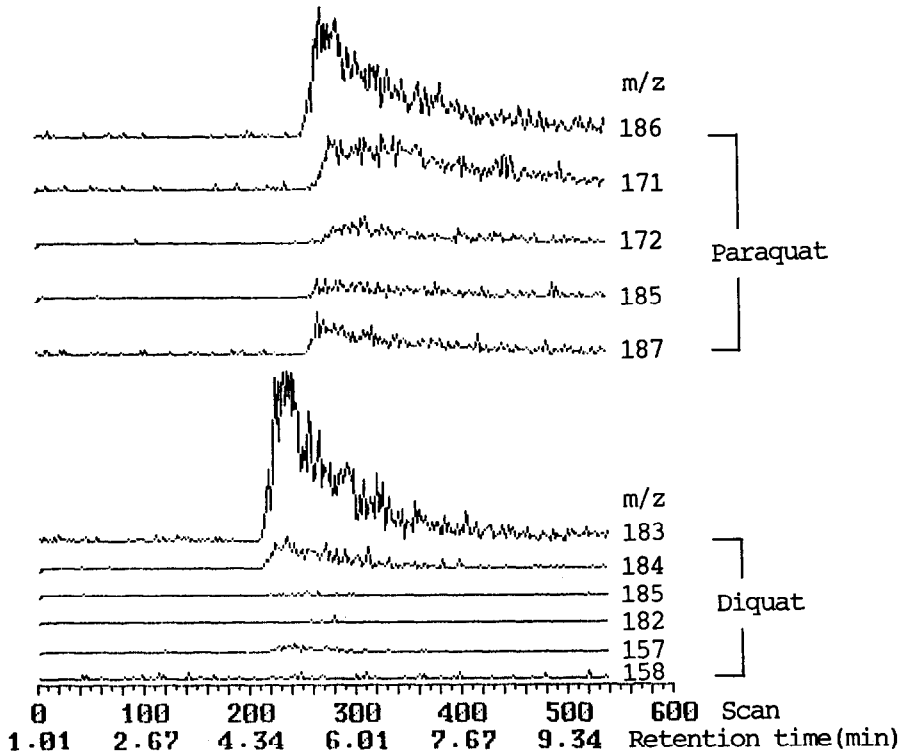

Fig. 4. Mass chromatograms of paraquat and diquat. The temperatures of the vaporizer, block and tip heater were 161, 310 and 320°C, respectively. Thermospray ionization mode; 200 ng of each sample injected.

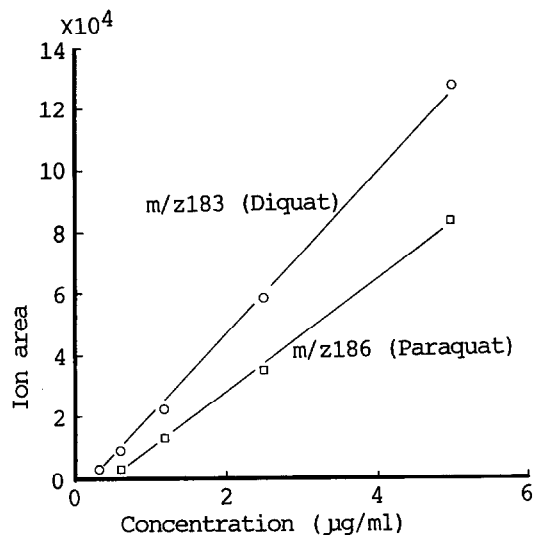

Fig. 5. Calibration graphs for paraquat and diquat. A 100-μl volume was injected; single determination by selected ion monitoring. The temperatures of the vaporizer, block and tip heater were 161, 310 and 320°C, respectively. Thermospray ionization mode. Ion area represents peak area.

the volume of sample injected was 100 μl, a linear response was observed over a concentration range 0.3–5 μg/ml; the detection limit was 20 ng (S/N = 3.5). The detection sensitivity changed, however, with variations in the temperature of the ion source block and vaporizer. The measurement error was about 10%. Quantitative analysis by this method was possible whit appropriate temperature control and when measurement of a known concentration of the compound was interspersed at frequent intervals.

The filament-on ionization mode was not appropriate for quantitative analysis because the sensitivity was lower than that of the thermospray ionization mode.

Sample preparation

Extraction methods have been reported in detail previsously [2,3,14,15,23–25], hence these were not included in this study. The extraction cartridge used was either a Sep-Pak C_{18} or a Bond Elut C_{18}, which

permitted direct injection into the LC–TSP-MS system. Extraction cartridges are suitable for practical use.

CONCLUSIONS

LC with UV detection has mostly been used for the determination of paraquat and diquat, whereas LC with diode-array detection has been used for the identification of these compounds. However, LC–TSP-MS enabled quantitative and qualitative analyses of paraquat and diquat to be achieved without the need for individual compound isolation and derivatization. The maximum ion intensities for mass spectrometry were obtained with the vaporizer at 160°C, the block at 310°C, and the tip heater at 320°C. Quantitative analysis was made possible by sensitive control of the temperature of the ion source block and vaporizer. A standard sample is measured regularly in order to obtain accurate readings.

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