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Note

Thermospray liquid chromatography–mass spectrometry of pesticides in river water using reversed-phase chromatography

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EEC Drinking Water Directive parameter 55, which concerns all pesticides, stipulates that individual pesticide levels in potable water must not exceed 100 ng l^{-1} and that the total level must be less than 500 ng l^{-1} [1].

By far the most highly-used class of pesticides is the herbicides in terms of both amounts applied and area treated. The two major classes of herbicides currently in use are the chlorophenoxy acids and the urons. Both classes of compound are sufficiently water-soluble and persistent to reach the aquatic environment and have been detected in surface, ground and drinking waters[2]. Chlorophenoxy acids are commonly analysed by gas chromatography–electron-capture detection (GC–ECD) or GC–mass spectrometry (MS) after derivatisation[3] but the analysis of the uron pesticides by GC–MS can be problematic in view of their thermal instability at the column temperatures typically used, and their relatively high polarity.

One potential approach to overcoming this problem whilst maintaining specificity of analysis reported the use of liquid chromatography–mass spectrometry (LC–MS) in conjunction with thermospray using a quadrupole mass spectrometer[4]. This study showed that carbamate and chlorophenoxy acid pesticides are amenable to analysis once the source conditions have been optimised and if an additional source of ionisation is used to complement the thermospray process. Spiked soil and lake water samples were analysed.

Our studies have concentrated on the use of thermospray LC–MS interfaced to a double-focusing magnetic sector instrument for the analysis of uron pesticides at environmentally-significant levels. The effects of varying source parameters have been investigated and optimised, and these conditions used to analyse a mixture of pesticides extracted from a river water sample.

EXPERIMENTAL

Mass spectra were obtained on a ZAB-1F (VG Analytical, Manchester, U.K.) mass spectrometer used in low-resolution mode for maximum sensitivity ($M/\Delta M$ typically less than 200). A 1090L liquid chromatograph (Hewlett-Packard, Winnersh, U.K.) was connected to the mass spectrometer via a thermospray interface (Vestec Corporation, Houston, TX, U.S.A.). Data was acquired into a Superincos Nova 4X data system (Finnigan MAT, Hemel Hempstead, U.K.).

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The heated thermospray source (Interion, Manchester, U.K.) was used in the discharge ionisation mode with a source block temperature of 250°C, tip temperature of 155°C and vapour temperature of 190°C. The spectrometer was scanned repetitively from m/z 110 to m/z 650 using a 2.5-s cycle time.

A mobile phase of methanol–aq. 0.1 *M* ammonium acetate (50:50, v/v) was filtered through a 2-μm membrane (Millipore, Bedford, MA, U.S.A.) and helium degassed. This mobile phase was used at a flow-rate of 0.75 ml min^{-1} during injection of the single pesticide standards.

Gradient elution for the fortified river water analyses was carried out using a linear programme of 40% methanol to 60% in 30 min and thereafter to 90% methanol in 10 min with aq. 0.1 *M* ammonium acetate as the secondary solvent system. The linear gradient programme for the water contamination analysis was from 30% methanol to 50% methanol in 5 min, held for 15 min, to 80% methanol in 15 min using the same flow-rate and secondary solvent as before. A reversed-phase S50DS1 25 cm × 0.49 cm column (Thames Chromatography, Maidenhead, U.K.) was used for all LC separations.

A standard pesticide solution was prepared containing metamitron, simazine, atrazine, chlortoluron, isoproturon and linuron at levels of 5 μg ml^{-1} in the eluent and this was used to fortify a 1-l river water sample to give a concentration of 5 $\mu g\, l^{-1}$ per component. An unfortified sample was also collected to act as a blank.

The water samples were passed through 0.7-μm glass-fibre filters (Whatman, Maidstone, U.K.) and then C_{18} bonded-phase cartridges (Millipore). The cartridges were eluted using 2 ml of methanol (Rathburn Chemicals, Walkerburn, U.K.) and the eluate reduced to 1 ml under a stream of nitrogen. A 20-μl aliquot was injected onto the LC column, corresponding to 100 ng of each component on-column, assuming 100% recovery.

A dichloromethane extract derived from river water known to be contaminated with a uron pesticide was analysed to provide confirmation of pollutant identity. Previous work by GM–MS suggested that the pesticide was isoproturon present at a level of about 20 μg ml^{-1} in the final extract. A 20-μl aliquot was injected for analysis by thermospray LC–MS, corresponding to 400 ng of pesticide on-column.

RESULTS

The effects of changing source parameters were investigated using PEG 600. At higher block temperatures when using discharge-assisted thermospray the $[M+H]^+$ adduct was formed preferentially, as has already been shown in other laboratories[5]. Using filament-assisted thermospray the $[M+NH_4]^+$ adduct predominated. Varying the tip temperature did not change the appearance of the spectra although a temperature of 155°C gave an optimum ion yield for the chosen mobile phase composition[6].

The result of varying the amount of analyte is illustrated in Figs. 1 and 2. The spectra consist of an $[M+H]^+$ adduct (m/z 203) and a fragment ion due to the loss of methyl from the protonated species ($[M+H-CH_3]^+\cdot$, m/z 188). Such losses have been reported previously in the LC–MS analysis of explosives ($[M+NH_4-CH_3]^{+\cdot}$[7] and pesticides ($[M+H-CH_3]^{+\cdot}$[8]). We have observed fragment ions in the TSP spectra of several pesticides[6], *e.g.* loss of chlorine from atrazine ($[M+H-Cl]^+\cdot$, m/z 181), simazine ($[M+H-Cl]^+\cdot$, m/z 167) and mecoprop ($[M+NH_4-ce-Cl]^+\cdot$, m/z 197),

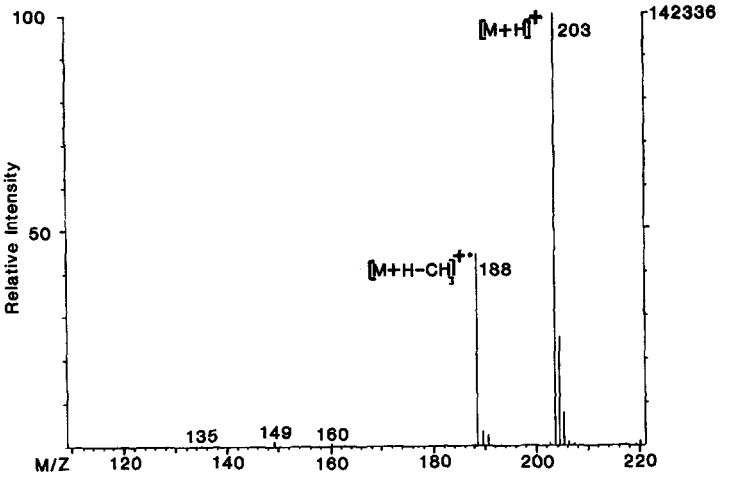

Fig. 1. Thermospray LC–MS spectrum of metamitron at 0.2 mg ml^{-1} in mobile phase (methanol–0.1 *M* ammonium acetate, 50:50 v/v,); flow-rate 0.75 ml min^{-1}; 20 μl injection.

loss of bromine from bromoxynil ($[M+NH_4-Br]^{+}\cdot$, *m/z* 216). loss of parachlorophenate from triadimefon ($[M+H-ClPhO]^{+}\cdot$, *m/z* 167) and losses from methiocarb ($[M+NH_4-CH_3]^{+}\cdot$, *m/z* 228; $[M+NH_4-CH_3S]^{+}\cdot$, *m/z* 197; $[M+H-CH_3S]^{+}\cdot$, *m/z* 179).

For all pesticides investigated the relative abundances of fragment ions were greatest at the lowest concentrations of analyte. Fig. 2 illustrates that a tenfold reduction in the amount of metamitron produced a fivefold increase in the $[M+H-CH_3]^{+}\cdot/[M+H]^{+}$ ratio. Another typical example of this effect was observed for dicamba in which chlorine was lost from the molecular adduct ion, as has been reported previously[8]. The relative intensities observed were, for 40 μg injected:

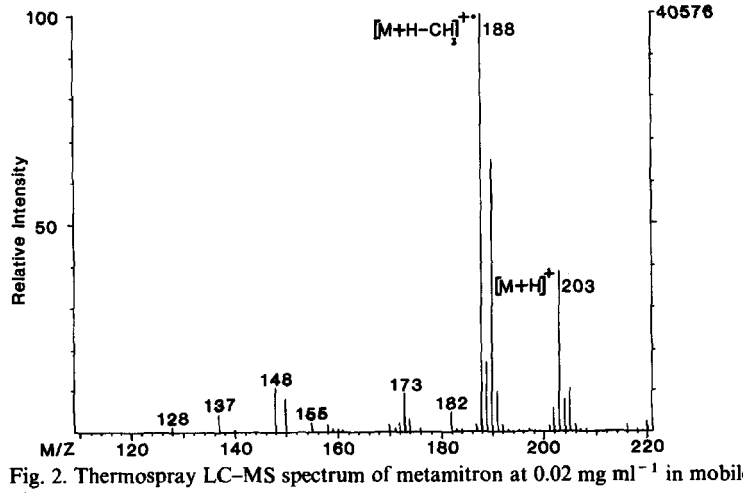

Fig. 2. Thermospray LC–MS spectrum of metamitron at 0.02 mg ml^{-1} in mobile phase, conditions as in Fig. 1.

$[M+NH_4]^+$, *m/z* 238 (60%); $[M+NH_4-Cl]^{+\cdot}$, *m/z* 203 (100%); $[M+H-Cl]^{+\cdot}$, *m/z* 186 (8%); $[M+NH_4-2Cl]^+$, *m/z* 168 (19%); $[M+H-2Cl]^+$, *m/z* 151 (1%); and for 400 ng injected: $[M+NH_4]^+$ (3%); $[M+NH_4-Cl]^{+\cdot}$ (26%); $[M+NH_4-2Cl]^+$ (100%); $[M+H-2Cl]^+$ (41%). The reason for this change with changing concentration is unknown, although similar losses of chlorine have been reported to result from pyrolysis in the TSP probe[9].

Fig. 3 shows the selected mass chromatograms obtained after the injection of an extract from a spiked river water. The chromatograms correspond to metamitron ($[M+H]^+$, *m/z* 203), simazine ($[M+H]^+$, *m/z* 202; $[M+H-Cl]^{+\cdot}$, *m/z* 167), atrazine ($[M+H]^+$, *m/z* 216; $[M+H-Cl]^{+\cdot}$, *m/z* 181), chlortoluron ($[M+H]^+$, *m/z* 213, 215), isoproturon ($[M+H]^+$, *m/z* 207) and linuron ($[M+NH_4]^+$, *m/z* 266; $[M+H]^+$, *m/z* 249; *m/z* 215 uncharacterised). An unidentified component exhibiting an *m/z* 167 ion was also observed at scan 605. The spectra compared favourably with previously-reported data[9,10,11] although we did not observe solvent adduct ions.

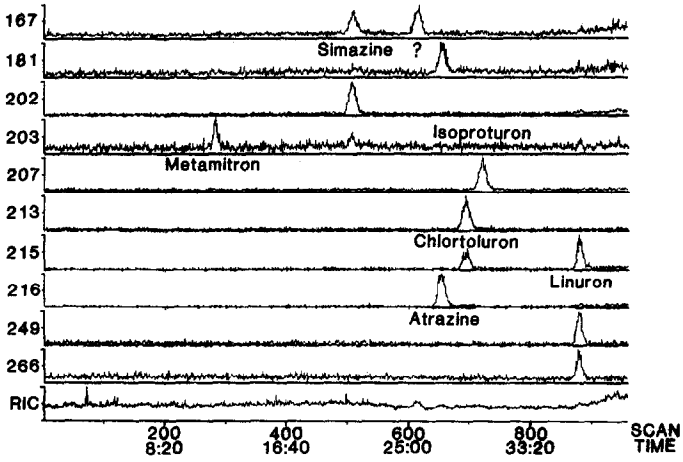

Fig. 3. Thermospray LC–MS chromatogram of 1-l river water extract spiked at 5 $\mu g\,l^{-1}$ with each pesticide component (methanol–0.1 *M* ammonium acetate (40:60) to (60:40) in 30 min to (90:10) in 10 min. Flow-rate 0.75 ml min^{-1}; 20 μl injection).

Mass chromatograms of similar intensity were obtained for the injection of the standard solution used to spike the water sample and indicated an ~80% recovery of the pesticides from the fortified water. Injection of the unfortified riverwater extract produced mass chromatograms which indicated the presence of low levels (<3 ng on-column; equivalent to about 150 ng l^{-1} in the original sample) of chlortoluron, atrazine, linuron and isoproturon although there was insufficient material to obtain recognisable full-scan spectra (approximately 20 ng on-column was necessary to achieve this).

Fig. 4 shows a mass chromatogram obtained from the analysis of a river water extract thought to contain high levels of isoproturon. The retention-time of a major peak exhibiting an ion of *m/z* 207 corresponded to that of an isoproturon standard (Greyhound Chemicals, Birkenhead, U.K.) and gave a similar mass spectrum

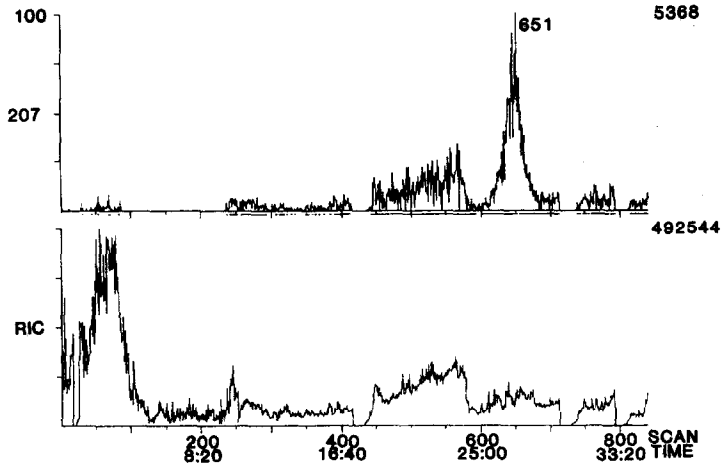

Fig. 4. Thermospray LC–MS chromatogram of 2-l river water extract in dichloromethane (methanol–0.1 *M* ammonium acetate (30:70) to (50:50) in 5 min, held 15 min, to (80:20) in 15 min; flow-rate 0.75 ml min^{-1}; 20 μl injection).

($[M + H]^+$, *m/z* 207). This provided confirmation of the suspected herbicide, isoproturon, in the river water extract.

CONCLUSIONS

The optimisation of source conditions and their effects on spectral appearance for a magnetic instrument have been found to be similar to previously published results obtained using quadrupole instruments. However, it seems unlikely that useful library searching can be performed because of the variation in relative intensities of fragments and molecular ions with analyte concentration. This has not been observed with quadrupole instruments and may be due to the high source potential involved (3 kV), partial pyrolysis of the sample, or a combination of these effects.

Application of LC–MS to analysis of riverwater extracts showed the absence of problematic interferences and allowed the detection of pesticides at levels of a few nanograms on-column. Concentration of extracts of 1-l samples to 0.1 ml and the injection of 20 μl onto the column should enable the detection of pesticides at concentrations equivalent to 10 ng l^{-1} in water samples by the use of TSP in selected-ion-monitoring mode. Due to the apparent changes in the relative intensities of TSP–MS ions with concentration, calibration curves obtained for SIM analysis will probably be non-linear leading to consequent problems with the precision of the analysis.

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