Journal of Chromatography, 562 (1991) 507–523
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5556

Mobile phase variations in thermospray liquid chromatography–mass spectrometry of pesticides

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ABSTRACT

The effect of four different mobile phase compositions with reversed-phase methanol–water (50:50) + 0.05 *M* ammonium acetate, methanol–water (50:50) + 0.05 *M* ammonium formate, acetonitrile–water (50:50) + 0.05 *M* ammonium acetate and acetonitrile–water (50:50) + 0.05 *M* ammonium formate were compared in filament-on thermospray liquid chromatography–mass spectrometry for the determination of carbamate and chlorotriazine pesticides. In the positive-ion mode, $[M + H]^+$ and $[M + NH_4]^+$ were generally the base peaks for the chlorotriazines and the carbamates, respectively. Depending on the mobile phase used, other adduct ions obtained corresponded to $[M + CH_3CN + H]^+$, $[M + CH_3OH + NH_4]^+$, $[M + CH_3COONH_4 + NH_4 - 2H_2O]^+$, $[M + CH_3CN + NH_4]^+$, $[M + CH_3COONH_4 + H - H_2O]^+$ and the dimer $[2M + H]^+$. In the negative-ion mode, $[M - H]^-$ and adducts with the ionizing additive $[M + CH_3COO]^-$ or $[M + HCOO]^-$ were obtained. Other ions for the carbamates carbaryl and oxamyl corresponded to $[M - CONHCH_3 + CH_3COOH]^-$ and $[M - CON(CH_3)_2 + HCOO]^-$, respectively. The variation of mobile phase composition provides additional structural information in thermospray liquid chromatography–mass spectrometry with no appreciable loss of sensitivity. Applications are reported for the determination of carbamate and chlorotriazine pesticides at the ng/g level in spiked and real soil samples, respectively.

INTRODUCTION

Thermospray liquid chromatography–mass spectrometry (TSP LC–MS) is a well established technique that uses a variety of LC eluents such as acetonitrile–water, methanol–water or cyclohexane mixtures [1–4]. Most of the work carried out with TSP LC–MS uses reversed-phase LC and requires the addition of an aqueous buffer solution, generally ammonium acetate, in order to result in the TSP ionization [5]. The TSP spectra as in the chemical ionization (CI) process, depend strongly on a variety of parameters, *e.g.* temperature, pressure and the geometry of ion source and interface [6]. In addition, the ionization is achieved with the use of filament-on or filament-off, and the relative abundance of the different fragment ions in the spectra is strongly dependent on the type and concentration of the analyte and the composition of the mobile phase. In general, it is accepted that for neutral compounds a gas-phase CI process takes place with the generation of ammonium and acetate ions as the reactant ions [4,7–9].

0378-4347/91/$03.50

The lack of structural information in TSP LC–MS spectra can be partly overcome by the complementary adducts obtained when using different eluent compositions. Ammonium formate has been evaluated as an ionizing additive in TSP LC–MS by several authors, with either lower [1] or similar [3,10,11] sensitivity to that of ammonium acetate, for a variety of pesticides. Other eluent compositions reported were 2% chloroacetonitrile in acetonitrile–water + 0.05 *M* ammonium acetate [6] and cyclohexane [4].

Owing to their thermal instability and polar functional groups, carbamate pesticides require derivatization to be amenable to GC. As an alternative, different LC methods have been employed, such as UV [12,13] electrochemical [14,15] and on-line NaOH hydrolysis with post-column reaction followed by fluorescence detection [16,17]. The use of LC with on-line MS has been employed with different interfacing systems, such as direct liquid introduction (DLI) [18], moving belt [19,20] and TSP [21–24]. Even the use of off-line tandem MS [25] and on-line with LC [26,27] has been reported. Other analytical techniques incidently employed include supercritical fluid chromatography [28,29]. For the analysis of triazines in environmental samples, although GC–MS [30,31] and GC–MS–MS [32] methods have been reported, the LC methods, generally with UV detection, are suitable for thermally labile triazines, such as cyanazine [33], and for analysing the most polar pesticides and the hydroxytriazines [34,35]. Other detectors used in LC were electrochemical [36,37], MS with TSP [3,4,35] or with DLI interfaces [38], and MS–MS [39] detectors have also been employed.

The overall objective of this study is to explore the influence of four different eluent compositions with reversed-phase LC eluents, containing methanol–water (50:50) + 0.05 *M* ammonium acetate, methanol–water (50:50) + 0.05 *M* ammonium formate, acetonitrile–water (50:50) + 0.05 *M* ammonium acetate and acetonitrile–water (50:50) + 0.05 *M* ammonium formate in positive- and negative-ion modes (PI and NI, respectively) during the generation of TSP spectra of chlorotriazines and carbamates. In addition, pesticide residues will be confirmed in soil samples.

EXPERIMENTAL

Chemicals

HPLC-grade water from Riedel-de-Haën (Seelze-Hannover, F.R.G.) and methanol and acetonitrile from Merck (Darmstadt, F.R.G.) were passed through a 0.45-μm filter (Scharlau, Barcelona, Spain) before use. The solvents, acetone, ethyl acetate, *n*-hexane, diethyl ether and dichloromethane, were pesticide grade obtained from SDS (Peypin, France). Analytical-reagent grade propoxur, carbaryl, carbofuran, oxamyl, α-naphthol, chlorpropham, atrazine, simazine and cyanazine were from Polyscience (Niles, IL, U.S.A.), Pirimicarb from Promochem (Wesel, F.R.G.) and the metabolites from Pirimicarb compounds V, (2-dimethylamino-5,6-dimethyl-4-hydroxypyrimidine), VI (2-methylamino-5,6-di-

methyl-4-hydroxypyrimidine) and VII (2-amino-5,6-dimethyl-4-hydroxypyrimidine) were gifts from P. Cabras (Cagliari, Italy). Florisil (100–200 mesh) was purchased from Merck. Ammonium acetate and ammonium formate were obtained from Panreac (Barcelona, Spain) and Fluka (Buchs, Switzerland). Labelled acetic acid was purchased from Cambridge Isotope Labs. (Innerberg, Switzerland).

Sample preparation

Sample pre-treatment for triazines was carried out using a procedure modified from that reported by Durand *et al.* [40]. For carbamate pesticides, sample pre-treatment was performed by the procedure described in ref. 41. Briefly, both methods include freeze-drying of soil samples, sieving through 120-μm mesh, and soxhlet extraction for 12 h with methanol or acetone–dichloromethane (50:50) for the triazines and the carbamates, respectively. After concentration in a rotary evaporator (35°C) to *ca.* 20–25 ml, the extract is carefully evaporated to dryness, dissolved in *n*-hexane, and cleaned up by Florisil eluted using 50% diethyl ether in *n*-hexane. After sample preparation is finished, methanol is added to yield a final volume of 0.5 ml for injection into the LC–MS system.

Chromatographic conditions

Eluent delivery was provided by 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. Four different LC mobile phase compositions were tested: methanol–water (50:50) + 0.05 *M* ammonium acetate, methanol–water (50:50) + 0.05 *M* ammonium formate, acetonitrile–water (50:50) + 0.05 *M* ammonium acetate and acetonitrile–water (50:50) + 0.05 *M* ammonium formate, at a flow-rate of 1 ml/min.

Mass spectrometric analysis

A Hewlett-Packard (Palo Alto, CA, U.S.A.) Model 5988A LC–TSP-MS quadrupole mass spectrometer and a Hewlett-Packard Model 59970C instrument for data acquisition and processing were employed. The TSP temperatures were: stem, 100°C; tip, 188°C, vapour, 270°C; ion source, 270°C. In all the experiments the filament-on mode (ionization by an electron beam) was used. In this mode of operation, conventional PI CI can be carried out using the vaporized mobile phase as the CI reagent gas [3]. Calibration of the TSP LC–MS instrument was achieved with a solution of hydrocortisone (5 mg), which was dissolved in an eluent containing 250 ml of acetonitrile–water (50:50) + 0.05 *M* ammonium formate. Ions for calibration in PI mode were at *m/z* 59, 141 and 363, corresponding to $[(CH_3CN)NH_4]^+$, $[(CH_3CN)_3NH_4]^+$ and $[M + H]^+$ (where M is the

molecular mass of hydrocortisone), respectively. In NI mode, the ions at m/z 91, 137 and 407, corresponding to $[(HCOOH)(HCOO)]^-$, $[(HCOOH)_2(HCOO)]^-$ and $[M + HCOO]^-$, were used. The hydrocortisone ions reported are in agreement with those reported elsewhere [9,42].

RESULTS AND DISCUSSION

In TSP LC–MS the nature of the reagent gas is determined by the composition of the LC eluent. Because volatile ionic modifiers, such as ammonium acetate or ammonium formate, are needed in most of the experiments, the demands of a suitable eluent can seriously interfere with the optimization of both PI and NI modes. The different clusters between the components of the LC eluent will determine the scan range. Obviously, the best solution is to use additives to the LC eluent that hardly influence the LC analysis, give good sensitivity and permit the scan range to begin at the lowest values. In these cases molecules with low molecular mass or fragments from other molecules at low masses can be efficiently identified. Because no complete survey has yet appeared of the different compositions of the reagent gas ions of the LC eluent, we started by investigating four different common eluent compositions in reversed-phase systems with PI and NI modes of operation. (Incidently, the use of labelled acetic acid was needed to identify unequivocally some adducts from the eluent.) The information obtained from these studies will be applied to the characterization of carbamate and triazine pesticides. When using different eluent mixtures, the adduct ion formation follows a general fomula that was recently proposed [43]:

$$[M + A + xB - yH_2O]^+$$

where M is the molecule of the analyte, A is the attached ion (either H^+ or NH_4^+), B is the attached molecule (CH_3OH, H_2O, CH_3CN or the ionizing additive) and x and y take the values of 0,1,2, etc. In NI mode, and as reported previously [3,9,10], $[M + CH_3COO]^-$, $[M + HCOO]^-$, $[M + CF_3COO]^-$ or $[M + CCl_3COO]^-$ are formed, depending on the ionizing additive used.

Reagent gas spectrum

Tables I and II list the relative abundances of the different reagent ions observed with acetonitrile–water (50:50) + 0.05 *M* ammonium acetate, acetonitrile–water (50:50) + 0.05 *M* ammonium formate, methanol–water (50:50) + 0.05 *M* ammonium acetate and methanol–water (50:50) + 0.05 *M* ammonium formate.

When considering PI mode mixtures, the formation of the different adducts follows the rules of the CI processes [44] and DLI LC–MS [45]. In general, the proton affinity values of ammonia are the highest, at 858 kJ/mol, so the predominant adduct ion will contain ammonia in most cases.

In the case of acetonitrile–water mixtures, acetonitrile exhibits a similar pro-

TABLE I

RELATIVE ABUNDANCES IN PI-TSP LC–MS OF SOLVENT IONS

Filament-on mode of operation; flow-rate, 1 ml/min. A = acetonitrile–water (50:50) + 0.05 *M* ammonium acetate; B = acetonitrile–water (50:50) + 0.05 *M* ammonium formate; C = methanol–water (50:50) + 0.05 *M* ammonium acetate; D = methanol–water (50:50) + 0.05 *M* ammonium formate.

m/z	Fragment (tentative identification)	A	B	C	D
18	$[NH_4]^+$	2	2	3	7
36	$[(H_2O)NH_4]^+$				15
50	$[(CH_3OH)NH_4]^+$			100	100
59	$[(CH_3CN)NH_4]^+$	40	100		
68	$[(CH_3OH)(H_2O)NH_4]^+$				42
77	$[(CH_3CN)(H_2O)NH_4]^+$	6	6		
78	$[(CH_3COONH_4)H]^+$			20	
82	$[(CH_3OH)_2NH_4]^+$				85
100	$[(CH_3CN)_2NH_4]^+$	100	100		
110	$[(CH_3COOH)\ (CH_3OH)\ (NH_4)]^+$			15	
118	$[(CH_3CN)_2(H_2O)NH_4]^+$	8	8		
141	$[(CH_3CN)_3NH_4]^+$	8	100		

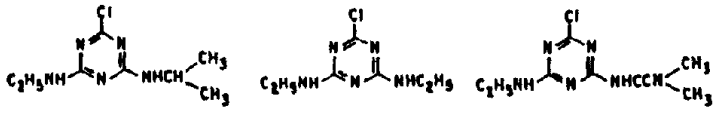
ton affinity (PA) value to that of acetic acid (797–798 kJ/mol), but higher than that of water (724 kJ/mol), so adducts between acetonitrile and ammonia predominate. When ammonium acetate is used, the base peak is at *m/z* 100 [46]. In the case of using methanol–water mixtures with ammonium acetate or ammoni-

TABLE II

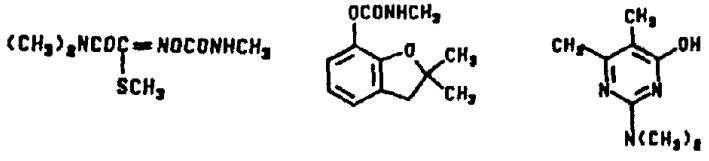
RELATIVE ABUNDANCES IN NI-TSP LC–MS OF SOLVENT IONS

Filament-on mode of operation; flow-rate, 1 ml/min. A = acetonitrile–water (50:50) + 0.05 *M* ammonium acetate; B = acetonitrile–water (50:50) + 0.05 *M* ammonium formate; C = methanol–water (50:50) + 0.05 *M* ammonium acetate; D = methanol–water (50:50) + 0.05 *M* ammonium formate.

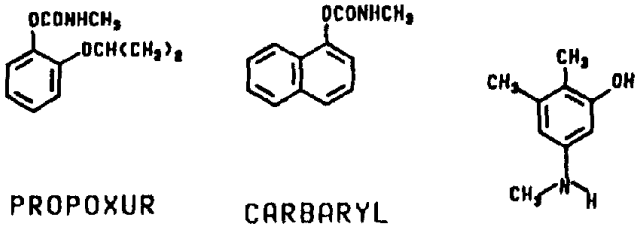
m/z	Fragment (tentative identification)	A	B	C	D
17	$[OH]^-$	1			
45	$[HCOO]^-$		2		2
59	$[CH_3COO]^-$	3		1	
91	$[(HCOOH)HCOO]^-$		100		100
91	$[(CH_3OH)(CH_3COO)]^-$			100	
95	$[(CH_3COOH)(H_2O)(OH)]^-$	2			
119	$[(CH_3COOH)(CH_3COO)]^-$	100		54	
137	$[(HCOOH)_2HCOO]^-$		100		85
137	$[(CH_3COOH)(H_2O)(CH_3COO)]^-$	2		11	
151	$[(CH_3COOH)(CH_3OH)(CH_3COO)]^-$			20	
179	$[(CH_3COOH)_2(CH_3COO)]^-$	5		3	



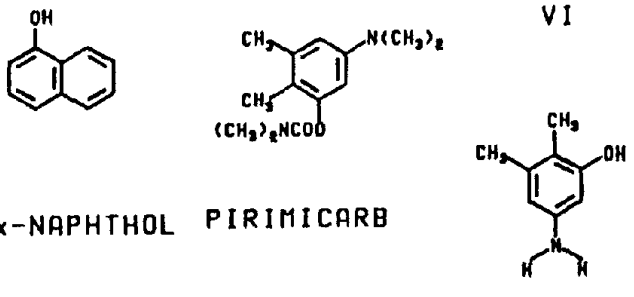
ATRAZINE SIMAZINE CYANAZINE



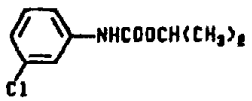
OXAMYL CARBOFURAN V



PROPOXUR CARBARYL VI



α-NAPHTHOL PIRIMICARB VII



CHLORPROPHAM

um formate as ionizing additives, the base peak is *m/z* 50, corresponding to the adduct between methanol and ammonia. When ammonium acetate is used a remarkable effect must be taken into consideration. In previous experiments of our group [11,47] and from other authors [48] a main peak with *m/z* 77 has been attributed to $[CH_3COONH_4]^+$. After taking into consideration a recent article which identifies quasimolecular ions in TSP LC–MS [43], we decided to use labelled acetic acid instead of ammonium acetate. The real composition obtained by using labelled acetic acid is shown in Table I, which indicates that the ion at *m/z* 77 had been wrongly assigned. This ion was never found again and can only be attributed to impurities in the purchased ammonium acetate.

The eluent mixtures used in NI mode are indicated in Table II. When we consider acetonitrile–water with ammonium acetate, it should be noticed that acetic acid has lower gaseous acidity than acetonitrile (1458 over 1557 kJ/mol), and consequently acetate adducts will predominate. The mechanism of anion formation follows the general rules of CI processes, and it is similar to that previously observed in acetonitrile–water and methanol–water in DLI LC–MS [44,49].

Carbamate pesticides

The molecular structures of the different carbamates and chlorotriazines used as model compounds are indicated in Fig. 1. The different adducts obtained with the four LC eluents in PI mode are indicated in Table III. Carbamates exhibit a lower PA value than ammonia (858 kJ/mol), so generally $[M + NH_4]^+$ is the base peak, as previously reported by other authors for TSP LC–MS [21–24]. In addition, the formation of $[M + H]^+$ is also relevant for some compounds, *e.g.* propoxur, which in our experiments exhibited relative abundance values of $[M + H]^+$ up to 40% compared with literature values from 69% [21] to 100% [22] in TSP LC–MS and 100% in DLI LC–MS [18]. Primicarb and its metabolites are an exception to the rule, and exhibit $[M + H]^+$ as base peak and no adducts with ammonia, thus indicating in this case a higher PA value than ammonia.

When analysing carbamates in PI mode, generally most authors have reported $[M + H]^+$ and $[M + NH_4]^+$ [21–24], and other adduct ions with the eluent, *e.g.* $[M + CH_3CN + H]^+$ and $[M + H]^+$, have also been reported in DLI LC–MS [18]. The ion at *m/z* 198 of chlorpropham corresponds to $[M - CH(CH_3)_2 + NH_4]^+$. The loss of the $CH(CH_3)_2$ giving *m/z* 180, has been previously observed for the same pesticide in LC–MS using a moving belt interface [20] and in DLI LC–MS [18]. In the case of the N-methylcarbamates carbaryl, carbofuran, propoxur and oxamyl, a new mechanism of formation of the $[M + 59]^{+\cdot}$ ion has been postulated recently with the formation of $[M + CH_3NH_2CO]^{+\cdot}$ instead of $[M + CH_3CN + NH_4]^+$ [50]. This ion is specific for the N-methylcarbamates, and it is not obtained with N-alkyl- or N-arylcarbamates. The tentative identification of the different ions of each pesticide are indicated at Table III. Differences

TABLE III

MAIN IONS AND RELATIVE ABUNDANCES OF CARBAMATE AND CHLOROTRIAZINE PESTICIDES IN FLOW-INJECTION PI-TSP-MS USING FOUR DIFFERENT MIXTURES AS THE CARRIER STREAM

Filament-on mode of operation; flow-rate, 1 ml/min. A = acetonitrile–water (50:50) + 0.05 *M* ammonium acetate; B = acetonitrile–water (50:50) + 0.05 *M* ammonium formate; C = methanol–water (50:50) + 0.05 *M* ammonium acetate; D = methanol–water (50:50) + 0.05 *M* ammonium formate.

Mol. wt.	Compound and ions (*m/z* and tentative identification)	A	B	C	D
201	Carbaryl				
	202 $[M + H]^+$	10	5	10	10
	219 $[M + NH_4]^+$	100	100	100	100
	251 $[M + CH_3OH + NH_4]^+$				2
	260 $[M + CH_3NH_2CO]^+$	16	25	15	
	261 $[M + CH_3COONH_4 + H - H_2O]^+$	20			
	278 $[M + CH_3COONH_4 + NH_4 - H_2O]^+$	20		5	
209	Propoxur				
	210 $[M + H]^+$	40	15	40	40
	227 $[M + NH_4]^+$	100	100	100	100
	268 $[M + CH_3NH_2CO]^+$	10	10	10	
213	Chlorpropham				
	198 $[M - CH(CH_3)_2 + NH_4]^+$	40		100	
	214 $[M + H]^+$			10	10
	231 $[M + NH_4]^+$	100	100	100	100
219	Oxamyl				
	220 $[M + H]^+$	2	10	10	5
	237 $[M + NH_4]^+$	100	100	100	100
	278 $[M + CH_3NH_2CO]^+$	3	10	5	
221	Carbofuran				
	222 $[M + H]^+$	10	40	40	45
	239 $[M + NH_4]^+$	100	100	100	100
	280 $[M + CH_3NH_2CO]^+$	5	20	10	
238	Pirimicarb				
	239 $[M + H]^+$	100	100	100	100
167	V				
	168 $[M + H]^+$	100	100	100	100
	209 $[M + CH_3CN + H]^+$	40	40		
	226 $[M + CH_3CN + NH_4]^+$	20			
	226 $[M + CH_3COONH_4 + NH_4 - 2H_2O]^+$			40	
	335 $[2M + H]^+$	20	10	10	10
153	VI				
	154 $[M + H]^+$	100	100	100	100
	195 $[M + CH_3CN + H]^+$	40	40		
	195 $[M + CH_3COONH_4 + H - 2H_2O]^+$			40	
	307 $[2M + H]^+$	10	10	15	15

(Continued on p. 515)

TABLE III (*continued*)

Mol. wt.	Compound and ions (m/z and tentative identification)	A	B	C	D
139	VII				
	140 $[M + H]^+$	100	100	100	100
	181 $[M + CH_3CN + H]^+$	20	20		
	181 $[M + CH_3COONH_4 + H - 2H_2O]^+$			90	
	199 $[M + CH_3COONH_4 + H - H_2O]^+$	30		40	
	279 $[2M + H]^+$	20	20	15	10
201	Simazine				
	202 $[M + H]^+$	100	100	100	100
	243 $[M + CH_3CN + H]^+$	35	45		
	261 $[M + CH_3COONH_4 + H - H_2O]^+$			15	
215	Atrazine				
	216 $[M + H]^+$	100	100	100	100
	257 $[M + CH_3CN + H]^+$	30	70		
	275 $[M + CH_3COONH_4 + H - H_2O]^+$	2		20	
240	Cyanazine				
	241 $[M + H]^+$	100	100	100	100
	282 $[M + CH_3CN + H]^+$	25	25		
	300 $[M + CH_3COONH_4 + H - H_2O]^+$			5	

in secondary adduct ions are noticeable and can easily be used for unequivocal identification of pesticides.

In NI mode, most of the carbamates did not show any response when 1 μg of sample was injected. Exceptions were oxamyl, carbaryl and its metabolite α-naphthol, and the metabolites of pirimicarb, V, VI and VII.

To illustrate the differences in adduct ion formation and sensitivity between PI and NI modes of operation, the PI mode spectra of carbaryl and oxamyl are shown in Fig. 2A and 2C, respectively, with $[M + NH_4]^+$ as the base peak in both cases. It is interesting to note the ion formed in NI mode for carbaryl (Fig. 2B) at m/z 203, which corresponds to $[M - CONHCH_3 + CH_3COOH]^-$. The carbanion $[M - CONHCH_3]^-$ is very stable under NI conditions: it has the α-naphthol structure and it has been previously described under conventional NICI conditions [51]. Similarly, for oxamyl (Fig. 2D) the base peak at m/z 193 is attributed to $[M - CON(CH_3)_2 + HCOO]^-$. Losses of $CON(CH_3)_2$ have been previously reported in NI-DLI LC–MS for different carbamates [18].

As regards to sensitivity, it should be pointed out that, in PI mode, the detection limits were 1 ng under full scan conditions, whereas in the NI mode sensitivities were generally 3 to 3.5 orders of magnitude lower, *e.g.* oxamyl (see Fig. 2C and D). Exceptions to this rule were the metabolites of pirimicarb, which had similar sensitivities in the two modes, carbaryl (Fig. 2A and B), which exhibited a

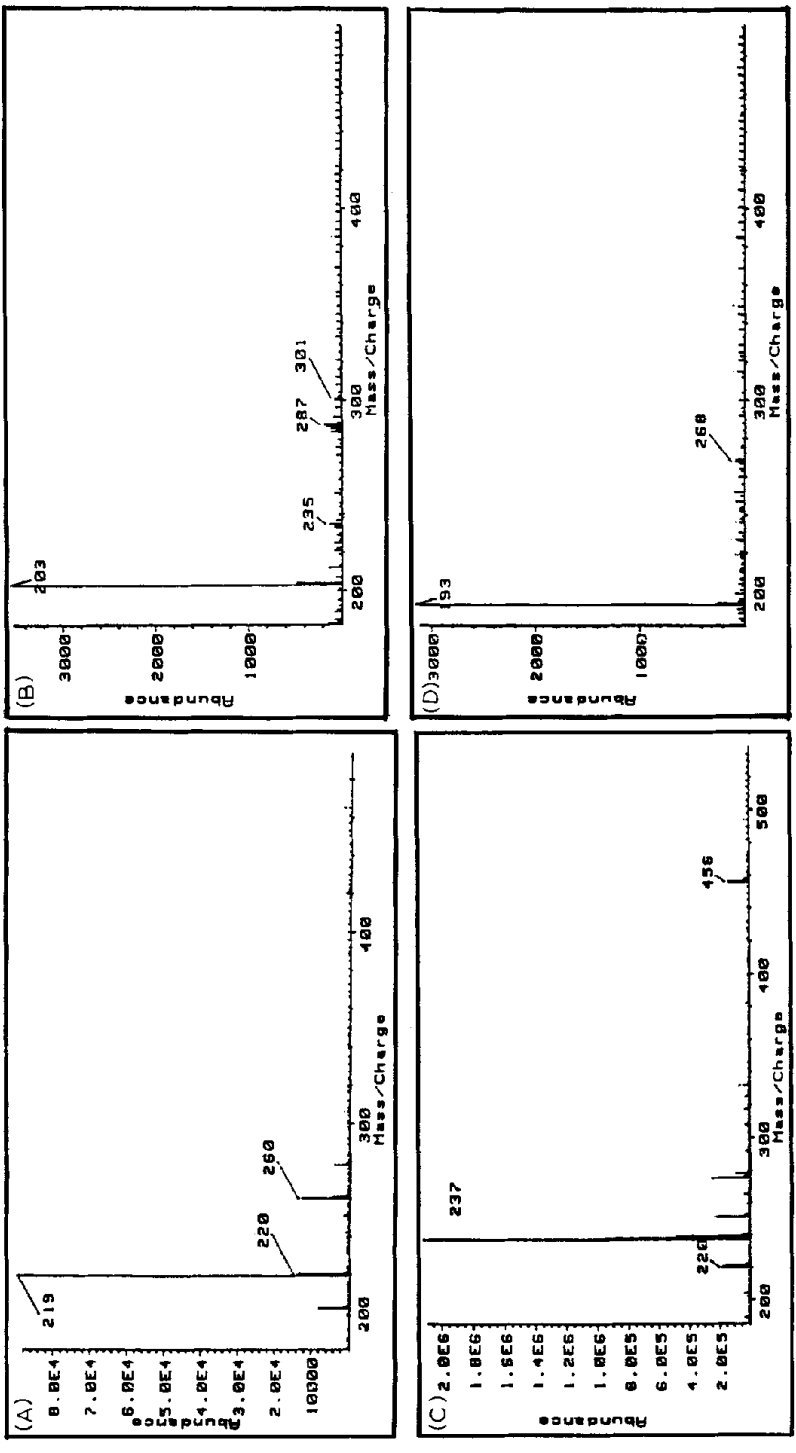


Fig. 2. Direct flow-injection TSP-MS spectra of carbaryl (A and B) and oxamyl (C and D) in PI mode (A and C) and NI mode (B and D). Eluents: methanol–water (50:50) + 0.05 *M* ammonium acetate (A and B), and acetonitrile–water (50:50) + 0.05 *M* ammonium formate (C and D); flow-rate, 1 ml/min; amount injected, 1 μg.

sensitivity 1.5 orders of magnitude lower in NI mode, and α-naphthol, which was only detected in NI mode.

These sensitivity values contradict previous results of Voyksner *et al.* [21], who reported for all carbamates sensitivities of 4 to 5 orders of magnitude better in PI than in NI mode TSP LC–MS. This large discrepancy is attributed to the use of a different mode of operation (filament-on in our experiments, filament-off in their work) and they optimized their thermospray conditions for PI mode, which led to larger differences in sensitivity between the two modes. In our experiment the sensitivity values in NI mode are one order of magnitude better than the NI values reported in ref. 21. In the case of carbaryl, for example we found the sensitivity in PI mode to be only 1.5 orders of magnitude better than in NI mode. This is attributed to the α-naphthol sructure, which can easily stabilize the negative charge under filament-on conditions. This mode of operation has a lot of points of similarity with conventional NICI [51], thus offering a considerable increase in sensitivity compared with filament-off for this type of compound.

The different information that can be obtained under NI conditions is illustrated in Fig. 3 for metabolite VII of pirimicarb, which exhibits base peaks at *m/z* 184 and 277, corresponding respectively to $[M + HCOO]^-$ and $[2M - H]^-$, depending on whether ammonium formate or ammonium acetate is used as the ionizing additive. In the case of ammonium acetate, the second most abundant ion at *m/z* 198 had a relative intensity of 90%, and corresponds to $[M + CH_3COO]^-$.

Chlorotriazine pesticides

The adduct ions obtained for the three chlorotriazines analysed in the four different LC eluents are indicated in Table III. Compared with our previous results [3,47] there is one remarkable difference. The fragment ion corresponding to $[M + CH_3COONH_4 + H - H_2O]^+$, which is observed for the chlorotriazines in abundances varying from 2 to 15% when ammonium acetate is used, was the base peak in our previous experiments using the same eluent mixtures but with a stem vapour temperature of 200°C. This discrepancy is attributed to a different vapour temperature, higher in the present experiments (270°C *vs.* 200°C). Consequently the formation of cluster adducts in the ion source is disfavoured, a phenomenon known to occur in gas-phase CI processes, *e.g.* in DLI LC–MS the number of solvent clusters decreased with increasing source temperature either in PI [52] or NI [49] mode. This change of vapour temperature makes no appreciable difference to the signals from lower mass ions, from scan values ranging from *m/z* 150 up to the value for the $[M + H]^+$ ion.

Chlorotriazines exhibited $[M + H]^+$ as the base peak in all the LC eluents used. This is attributed to these analytes having higher PA values than ammonia (858 kJ/mol) [44]; as a consequence, $[M + NH_4]^+$ is not formed [3,47]. For example, the PI mode TSP-MS spectra of cyanazine using two different eluents are reported in Fig. 4A and B. Although the base peaks correspond to $[M + H]^+$

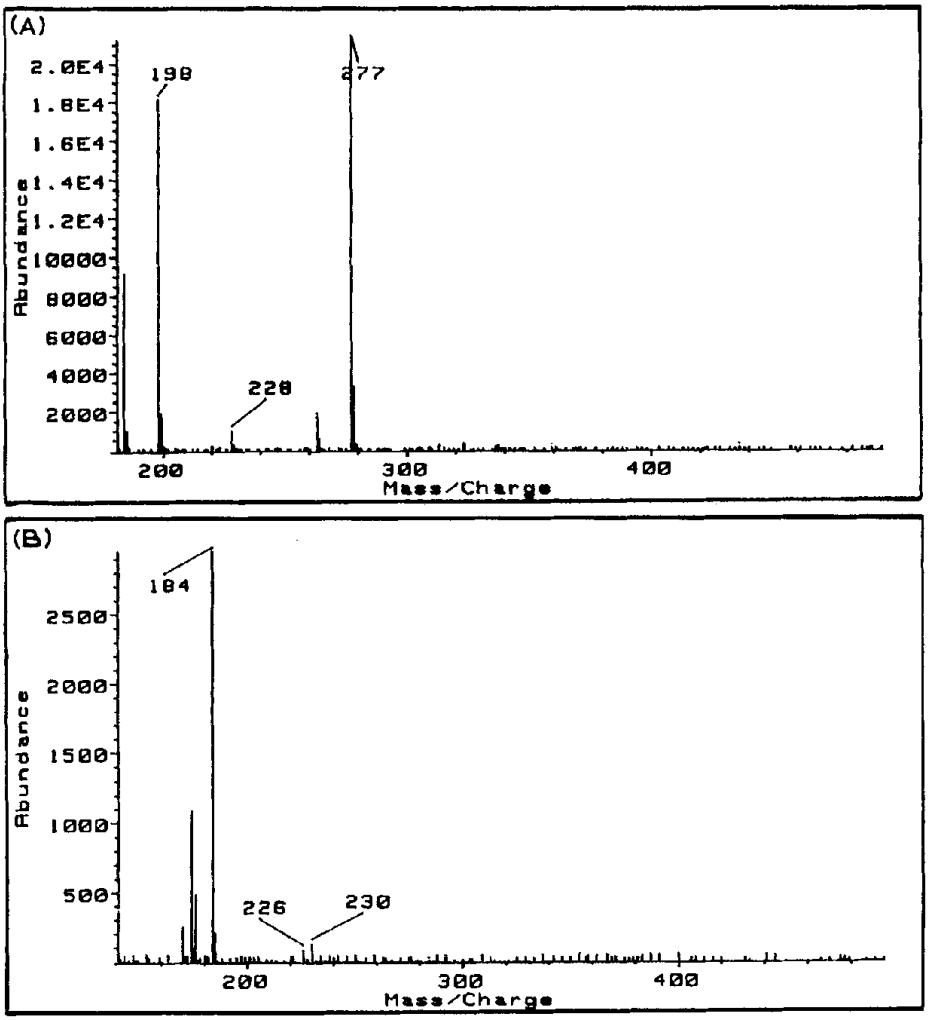


Fig. 3. Direct flow-injection TSP-MS spectra of metabolite VII of pirimicarb in NI mode of operation. Eluent, methanol–water (50:50) + 0.05 *M* ammonium acetate (A) and ammonium formate (B); flow-rate, 1 ml/min; amount injected, 1 μg.

in both cases, the second most abundant ions are at either *m/z* 282 or 300, corresponding to $[M + CH_3CN + H]^+$ or $[M + CH_3COONH_4 + H - H_2O]^+$, respectively.

In the NI mode of operation, $[M]^{-\cdot}$ is obtained as the base peak for cyanazine, with sensitivities one order of magnitude lower than in PI mode. Fig. 4C shows the NI-TSP-MS spectrum in an eluent consisting of acetonitrile–water (50:50) + 0.05 *M* ammonium acetate. In this particular spectrum, two peaks are observed at *m/z* 240 and 275 corresponding to the base peak $[M]^{-\cdot}$ and the second most

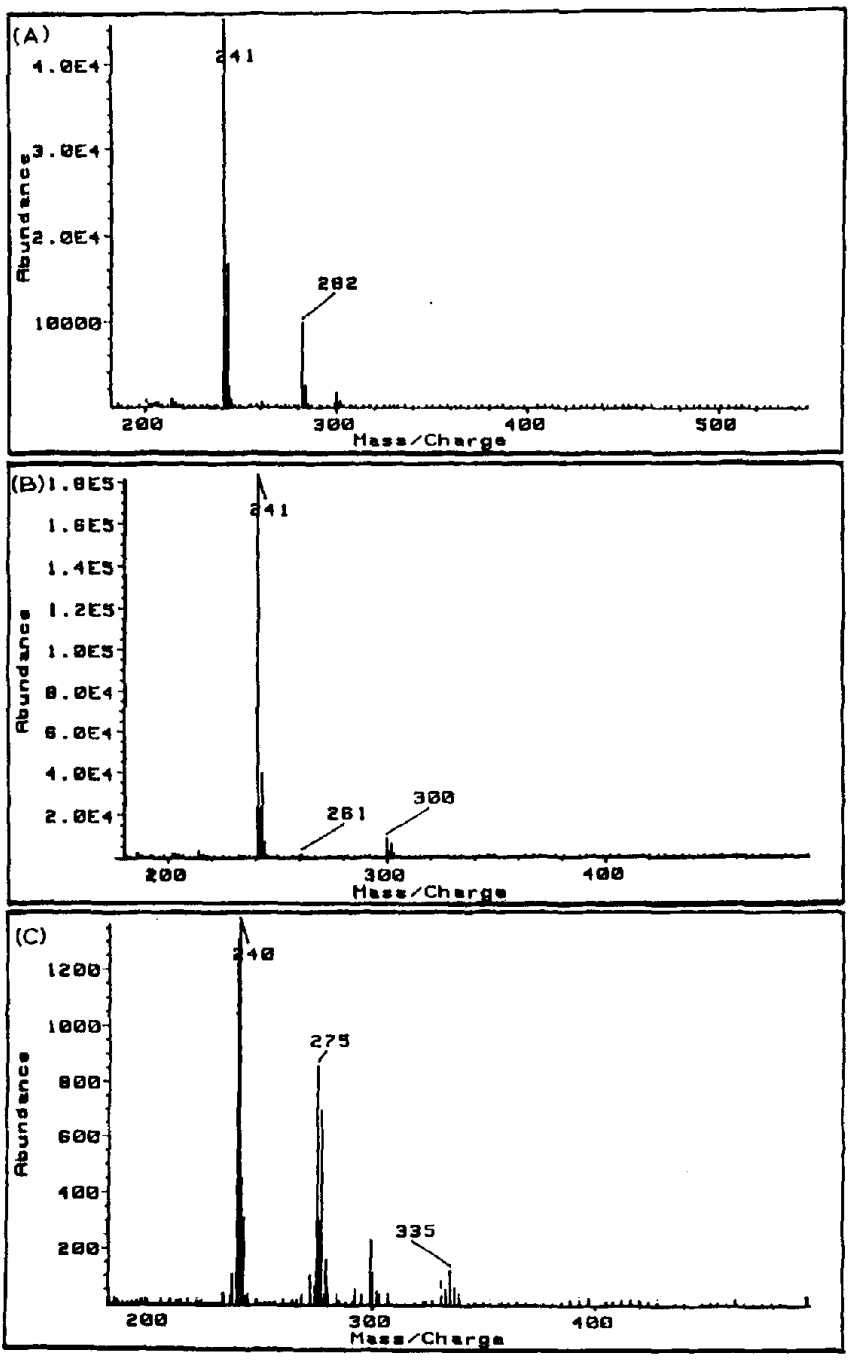


Fig. 4. Direct flow-injection TSP-MS spectra of cyanazine in PI (A and B) and NI modes (C) of operation. Eluents: acetonitrile–water (50:50) + 0.05 *M* ammonium acetate (A and C); and methanol–water (50:50) + 0.05 *M* ammonium acetate (B).

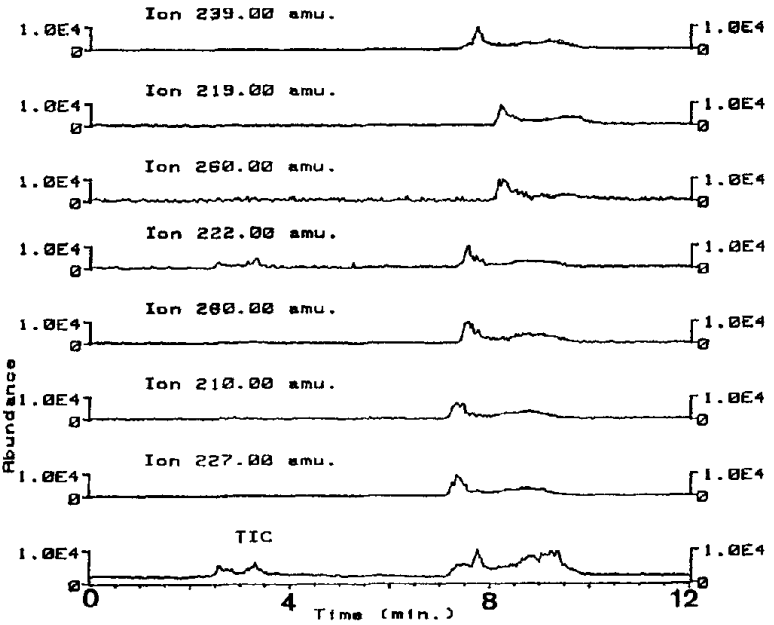


Fig. 5. Total ion current and selected-ion chromatograms in PI-TSP LC–MS of a soil sample spiked with carbamate insecticides at 1 μg/g. Diagnostic ions and compounds identified were at *m/z* 239 (pirimicarb), 219 and 260 (carbaryl), 222 and 280 (carbofuran) and 210 and 227 (propoxur). Mobile phase: gradient elution of acetonitrile–water from (30:70) + 0.1 *M* ammonium acetate to (65:35) in 7 min. Flow-rate, 1 ml/min; column packing, 5 μm LiChrospher 100 RP-18.

abundant ion $[M + Cl]^-$, respectively. The formation of this chloride-attachment ion, previously noticed for chlorine-containing pesticides in DLI LC–MS [49] and in TSP LC–MS [4,11,46,47], is presumably attributable to the availability of Cl^- from pyrolysis of the analyte.

Application to the determination of soil residues

Soil samples were spiked with four different carbamates, propoxur, carbaryl, carbofuran and pirimicarb, at the 1 μg/g level. Fig. 5 shows the total ion current (TIC) and selected-ion chromatograms in PI mode LC–TSP-MS of soil samples after the pre-treatment described in ref. 41. The ions monitored corresponded to *m/z* 239 (pirimicarb), 219 and 260 (carbaryl), 222 and 280 (carbofuran) and 210 and 227 (propoxur). The ion at *m/z* 239 is the diagnostic ion for pirimicarb, but it is also obtained with carbofuran (see Table III). For pirimicarb this is the only ion obtained in the TSP spectrum, and it exhibits a much greater intensity than the *m/z* 239 for carbofuran. So, to confirm assignments, ions at *m/z* 222 and 280 are used for carbofuran and the ion at *m/z* 239 is used only for pirimicarb identification.

Real soil samples from the Ebro delta (Tarragona, Spain) polluted with chlo-

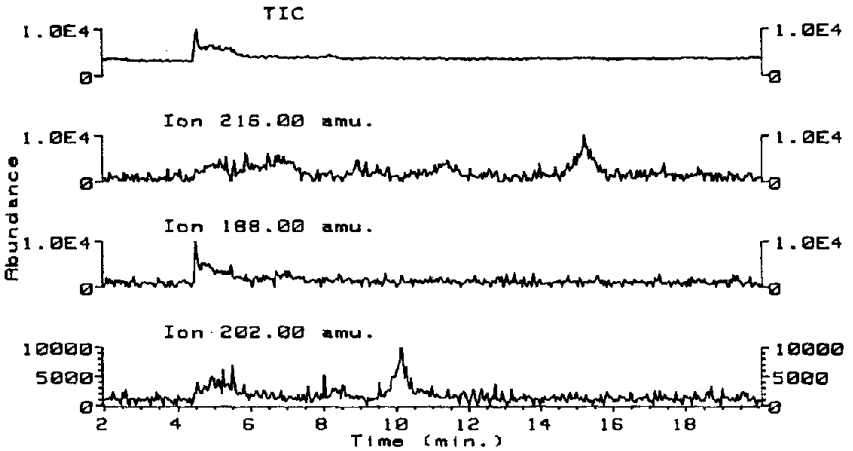


Fig. 6. Total ion current and selected-ion chromatograms in PI-TSP LC–MS of a real soil sample containing desethylatrazine, atrazine and simazine, at concentrations of 29, 344 and 17 ng/g, respectively. Compounds identified are: desethylatrazine, simazine and atrazine, at m/z 188, 202 and 216, respectively, corresponding to $[M + H]^+$ ions. Mobile phase, methanol–water (60:40) + 0.05 M ammonium formate; flow-rate, 1 ml/min; column packing, 5 μm LiChrospher 100 RP-18.

rotriazine pesticides were pretreated by a slightly modified procedure from that described elsewhere [40]. The chlorotriazines were applied 6.5 months earlier. The corresponding TIC and selected-ion chromatograms obtained under PI mode LC–TSP-MS are shown in Fig. 6. The ions monitored corresponded to m/z values of 188, 216 and 202, which are the $[M + H]^+$ ions of desethylatrazine, atrazine and simazine, respectively. The concentrations (ng/g) in the soil sample were 29, 344 and 17 for desethylatrazine, atrazine and simazine, respectively. Desethylatrazine, the main breakdown product of atrazine, has been previously observed in soil samples of the same area [40].

Detection limits, under full scan conditions in PI mode, were 1 and 10 ng, respectively, for the carbamates and chlorotriazines analysed in the soil samples. These limits can, in practice, be reduced to the picogram level by employing selected-ion monitoring. For most of the soil samples where the pesticides are being applied, the sample preparation method and TSP LC–MS analysis described in this paper will be sufficient to allow detection at the 1–5 ng/g level.

CONCLUSIONS

The identification potential of TSP LC–MS can be easily extended by using different LC eluent mixtures in PI-TSP LC–MS and NI-TSP LC–MS. The formation of adducts with the analyte depends on the solvent used. By combining the information obtained with two or more different LC eluents an unambiguous molecular weight assignment of an unknown pesticide is feasible. Four LC eluent

mixtures containing different combinations of methanol, acetonitrile, water, ammonium formate and ammonium acetate have been used for the characterization of carbamate and chlorotriazine pesticides and different breakdown products, such as the metabolites of pirimicarb and α-naphthol.

Sensitivities were, generally, 3–3.5 orders of magnitude better in PI mode than in NI mode for carbamates. Exceptions were: the metabolites of pirimicarb, with a similar sensitivity in both PI and NI modes; carbaryl, which exhibited a sensitivity 1.5 orders of magnitude lower in NI than in PI mode; and α-naphthol, which was detected only in NI mode. For the chlorotriazines, cyanazine showed a sensitivity one order of magnitude better in PI than in NI mode.

Further experiments will be conducted with other groups of pesticides and their corresponding breakdown products, *e.g.* organophosphorus pesticides and their oxygen analogues. Their response and fragmentation will be evaluated in both PI and NI modes of operation by using a variety of LC eluent compositions.

ACKNOWLEDGEMENTS

G.D. has a grant from the Commission of the European Communities (ST2*-0488). N. de B. is the recipient of a fellowship from the Community Bureau of Reference (BCR) of the Commission of the European Communities (CEE). This work has been supported by the Ministerio de Educación y Ciencia and the BCR of the CEE. P. Cabras (Cagliari, Italy) is thanked for providing metabolites V, VI and VII of pirimicarb. R. Alonso and J. Abián (C.S.I.C.) are thanked for laboratory assistance.

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