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Determination of quaternary amine pesticides by thermospray mass spectrometry

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ABSTRACT

Positive-ion (PI) thermospray mass spectrometry (TSP-MS) with methanol–water (50:50) + 0.05 *M* ammonium formate as eluent was used for the characterization of the quaternary amine pesticides paraquat, difenzoquat, diquat, mepiquat and chlormequat and gave as base peaks $[Cat + H]^{+\bullet}$, $[Cat - CH_3 + H]^{+\bullet}$, $[Cat]^{+\bullet}$, $[Cat]^{+\bullet}$ and $[Cat]^{+\bullet}$, respectively. A postcolumn ion-pair extraction system was developed for the determination of difenzoquat from water samples whereas the other quaternary amine pesticides were not extracted. An aqueous mobile phase with various sulphonate counter ions such as dodecanesulphonic acid, methyl orange, Blue Acid 113, Mordant Red 9 and sodium picrate was tested in combination with an extraction solvent containing cyclohexane–dichloromethane–*n*-butanol (45:45:10) with UV diode-array spectra and PI TSP-MS detection. Applications are reported for the TSP-MS determination of 500 μg/l of difenzoquat in spiked water samples using the postcolumn extraction system. Diquat and paraquat were determined at levels of 0.10–0.17 μg/g in soil samples from the Ebro delta (Tarragona, Spain) using a reversed-phase eluent containing 0.05 *M* ammonium formate.

INTRODUCTION

The determination of quaternary ammonium compounds is tedious and involves either multi-step extraction and derivatization processes prior to GC determination [1,2] or cation-exchange LC and UV detection [1,3,4]. Of the different quaternary amine pesticides, mainly paraquat and diquat have been determined. The EPA method 549 was developed for the isolation of diquat and paraquat from water samples and involves solid-phase ion-pair extraction with further analysis using LC–diode-array detection at 308 and 257 nm [5]. Ion-pair formation in LC with UV detection is a common method for the determination of quaternary ammonium compounds, *e.g.*, by using as counter ions either sodium 1-heptanesulphonate or sodium *p*-xylenesulphonate and it has been applied to the determination of diquat and paraquat residues in potatoes [3] and to industrial cationic surfactant homologues [6], respectively.

Ion pairs can be extracted from a reversed-phase eluent by using appropriate extraction solvents. The extraction ability of quaternary ammonium compounds, which form an extractable ion pair with anionic dyes, has been reported [7]. In the last few years, ion-pairing systems have been coupled on-line to LC. In such systems, by using continuous-flow post-column extraction and detection, the LC eluent is segmented by an inmiscible solvent, containing a UV–Vis-absorbing or fluorescent counter ion. Ion-pair formation takes place in an extraction coil. Before detection is possible, the two phases

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are separated by means of a phase separator, which is the most critical part of the postcolumn extraction system. The use of a sandwich phase separator permitted the determination of cationic surfactants in environmental samples with UV or fluorescence detection [8]. Vouros *et al.* [9] were the first to incorporate an ion-pair extraction as an on-line chemical step before MS using a moving-belt interface. Recently, we have also reported preliminary results on the postcolumn ion-pair extraction of difenzoquat using thermospray (TSP) MS detection [10]. On-line postcolumn extraction systems can significantly enhance the power of LC–MS, as they allow the chromatographic part to be operated separately from the MS detection and therefore permitting to use non-volatile salts, *e.g.*, phosphate buffers, which cannot be used in conventional LC–MS work [11].

Diquat and paraquat have also been determined using reversed-phase systems in LC–TSP-MS, although chromatographic tailing is evident owing to the restrictive chromatographic conditions [12–15]. Fast atom bombardment (FAB) tandem mass spectrometry (MS–MS) [16] has also been used for the characterization of such group of pesticides.

The purposes of this work were to use TSP-MS for the characterization of a variety of quaternary amine pesticides which are of importance within the monitoring programmes of pesticides in environmental matrices [1], such as chlormequat, difenzoquat and mepiquat, in addition to the more often studied diquat and paraquat, to test the postcolumn ion-pair extraction system for these compounds and to evaluate its extraction efficiency with different counter ions prior to MS detection, achieving an independent chromatography from the MS detection, and to apply LC–TSP-MS to the determination of diquat and paraquat residues in real soil samples.

EXPERIMENTAL

Chemicals

HPLC-grade water, methanol and acetonitrile from Merck (Darmstadt, Germany) were passed through a 0.45-μm filter (Waters Chromatography Division, Millipore, Bedford, MA, USA) before use. Cyclohexane and dichloromethane were of pesticide grade obtained from SDS (Peypin, France). Ammonium formate was supplied by Fluka (Buchs, Switzerland). Hydrochloric acid was of analytical-reagent grade from Merck. The sulphonate counter ions used were dodecanesulphonic acid, methyl orange, Blue Acid 113, Mordant Red and sodium picrate and were gifts from F. Ventura (Barcelona Water Supply, Barcelona, Spain). Analytical-reagent grade paraquat, diquat, difenzoquat, mepiquat and chlormequat were purchased from Promochem (Wesel, Germany).

Sample preparation

Sample pretreatment for the determination of paraquat and diquat in soil samples was carried out using a slight modification of the procedure reported by Needham *et al.* [17]. The procedure includes freeze-drying of soil samples and sieving (120 μm). A 10-g amount of soil was wetted with 10 ml of water and 10 ml of 6 *M* HCl were added. The samples were allowed to soak for 30 min, then sonicated for 20 min. Subsequently they were filtered through a Whatman glass-fibre filter-paper and the acidic solutions were extracted with 2 × 20 ml of dichloromethane. The aqueous layer was then evaporated to dryness under nitrogen. Finally, methanol was added to yield a final volume of 50 μl and 20 μl were injected into the LC–MS system.

Chromatographic and postcolumn extraction conditions

Eluent delivery was provided by a high-pressure pump (Waters Model 510). Injection was carried out with a Model 7125 injection valve with a 20-μl loop (Rheodyne, Cotati, CA, USA). A LiChroCART cartridge column (12.4 cm × 4.0 mm I.D.) packed with 5-μm LiChrospher 100 RP-18 (Merck) was used. In reversed-phase experiments, an eluent mixture of methanol–water (50:50) + 0.05 *M* ammonium formate was used at a flow-rate of 1 ml/min.

The postcolumn extraction system has been described elsewhere [8,11] and consisted of a laboratory-made sandwich phase separator equipped with two stainless-steel blocks with and

without a PTFE disc with a groove. The extraction solvent was delivered by a second high-pressure pump (Waters Model 510). A schematic diagram of the experimental set-up is given in ref. 11. The aqueous phase contained acetonitrile–water (60:40) + $1 \cdot 10^{-4}$ *M* of sulphonate counter ion and the organic phase was cyclohexane–dichloromethane–*n*-butanol (45:45:10) at a flow-rate of 1 ml/min. The organic phase was added to the aqueous phase via a Valco (Houston, TX, USA) T-piece with a 0.25-mm bore. The extraction took place in a 1.5 m × 0.8 mm I.D. stainless-steel capillary (helix diameter 40 mm). After separation of the phases, the analytes together with the organic normal phase were introduced either into the UV or the TSP-MS detection system. A rapid-scanning UV–Vis detector was obtained from Barspec (Rehovot, Israel). With this phase separator, a purely organic normal phase can be obtained. The organic flow through the detector was regulated by means of a PTFE capillary equipped with a restrictor.

Mass spectrometric analysis

A Hewlett-Packard (Palo Alto, CA, USA) Model 5988A thermospray quadrupole mass spectrometer and a Hewlett-Packard Model 59970C instrument for data acquisition and processing were employed. The temperatures of the TSP were 100, 188 and 270°C for the stem, vapour and ion source, respectively. In all experiments the filament was on. Full-scan conditions were used in most of the experiments, with scanning from *m/z* 90 to 500. In the analysis of spiked water and soil samples, the selected-ion monitoring (SIM) mode was used. Ions used for monitoring were *m/z* 235 and 249 (difenzoquat), 172 and 187 (paraquat) and 157 and 184 (diquat).

RESULTS AND DISCUSSION

TSP–MS characterization

The flow-injection analysis and the TSP spectra of the quaternary amine pesticides obtained in the positive-ion (PI) mode of operation and using methanol–water (50:50) + 0.05 *M* ammonium formate as eluent are shown in Figs. 1

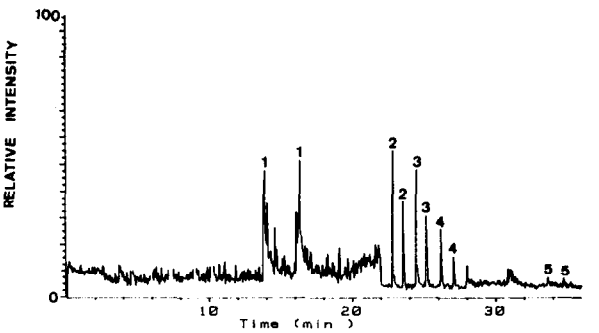

Fig. 1. Total ion current chromatogram obtained under flow-injection analysis of (1) mepiquat, (2) paraquat, (3) diquat, (4) difenzoquat and (5) chlormequat. Carrier stream, methanol–water (50:50) + 0.05 *M* ammonium formate; flow-rate, 1 ml/min; TSP temperatures, 100, 188 and 270°C for the stem, vapour and ion source, respectively.

and 2. The base peaks for paraquat, diquat, difenzoquat, mepiquat and chlormequat were at *m/z* 187, 184, 235, 114 and 122, corresponding to $[Cat + H]^{+\bullet}$, $[Cat]^{+\bullet}$, $[Cat - CH_3 + H]^{+\bullet}$, $[Cat]^{+\bullet}$ and $[Cat]^{+\bullet}$, respectively. The formation of the different ions under TSP conditions for difenzoquat [10] and paraquat [12] is usually dependent on the TSP temperature. In addition, the composition of the mobile phase has been reported to be a key factor in the ion formation of various pesticides under TSP conditions [13,18]. In this work, and comparing our results with literature data obtained using a TSP interface [12], it was noted that the base peak for paraquat was previously obtained at *m/z* 186. We attribute this discrepancy to the mobile phase composition, since in ref. 12 a mobile phase of methanol–water (80:20) + 0.1 *M* ammonium acetate (adjusted with trifluoroacetic acid to pH 5 as buffer) was used. Jones *et al.* [13] also reported relevant information; the base peak for paraquat was obtained at *m/z* 186 and 187 when water and ammonium acetate, respectively, were used as post-column solvents. It was proposed by Vestal [14] that the attachment of a hydride ion forms the *m/z* 187 ion. By MS–MS it was demonstrated that losses of CH_4 and a methyl radical occurred for *m/z* 186 and 187 after a prior loss of 15 u [13].

The results obtained in this work follow similar fragmentation patterns as previously published by Schmelzeisen-Redeker *et al.* [15], who

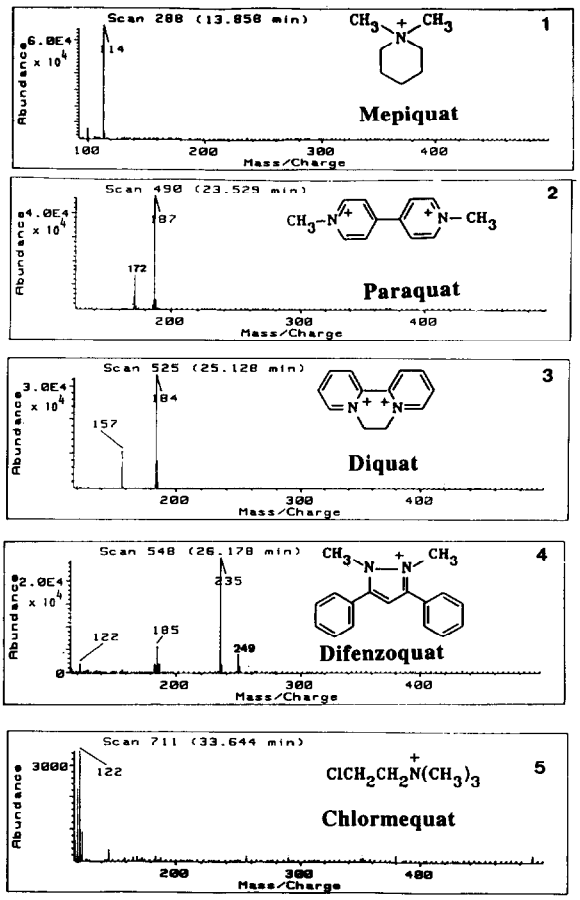

Fig. 2. TSP mass spectra of the compounds in Fig. 1. Conditions as in Fig. 1.

carried out the TSP-MS characterization of diquaternary salts using pure water as eluent in the TSP jet. Singly charged ions were formed, $[Cat - CH_3]^{+\bullet}$ ions resulting from dealkylation reactions being the most frequently found ions in the spectra [15].

The formation of an m/z 186 ion has also been observed in particle beam LC–MS using electron impact ionization, although in this instance the base peak corresponded to m/z 171 (due to a loss of CH_3) [19], and indicates that the absence of ammonium acetate gives as base peak the m/z 186 ion. The discrepancy between our results and the values reported in ref. 12, also obtained using a TSP interface, can be attributed to the use of a different mobile phase, thus giving the possibility of the formation of more hydride ions in our case as ammonium formate is used and consequently m/z 187 is favoured over m/z 186. Yoshida *et al.* [12] also observed the ion at m/z 187, although always with a lower abundance than that at m/z 186, thus indicating the presence of both ions (probably due to the presence of ammonium acetate).

On comparing our results with the use of FAB-MS [16,20], it can be seen that some of the ions formed match, *e.g.*, those at m/z 171, 184 and 249 for paraquat, diquat and difenzoquat, respectively, although their relative abundances in the spectra differ. These similarities in fragmentation between the two techniques indicate that TSP-MS should be considered not only as a gas-phase chemical ionization-dependent technique but also as a solution chemistry method, in a similar manner to FAB-MS [20].

Mepiquat and chlormequat were characterized for the first time by TSP-MS in this work. Owing to the low molecular masses of these two compounds, it is important to start scanning at m/z of 100, so both compounds can be identified. This may be a problem when analysing real samples owing to matrix interferences.

Postcolumn extraction

As mentioned in the Introduction, it was the purpose of this work to achieve an independent

TABLE I

AVERAGE RECOVERIES AND RELATIVE STANDARD DEVIATIONS (R.S.D.s) FOR THE DETERMINATION OF QUATERNARY AMINE PESTICIDES FROM WATER AND SOIL SAMPLES

Compound	Water samples (postcolumn extraction)		Soil samples	
	Average recovery (%)[a]	R.S.D. (%)	Average recovery (%)[a]	R.S.D. (%)
Difenzoquat	81	7	n.r.[b]	
Diquat	17	21	85	7
Paraquat	n.e.[c]		91	4

[a] $n = 5$.
[b] Not reported.
[c] Not extractable.

chromatography from the TSP-MS detection, so a postcolumn extraction system for difenzoquat with appropriate sulphonate counter ions was developed. This is an extension to the method developed by De Ruiter *et al.* [8] for the analysis of cationic detergents with sulphonate counter ions via postcolumn ion-pair formation and subsequent extraction. Various sulphonated counter ions (methyl orange, dodecanesulphonate, Acid Blue 113 and Mordant Red 9) [21] were tested. All these sulphonates showed good extraction efficiencies of 80% for difenzoquat (see Table I) when it was extracted from an aqueous phase [acetonitrile–water (60:40) containing $1 \cdot 10^{-4}$ *M* of the sulphonate] into an organic phase [cyclohexane–dichloromethane–*n*-butanol (45:45:10)].

Fig. 3 shows the different postcolumn extraction UV spectra obtained by direct flow injections of difenzoquat using a carrier stream of acetonitrile–water (A) and via the postcolumn extraction system, where difenzoquat was extracted as an ion pair with dodecanesulphonic acid (B), methyl orange (C), Blue Acid 113 (D) and Mordant Red 9 (E). The limit of detection after the postcolumn extraction under full-scan conditions was 100 ng, which is comparable to that obtained with postcolumn reduction using alkaline sodium dithionite and UV detection [1,4]. The UV spectra of difenzoquat in the carrier stream and in the extraction solvent exhibited maxima at 254 nm, being slightly different on the blue side of the spectrum, thus indicating that the UV spectra are dependent on the eluent composition and that the ion pair was extracted. Although the observed changes in both UV spectra could prove the formation of the ion pair (difenzoquat–sulphonate complex) and its extraction into the organic phase, it could not be proved by TSP-MS and always gave the two ions at *m/z* 235 and 249. When using Acid Blue 113 the background TSP spectra contained a peak at *m/z* 344, probably corresponding to a cleavage near the azo group. This fragment was also observed in FAB-MS experiments [21].

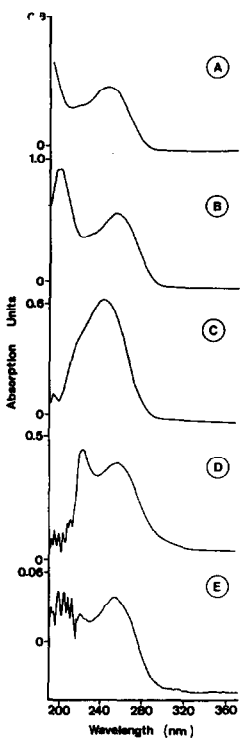

Fig. 3. UV mass spectra of difenzoquat obtained using (A) a reversed-phase eluent of acetonitrile–water (60:40), and after the postcolumn extraction system using cyclohexane–dichloromethane–*n*-butanol (45:45:10) at 1 ml/min of an aqueous ion pair containing $1 \cdot 10^{-4}$ *M* of (B) dodecanesulphonic acid, (C) methyl orange, (D) Blue Acid 113 and (E) Mordant Red.

Although the extraction efficiency for difenzoquat was 81%, for diquat it was only *ca.* 17% and paraquat was not extracted at all (see Table I). Other problems encountered were the absence of a chromophore for mepiquat and chlormequat. As other alternatives for ion-pair extraction, picrate counter ion is known to offer relatively high extraction constants [22,23] and so it was also tested. A mobile phase containing sodium picrate at $1 \cdot 10^{-4}$ *M* (at pH 8) was used in combination with the common extraction solvent. Also in this instance none of the quaternary amine pesticides was extracted, with the exception of difenzoquat, which showed a similar extraction efficiency as previously. A drawback in these experiments was the relatively high noise level, due to the partial extraction of picrate into the organic phase.

As the use of picrate did not represent an improvement for the determination of quaternary amine pesticides, it was decided to use one of the previous sulphonate ion pairs, such as Acid Blue 113, in combination with the post-

column extraction solvent described earlier. The analysis of a spiked water sample using TSP-MS detection is shown in Fig. 4. The SIM mode was used for the ions corresponding to m/z 235 and 249. The method permitted the determination of 500 μg/l of difenzoquat in spiked river water samples, thus representing an absolute amount of 10 ng of difenzoquat detected.

Environmental analysis

The use of the different quaternary ammonium pesticides within European countries has recently been reported [24]. They are currently applied as herbicides (*e.g.*, paraquat) and as growth regulators (*e.g.*, chlormequat). After application to the soil, paraquat and diquat are good examples of compounds with a cationic structure that can be sorbed into clay minerals in a process that is dependent on the cation-exchange capacity of the clay. They can be rapidly sorbed between the layers of the clay platelets and as a consequence they exhibit low mobility [25].

Paraquat and diquat are being currently applied in fields located at the Ebro delta (Tarragona, Spain). Two environmental soils from this area containing paraquat and diquat were analysed following the analytical protocol described here and good recoveries were obtained (Table I). Fig. 5 shows the SIM traces obtained using LC–TSP-MS for these two soil samples corresponding to (A) paraquat with ions at m/z 172 and 187 and (B) diquat with the ions at m/z 157 and 184. The samples analysed contained 0.10 and 0.17 μg/g of paraquat and diquat, respectively. This corresponded to an injection of 400–600 ng of each compound into the analytical column.

Calibration graphs for paraquat and diquat were constructed by using the SIM mode. They were linear between 100 and 1200 ng. Volumes of 20 μl of each of five standard solutions of paraquat and diquat concentrations from 5 to 60 ng/μl were injected ($n = 3$) into the system.

The chromatographic traces in Fig. 5 exhibit tailing, similarly as reported by Yoshida *et al.* [12]. In contrast, the postcolumn extraction chromatogram in Fig. 4 for difenzoquat is much better. Two comments can be made: first, the tailing observed under reversed-phase conditions is a common behaviour for these compounds when using either with UV [6] or TSP-MS detection [12]. This could be avoided by using another type of column, *e.g.*, a cyano- or amino-

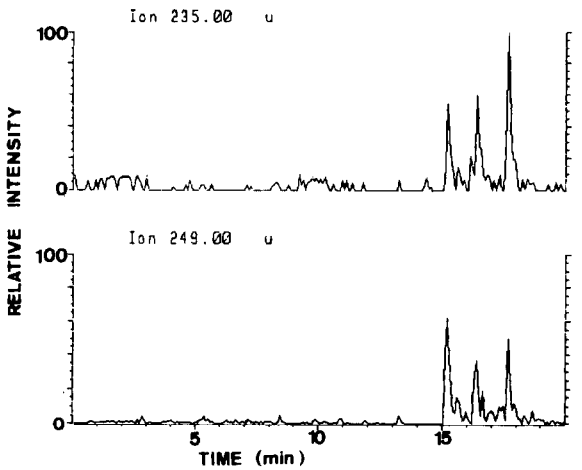

Fig. 4. TSP-MS under SIM conditions for three repetitive injections of a river water solution spiked with 500 μg/l of difenzoquat after postcolumn extraction with cyclohexane–dichloromethane–*n*-butanol (45:45:10) at 1 ml/min of an aqueous phase of acetonitrile–water (60:40) and $1 \cdot 10^{-4}$ *M* of Blue Acid 113. TSP temperatures as in Fig. 1.

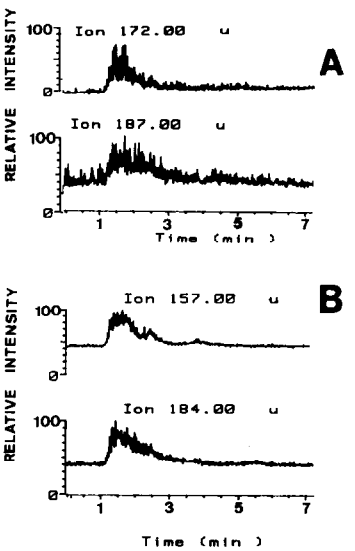

Fig. 5. LC–TSP-MS under SIM conditions for a soil sample from the Ebro delta containing 0.10 and 0.17 μg/g of paraquat and diquat, respectively. Ions monitored were at m/z values of (A) 172 and 187 (paraquat) and (B) 157 and 184 (diquat). LC column, 5-μm LiChrospher 100 RP-18; LC mobile phase, methanol–water (50:50) + 0.05 *M* ammonium formate; flow-rate: 1 ml/min; TSP temperatures as in Fig. 1.

bonded phase with a chloroform–methanol–acetonitrile mixture in combination with a post-column extraction system, as described previously for cationic surfactants [8]. However, this approach could not be used for paraquat and diquat owing to the non-extraction of these two compounds under the above-described analytical conditions. The use of an ion-pair system without postcolumn extraction will evidently damage the MS source owing to the introduction of non-volatile salts. The use of an ion-exchange column with an ionic strength gradient mixture has also been described by Vestal [14].

CONCLUSIONS

LC–TSP-MS was used for the characterization of a variety of quaternary ammonium pesticides. Under reversed-phase conditions and using ammonium formate as an ionizing additive, 1–2 μg of each compound could be positively identified when injected under full-scan conditions. A postcolumn ion-pair system using different types of counter ions was developed for the characterization of difenzoquat, thus permitting the extraction and determination of this pesticide in water samples. This system could not be used with the other quaternary amine pesticides. The determination of paraquat and diquat in soil samples was feasible by using LC–TSP-MS with SIM and with the two main ions of each compound.

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