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Evaluation of eluents in thermospray liquid chromatography–mass spectrometry for identification and determination of pesticides in environmental samples

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ABSTRACT

The influence of different eluents in positive and negative ion mode thermospray liquid chromatography–mass spectrometry was studied with several groups of pesticides, including carbamates, chlorotriazines, phenylureas, phenoxy acids and organophosphorus and quaternary ammonium compounds, and the corresponding degradation products. Using the positive ion mode in combination with reversed-phase eluents the base peaks generally corresponded either to $[M + H]^+$ for the chlorotriazines and their hydroxy metabolites or to $[M + NH_4]^+$ for the carbamates, the phenylureas, the organophosphorus pesticides and their oxygen analogues. In the negative ion mode different processes such as (dissociative) electron-capture and anion attachment mechanisms occurred. Fragment ions such as $[M - CONHCH_3]^-$ for the carbamates, $[M - H]^-$ for the chlorotriazines, phenylureas and chlorinated phenoxy acids and $[M]^{\cdot -}$, $[M - R]^-$ (R being a methyl or ethyl group) for organophosphorus pesticides were usually formed. Depending on the eluent additive used (ammonium acetate, ammonium formate and/or chloroacetonitrile), three different adduct ions were formed: $[M + CH_3COO]^-$, $[M + HCOO]^-$ and $[M + Cl]^-$.

Normal-phase eluents with cyclohexane, *n*-hexane and/or dichloromethane provided more structural information and enhanced the response of several compounds. The positive ion mode was useful for the detection of chlorinated phenoxy acids and chlorophenols which could not be detected in the positive ion mode using reversed-phase systems. The base peaks generally corresponded to $[M]^{\cdot +}$, $[M + H]^+$ or $[M - Cl]^+$.

For the characterization of difenzoquat, a quaternary ammonium pesticide of which trace level analysis is troublesome, a post-column ion-pair extraction system was used. An aqueous mobile phase with a sulphonate-type counter ion was applied and an extraction solvent containing cyclohexane–dichloromethane–*n*-butanol (45:45:10) was used in thermospray liquid chromatography–mass spectrometry. Illustrative examples of the determination of residue levels of pesticides in soil matrices are shown.

INTRODUCTION

The determination of thermally labile and/or polar pesticides, which is troublesome by gas chromatography(GC)–mass spectrometry (MS), can be partly achieved

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by using liquid chromatography (LC)–MS. During the last decade, LC–MS with different interfaces such as the moving belt, direct liquid introduction (DLI), thermospray (TSP) and particle beam, has developed into a well established technique with practical applications for characterizing different groups of pesticides, *e.g.*, triazines [1–4], organophosphorus pesticides [5–7], chlorinated phenoxy acids [2,3,8], phenylureas [2,7,9] and carbamates [10–12]. Recently, two monographs [13,14] have been published on the use of LC–MS systems in pesticide analysis.

Among the different interfaces, TSP is the most popular nowadays in reversed-phase (RP) where acetonitrile–water or methanol–water mixtures containing a volatile buffer (ammonium acetate) are used as the eluent. One of the drawbacks of TSP-LC–MS is the lack of structural information that can be obtained. Normally the positive ion (PI) mode is used and the only ions which are usually formed are the protonated molecular ion $[M+H]^+$ and/or an adduct ion $[M+NH_4]^+$. Different approaches have been described for improving fragmentation, such as the use of the PI and the negative ion (NI) mode [6,7,15], the use of ammonium acetate, ammonium formate or chloroacetonitrile as eluent additives [2,8,15] and normal-phase (NP) eluents (*e.g.*, cyclohexane [3,16]). As instrumental procedures to induce fragmentation, the use of a repeller electrode operated at high voltage comparable to collision-induced dissociation (CID) [17] and CID TSP-LC–MS–MS [18] has been reported. These techniques have been demonstrated to provide adequate information for the identification and determination of pesticides in environmental samples.

The objective of this work was to study the potential of obtaining more structural information in filament-on TSP-LC–MS for the identification and determination of pesticides in environmental samples. Case studies are used to describe the possibilities for organophosphorus pesticides and their oxygen analogues, carbamates and their corresponding hydroxypirimidine metabolites, chlorotriazines and hydroxytriazines, chlorinated phenoxy acids, chlorophenols and phenylurea herbicides. A post-column extraction system, equipped with a sandwich phase separator, for the ion-suppressed analysis of chlorinated phenoxy acids and the ion-pair extraction of quaternary ammonium pesticides will be described.

EXPERIMENTAL

Chemicals

High-performance liquid chromatographic-grade water from Riedel-de Haën (Seelze-Hannover, Germany) and methanol, acetonitrile and chloroacetonitrile from Merck (Darmstadt, Germany) were passed through a 0.45-μm filter (Scharlau, Barcelona, Spain) before use. Acetone, ethyl acetate, *n*-hexane, diethyl ether and dichloromethane were of pesticide grade, obtained from SDS (Peypin, France). Analytical-reagent grade carbaryl, oxamyl, atrazine, simazine, cyanazine, fonofos, fensulfothion, trichlorfon, parathion-ethyl, phosmet, chlorpyrifos, (2,4-dichlorophenoxy)-acetic acid (2,4-D), (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T), (2,4,5-trichlorophenoxy)propionic acid (Silvex), monuron and diuron were purchased from Polyscience (Niles, IL, U.S.A.), difenzoquat from Promochem (Wesel, Germany) and linuron from Riedel-de Haën. Fenitrothion was a gift from Sumitomo (Osaka, Japan) and fenitrooxon was synthesized [19]. Florisil (100–200 mesh) was purchased from Merck. Ammonium acetate and ammonium formate were supplied by Panreac (Barcelona, Spain) and Fluka (Buchs, Switzerland), respectively.

Sample preparation

Sample pretreatment for the determination of organophosphorus and phenylurea pesticides in soil was carried out using a modification of the procedure reported by Durand *et al.* [20]. The procedure includes freeze-drying of soil samples, filtering (120 μm) and Soxhlet extraction for 12 h with methanol. After concentration to *ca.* 20–25 ml in a rotary evaporator (35°C), the extract was carefully evaporated to dryness. Subsequently, the residue was dissolved in *n*-hexane and cleaned up on Florisil with elution using diethyl ether–*n*-hexane (50:50). Finally, methanol was added to a final volume of 0.5 ml and 20 μl of this solution were injected into the LC–MS system.

Chromatographic conditions

Chromatography was performed with two Model 510 high-pressure pumps coupled with a Model 680 automated gradient controller (Waters Chromatography Division, Millipore, Bedford, MA, U.S.A.) and a Model 7125 injection valve with a 20-μl loop from Rheodyne (Cotati, CA, U.S.A.). LiChrocart cartridge columns (12.5 cm × 4.0 mm I.D.) packed with 5-μm LiChrospher 100 RP-18 from Merck were used. The following eluents were tested: methanol–water (50:50) containing 0.05 *M* ammonium acetate, methanol–water (50:50) + 0.05 *M* ammonium formate, acetonitrile–water (50:50) containing 0.05 *M* ammonium formate and acetonitrile–water–chloroacetonitrile (49:49:2) containing 0.05 *M* ammonium acetate at a flow-rate of 1 ml/min. Cyclohexane and *n*-hexane were used at 0.7–0.8 ml/min. All solvent compositions are represented volume-to-volume.

Mass spectrometric analysis

A Hewlett-Packard (Palo Alto, CA, U.S.A.) Model 5988A TSP-LC–MS quadrupole mass spectrometer and a Hewlett-Packard Model 59970C instrument for data acquisition and processing were employed. For most of the RP-LC conditions, the temperatures of the TSP system were 100, 188 and 270°C for the stem, tip and vapour and ion source, respectively. Some experiments were carried out at a vapour and ion source temperature of 200°C, keeping the other temperatures as above. When NP or post-column extraction systems were used, the temperatures of the TSP system were 86, 188 and 200°C, respectively. In all the experiments, the filament was on.

RESULTS AND DISCUSSION

Positive and negative ion modes

In most TSP-LC–MS systems methanol–water or acetonitrile–water mixtures containing a volatile buffer (ammonium acetate) are used to enhance ionization in the PI mode resulting in the formation of combinations of $[M+H]^+$ and $[M+NH_4]^+$ ions as base peaks. As a result, relatively simple mass spectra are obtained, which means that this mode is nowadays used for routine analysis.

The NI mode, however, is not as frequently applied as the PI mode, probably because the response is much more compound dependent than in the PI mode, although the mechanisms involved such as anion attachment and proton abstraction have been pointed out to be predominant [21] and similar to conventional negative chemical ionization (NCI) and DLI-LC–MS. These processes, *e.g.*, resonance and dissociative electron capture, proton transfer and anion attachment, and parameters

TABLE I

IMPORTANT FRAGMENTS AND RELATIVE INTENSITIES OBSERVED UNDER RP-TSP-LC-MS CONDITIONS

Carrier stream: methanol–water or acetonitrile–water* with different eluent additives (A = 0.05 *M* ammonium acetate, B = 0.05 *M* ammonium formate, C = 2% chloroacetonitrile). Filament-on mode of operation.

Mol. wt.	Compounds and ions (*m/z* and tentative identification)		A	B	C
Carbamate pesticides					
201	Carbaryl				
	202	$[M+H]^+$	10	10	n.i.[a]
	203	$[M-CONHCH_3+CH_3COOH]^-$	100		
	219	$[M+NH_4]^+$	100	100	
	260	$[M+CH_3NH_2CO]^+$	15		
219	Oxamyl				
	193	$[M-CON(CH_3)_2+HCOO]^-$		100	n.i.
	220	$[M+H]^+$	10	5	
	237	$[M+NH_4]^+$	100	100	
*Chlorinated phenoxy acids**					
220	2,4-D				
	219	$[M-H]^-$	30	30	10
	255	$[M+Cl]^-$	2		100
	265	$[M+HCOO]^-$		100	
	279	$[M+CH_3COO]^-$	100		10
	315	$[M+(CH_3COOH)\cdot Cl]^-$			10
268	Silvex				
	267	$[M-H]^-$	40	40	10
	269	$[M+H]^-$	50	50	7
	303	$[M+Cl]^-$	5	5	50
	313	$[M+HCOO]^-$		100	2
	327	$[M+CH_3COO]^-$	100		2
Chlorotriazine herbicides					
215	Atrazine				
	216	$[M+H]^+$	100	100	n.i.
	257	$[M+CH_3CN+H]^+$	30*	70*	
	275	$[M+CH_3COONH_4+H-H_2O]^+$	20		
240	Cyanazine				
	240	$[M]^{\cdot -}$	100	100	n.i.
	241	$[M+H]^+$	100	100	
	275	$[M+Cl]^-$	60	60	
	282	$[M+CH_3CH+H]^+$	25*	25*	
Organophosphorus pesticides					
256	Trichlorfon				
	205	$[M-HCl-CH_3]^-$	40	40	
	241	$[M-CH_3]^-$	20	20	7
	291	$[M+Cl]^-$	100	100	100
	301	$[M+HCOO]^-$		20	
	315	$[M+CH_3COO]^-$	20		
	351	$[M+(CH_3COOH)\cdot Cl]^-$			10

TABLE I *(continued)*

Mol. wt.	Compounds and ions (*m/z* and tentative identification)		A	B	C
277	Fenitrothion				
	141	$[FG]^-$	15	15	n.i.
	262	$[M-CH_3]^-$	15	15	
	277	$[M]^{\cdot -}$	100	100	
	278	$[M+H]^+$	30	30	
	295	$[M+NH_4]^+$	100	100	
Phenylurea herbicides					
198	Monuron				
	199	$[M+H]^+$	30	30	n.i.
	216	$[M+NH_4]^+$	100	100	
	233	$[M+Cl]^-$	15	15	
	243	$[M+HCOO]^-$		100	
	257	$[M+CH_3COO]^-$	100		
	275	$[M+CH_3COONH_4+NH_4-H_2O]^+$		50	
248	Linuron				
	247	$[M-H]^-$	100	100	n.i.
	249	$[M+H]^+$	20	20	
	266	$[M+NH_4]^+$	100	100	
	283	$[M+Cl]^-$	80	80	
	307	$[M+CH_3COONH_4+NH_4-H_2O]^+$		40	

[a] Not investigated.

such as pressure and temperature, impurities and radical species in the reagent gas and source characteristics, are involved in the ion formation in the NI mode of TSP-LC–MS.

When comparing the PI and NI modes for the different groups of pesticides, both qualitative and quantitative aspects should be considered. With respect to structure identification, the similarity between the TSP-LC–MS results in the PI and in the NI mode should be studied.

Table I shows the main ions obtained in the PI and NI modes for selected pesticides of different chemical groups and with the equipment described under Experimental. In the NI mode the ions formed depended on the compound and eluent used, whereas in the PI mode the different groups of pesticides behaved more similarly.

From Table I it can be seen that in the PI mode the $[M+H]^+$ (for chlorotriazines) and the $[M+NH_4]^+$ (for carbamates, organophosphorus pesticides, phenylurea herbicides) ions are the base peaks in the TSP mass spectra. Exceptions are those compounds having a higher proton affinity than the ammonium ion, as a result of their pyrimidinyl, amidate and pyrimidine structure, such as the organophosphorus pesticides, diazinon or fenamiphos [22,23], and the carbamates, pirimicarb and their pyrimidine metabolites [12], which exhibit $[M+H]^+$ as the base peak. The phenoxy acids and their degradation products, the chlorophenols, are hardly ionized in the PI mode because of the relative high electronegativity of these solutes. Because the quaternary ammonium compounds are already ionized, the M^+ ion is the base peak.

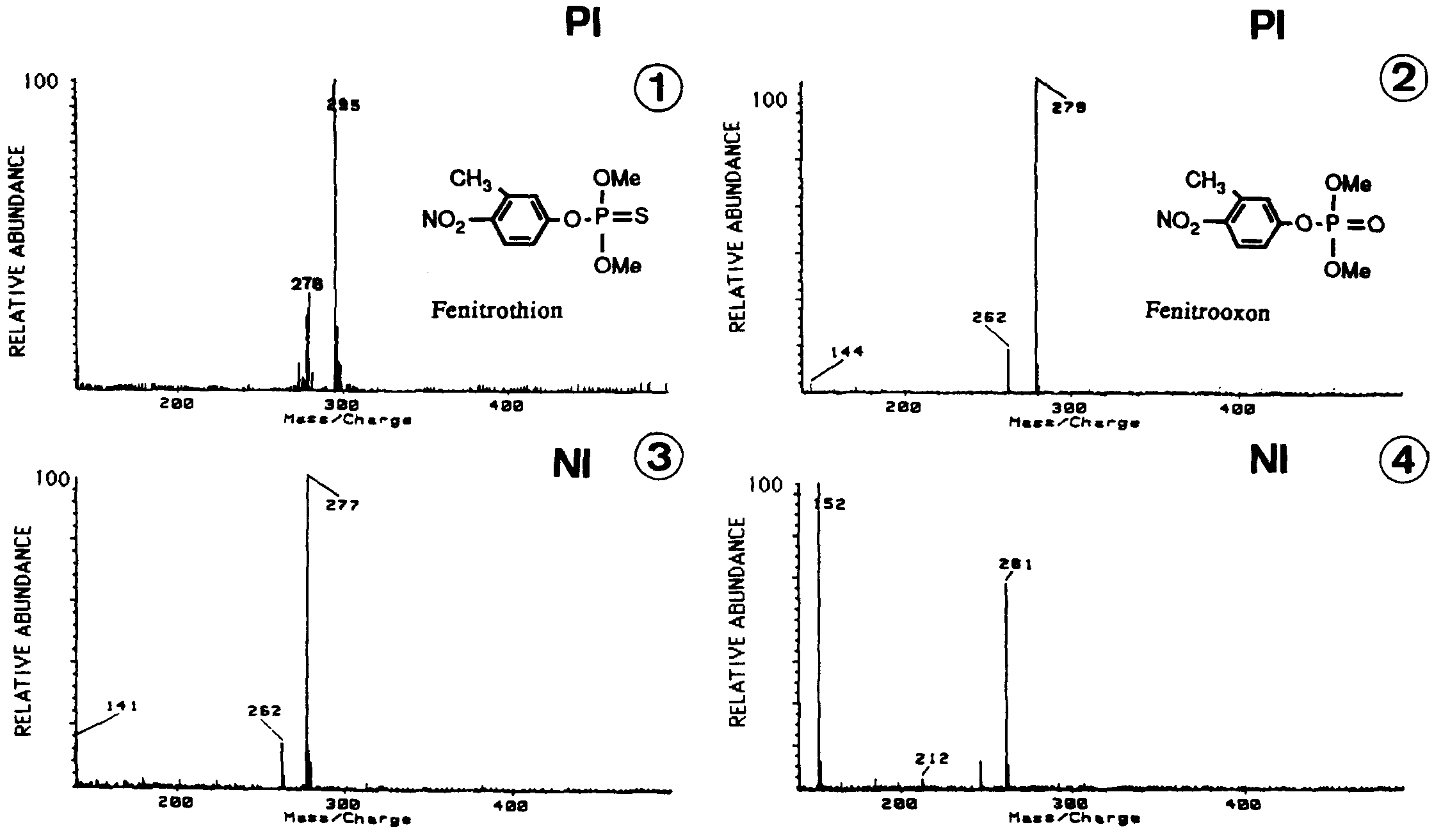

Fig. 1. Flow injection TSP mass spectra in the PI (spectra 1 and 2) and NI (spectra 3 and 4) modes for fenitrothion and fenitrooxon. Amount injected, *ca.* 1 μg; carrier stream, methanol–water (50:50) + 0.05 *M* ammonium formate; flow-rate, 1 ml/min; TSP vapour and ion source temperature, 270°C. Me = Methyl.

In the NI mode, the formation of anions depends on the chemical structure of the pesticide. For most of the carbamates, hardly any signal in the NI mode was obtained when 1 μg of sample was injected. Carbamates such as carbaryl and oxamyl exhibited anions like $[M-CONHCH_3]^-$, $[M-CONHCH_3+CH_3COOH]^-$ and $[M-CON(CH_3)_2+CH_3COOH]^-$, which correspond to the cleavage of the alkyl side-chain or the aromatic ring, just as observed in DLI-LC–MS [24]. For cyanazine, a chlorotriazine herbicide, $[M]^{\bullet -}$ was normally obtained as the base peak in combination with the chloride attachment $[M+Cl]^-$ ion, as a second abundant ion [12]. Typical NCl mechanisms were observed for the organophosphorus pesticides. The specific fragments were $[M-R]^-$, R being methyl or ethyl, $[FG]^-$ (FG being the functional group fragment of the organophosphorus pesticide), $[M]^{\bullet -}$, $[M-Cl]^-$, the phenolate and thiophenolate anions and adducts with the ionizing additive [6,7,25]. For the phenylurea herbicides, $[M-H]^-$ and $[M+CH_3COO]^-$ ions were, in general, the base peaks, while $[M+Cl]^-$ was a minor ion [7,26]. Finally, for the chlorinated phenoxy acids, anions such as $[M-H]^-$, $[M+H]^-$, $[M+CH_3COO]^-$ and $[M+Cl]^-$ were obtained. In this instance the base peak depended strongly on the eluent used [2,3,8,16].

In Fig. 1 an example is shown of the information that can be obtained by using both the PI and NI modes. The TSP mass spectra of fenitrothion and fenitrooxon are shown. The base peaks of fenitrothion and fenitrooxon in the PI mode correspond to the $[M+NH_4]^+$ ion with m/z values of 295 and 279, respectively. Other ions correspond to the $[M+H]^+$ ion at m/z 278 for fenitrothion and at m/z 262 for fenitrooxon. Some of the fragment ions observed for fenitrothion in NI-TSP–MS are similar to those reported in NCI-MS [27] such as fragment ions at m/z 141, 262 and 277 corresponding to the $[FG]^-$, $[M-CH_3]^-$ and $[M]^{\bullet -}$ ions, respectively. With fenitrooxon, the phenolate anion at m/z 152, corresponding to $[OC_7H_6NO_2]^-$, is the base peak. The second abundant ion corresponds to $[M]^{\bullet -}$ at m/z 261. Hence, by using both

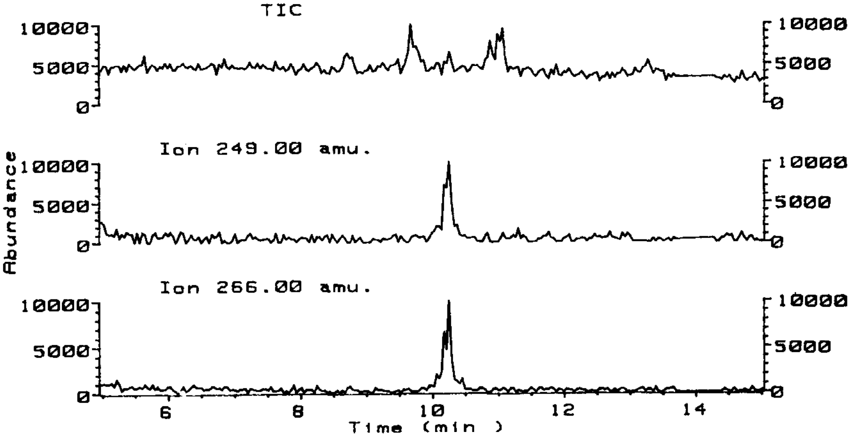

Fig. 2. Total ion current and selected ion chromatograms in PI mode for a real soil sample from the Ebro Delta containing 0.5 μg/g of linuron, collected 1 month after pesticide application. Monitored ions correspond to $[M+H]^+$ and $[M+NH_4]^+$, with m/z values of 249 an 266, respectively. Mobile phase, methanol–water (80:20) + 0.05 *M* ammonium formate; flow-rate, 1 ml/min; TSP vapour and ion source temperature, 270°C.

modes of operation, a complete and unequivocal identification of both organophosphorus pesticides is achieved.

In Fig. 2 the total ion current and the selected ion chromatograms are shown for a soil sample polluted with 0.5 μg/g of linuron, applied as a herbicide 1 month before the sample was taken. The presence of linuron is clearly shown in the sediment sample.

The second aspect, in comparing the PI and NI modes in TSP-LC–MS, is the quantitative potential of each mode. In Table II, the detection limits using RP eluents for the various groups of pesticides are shown. In general, the PI mode is recommended for the analysis of compounds with proton affinity values higher than or close to that of ammonia, such as carbamate insecticides and chlorotriazine and phenylurea herbicides. For the organophosphorus pesticides from the parathion group (phosphorothionates with an aromatic structure like parathion), although they also exhibit proton affinity values close to ammonia, they can easily stabilize the negative charge under NI conditions, owing to the chemical structure. Hence, the sensitivities for the PI and the NI modes are approximately identical. With the oxygen analogues, because the oxo group enhances the proton affinity in comparison with the thio group, the sensitivity is one order of magnitude better in PI compared with the NI mode. For the chlorinated phenoxy acids and chlorophenols the sensitivity in the NI mode is much better than that in PI mode. This is due to their proton affinity values, which are much lower than that of ammonia, so no signal is obtained under PI conditions.

TABLE II

DETECTION LIMITS (FULL SCAN) FOR THE DIFFERENT GROUPS OF PESTICIDES WITH TSP-LC–MS

LC eluent: methanol–water (50:50) or (see footnote) acetonitrile–water (50:50) + 0.05 M ammonium acetate or 0.05 M ammonium formate

Pesticides	Detection limit (ng)	
	PI	NI
Organophosphorus pesticides (parathion group)	20–50	50–70
Organophosphorus pesticides (oxygen analogues) (paraoxon group)	1–2	50–70
Carbamates (in general)	1–2	>200
Hydroxypyrimidine and pirimicarb metabolites	1–2	1–2
Chlorotriazines + hydroxytriazines (except cyanazine)	5–10	100
Chlorinated phenoxy acids[a]	>200[a]	1–10
Chlorophenols[a]	>200[a]	1–10
Phenylurea herbicides	2–5	10–20
Quaternary ammonium compounds[a] (only difenzoquat)	~100	n.m.[b]

[a] Acetonitrile–water (50:50) eluent.

[b] Not measured.

Influence of (ionizing) additives in TSP-LC-MS eluents

The lack of structural information observed for some of the tested pesticide groups is the main limitation of TSP-LC-MS. A possible solution is the use of eluent additives to form adducts which provide more information for molecular weight assignment and/or structure identification. In the PI mode the formation of adduct ions in TSP-LC-MS follows a general rule as was recently proposed by Maeder [28]. The masses of these adduct ions can be described by the equation

$$M_{\text{adduct}} = [\text{M}+\text{A}+x\text{B}-y\text{H}_2\text{O}]^+ \qquad (1)$$

where M is the molecular weight of the analyte, A is the attached ion (H^+ or NH_4^+), B is the attached molecule (CH_3OH, H_2O, CH_3CN) and x and y can have the values 0, 1, 2, etc.

Changing the eluent from acetonitrile–water to methanol–water or using ammonium formate or ammonium acetate as ionizing additives will not change the base peaks of the different pesticides. Significant differences are observed, however, for the other fragment ions. The $[M+CH_3CN+H]^+$ and $[M+CH_3CN+NH_4]^+$ ions showed relative abundances of 40–70% for the carbamates and the chlorotriazines when acetonitrile–water mixtures were used [12]. When ammonium acetate was used as an ionizing additive with acetonitrile–water or methanol–water mixtures, adduct ions such as $[M+CH_3COONH_4+NH_4^+-2H_2O]^+$ or $[M+CH_3COONH_4^++H-H_2O]^+$ appeared in the spectra. Although the base peak was normally the same, for the chlorotriazines different base peaks were observed when the vapour temperature of the TSP interface was changed. At 200°C the $[M+CH_3COONH_4+H-H_2O]^+$ ion was the base peak using ammonium acetate as ionizing additive [2], but at 270°C the $[M+H]^+$ ion was the base peak, because of the thermally induced dissociation in the ion source.

In the NI mode, different adduct ions have been reported for a given additive

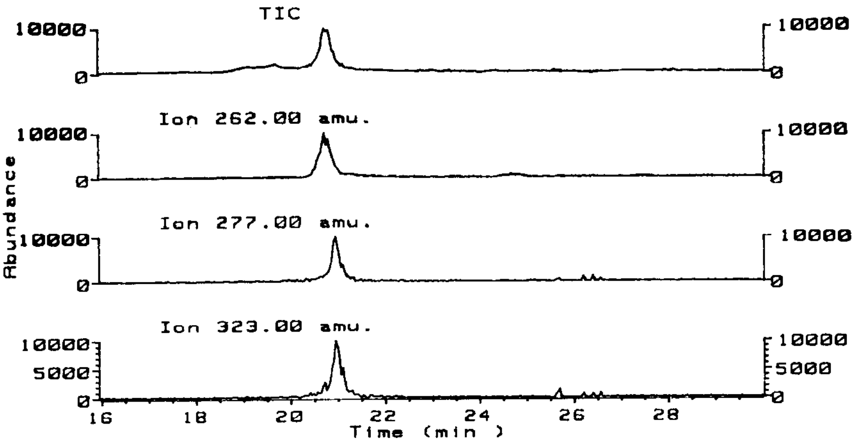

Fig. 3. Total ion current and selected ion chromatograms in NI mode for a real soil sample from the Ebro Delta containing 0.8 μg/g of fenitrothion, collected 2 days after aircraft application. Ions monitored correspond to $[M-CH_3]^-$, $[M]^{\cdot-}$ and $[M+HCOOH]^-$, with m/z values of 262, 277 and 323, respectively. Chromatographic conditions as in Fig. 2; TSP vapour and ion source temperature, 200°C.

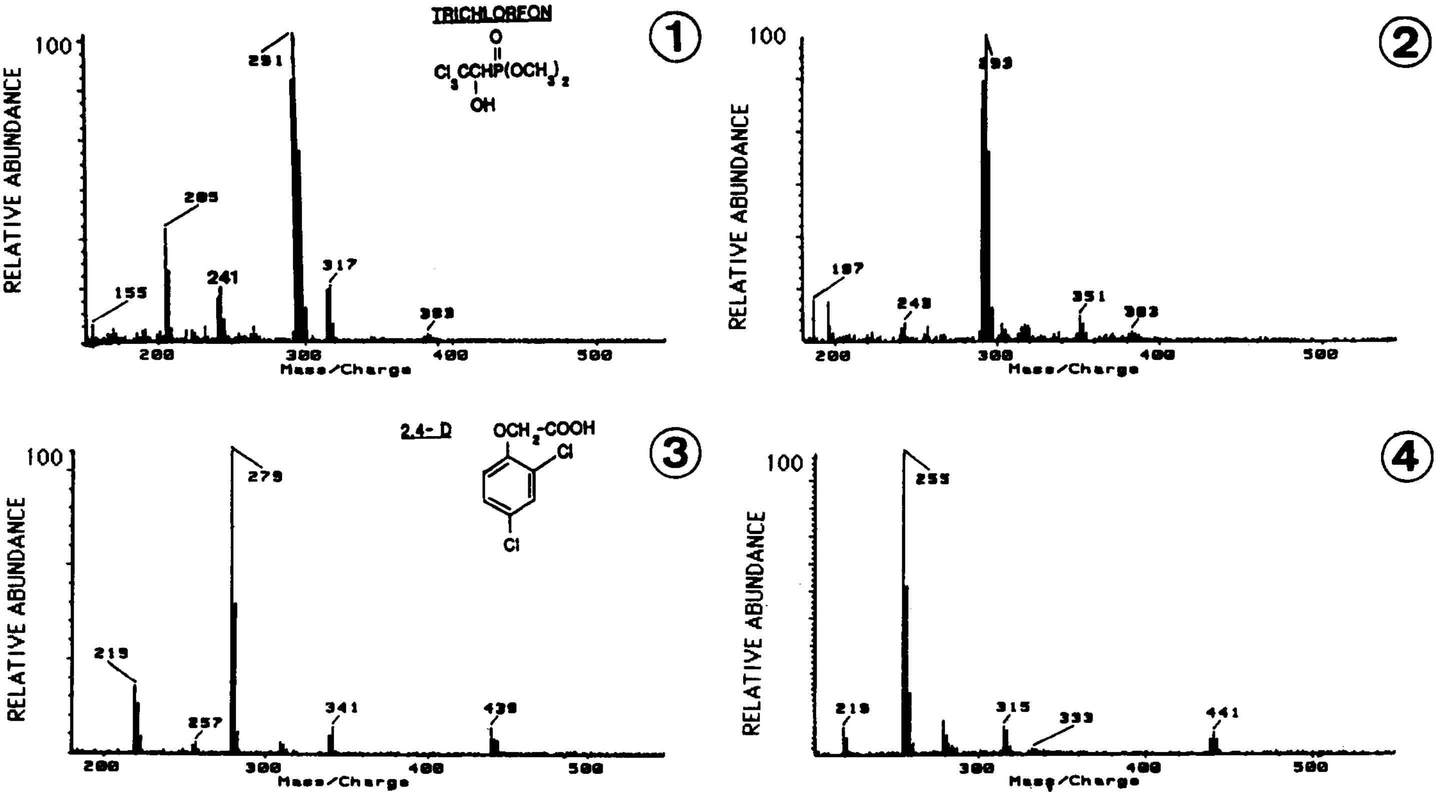

Fig. 4. Flow-injection NI mode TSP-MS spectra of trichlorfon (spectra 1 and 2) and 2,4-D (spectra 3 and 4). Carrier stream, (1, 3) acetonitrile–water (50:50) + 0.05 *M* ammonium acetate and (2,4) acetonitrile–water–chloroacetonitrile (49:49:2) + 0.05 *M* ammonium acetate; flow-rate, 1 ml/min; amount injected, 1 μg; TSP vapour and ion source temperature, 200°C.

depending on the eluent. Because ammonium acetate is the most frequently applied buffer, the $[M+CH_3COO]^-$ ion is the most cited adduct ion. Adducts with formate have also been reported [2,12,15] to yield additional molecular weight information. An example is the analysis of a soil sample from the Ebro Delta (Tarragona, Spain), where fenitrothion is currently applied as an insecticide [29] (Fig. 3). The $[M-R]^-$ anion, in this instance the $[M-CH_3]^-$ ion, seems to be typical of the organophosphorus pesticides of the parathion group. A third ion, corresponding to $[M+HCOOH]^-$, was also used in this instance for confirmation purposes and it is an attachment ion obtained in this case and not in the spectra in Fig. 1. This has been previously explained for this compound in NI-TSP–MS and it is due to the fact that a lower vapour temperature (200°C in Fig. 3 *versus* 270°C in Fig. 1) produces higher abundances of anion attachment ions [25]. These ions provide adequate proof of the presence of fenitrothion in sediment. In PI TSP-LC–MS only two ions, corresponding to the $[M+H]^+$ and $[M+NH_4]^+$ ions, were observed, so less structural information was obtained than in the NI mode.

The applicability of chloride-attachment phenomena for obtaining complementary structural information using chloracetonitrile in the eluent has proved to give satisfactory results for chlorine-containing compounds [8]. In the NI mode, the mentioned additives (ammonium acetate, ammonium formate and chloracetonitrile) all provide additional adduct ion information for compounds with a good signal in the NI mode, such as chlorinated phenoxy acids and chlorophenols [2,3,8,15]. As an example, the TSP mass spectra of trichlorfon and 2,4-D are given in Fig. 4. A mixture of acetonitrile and water, containing ammonium acetate as the buffer, was used with and without the addition of 2% chloroacetonitrile. For trichlorfon, an organophosphorus pesticide containing a chlorine atom, the $[M+Cl]^-$ ion (m/z 291) is already the base peak even without the addition of chloracetonitrile. This ion can be due to self-attachment of chlorine to the molecule by pyrolysis in the TSP aerosol of this thermally labile compound [6]. The other anions found for trichlorfon which have also been reported in NCI DLI-LC–MS [5] corresponded to $[M-HCl-CH_3]^-$, $[M-CH_3]^-$ and $[M+CH_3COO]^-$ at m/z 205, 241 and 315, respectively.

For 2,4-D, the base peak without the addition of chloracetonitrile corresponds to $[M+CH_3COO]^-$. As the molecule also contains a chlorine atom, a chloride attachment ion at m/z 255 was observed, but because 2,4-D itself is less thermolabile than trichlorfon this chloride attachment peak was not the base peak. Adding chloroacetonitrile to the eluent changed both spectra. For trichlorfon, the base peak remained the same, but the other ions nearly disappeared, indicating that only chloride attachment was taking place. For 2,4-D the new base peak was the $[M+Cl]^-$ ion and the other ions were relatively weak, as in the case of trichlorfon. Additional chlorine anion attachment ions at m/z 351 and 315 for trichlorfon and 2,4-D, respectively, are obtained when chloroacetonitrile is added to the eluent, which corresponds to the $[M+(CH_3COOH)\cdot Cl]^-$ ion, as reported previously for 2,4-D [8].

Normal-phase eluents

In most instances RP eluents are used in TSP-LC–MS and only a few applications have been reported using NP eluents such as *n*-hexane, cyclohexane or dichloromethane [3,16]. The necessity for using NP-LC for a number of analyses results in a wider application range of TSP-LC–MS. Moreover, the detection possibilities of

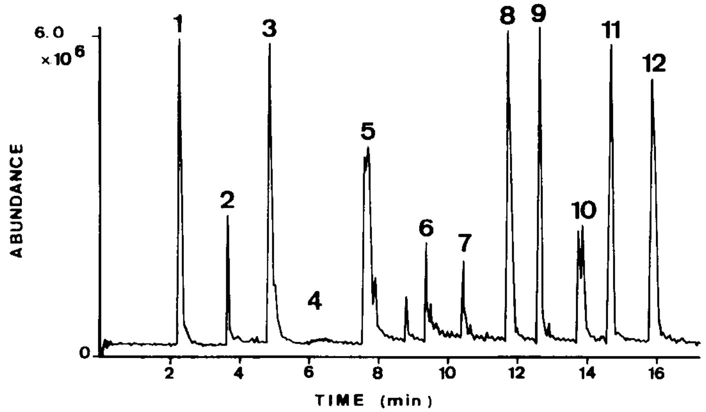

Fig. 5. Flow-injection PI mode TSP-MS reconstructed ion current of several pesticides and chlorophenols (*ca.* 500 ng each) using cyclohexane as carrier stream. Compounds: 1 = 2,4-dichlorophenol; 2 = Silvex; 3 = trichlorfon; 4 = pentachlorphenol; 5 = phosmet; 6 = 2,4-D; 7 = 2,4,5-T; 8 = parathion-ethyl; 9 = 2,4,5-trichlorophenol; 10 = fensulfothion; 11 = fonofos; 12 = chlorpyrifos. Flow-rate, 0.7 ml/min; TSP vapour and ion source temperature, 200°C.

TSP-LC–MS will be improved, as additional structural information and/or greater sensitivity can be obtained.

The main advantage of NP eluents in TSP-LC–MS is the improved response in this mode for compounds with high electronegativity, *e.g.*, chlorophenols and chlorinated phenoxy acids. The detection limits for these compounds in the NI and PI modes, using an NP eluent, were 1–10 and 2–20 ng, respectively. The total ion current trace of individual test compounds (organophosphorus pesticides, chlorophenols and chlorinated phenoxy acids) injected one after the other in the PI-TSP-MS system is shown in Fig. 5. Cyclohexane was used as the carrier stream and reagent gas. The chlorophenols and chlorinated phenoxy acids did not show any response when the same amount was injected as in an RP eluent using the PI mode.

In Fig. 6 the PI and NI-TSP mass spectra of chlorpyrifos in cyclohexane and parathion-ethyl and 2,4,5-T in dichloromethane are shown. The observed base peaks in both modes can be explained by the similarity of the chemical ionization process in TSP-LC–MS and DLI-LC–MS. This similar behaviour was even more pronounced when TSP operation was carried out without a buffer and with the filament on. In this case $[M+NH_4]^+$ ions were not formed at all. When the different PI-TSP spectra in Fig. 6 are compared, it is obvious that the base peak was the $[M+H]^+$ ions for the organophosphorus pesticides, chlorpyrifos and parathion-ethyl, and the $[M]^{\cdot+}$ ion for 2,4,5-T. In PI-DLI with RP eluents normally the $[M+H]^+$ ion is the base peak for the organophosphorus pesticides [5] and the $[M]^{\cdot+}$ ion is observed for chlorinated phenoxyacids, with relative intensities of *ca.* 10% [24].

Further, it can be seen that the NI-TSP spectra offer significantly more structural information than PI-TSP spectra. In NI-TSP-MS the spectra were similar to those obtained by conventional NCl-MS and DLI-LC–MS using the NI mode. Chemical ionization processes such as dissociative electron capture, resonance electron capture, proton transfer and anion attachment occur. For chlorpyrifos, most of the ions were observed in NCl-MS. The ions observed were the functional group fragment of the organophosphorus pesticide $[FG]^-$, $[M-(C_2H_5O)_2PO]^-$, $[M-HCl]^-$ and $[M]^{\cdot -}$. They had relative abundances of 48, 61, 100 and 12%, respectively, in NCl-MS [27], which is consistent with the TSP mass spectra in Fig. 6. For parathion-ethyl, the base peaks in NCl-MS or DLI-LC–MS [30] corresponded to the thiophenolate anion at *m/z* 154, whereas the other ions exhibited relative abundances below 30%. In TSP-LC–MS the abundance of the ions was not the same as in DLI-LC–MS or NCl-MS. Moreover, the base peak was the $[M-R]^-$ ion (at *m/z* 262) and about 15% was the $[M-H+Cl]^-$ ion, corresponding to a mixed mechanism of proton abstraction and chloride attachment. The $[M-R]^-$ ion was also observed as the base peak for most of the organophosphorus pesticides when 1% chloroacetonitrile was added to an RP eluent in DLI-LC–MS [5]. The 15% relative abundance of the chloride adduct was due to the use of dichloromethane as reagent gas, which favours the addition of a chlorine atom. For 2,4,5-T, the base peak was the $[M+Cl]^-$ ion, at $m/z = 289$. This can be explained by the relatively high concentration of chlorine available in the ion source available from the dichloromethane combined with the pyrolytic behaviour of the analyte itself, in a similar manner to that mentioned previously for aromatic chlorine-containing compounds in NI-TSP-LC–MS [8,15,21,26]. The $[M+Cl]^-$ ion has previously been observed in DLI-LC–MS [31], with abundances of up to 60% with RP systems in TSP-LC–MS [2,3,8]. Abundances up to 100% were found when 2% chloracetonitrile was added to an RP-LC eluent [8,15].

Post-column extraction strategies

In TSP-LC–MS, normally RP eluents containing volatile buffers (*e.g.*, ammonium acetate or ammonium formate) are used. However, in many pesticide analyses non-volatile buffers and/or ion-pairing reagents are applied. The latter system can be combined with TSP-LC–MS using a post-column extraction system. The applicability of this system is demonstrated with the ion-suppressed liquid–liquid extraction of chlorinated phenoxy acids and with the ion-pair extraction of difenzoquat, one of the quaternary ammonium compounds used as a herbicide nowadays. A schematic diagram of the experimental set-up has been described [16]. The phase separator in this system allows an efficient separation of an organic and an aqueous phase, so a purely organic phase can be obtained which can be directly introduced into the mass spectrometer by means of the TSP interface. The flow of the organic solvent is regulated by a PTFE capillary and a restrictor. For the ion-suppressed extraction of chlorinated phenoxy acids, an RP-LC separation on a C_{18} column is applied with an eluent consisting of acetonitrile–water (50:50) containing 0.1 *M* phosphate buffer (pH 2.5), ensuring that the acids were non-dissociated, at a flow-rate of 1 ml/min. The post-column extraction was performed with dichloromethane–cyclohexane–*n*-butanol (45:45:10) at a flow-rate of 1 ml/min. In this way the chlorinated phenoxy acids were extracted into the organic phase and subsequently introduced into the TSP-LC–MS system. The extraction efficiencies for 2,4-D, 2,4,5-T and Silvex were 100%, 70% and 60%, respectively.

The analysis of quaternary ammonium compounds is tedious and involves either multi-step extraction and derivatization processes prior to GC determination [32,33] or cation-exchange LC and UV detection [32,34,35]. Owing to problems with separation and detection [32], methods based on fast atom bombardment MS–MS

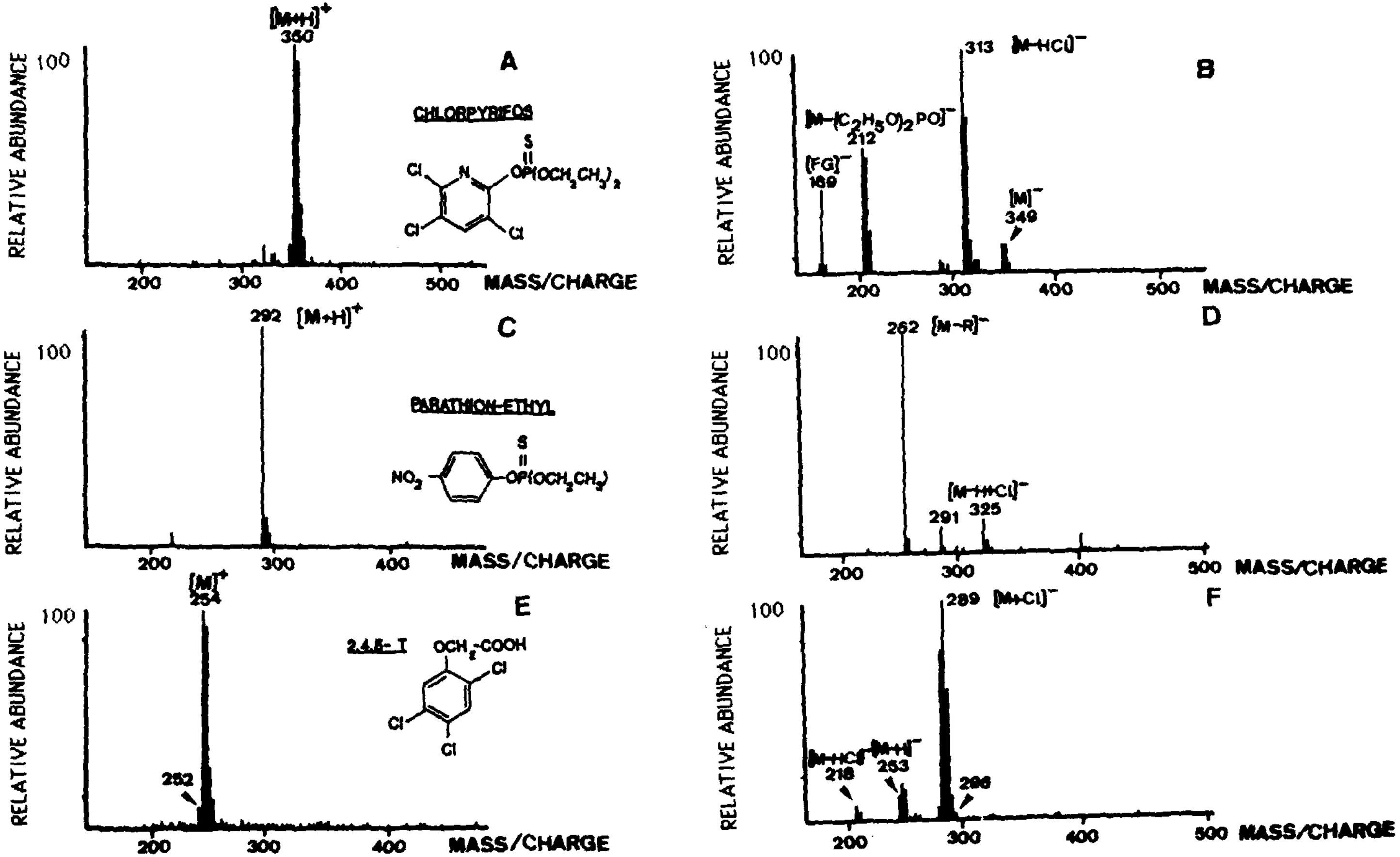

Fig. 6. Flow-injection PI (A, C, E) and NI (B, D, F) mode TSP mass spectra of chlorpyrifos (A, B), parathion-ethyl (C, D) and 2,4,5-T (E, F). Carrier stream for compounds A and B, cyclohexane, and for C–F, dichloromethane; flow-rate, 0.7 ml/min; amount injected, 500 ng; TSP vapour and ion source temperature, 200°C.

[36] and TSP-LC–MS [37] have also been reported. The method reported here involves the use of a post-column extraction system of difenzoquat with appropriate sulphonate counter ions. This is a sequel to the method developed by De Ruiter *et al.* [38] for the analysis of cationic detergents with sulphonate counter ions via post-column ion-pair formation and subsequent extraction. Several sulphonated counterions (methyl orange, dodecane sulphonate, Acid Blue 113 and Mordant-Red 9 [39]) were tested. All these sulphonates showed good extraction efficiencies (80–100%) with difenzoquat when it was extracted from an aqueous phase [acetonitrile–water (60:40) containing $1\cdot10^{-4}$ *M* of the sulphonate] to an organic phase [cyclohexane–dichloromethane–*n*-butanol (45:45:10)]. In Fig. 7 the TSP mass spectra obtained with flow injection of difenzoquat using a carrier stream of acetonitrile–water (A) and via the post-column extraction system, where difenzoquat is extracted as an ion pair with Acid Blue 113 (B), are shown. The limit of detection after the post-column extraction and under full scan conditions was 100 ng, which is comparable to that obtained by post-column reduction using alkaline sodium dithionite and UV detection [32,35]. 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 UV spectra are dependent on the eluent composition [40]. 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, nothing could be detected by TSP-MS.

The TSP mass spectra of difenzoquat in the PI mode showed a base peak at *m/z* 235 and a second abundant ion at *m/z* 249, corresponding to the cations $[(C_{17}H_{17}N_2)$

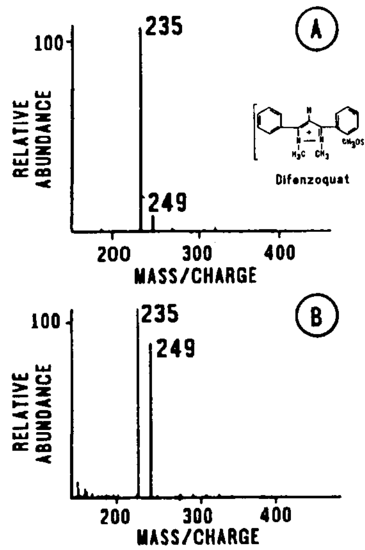

Fig. 7. TSP-mass spectra of difenzoquat obtained (A) using a reversed-phase eluent of acetonitrile–water and (B) after the post-column extraction system with cyclohexane–dichloromethane–*n*-butanol (45:45:10) at 1 ml/min; TSP vapour and ion source temperature, 200°C, with stem temperatures of (A) 100°C and (B) 86°C.

$-CH_3+H]^+$ and to $[(C_{17}H_{17}N_2)]^+$, respectively. The relative abundance of the latter was higher when the post-column ion-pair extraction system was employed (Fig. 7B). This can be explained by the lower vaporizer temperature used in the post-column set-up. The lower vaporizer temperature was needed for a stable signal, because the higher volatility of the extraction solvent is lower than that of water. A consequence of this lower vaporizer temperature is a decreased thermally induced dissociation. Hence the ion at *m/z* 249 had a higher abundance in the extraction set-up. The observed mass spectra match closely those obtained in fast atom bombardment (FAB)-MS experiments [36]. The background TSP spectra contained a peak at *m/z* 344, resulting from Acid Blue 113, and corresponding to a cleavage near the azo group. This fragment was also observed in FAB-MS experiments [39].

CONCLUSIONS

The features of different eluents for pesticide confirmation using both the PI and NI mode in TSP-LC–MS have been discussed. The influence of either the PI or NI mode on the sensitivity depends, in general, on the compound of interest. For chlorinated phenoxy acids, the NI mode is recommended using RP eluents whereas for carbamates and chlorotriazines the PI mode is preferred. For the other groups of pesticides the differences are not so pronounced and, consequently, both modes can be used. With different eluents significant differences are observed in the PI mode for the adduct ions, although the base peak is always the $[M+NH_4]^+$ ion. When the NI mode is used the base peak generally changes to the anion attachment peak of the eluent additive used. Different eluent additives can be applied in both the PI and NI modes to obtain more information for identification of the different groups of pesticides.

The use of NP eluents in TSP-LC–MS offers the advantage of improved detection in the PI mode for electronegative compounds such as chlorinated phenoxy acids. In the NI mode additional structural information is obtained and the fragment ions show a great resemblance to the ions observed under NCI-MS conditions.

The post-column ion-pair extraction system increases the selectivity and allows the introduction of polar herbicides in TSP-LC–MS. However, there is still a need to improve the analytical system for the analysis of quaternary ammonium compounds by LC–MS with ion-pair extraction, because the volatility of the ion-pairing reagent should be chosen carefully.

The possibilities of using different LC mobile phase compositions in both the PI and NI modes and their application to the identification and determination of pesticide residues at the ppb level in soil samples have been demonstrated.

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