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Temperature and extraction voltage effect on fragmentation of organophosphorus pesticides in liquid chromatography–atmospheric pressure chemical ionization mass spectrometry

S. Lacorte*, C. Molina, D. Barceló

Department of Environmental Chemistry, CID-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain

Abstract

Liquid chromatography coupled to mass spectrometry with atmospheric pressure chemical ionization is an excellent technique for analysis of organophosphorus pesticides which are not gas chromatography amenable or pose problems with the use of diode array detection or an ionspray interface. This study was directed to evaluate the effect of the probe temperature and extraction voltage on sensitivity and fragmentation of several organophosphorus pesticides. Five different temperatures, varying from 100 to 500°C and five extraction voltages, from 10 to 60 V were applied. In first instance, all the studied compounds were characterized at 3–5 different ions under each experimental condition. From this qualitative information, together with the relative abundances of each ion, the theory of information was applied to objectively distinguish the condition(s) that gave more structural information. For pesticides of the parathion group, an intense fragmentation was observed at all extraction voltages, while sensitivity decreased with increasing extraction voltage. In general, higher structural information was obtained when increasing the extraction voltage, compared to an increase of temperature. However, temperatures of 400–500°C produced the highest sensitivity for the majority of the pesticides. Extraction voltages of 40 to 60 V produced ions at low m/z, which could not be used for identification purposes. The optimum conditions, with regard to best sensitivity and structural information were used to calculate the recoveries of the studied pesticides. Two solid-phase extraction phases, LiChrolut EN and Isolute ENV, were used to preconcentrate 200 ml of groundwater spiked at levels of 0.2 μg/l. Problems arose during attempts to recover those compounds which exhibit high vapor pressure.

Keywords: Water analysis; Interfaces, LC–MS; Atmospheric pressure chemical ionization; Shannon–Weaver entropy; Pesticides; Organophosphorous compounds

1. Introduction

The main problem of introducing pesticides into the environment deal with presence of residues in surface and ground water, where they can cause lethal effects to non-target organisms as well as degrading to form more toxic transformation products (TPs) [1]. As a consequence, methods which permit the unequivocal identification and confirmation of pesticides along with their TPs in the environment in a quick and reliable way are emerging. Gas chromatography (GC) [2] provides very good sensitivity although the polar pesticides, the non-volatile and thermally labile, which are often the TPs, cannot be analyzed without a previous derivatization step. To overcome the problem, liquid chromatography (LC) based techniques have been successfully applied to determine a wider range of

*Corresponding author.

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pesticides. LC with diode array detection (DAD) has the advantage that it is very sensitive, provided an efficient preconcentration technique is used, and can render absorbance spectral information which can be used for identification purposes [3]. LC coupled to mass spectrometry (LC–MS) is more selective and can solve the problem of determination of pesticides which lack a chromophore, of coeluting pesticides or presence of interferences, mainly due to humic and fulvic material. Among other interfaces, the most common interfaces used for pesticide analysis have been the particle beam (PB) [4,5] and thermospray (TSP) [6–10] interfaces, but nowadays there is a general tendency to use atmospheric pressure ionization (API) interfaces which provide very good sensitivity and produce abundant fragmentation of the analytes via collision induced dissociation (CID), which is effectively used to identify unknowns from environmental matrices. Whereas electrospray (ESP) needs special optimization and equipment by working at low flows [11], by splitting the LC eluent [12,13], or by applying heat at the tip of the ESP interface [14], ionspray (ISP) and atmospheric pressure chemical ionization (APCI) have been extensively used for pesticide monitoring [15–19]. These two techniques permit the achievement of limits of detection (LODs) of a few ng/l, and the linear response range over two orders of magnitude [15–17]. However, the fragmentation and sensitivity of the APCI source depends highly on the probe temperature and extraction voltage applied. Previous work [20] indicates the effect of various variables, e.g., flow-rate, capillary heat, gas pressure, capillary voltage among others, on fragmentation and sensitivity. In that study, the fractional modeling with center points [21,22] and the response surface modeling [21,23] were used to identify the factors which produced a significant change in response. Another study carried out by Spliid and Koppen [24] indicated the effect of corona current, sheath gas flow, vaporizer and capillary temperature on sensitivity measured as signal-to-noise ratio. However, in this study a different approach was used to evaluate the response of the APCI interface at different conditions of probe temperature and extraction voltage. It consisted in ''quantifying'' the degree of fragmentation of each condition by applying the Shannon–Weaver entropy [25,26]. A further parameter which was checked for optimization was the LC flow-rate. It has been previously described the effect of this parameter upon sensitivity, and it is linked to the mobile phase composition [27].

Another difficulty which arises for pesticide determination in environmental waters is related to the extraction of pesticides from the water in an efficient way. Liquid–liquid extraction has been widely used [28,29], but nowadays, there is a general tendency to use liquid–solid extraction (LSE) both by off-line with C_{18} or polymeric nature disks or cartridges [30,31] or by coupling LSE on-line with the chromatographic system [32,33]. Among the different types of pesticides, compounds which exhibit more analytical difficulties for being determined from water samples include the ones which have high polarity and volatility. The polymeric nature sorbents Isolut-ENV and LiChrolut EN were tested and the recoveries of twelve pesticides were calculated. These two phases are high porosity polymeric materials which have a large active surface area which produces a high retention of the analytes. Puig and Barceló [34] have carried out a comparative study of both phases with on-line LSE–LC–DAD of phenolic compounds, and indicate recoveries of 55 to 67% for phenols and catechols, a highly polar compounds which pose problems for retention with other more common phases, such as C_{18} or PLRPs.

The specific objectives of the present study were (i) to characterize the pesticides under study under different experimental voltages by LC–APCI-MS in positive ionization (PI) mode, (ii) to optimize the flow-rate which enhance sensitivity for all the studied pesticides, (iii) to study the effect of probe temperature and extraction voltage on LC–APCI-MS fragmentation and evaluate it by calculating the Shannon–Weaver entropy at each condition and (iv) to study the recovery of selected pesticides after preconcentration of groundwater onto polymeric based cartridges. In this study, special attention was given to the pesticides of the parathion-group, which do not exhibit sufficient sensitivity under ESP [16] and to naled, metamidophos and acephate, for which there is not a well documented method due to their high polarity or lack of chromophore. Moreover, emphasis was also given to the determination of trichlorfon which cannot be determined with LC–DAD and suffers thermal degradation under GC and

also under TSP, caused by temperatures higher than 200°C. In the present case, the APCI source was applied at low probe temperature to see to what extent the method was capable of detecting this compound.

2. Experimental

2.1. Chemicals and reagents

Pesticides acephate, azinphos-ethyl, fenitrothion, fensulfothion, fenthion, metamidofos, naled, paraoxon-methyl, parathion-methyl, trichlorfon, vamidothion and vamidothion sulfoxide were obtained from Promochem (Wesel, Germany). Pesticide grade acetonitrile, methanol and water (Merck, Darmstadt, Germany) were filtered through a 0.45 μm (Millipore, Bedford, MA, USA) filter before use. Acetic acid was obtained from Fluka (Buchs, Switzerland). Stock standard solutions were prepared in methanol, from which series of dilutions were performed to obtain working solutions dissolved with the mobile phase.

2.2. Liquid chromatography–mass spectrometry

The eluent was delivered by a Waters 616 pump coupled to a Waters 600MS gradient controller (Waters, Milford, MA, USA). Analyses were performed with a methanol and water with 0.1 *M* acetic acid mobile phase following the gradient from methanol–water (30:70) to methanol–water (90:10) in 30 min at a flow-rate of 1.2 ml/min. The analytical column was a Symmetry cartridge column (250 mm×4.6 mm I.D.) packed with 5 μm C_8 (Waters).

The gradient LC system was coupled to a VG Platform ESP from Micromass (Manchester, UK) equipped with an APCI source. The source consists of a heated probe through which the LC eluent is heated and converted to an aerosol, which is rapidly evaporated at the probe tip. A nebulizing gas set at 10 l/h flows directly through the probe tip to maximize the efficiency of the nebulization. Afterwards, this aerosol is flushed towards a counter electrode (held at 3 kV), process which is assisted by a drying gas (set at 300 l/h). Ionization takes place by applying an extraction voltage between 10 and 130 V at the sample cone, with the skimmer held at ground potential. An increase of this voltage produces an acceleration of the ions, which gain in internal energy and extends the fragmentation of these compounds, producing spectra similar to those obtained with MS–MS.

2.3. Optimization of analytical parameters

2.3.1. Flow-rate

Infusion analysis of each individual pesticide was performed with LC–APCI-MS, at flow-rates varying from 0.2 to 1.4 ml/min, at water–methanol (50:50). Acquisition was done at an extraction voltage of 20 V and the probe temperature was maintained at 400°C. It was seen that the optimum flow-rate oscillated between 1 to 1.2 ml/min, with the latter enhancing sensitivity for most of the studied pesticides. Therefore, a flow-rate of 1.2 ml/min was set for all the further experiments.

2.3.2. Temperature and extraction voltage

To study the effects of temperature and extraction voltage on ion abundance and fragmentation in the LC–APCI-MS mass spectra, twelve pesticides were analyzed at (i) probe temperatures of 100, 200, 300, 400 and 500°C, keeping the extraction voltage at 20 V and (ii) extraction voltages of 10, 20, 30, 40 and 60 V, maintaining the temperature constant at 400°C. In all cases, the ion source was maintained at 150°C, the HV lens were set at 0.3 kV and the focus voltage at 30–40 V. Acquisition was performed in PI with the scan mode from m/z 20 to 450 at a rate of 1 s/scan.

Under each experimental condition, the Shannon–Weaver entropy [26], used in the theory of information, was used to elucidate the disarrangement of the ions or, in other words, to establish the probe temperature or extraction voltage that gave maximum information as regards to fragmentation. This function has a minimum (when only one ion is present at a certain condition), and a maximum (when all the ions are different). To do so, and after identification of each ion, the fragmentation pattern of each compound under the different experimental

conditions along with the relative abundance of each ion were used to calculate the Shannon entropy (H) [25,26] which is defined by the equation:

$$H(P) = -\sum Pi \log_2 Pi$$

where Pi is:

$$Pi = n(i)/N, \quad \text{being } \sum Pi = 1$$

where n is the relative abundance of each ion and N the sum of relative abundances at each variable. The units in which the uncertainty is expressed are determined by the base of the logarithm in the former equation. For the binary logarithm, $b=2$, the unit is a bit of information [35]. This expression can be used to objectively evaluate which condition enhanced fragmentation. For instance, if at a certain condition of temperature we have only two ions and both are equifrequent, the uncertainty is 50%, two alternatives which are both possible, and the certainty, is in this case 1 bit. With four ions with the same relative abundance, the information provided will be 2 bits. This unit can well be applied as an expression of fragmentation of a certain compound at a certain condition of temperature or at a certain extraction voltage. The simplest way to calculate the fragmentation of a compound at a certain analytical condition, is to compare the bits in that condition with the maximum information which would be represented by the presence of all ions at a maximum abundance and corresponds to $H(\max)=\log_2 X$, where X is the number of ions of a certain compound at a certain probe temperature (or extraction voltage). However, in the present case it is more interesting to compare the bits obtained at one analytical condition with the ones obtained at the other conditions. For instance, if we take as example the relative abundances of a compound at the five different temperatures considered, we get in each case the bits which indicate the ''amount'' of fragmentation. The minimum information will correspond to that temperature which produces only one ion. In that case,

$$\lim_{p\to 0} P \log_2 P = 0$$

and it indicates minimum structural information.

2.4. Sample preparation

200 ml of groundwater samples, acidified at pH 3, were spiked to a final concentration of 0.2 μg/l. Immediately after, the pesticides were extracted with the ASPEC XL (Gilson, Villiers-le-Bel, France) to avoid degradative processes. A Model 306 LC pump (Gilson), which was connected on-line to the ASPEC XL, was used to automatically dispense the water samples through the LSE cartridges. A Model 817 switching valve (Gilson) was used to select each water sample. Disposable 6 ml cartridge columns from Isolut International Sorbent Technology (Hengoed, UK) packed with 200 mg of ENV (Merck) or 200 mg of LiChrolut-EN (Supelco, Bellefonte, PA, USA) were used. The pre-columns were conditioned with 2.5 ml of methanol and 2.5 ml of water at a flow-rate of 5 ml/min. The preconcentration step was performed at a flow-rate of 10 ml/min. Immediately after, the cartridges were dried for 10 min with a Baker Spe 12G apparatus which was connected to a vacuum system which operated at 15 p.s.i. (negative pressure) (1 p.s.i. = 6894.76 Pa). Elution was performed with 2×2 ml of methanol, letting the solvent soak the cartridge for 1 min before applying the vacuum. A waiting time of 5 min was performed between the two aliquots. The excess of solvent was evaporated under a gentle stream of nitrogen to a final volume of 200 μl. Samples were never allowed to dry in order to avoid evaporation of the more volatile compounds. The extracts were stored at −20°C until analysis. Before injecting those samples, they were diluted with 200 μl of HPLC-grade water to a final solution similar to the initial composition of the mobile phase, in order to attain better peak efficiency. In each case, 20 μl of sample was injected into the MS system at an extraction volatge of 20 V both in scan mode and in selected ion monitoring (SIM) mode.

Recoveries were calculated by external standard comparison with direct injection using the ion chromatogram from the total ion current. The system was linear from 0.0125 to 5 μg/l, with coefficients of correlation in the order of 0.99 for all the studied compounds. The LODs were calculated from the calibration curve, taking a ratio S/N of three times (three times the standard deviation from the origin).

3. Results

3.1. Mass spectra information

One of the main advantages of LC–APCI-MS for multiresidue analysis deals with the production of ions as a consequence of reactions of the analyte with the solvent vapor kept at high pressure. The majority of ion–molecule reactions in APCI involve gas-phase acid–base chemistry. This produces a highly efficient ionization and produce abundant fragmentation (more than three ions) which is useful for identification purposes. Table 1 indicates the ions formed and their abundances at 20 and 40 V, keeping the probe temperature at 400°C. Ion formation agrees with results reported by previous papers obtained with LC–TSP-MS [9] and LC–ESP-MS [13,16]. Of the studied compounds, the phosphorotioates (fenitrothion, fenthion, parathion-methyl, paraoxon-methyl, vamidothion and its sulfoxide) underwent strong fragmentation at 20 V with the formation of molecule specific fragment ions as base peaks. This represents an advantage since it is possible to characterize these compounds at low extraction voltages and thus obtain maximum sensitivity. For these pesticides, a breakage of the phosphate (tiophosphate) bond leads to the formation of fragment ions which corresponded to losses of diagnostic ions, e.g., $[M-(CH_3O)_2PO]^+$ or $[M-(CH_3O)_2PS]^+$ (fenitrothion, vamidothion) or diagnostic ions themselves at m/z 93, 109 or 125 (paraoxon and parathion-methyl). At 20 V $[M+H]^+$ was also formed and occasionally $[M+Na]^+$ was present in lower amounts, which could be useful for compound identification. In general, an increase of the extraction voltage from 20 to 40 V produced a shift on the formation of ions of higher to lower mass (see Table 1) such as $[CH_3S]^+$ or $[POS]^+$, which alone cannot be used for identification purposes. However, in some cases these ions appeared as base peaks, corroborating the idea of Bruins [36], who indicated that the relative abundances of small fragment ions highly depends on the voltage difference between the sample orifice and the skimmer. In general terms, it was found that an increase of the extraction voltage produced an increase of the number of ions formed. As an example, fensulfothion formed four ions at 20 V, while at 40 V, eight ions were formed which derive from the breakage of the $[M+H]^+$ adduct. In all cases, the presence of solvent adduct ions or fragments originated from solvent adducts was null, which permitted an easy interpretation of the APCI-MS mass spectra.

In order to study the stability of the signal with regard to the relative abundance of each ion, five consecutive injections were performed at 400°C and 20 V. The relative standard deviation was calculated for all the fragments formed of each compound and values below 5% were obtained. This indicates that ion formation, as well as the stability of the ion source is highly repetitive and therefore, changes in spectra can be due to external factors, such as the presence of interferences when environmental samples are being analyzed or variations due to a dirty source [12].

3.2. Optimization of LC–APCI-MS variables: temperature and extraction voltage

Mass spectra of the APCI source strongly depend on the extraction voltage applied and on the probe temperature. It is well known the fact that an increase of the extraction voltage enhance fragmentation via the phenomenon of collision induced dissociation (CID) which occurs at the sampling orifice and expansion region of the APCI source. This process is very useful because it aids identification of unknowns in a simple way, since the spectra generated are very similar to those obtained by MS–MS. The probe temperature is related to the ionization efficiency and will mainly affect sensitivity. It has been previously described the effect of the vaporizer and gas-phase temperature of the TSP interface [37] on sensitivity and fragmentation.

To evaluate the effect of the probe temperatures and the extraction voltages on fragmentation, the Shannon–Weaver entropy was applied since it permits one to ''quantify'' the degree of fragmentation of each pesticide under each experimental condition. Most works related to the optimization of LC–MS variables, one evaluates the fragmentation pattern by comparing the spectra of one compound under different situations. However, this leads to an imprecision and often it is not possible to predict which

Table 1
Characterization of the organophosphorus pesticides under study with LC–APCI-MS at extraction voltages of 40 and 20 V

Compound	m/z	Abundance (%)		Tentative identification
		40 V	20 V	
Acephate	49	100	0	$[CH_3SH+H]^+$ or $[HPO+H]^+$
PM 183	95	68	0	$[(CH_3S)POH]^+$
	125	20	0	$[(CH_3S)(CH_3O)PO]^+$
	143	38	100	$[(CH_3S)(CH_3O)PO(OH)+H]^+$
	184	0	10	$[M+H]^+$
	206	0	16	$[M+Na]^+$
Azinphos-ethyl	77	67	0	$[C_6H_4+H]^+$
PM 345	105	35	8	$[C_6H_4N_2+H]^+$ or $[C_6H_4CO+H]^+$
	132	100	58	$[C_6H_4N_3CH_2+H]^+$
	160	15	100	$[M-(OCH_2CH_3)_2PS_2)]^+$
	368	0	11	$[M+Na]^+$
Fenitrothion	109	0	25	$[(CH_3O)_2PO]^+$
PM 277	122	72	30	$[C_6H_4CH_3S]^+$
	125	70	62	$[(CH_3O)_2PS]^+$
	138	100	100	$[C_6H_4CH_3NO_2+H]^+$
	248	11	78	$[M-NO+H]^+$
	262	0	12	$[M-CH_3]^+$
	278	0	7	$[M+H]^+$
	300	0	69	$[M+Na]^+$
Fensulfoton	94	6	0	$[(CH_3CH_2O)P(OH)+H]^+$
PM 308	140	23	0	$[C_6H_4(CH_3S)OH]^+$
	157	100	14	$[C_6H_4(CH_3S)(OH)_2]^+$
	173	57	0	$[C_6H_4(CH_3S)(OH)(SH)]^+$
	219	38	0	$[C_6H_4(CH_3S)(OH)(POS)]^+$
	235	75	0	$[C_6H_4(CH_3S)(OH)(POS)O)]^+$
	281	25	14	$[M-(CH_2)2]^+$
	309	9	100	$[M+H]^+$
	331	0	21	$[M+Na]^+$
Fenthion	153	68	0	$[M-(CH_3O)_2PS]^+$
PM 278	216	100	0	$[C_6H_3CH_3SCH_3POS]+$ or $[M-CH_3SCH_3]^+$
	231	93	21	$[M-CH_3S]^+$
	279	0	100	$[M+H]^+$
	301	0	15	$[M+Na]^+$
Metamidophos	47	11	0	$[PO]^+$ or $[CH_3S]^+$
PM 141	79	5	0	$[POS]^+$
	94	100	68	$[(CH_3S)PO]^+$ or $[M-CH_3S]^+$
	125	25	28	$[(CH_3S)(CH_3O)PO]^+$
	142	0	100	$[M+H]^+$
Naled	127	n.d.	100	$[(CH_3O)_2P(OH)_2]^+$
PM 380	177	n.d.	16	n.i.
Paraoxon-methyl	65	27	0	$[P(OH)_2]^+$
PM 247	80	45	0	$[(CH_3O)_2OH+H]^+$
	93	100	24	$[(CH_3O)_2P]^+$
	109	33	100	$[(CH_3O)_2PO]^+$
	248	6	30	$[M+H]^+$

Table 1. Continued

Compound	m/z	Abundance (%)		Tentative identification
		40 V	20 V	
Parathion-methyl	65	40	0	$[P(OH)_2]^+$
PM 263	93	59	0	$[(CH_3O)_2P]^+$
	109	79	100	$[(CH_3O)_2PO)]^+$
	125	100	0	$[(CH_3O)_2PS]^+$
	154	15	17	$[C_6H_4NO_2S]^+$
	234	8	62	$[M-NO+H]^+$
	264	0	19	$[M+H]^+$
Trichlorfon	79	12	9	$[(CH_3O)POH]^+$
PM 257	109	100	45	$[(CH_3O)_2PO]^+$
	111	0	100	$[(CH_3O)_2PO+H]^+$
	221	0	35	$[M-Cl]^+$
	223	0	20	$[M-Cl]^+$
	257	0	46	$[M]^+$
	259	0	58	$[M]^+$
Vamidothion	58	41	0	$[CH_3NHCO]^+$
PM 287	87	59	0	$[CH_3NHCO(CH_2CH_3)]^+$
	118	100	0	$[CH_3NHCO(CHSCH_3)]^+$
	146	89	100	$[M-(CH_3O)_2POS]^+$
	288	0	6	$[M+H]^+$
	310	25	38	$[M+Na]^+$
Vamidothion sulfoxide	58	27	5	$[CH_3NHCO]^+$
PM 303	86	0	100	$[CH_3NHCO(CH_2CH_3)+H]^+$
	87	38	0	$[CH_3NHCO(CH_2CH_3)]^+$
	109	54	0	$[(CH_3O)_2PO]^+$
	125	8	0	$[(CH_3O)_2PS]^+$
	162	0	30	$[M-(CH_3O)_2POS]^+$
	169	55	41	$[(CH_3O)_2POS(CH_2)_2]^+$
	201	8	33	$[(CH_3O)_2POS(CH_2)_2SO]^+$
	241	100	27	$[M-(CH_3O)_2]^+$
	304	0	10	$[M+H]^+$
	326	0	68	$[M+Na]^+$

The percentage of abundance of each ion and the tentative identification is specified. Analytical conditions are described in Section 2. n.i. = Not identified.

condition is closest to the maximum response, related to fragmentation. Table 2 indicates for each compound the temperature which provided most fragmentation calculated by the Shannon–Weaver entropy with bits indicated in parentheses. It can be observed that for most of the compounds, an increase of the probe temperature produced an enhancement of fragmentation, being the optimum temperature between 400 and 500°C. However, for naled, paraoxon-methyl, trichlorfon and vamidothion sulfoxide, the maximum fragmentation was at 200–300°C. This decrease in *H* can be explained by the disappearance or decrease in abundances of some of the fragment ions due to the rise of the probe temperature. With regard to the ions formed, two different behaviors were noticed: (i) most of the compounds (acephate, fensulfothion, fenthion, trichlorfon, vamidothion and vamidothion sulfoxide) produced thermally stable ions that did not decompose at increasing the probe temperature and remained as base peaks. Both fensulfothion and fenthion have a high proton affinity which produces $[M+H]^+$ which is thermally stable. Moreover, the compounds that exhibit the P=S bond are more stable than the ones that have

Table 2
Temperature and voltage conditions which give most information as regards pesticide fragmentation and more sensitivity (calculated from areas of the TIC)

Compound	Temperature		Voltage	
	Fragmentation	Sensitivity	Fragmentation	Sensitivity
Acephate	500 (0.99)	200	40 (1.78)	20
Azinphos-ethyl	400 (1.71)	400	40 (1.72)	30
Fenitrothion	500 (2.28)	500	All voltages	40
Fensulfothion	500 (1.49)	400	60 (2.66)	20
Fenthion	400 (0.99)	400	40 (1.56)	40
Metamidophos	400 (1.73)	400	60 (1.47)	20
Naled	200 (0.65)	500	n.d.	n.d.
Paraoxon-methyl	200 (1.56)	400	40 (2.14)	20
Parathion-methyl	500 (1.46)	400	30 (2.30)	20
Trichlorfon	200 (2.52)	200	20 (2.53)	30
Vamidothion	500 (0.98)	400	40 (2.44)	30
Vamidothion sulfoxide	200 (2.17)	400	20 (2.53)	20

Numbers in parentheses indicate the information (=fragmentation) of each compound at the temperature indicated. Maximum information is log (5)=2.32 which corresponds to the five different temperature and voltage conditions studied.
n.d.=Not detected.

the P=O group, since more energy is needed to break the former bond and (ii) an increase of the temperature was related to the production of more ions and the base peak varied. Taking fenitrothion as example, at 200°C, the molecular ion was the base peak, which was thermally labile and at 300°C this peak decomposed to give the fragment ion at m/z 248, which corresponded to a transposition reaction with a loss of a nitro group, which remained as base peak. Other fragment ions corresponded to typical diagnostic ions of organophosphorous (OP) pesticides, at m/z 125 and the fragment at m/z 138 (see Fig. 1). In this case, a low probe temperature can be used to obtain molecular peak information.

Fig. 2 represents the values of the entropy of fensulfothion. It can be observed that the H has a minimum at 100°C which corresponds to the formation of only two ions, $[M+H]^+$ as base peak and $[M+Na]^+$ with an abundance of 8%. At increasing the probe temperature, the base peak remains stable at m/z 309, but from this protonated molecule, other fragment ions of lower m/z were generated, thus increasing the identification potential. At a probe temperature of 300°C, a plateau was reached and it corresponded to the maximum fragmentation obtained: all the four ions identified were present. This corresponded to the maximum entropy, which is defined by $H(\max)=\log_2 4=2$. For all the probe temperatures considered, the one which gave H closer to this value corresponded to that temperature which produced most fragmentation. This means that a higher entropy is translated in a higher number of ions and a higher abundance, in other words, maximum structural information. The presence of the $[M+H]^+$ at high probe temperature indicated that fragmentation via thermal degradation did not occur during the nebulization/ionization process [38]. The same graph in Fig. 2 indicates the sensitivity of fensulfothion under different temperature conditions, measured as the peak area obtained from the ion chromatogram of m/z 309. In this case, the maximum sensitivity was at 400°C.

Therefore, besides fragmentation, an increase in the probe temperature may affect sensitivity. Table 2 also indicates the temperature which provides maximum sensitivity, calculated from the areas of the total ion chromatogram (TIC). Azinphos-ethyl, fenitrothion, fensulfothion, fenthion, metamidofos, parathion and vamidothion produced an increase of fragmentation and of sensitivity upon an increase of temperature, corroborating the results of Kawasaki et al. [39], who reported an increase in the signal-to-noise ratio of several pesticides with an increase of vaporizer temperature of the APCI interface, reaching a maximum at 250°C. A similar effect was noted by Allen and Vestal [40] when optimizing the signal

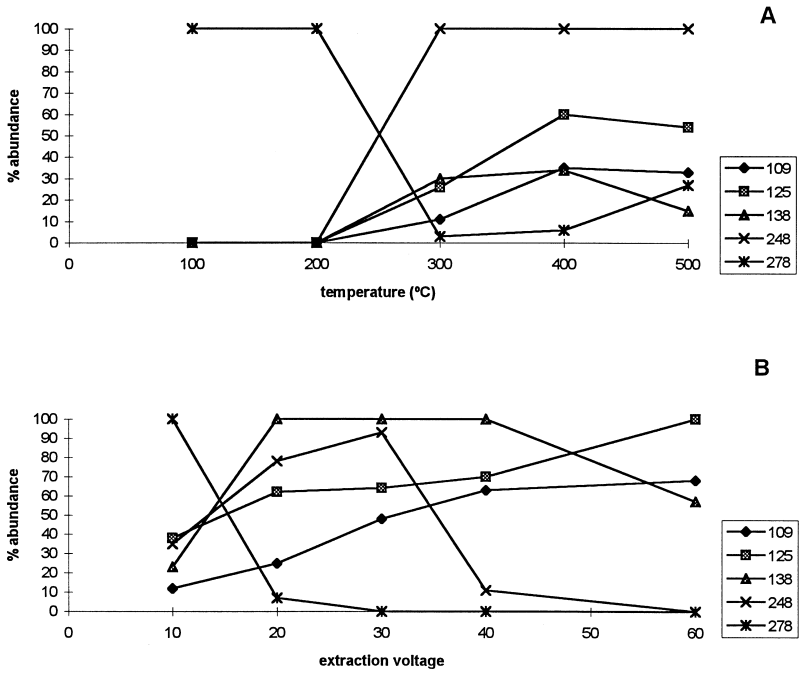

Fig. 1. Evolution of the formation of ions of fenitrothion under different conditions of (A) probe temperature and (B) extraction voltage.

intensity of leucine enkephalin at increasing the source temperature of a LC–ESP-MS interface. This can be attributed to the fact that the pesticides can vaporize without undergoing decomposition, as stated by Niessen and Tinke in a recent review [41]. Acephate and trichlorfon were the two compounds which showed higher sensitivity at 200°C. Acephate decomposes on heating, and therefore, at temperatures greater than 300°C there was a sharp decay in sensitivity. It is worth mentioning the case of trichlorfon: at higher temperatures a higher signal is obtained. Despite this fact, trichlorfon also decomposes at temperatures above 200°C and it is rapidly converted to dichlorvos which is seen by the presence of an ion at m/z 221. Naled, paraoxon-methyl and vamidothion sulfoxide presented 2–3 times higher sensitivity at 400–500°C, while highest fragmentation was observed at 200°C. All these compounds have a P=O bond, which is fragmented even at low probe temperatures.

The Shannon–Weaver entropy was also applied to determine which extraction voltage produced maxi-

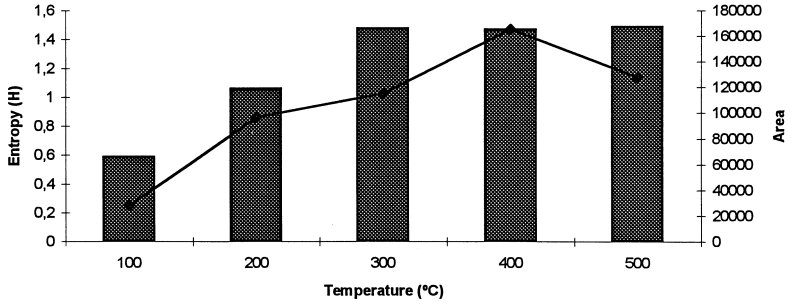

Fig. 2. Shannon–Weaver entropy of fensulfothion (in bits), calculated at the different probe temperatures (graphic of bars). The line indicates the sensitivity at the different experimental temperatures, calculated from the ion chromatogram at m/z 309.

mum fragmentation. Table 2 indicates the extraction voltage that produced most fragmentation and the ones which gave best sensitivity for each studied compound. In general, maximum sensitivity was obtained between 20 V and 30 V and at 40–60 V fragmentation was enhanced, thus lowering the sensitivity. It is important to mention that extraction voltage above 20 V produced more fragment ions in comparison to an increase of the probe temperature. Extraction voltages of 10 V may be insufficient to extract the ions from the vaporizer to the analyzer and in many cases, there was a formation of a sodiated adduct as the second most abundant peak. This adduct decreased sharply at increasing the extraction voltage and since it is a low energy bond, it was cleaved at extraction voltages higher than 30 V. The presence of this adduct can be used for identification purposes. In contrast to what was found when increasing the probe temperature, an increase of the extraction voltage produced a fragmentation of the molecular peak to form ions of low m/z values and there was a shift of the base peak from high to low m/z values. The extraction cone voltage acts like a collision induced reaction. This means that at identical tuning conditions and N_2 flow, an increase of temperature has a stronger effect on sensitivity while an increase of extraction voltage enhances fragmentation.

In this study it was found that an enhancement of fragmentation produced by an increase of the extraction voltage corresponded to a decrease in sensitivity, a similar result to that obtained by Pleasance et al. [27]. This effect can be explained by a maximum ion transmission at low extraction voltages. Fig. 3 represents the variations of the peak area of the TIC of paraoxon-methyl at increasing extraction voltage. The trend followed by most of the OP pesticides was similar, with slight changes in the curve profile, to that illustrated for paraoxon-methyl. In the same figure, the Shannon–Weaver entropy indicates the effect of the extraction voltage on the fragmentation. It can be observed that the maximum value of H was at an extraction voltage of 40 V where five ions were formed, and at 60 V, the base peak shifted from m/z 109 (40 V) to m/z 39. Fenitrothion behaved similarly to paraoxon-methyl. Fig. 1 also shows an example of ion formation of fenitrothion at different voltages. An increase in the cone voltage produced a decrease of the protonated molecular ion whereas fragment ions of lower mass increased, and at 30 V, the base peak corresponded to m/z 138. This indicates that the extraction voltage has a stronger effect on fragmentation than the temperature. Extraction voltages higher than 40 V produced a strong fragmentation and neither the protonated molecule nor the fragment ion at m/z 248 were formed, and instead it was possible to monitor the ions at m/z 138 and 125, the latter being the base peak at 60 V. As a result, applying a strong extraction voltage will produce only diagnostic ions, characteristic of each family of pesticides, which are not so straightforward for compound identification.

From the information obtained up to now, it was seen that the ions formed through application of heat differed to those obtained by CID. This latter effect was more important for enhancing fragmentation and ions of low m/z were formed, which were not found even at high probe temperatures. As an example,

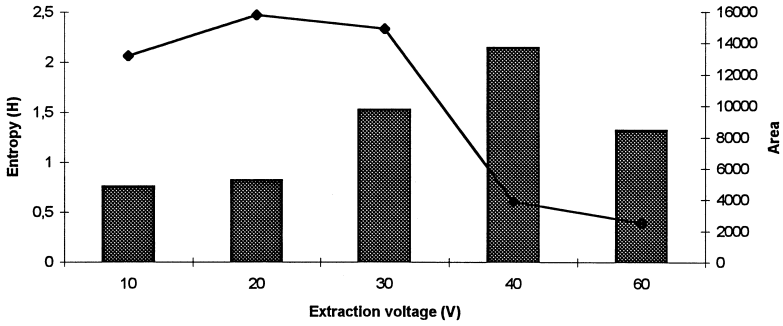

Fig. 3. Shannon–Weaver entropy of paraoxon-methyl (in bits), calculated at the different extraction voltages (bars). The line indicates the sensitivity at the different experimental extraction voltages, calculated from the ion chromatogram at m/z 109.

metamidofos, which produced an ion at m/z 94 at 500°C while a fragment ion at m/z 47 was formed at 60 V. Vamidothion formed a thermally stable ion at m/z 146 and it was fragmented at 40 and 60 V to produce ions at m/z 118 and 58, respectively. Moreover, for compounds with high proton affinities, the protonated molecule can remain stable at increasing probe temperature, but it is easily fragmented at extraction voltages above 30 V, as in the case of fensulfothion.

3.3. Recovery studies

The recoveries of the studied pesticides are shown in Table 3. Fig. 4 indicates the TIC after LSE–LC–APCI-MS of a groundwater sample. In general, higher recoveries are obtained using LiChrolut EN for compounds which have low water solubility and low vapor pressure. Compounds which have a high vapor pressure or high Henry's Law Constant, which indicates the volatilization potential of a compound in solution (see Table 4), such as fenitrothion, metamidofos and naled, were poorly recovered due to a double drying effect, during the elimination of water of the cartridges and during the evaporation of the final extract. Currently, on-line methodologies are being applied to efficiently recover these compounds. Moreover, fenthion could be recovered at low levels from the water sample. This compound can hydrolyze in water during the time needed to carry out the preconcentration step. Trichlorfon was recovered at 46% when using the LiChrolut cartridges. However, this compound was monitored at m/z 109, which can belong to both trichlorfon and dichlorvos, and therefore, the high recovery values were attributed to the formation of dichlorvos during the vaporization of the compound in the APCI probe. Vamidothion sulfoxide presented lower recoveries than its parental form, vamidotion, since this compound is more polar than vamidothion, and it was expected to have a lower breakthrough volume. The preconcentration of 50 to 100 ml of water would, most probably, enhance recovery at the expense of sensitivity.

4. Conclusions

LC–APCI-MS in PI mode permitted the determination of twelve pesticides, including polar compounds and the parathion group which cannot be determined with ESP-MS. However, for compounds that suffer thermal degradation, such as trichlorfon, ESP-MS appears a more suitable technique. The pesticides under study were characterized under different probe temperatures and extraction voltages. It was found that for compounds with high proton affinity there was a formation of the $[M+H]^+$ as base peak but for others, compound specific fragment ions were formed. In all cases, more fragmenta-

Table 3
Percentage recovery and standard deviation of the studied pesticides after preconcentration of 200 ml of groundwater spiked at 0.2 μg/l on ENV and LiChrolut cartridges

Compound	m/z	Recovery (%)		LOD (pg)
		ENV	LiChrolut	
Acephate	143	154±6	125±15	104
Azinphos-ethyl	160	63±17	132±9	50
Fenitrothion	125	58±10	76±10	120
Fensulfothion	157	95±5	122±5	30
Fenthion	231	21±23	32±12	200
Metamidophos	94	31±11	24±15	60
Naled	127	n.d.	n.d.	250
Paraoxon-methyl	234	56±11	69±12	180
Parathion-methyl	109	46±6	76±5	210
Trichlorfon	109	n.d.	46±15	70
Vamidothion	146	72±10	83±15	110
Vamidothion sulfoxide	241	46±3	106±3	80

Aquisition was performed in the SIM mode at the mass indicated, and the LODs were calculated from direct injection of a standard.

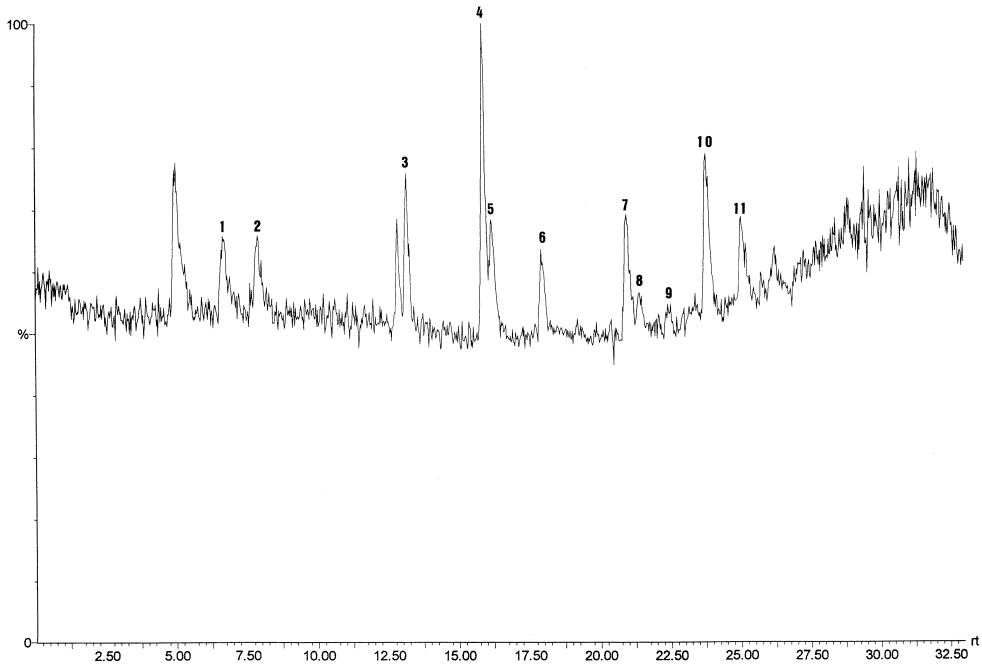

Fig. 4. LC–APCI-MS chromatogram in positive ionization mode which corresponds to spiked groundwater (at a level of 0.2 μg/l) preconcentrated onto LiChrolut EN cartridges. Acquisition was performed in scan mode. Peak identification: 1=metamidofos, 2=acephate, 3=vamidothion sulfoxide, 4=vamidothion, 5=trichlorfon, 6=paraoxon-methyl, 7=fensulfothion, 8=parathion-methyl, 9=fenitrothion, 10=azinphos-ethyl and 11=fenthion. rt=Retention time (min).

tion was obtained at 40 V, which produced a shift of the base peak from high to lower m/z values and at 60 V strong fragmentation was produced and in some cases, only diagnostic ions of OP pesticides were formed. The increase in fragmentation was produced at the expense of sensitivity. The present study indicated that besides the extraction voltage, probe temperatures between 400 and 500°C lead to an increase in fragmentation in thermally labile compounds or with low proton affinities, but in most

Table 4
Physico-chemical properties (water solubility, vapor pressure and Henry's Law Constant) of the studied organophosphorus pesticides which influence LC–APCI-MS fragmentation

Compound	Water solubility (g/l)	Vapor pressure (mPa) (20–25°C)	HLC (Pa m^3/mol)
Acephate	790	0.23	0.00029
Azinphos-ethyl	0.044	0.32	7.27
Fenitrothion	0.021	18	857
Fensulfothion	n.f.	n.f.	n.f.
Fenthion	0.0042	0.37	88
Metamidophos	>200	2.3	0.015
Naled	Insoluble	266	Very high
Paraoxon-methyl	n.f.	n.f.	n.f.
Parathion-methyl	0.055	0.2	3.6
Trichlorfon	120	0.21	0.0017
Vamidothion	4000	Negligible	Low
Vamidothion sulfoxide	n.f.	n.f.	n.f.

n.f.=Not found.

cases, the base peak remained invariable. This effect was evaluated by applying the Shannon–Weaver entropy, which permitted us to compare the extend of fragmentation under different experimental conditions. As a general rule, it was found that the probe temperature has a greater effect on compound sensitivity than on fragmentation. A compromise between sensitivity and structural information has to be defined and as a result, the probe temperature and extraction voltage have to be optimized depending on the aim of each study. Optimum LC flow-rate in terms of sensitivity was of 1.2 ml/min. The optimum conditions as regards to flow-rate, probe temperature and extraction voltages (1.2 ml/min, 400°C and 20 V, respectively) were used to calculate the recoveries of selected pesticides. It was found that the use of cartridges filled with Isolut ENV and LiChrolut EN showed recoveries that varied from 132 to 63%. Worst results were attributed to losses during the drying and vaporization step and to high polarity of some of the compounds. LC–APCI-MS accomplished the detection below the limits imposed by the European Union.

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