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Comparison of automated on-line solid-phase extraction followed by liquid chromatography–mass spectrometry with atmospheric pressure chemical ionization and particle beam mass spectrometry for the determination of a priority group of pesticides in environmental waters

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Abstract

Atmospheric pressure chemical ionization (APCI) in both positive and negative ionization modes and particle beam (PB) in electron impact (EI) and chemical ionization in positive (PCI) and negative (NCI) modes mass spectrometry (MS) coupled to high-performance liquid chromatography (HPLC) are compared in the determination of a group of pesticides. The method was combined with an automated on-line solid-phase extraction (SPE) step by preconcentrating 200 ml of sample through C_{18} pre-columns in order to perform the determination of these pesticides at low levels. Calibration graphs were constructed with time-scheduled selected ion monitoring (SIM) and using the internal standard method; for APCI under positive ion (PI) mode limits of detection (LODs) were between 0.8 and 4 ng l^{-1} and under negative ion (NI) mode of operation, between 4 and 20 ng l^{-1}; for PB the LODs were between 0.05 to 0.2 μg l^{-1} under EI conditions and from 0.02 to 0.1μg l^{-1} under chemical ionization in both positive and negative acquisition modes. The study demonstrates the higher sensitivity of HPLC–APCI-MS compared with HPLC–PB-MS and the potential of both techniques for confirming the presence of contaminants in environmental matrices. The developed methods were validated by participating in Aquacheck inter-laboratory exercises. © 1998 Elsevier Science B.V.

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1. Introduction

In the last few years, the on-line combination of liquid chromatography (LC) and mass spectrometry (MS) has become a robust and routinely applicable tool in environmental laboratories [1–4]. High-performance liquid chromatography (HPLC)–MS is the preferred technique for the identification and quantification of polar and thermally labile compounds that are not GC amenable. The initial incompatibility between these two analytical techniques has been solved by different interfaces. Thermospray (TSP) is an interface which has been widely used [5–8] although is not very useful for the identification of unknown compounds because of its poor structural information. Particle beam (PB) is specially interesting for identifying unknown compounds [9–15], despite its limitations in the quantification purposes and lack of sensitivity at trace levels. Electrospray (ESP) has become, in the last few years, a widely

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applicable and very sensitive soft ionization technique [16–22], this interface is very useful for polar and thermally labile compounds but semipolar analytes as the parathion group are not easily ionized [16,23]. Atmospheric pressure chemical ionization (APCI) has recently become the most universal technique for environmental analysis due to the high sensitivity and the possibility of detecting a broad range of analytes [19,21,23–29].

A great advantage of the APCI interface is the property of inducing fragmentation of the primary ions by the application of an appropriate voltage value, so APCI offers the possibility of obtaining additional structural information; this mode of operation is termed cone voltage fragmentation (CVF) or pre-analyzer collision-induced dissociation (CID) [30,31] and is based on the application of a voltage to one of the cones of an API source resulting in the dissociation of the quasi-molecular ion.

The PB interface enables the coupling with LC to conventional EI- and CI-MS procedures. This interface is mainly used for identifying non-target compounds in real-world samples because it provides structurally significant ions through the EI ionization [10–12,14,15], despite the limited linearity and sensitivity of the response. To solve these disadvantages different approaches have been developed such as the use of a carrier additive in LC eluent [32–34] or the isotope dilution [35,36].

Since PB can provide both EI and CI spectra it is a powerful technique for an unambiguous identification of non target compounds; but, in some cases, non-linear response and poor sensitivity limit this technique for the quantification of contaminants at trace levels. APCI is a sensitive and widely applicable technique which gives primarily molecular mass information with the ability to provide structural information by increasing the voltage. So LC–APCI-MS is a valuable technique for both structural confirmation and quantitative analysis of target compounds in environmental samples.

The detection limits achieved with the use of LC–MS with both interfaces, PB and APCI, will often not be sufficiently low, so it became clear that sample preconcentration is necessary in order to reach the low detection limits required in environmental analysis. An on-line trace enrichment procedure such as solid-phase extraction (SPE) improves analyte detectability [37].

In this study APCI- and PB-MS in both positive and negative modes coupled to HPLC have been used for the determination of a group of pesticides of different chemical nature. The two interfaces are evaluated for their suitability for the analysis of the compounds under study. The selection of the different analytes was based on the use of some of them in the region of the Ebro delta for the rice and corn cultivation and also on their presence in the priority lists of pesticides. The analytical methods were validated by analyzing samples distributed by the Aquacheck inter-laboratory program (WRC, Medmenham, UK).

2. Experimental

2.1. Materials

Fifteen different pesticides are included in this study, which belong to different groups, such as organophosphorous pesticides (fenitrothion, malathion, parathion-ethyl and vamidothion), triazines (ametryn, atrazine, prometryn and terbutryn), diazines (bentazone), phenylureas (isoproturon), chlorophenoxy acids (MCPA and mecoprop), phenolic compounds (dinoseb and 4-NP) and thiocarbamates (molinate). All of them were from Riedel-de Häen (Seelze, Germany). 1000 mg l^{-1} stock solutions were prepared by weighing and dissolving each pesticide in methanol and storing them at 4°C. Working standard solutions were prepared by diluting the stock solutions in methanol and they were stored in the same way. The chemical structures of all the compounds studied are shown in Fig. 1.

For APCI the internal standards were dinoterb from Riedel-de Haën when the acquisition was in the negative ion mode and terbuthylazine from Promochem (Wesel, Germany) when the acquisition was in the positive ion mode. For PB the internal standard was fluomethuron from Riedel-de Häen, for both EI and chemical ionization (CI) in both positive and negative ion modes. The structures of the internal standards are also shown in Fig. 1.

HPLC grade methanol was obtained from Scharlau (Barcelona, Spain). Ultra-pure water was prepared by ultrafiltration with a Milli-Q water purification system (Millipore, Bedford, MA, USA). Ammonium acetate was from Panreac (Montcada i Reixac,

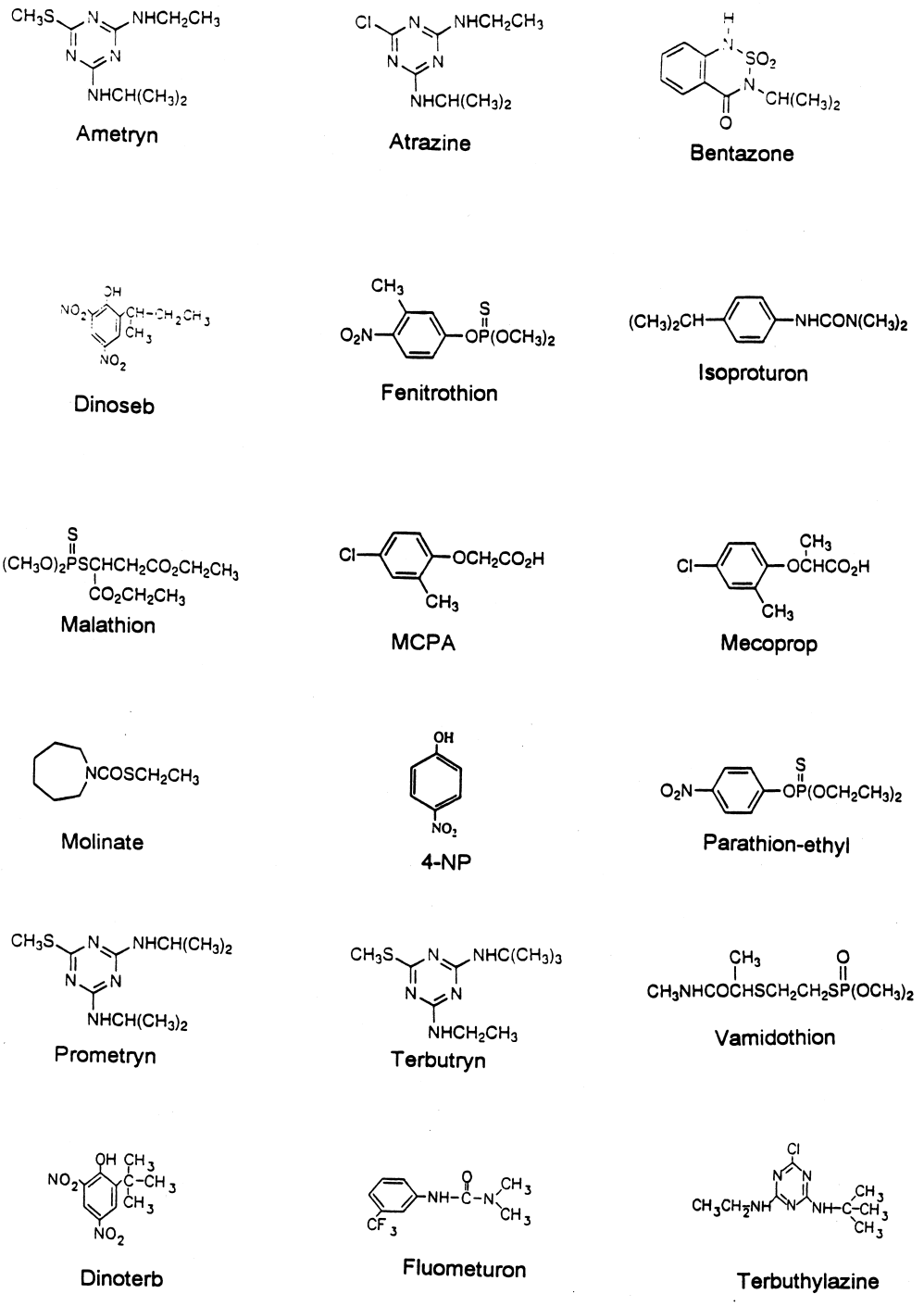

Fig. 1. Chemical structures of the pesticides studied and the internal standards.

Spain) and the solution prepared in Milli-Q purified water was acidified to pH 5 by adding acetic acid from Probus (Badalona, Barcelona, Spain). This solution was filtered through a 0.45 μm nylon filter prior to use.

Both helium for the PB interface and for degassing the LC solvents and the methane reagent gas for chemical ionization were 99.995% pure and supplied by Carburos Metálicos (Tarragona, Spain). Nitrogen for the APCI interface was 99.998% pure and it was supplied by Air Liquide (Barcelona, Spain).

2.2. Experimental conditions

2.2.1. LC–APCI-MS

The eluent was delivered by a gradient system from Waters 616 pumps controlled by a Waters 600S (Waters, Milford, MA, USA).

Separations were performed on a 200 mm×4.6 mm I.D. stainless-steel analytical column packed with 5 μm Spherisorb ODS2 (Teknokroma, Barcelona, Spain). Elution was accomplished using methanol–0.1 *M* ammonium acetate (acidified to pH 4.5 with acetic acid) at a flow-rate of 0.8 ml min^{-1} with the following gradient: from 30:70 to 60:40 in 20 min, then to 70:30 in 20 min and from there to 90:10 in 5 min. Return to initial conditions was carried out in 5 min.

For the mass spectrometric analysis a VG Platform from Micromass (Manchester, UK) was used. The system was equipped with an APCI interface. The experimental conditions were as follows: the cone and corona voltages were set at values of 20 V and 3.5 kV, respectively. The ion source temperature was set at 180°C and the probe temperature was 400°C. A nitrogen flow-rate of 10 l/h was used for the nebulization and the flow-rate for the drying nitrogen was 200 l/h. Chromatograms were recorded under time-scheduled selected-ion monitoring (SIM) conditions in both positive and negative acquisition modes for quantitation. Full-scan (from m/z 64 to 400) conditions were also used. The instrument control and data processing were carried out through a MegaLinx software.

2.2.2. LC–PB-MS

A Hewlett-Packard (Palo Alto, CA, USA) 1090 liquid chromatograph equipped with a six-port rotary valve and an autosampler was used. The system was controlled by a Workstation HP 79994A.

The analytical separations were performed using the same column and gradient as for LC–APCI-MS but in this case at a flow-rate of 0.4 ml min^{-1}.

A Hewlett-Packard 5989 A MS Engine, equipped with a dual EI/CI source was connected to the outlet of the LC via a Hewlett-Packard PB interface through a 50 cm×0.12 mm stainless-steel capillary tubing. The interface conditions were as follows: the desolvation chamber temperature was set at 65°C and the helium nebulizer pressure at 60 p.s.i. (1 p.s.i.= 6894.76 Pa). Under CI conditions, methane was used as the reagent gas for PCI and NCI. For EI ionization, the ion source block and the quadrupole temperatures were set at 250°C and 100°C, respectively and for CI the reagent gas pressure was kept at 1.0 Torr for PCI and 1.3 Torr for NCI and the ion source temperatures were 250°C for PCI and 200°C for NCI (1 Torr=133.322 Pa). The ionization energy was 70 eV. The MS was tuned to m/z 69, 219 and 502 for EI, to m/z 219, 414 and 652 for PCI and m/z 264, 414 and 633 for NCI corresponding to perfluorotributylamine (PFTBA). Chromatograms were recorded under time-scheduled selected ion monitoring SIM. In full-scan mode acquisition, spectra were acquired in the mass range 64–400 u at a scan rate of 2.32 scans s^{-1} for EI, from 81 to 400 u for PCI at 2.43 scans s^{-1} and from 64 to 400 u at 2.32 scans s^{-1} for NCI. The instrument control and data processing were performed by an HP UX 59944C data system.

2.3. Sample preparation

For APCI, the automated SPE device, OSP-2, (Merck, Darmstadt, Germany) was connected on-line with the gradient pumps. A LiChroGraph Model L-600A intelligent pump (Merck–Hitachi) was used to deliver the solvents to condition the pre-columns and the samples which contained the pesticides. The pre-columns were conditioned by flushing 5 ml of methanol and then 5 ml of HPLC grade water at 1 ml min^{-1}. Tap water volumes of 200 ml spiked with the pesticides and the corresponding internal standard were preconcentrated on disposable pre-columns (Merck) prepacked with 10 μm LiChrospher Si100 RP-18, at a flow-rate of 5 ml min^{-1}. Water samples

were acidified to pH 3, by the addition of hydrochloric acid, and filtered through 0.45 μm filters (Millipore) before preconcentration.

For PB, trace enrichment process was performed on a holder and a 10×2.0 mm cartridge packed with C_{18} (Spark Holland, Emmen, Netherlands). An Applied Biosystems (Ramsey, USA) pump was used to deliver the sample and wet the pre-column. The pre-column tubing and sorbent were first washed with 5 ml of methanol at a flow-rate of 4 ml min^{-1}. The samples were treated in the same way as for APCI.

Validation of the system was carried out by participating in an inter-laboratory calibration study for herbicides and organophosphorous compounds organized by Aquacheck (WRC, Medmenham, UK). The protocol of preparation for these samples involves spiking a water sample with a solution that contains the herbicides and the organophosphorous pesticides at an unknown concentration at a level imposed by the organization in order to determine the levels of these pesticides.

3. Results and discussion

3.1. Mass spectral information

The evaluation of the spectral information was carried out using direct injection of each pesticide in a concentration of 50 mg l^{-1} into a carrier stream of methanol–0.1 *M* ammonium acetate (50:50). The amount of each pesticide injected was 1 μg under full-scan conditions. The mass spectrum of each compound was recorded with the two interfaces, APCI and PB, and the results were compared.

3.1.1. APCI

3.1.1.1. Positive acquisition

Table 1 presents the major ions obtained with APCI under the positive acquisition mode. Those results were obtained for a cone voltage of 20 V. The spectra of all the pesticides were also obtained at a voltage of 40 V; from the results obtained through this study it should be pointed that the higher the cone voltages the greater the degree of fragmentation and so no molecular mass information is obtained. The cone voltage of 20 V provides enough structural information and the sensitivity was higher than for 40 V, so the lower value was used for further studies.

As it can be observed in Table 1, the phenolic compounds (dinoseb and 4-NP) and the chlorophenoxy acid MCPA were not detected in the positive acquisition mode, and for mecoprop the sensitivity was very low. Another important feature is that the mass spectra for the triazines, phenylureas and molinate showed molecular mass information through the quasi-molecular ions, $[M+H]^+$, as the base peak but little fragmentation is observed; this route of ionization can be related with the high proton affinity of those compounds in the gas phase. Molinate also showed the peak at *m/z* 126 which can be assigned to the loss of the group $[SCH_2CH_3]$, isoproturon showed a peak at *m/z* 72 which corresponds to the fragment $[(CH_3)_2NCO]^+$ which is typical of a group of phenylureas including chlortoluron or diuron as was previously reported by other authors although they use PB [10]. For the organophosphorous compounds an important fragmentation was observed and the corresponding ions were compound dependent [23]. Some of the compounds under study present the sodium adduct ion, $[M+Na]^+$; the presence of Na^+ ions can be attributed as an impurity in the methanol solution employed as the mobile phase, or as sodium in the metal tubing or needle assembly [16,23,29].

3.1.1.2. Negative acquisition

Table 1 presents the major ions obtained under the negative acquisition mode. As can be observed, the triazines and molinate did not show any response under NI acquisition and isoproturon gave a low response, so it can be pointed that those pesticides have been reported to offer better sensitivity under PI than NI conditions. The $[M-H]^-$ ion is the base peak for the acidic herbicides under study, bentazone, the phenolic compounds (dinoseb and 4-NP) [38] and the chlorophenoxy acids (MCPA and mecoprop); for the chlorophenoxy acids the formation of an ion at *m/z* 141, which can be attributed to the loss of the corresponding alkanecarboxylic acid, also was observed; this behaviour was previously reported by other authors although they use ESP-MS [18].

For the organophosphorous compounds of the parathion group (parathion-ethyl and fenitrothion)

Table 1
Important mass spectral fragments and their relative abundances obtained by FIA-APCI-LC–MS under positive and negative acquisition

Compound	M_w	PCI		NCI	
		m/z and tentative ions	R.A. (%)	m/z and tentative ions	R.A. (%)
Ametryn	227	228 $[M+H]^+$	100	n.d.[1]	
Atrazine	215	216 $[M+H]^+$	100	n.d.	
Bentazone	240	120 $[C_6H_4NH_2CO]^+$	100	239 $[M-H]^-$	100
		241 $[M+H]^+$	22		
Dinoseb	240	n.d.[1]		239 $[M-H]^-$	100
Fenitrothion	277	248 $[M-NO+H]^+$	100	152 $[C_6H_3ONO_2CH_3]^-$	100
		124 $[PS(OCH_3)_2-H]^+$	94	168 $[SC_6H_3NO_2CH_3]^-$	52
		262 $[M-CH_3]^+$	19	262 $[M-CH_3]^-$	39
				276 $[M-H]^-$	12
Isoproturon	206	207 $[M+H]^+$	100	205 $[M-H]^-$	100
		229 $[M+Na]^+$	26		
		72 $[(CH_3)_2NCO]^+$	4		
Malathion	330	285 $[M-C_2H_5O]^+$	100	157 $[(CH_3O_2)PS_2]^-$	100
		127 $[M-(CH_3O)_2PS_2)-(C_2H_6O)]^+$	38		
		353 $[M+Na]^+$	36		
		331 $[M+H]^+$	29		
MCPA	200	n.d.		199 $[M-H]^-$	100
Mecoprop	214	228 $[M+14]^+$	100	213 $[M-H]^-$	100
				141 $[M-CH_3CHCO_2H]^-$	72
Molinate	187	188 $[M+H]^+$	100	n.d.	
		126 $[M-SC_2H_5]^+$	27		
4-NP	139	n.d.		138 $[M-H]^-$	100
Parathion-ethyl	291	262 $[M-C_2H_5]^+$ or $[M-NO+H]^+$	100	138 $[O_2NC_6H_4O]^-$	100
		110 $[(CH_3O)_2POH)]^+$	82	154 $[(C_2H_5O)_2PSH)]^-$	53
		234 $[M-(C_2H_5)_2]^+$	44	169 $[(C_2H_5O)_2POS)]^-$	41
		276 $[M-CH_3]^+$	27	262 $[M-CH_2CH_3]^-$	19
		292 $[M+H]^+$	4	122 $[C_6H_4NO_2]^-$	16
				290 $[M-H]^-$	9
Prometryn	241	242 $[M+H]^+$	100	n.d.	
Terbutryn	241	242 $[M+H]^+$	100	n.d.	
		186 $[M-C_4H_7]^+$	8		
Vamidothion	287	146 $[CH_3NHCOCH_3CHSCH_2CH_2]^+$	100	141 $[(CH_3O)_2POS]^-$	100
		288 $[M+H]^+$	27	272 $[M-CH_3]^+$	24
		310 $[M+Na]^+$	20		

The amount injected was 1 μg.
[1] n.d.: No spectra detected under experimental conditions.

the sensitivity is better in NI than in PI mode as was previously reported [23]. For fenitrothion, the ion at m/z 168 corresponds to the thiophenolate ion; the formation of this ion instead of the phenolate ion is due to the strong acidity of it in the gas phase and there is a transfer from the aromatic moiety from the oxygen to the sulphur atom; this fact was also observed by other authors using GC–MS [39,40].

3.1.2. PB

3.1.2.1. EI ionization

Table 2 shows the major ions obtained under EI ionization and using a carrier stream of methanol–0.1 *M* ammonium acetate (50:50). As can be observed, the ionization is compound dependent and an important fragmentation is usually observed for most of the compounds. The corresponding fragments to the ions are widely explained in another paper [41]. In general, EI provides more structural informative spectra than APCI, except for organophosphorous pesticides for which in their APCI spectra in both positive and negative acquisition modes different

Table 2
Important mass spectral fragments and their relative abundances obtained by PB under electron impact ionization

Compound	M_w	m/z and tentative ions	R.A. (%)
Ametryn	227	227 $[M]^+$	100
		212 $[M-CH_3]^+$	55
		170 $[M- C_3H_8NH]^+$	36
Atrazine	215	200 $[M-CH_3]^+$	100
		216 $[M+H]^+$	66
Bentazone	240	120 $[C_6H_4NH_2CO]^+$	100
Dinoseb	240	211 $[M-CH_2CH_3]^+$	100
Fenitrothion	277	138 $[O_2NC_6H_4O]^+$	100
		248 $[M-NO]^+$	71
		277 $[M]^+$	29
Isoproturon	206	72 $[(CH_3)_2NCO]^+$	100
		206 $[M]^+$	36
Malathion	330	173 $[M-(CH_3O)_2PS_2]^+$	100
		127 $[M-(CH_3O)_2PS_2)-(C_2H_6O)]^+$	88
MCPA	200	141 $[M-CH_2COOH]^+$	100
		200 $[M]^+$	63
Mecoprop	214	142 $[M-CH(CH_3)COO]^+$	100
		214 $[M]^+$	61
Molinate	187	126 $[M-SC_2H_5]^+$	100
		187 $[M]^+$	26
4-NP	139	109??	100
		139 $[M]^+$	40
Parathion-ethyl	291	109 $[C_2H_50PO_2H]^+$	100
		291 $[M]^+$	54
Prometryn	241	226 $[M-CH_3]^+$	100
		184 $[M-NCH(CH_3)_2]^+$	93
		241 $[M]^+$	64
Terbutryn	241	226 $[M-CH_3]^+$	100
		185 $[M-C_4H_8]^+$	93
		241 $[M]^+$	64
Vamidothion	287	87 $[CH_3NHCOC_2H_5]^+$	100
		146 $[CH_3NHCOCH_3CHSCH_2CH_2]^+$	31
		109 $[(CH_3O)_2PO]^+$	14

The injected amount was 1 μg.

ions appear. The advantage of using EI is that it generates library searchable spectra and thus it is helpful for analyte identification.

3.1.2.2. CI

Mass spectra were initially recorded in positive-ion CI mode. The most important mass spectral fragments are shown in Table 3. As can be observed, bentazone, dinoseb, MCPA, mecoprop, molinate and 4-NP did not show any response under experimental conditions. For the other compounds under study, the fragmentation patterns obtained through PCI conditions is similar with those obtained by APCI in positive acquisition mode except for the organophosphorous pesticides (fenitrothion, malathion, parathion-ethyl and vamidothion) which showed a higher fragmentation under APCI. It is important to remark that the PCI spectrum of most of pesticides showed the presence of the protonated molecule, $[M+H]^+$, so molecular mass information can be obtained and this can be used for the unequivocal identification.

Table 3 presents the major ions obtained under the negative acquisition mode, NCI. As can be observed, bentazone, molinate and 4-NP did not show any response. Except for the chlorophenoxy acids (MCPA and mecoprop) and the organophosphorous malathion and vamidothion, the other pesticides showed the $[M-H]^-$ or $[M]^-$ ion as the base peak which is helpful for molecular mass information. The fragmentation of some of the pesticides obtained by NCI differs significantly from that achieved in APCI under negative mode, in particular for organophosphorous pesticides a greater degree of fragmentation is observed by APCI. On the other

Table 3
Important mass spectral fragments and their relative abundances obtained by PB under chemical ionization in positive and negative ionization modes

Compound	M_w	PCI		NCI	
		m/z and tentative ions	R.A. (%)	m/z and tentative ions	R.A. (%)
Ametryn	227	228 $[M+H]^+$	100	226 $[M-H]^-$	100
Atrazine	215	216 $[M+H]^+$	100	214 $[M-H]^-$	100
Bentazone	240	n.d.[1]		n.d.[1]	
Dinoseb	240	n.d.		141	100
Fenitrothion	277	248 $[M-NO+H]^+$	100	276 $[M-H]^-$	100
Isoproturon	206	207 $[M+H]^+$	100	205 $[M-H]^-$	100
Malathion	330	127 $[M-(CH_3O)_2PS_2)-(C_2H_6O)]^+$ 173 $[M-(CH_3O)_2PS_2]^+$ 331 $[M+H]^+$	100 82 31	172 $[M-(CH_3O)_2PS_2-H]^-$ 157 $[(CH_3O)_2PS_2]^-$	100 75
MCPA	200	n.d.		141 $[M-CH_2COOH]^-$	100
Mecoprop	214	n.d.		141 $[M-CH_3CHCO_2H]^-$	100
Molinate	187	n.d.		n.d.	
4-NP	139	n.d.		n.d.	
Parathion-ethyl	291	262 $[M-C_2H_5]$ 292 $[M+H]^+$	100 27	291 $[M]^-$ 154 $[(C_2H_5O)_2PSH)]^-$	100 16
Prometryn	241	242 $[M+H]^+$	100	240 $[M-H]^-$	100
Terbutryn	241	242 $[M+H]^+$ 186 $[M-C_4H_7]^+$	100 36	240 $[M-H]^-$	100
Vamidothion	287	146 $[CH_3NHCOCH_3CHSCH_2CH_2]^+$ 88 $[CH_3NHCOC_2H_6]^+$	100 87	272 $[M-CH_3]^-$	100

The injected amount was 1 μg.
[1] n.d.: No spectra detected under experimental conditions.

hand, for the triazine compounds no response was obtained neither by PB nor by APCI under NCI conditions. Additional explanation of the fragments in both PCI and NCI are widely explained in a previous paper [41].

From the obtained results by PB and APCI it can be pointed that LC–PB-MS appears to have high potential as an identification method, because it generates the EI library searchable spectra and the CI spectra, being a very valuable tool for the unequivocal identification of the compounds. LC–APCI-MS is also a valuable technique for structural information and for this interface the protonated or deprotonated molecule, for PI or NI, respectively, are usually obtained in the mass spectrum; however the fragmentation is sometimes not sufficient to completely elucidate an unknown analyte.

3.2. On-line SPE–HPLC–MS

The determination of pesticides in real water samples by HPLC involves the use of a trace enrichment step in order to reach the levels required for environmental samples. The performance of the methods was checked for tap water and so 200 ml of sample spiked with the solution of pesticides was analyzed. A volume of 200 ml was selected for further studies despite the fact that breakthrough of some of the analytes occurs with the subsequent low recoveries. Quantitation was performed with the internal standard method as was carried out in previous papers [29], using the SIM mode, so the base peak of each compound was selected.

3.2.1. APCI

Terbuthylazine and dinoterb, in a concentration of 0.05 ng ml^{-1}, were used as internal standards for PI and NI acquisition modes, respectively. The corresponding time-scheduled SIM conditions are shown in Table 4. The linearity of the response was checked in the range from 0.002 to 0.25 ng ml^{-1} using both positive and negative modes of ionization. The limits of detection (LODs) were calculated by using a signal-to-noise ratio of 3. The ranges of linearity, correlation coefficients and LODs obtained by APCI in both modes are given in Table 5. The repeatability of the method, expressed as relative standard deviation (R.S.D.) and calculated at spiking level of 0.08 ng ml^{-1} ($n=4$) was between 3% and 30% for PI and from 4% and 23% for NI. Bentazone gave no response under these conditions neither PI nor NI and this is due to its low sensitivity.

It can be pointed that although the quantification was carried using SIM acquisition mode in order to obtain lower detection limits full-scan acquisition mode can be used in order to identify the com-

Table 4
Time-scheduled SIM conditions for on-line SPE–APCI-MS in positive and negative ion modes

Compound	PI		NI	
	m/z	Acquisition window (min)	m/z	Acquisition window (min)
Bentazone	120	0–20	239	0–30
Vamidothion	146	0–20	n.d.	–
4-NP	n.d.[1]	–	138	0–30
MCPA	n.d.	–	199	0–30
Mecoprop	n.d.	–	213	0–30
Dinoseb	n.d.	–	239	0–30
Atrazina	216	20–28	n.d.	–
Isoproturon	207	20–28	n.d.	–
Ametryn	228	27–37	n.d.	–
Malathion	285	27–37	157	27–50
Fenitrothion	248	27–37	152	27–50
Molinate	188	27–37	n.d.	–
Prometryn	242	32–50	n.d.	–
Terbutryn	242	32–50	n.d.	–
Parathion-ethyl	262	32–50	138	27–50

[1] n.d.: Not detected under experimental conditions.

Table 5
Calibration data and LODs obtained by APCI using SIM under PI and NI ionization modes after the preconcentration of 200 ml of sample

Compound	PI			NI		
	r^2	Linearity range ($\mu g\ l^{-1}$)	LOD ($\mu g\ l^{-1}$)	r^2	Linearity range ($\mu g\ l^{-1}$)	LOD ($\mu g\ l^{-1}$)
Ametryn	0.997	0.002–0.25	0.001		n.d.	
Atrazine	0.997	0.002–0.25	0.0008		n.d.	
Dinoseb		n.d.		0.994	0.01–0.25	0.004
Fenitrothion		n.d.		0.973	0.02–0.25	0.01
Isoproturon	0.999	0.004–0.25	0.002		n.d.	
Malathion	0.999	0.01–0.25	0.004	0.930	0.02–0.25	0.01
MCPA		n.d.		0.999	0.04–0.25	0.02
Mecoprop		n.d.		0.995	0.02–0.25	0.01
Molinate	0.998	0.002–0.25	0.001		n.d.	
4-NP		n.d.		0.917	0.01–0.25	0.02
Parathion-ethyl		n.d.		0.996	0.01–0.25	0.005
Prometryn	0.995	0.002–0.25	0.0008		n.d.	
Terbutryn	0.996	0.002–0.25	0.0008		n.d.	
Vamidothion	0.998	0.002–0.25	0.004		n.d.	

pounds. In Fig. 2 typical chromatograms obtained for a 200 ml of tap water sample spiked at 0.04 ng ml^{-1} level under time-scheduled SIM in positive and negative acquisition mode are shown.

The method was validated by analyzing samples distributed by the Aquacheck inter-laboratory program. A mixture of two different samples containing herbicides and organophosphorous pesticides was analyzed, under PI and NI conditions. The obtained results have been compared with those given by Aquacheck. For this sample three different measurements were carried out and Table 6 shows the average value of those determinations and the percentage of error referred to the certified values reported by Aquacheck. The results obtained are evaluated according to the limits imposed by the organization and it could be observed that they are outside of the acceptable ranges (17%) but they were only marginally above the limits set by the Association of Official Analytical Chemists (AOAC) (22%) [42,43].

3.2.2. PB

For PB the calibration graphs were constructed in the same way as for APCI and this study was carried out for EI and CI in both positive and negative acquisition modes. In this case the internal standard for quantification purposes was fluomethuron in a concentration of 5 ng ml^{-1} for EI and 10 ng ml^{-1} for CI. The corresponding time-scheduled SIM conditions are shown in Table 7.

For EI, the linearity of the response was checked in the range from 0.1 to 10 ng ml^{-1} and the LODs were estimated by a signal-to-noise of 3. The ranges of linearity, LODs and correlation coefficients obtained by EI are given in Table 8. The repeatability of the method expressed as R.S.D.s and calculated at spiking level of 1 ng ml^{-1} was between 7% and 28%. For PCI and NCI the linearity was examined for the concentration range from 0.05 to 10 ng ml^{-1}; the corresponding results obtained by CI in both ionization modes are summarized in Table 8. From the obtained results it could be observed that the sensitivity for some of the compounds is better for chemical ionization in both acquisition modes than for EI. The repeatability of the method was found to be between 12% and 34% for PCI and between 10% and 24% for NCI. The use of an internal standard improves the linearity of the responses and enables the compounds to be quantified whereas limited linearity is demonstrated without using the internal standard [15]. It should be also pointed that with PB only some of the compounds could be determined due to the low sensitivity.

Fig. 3 shows the chromatograms obtained for a 200 ml of tap water spiked at 0.5 ng ml^{-1} level under SIM acquisition and obtained by EI and CI in both positive and negative modes.

The samples distributed by Aquacheck inter-lab-

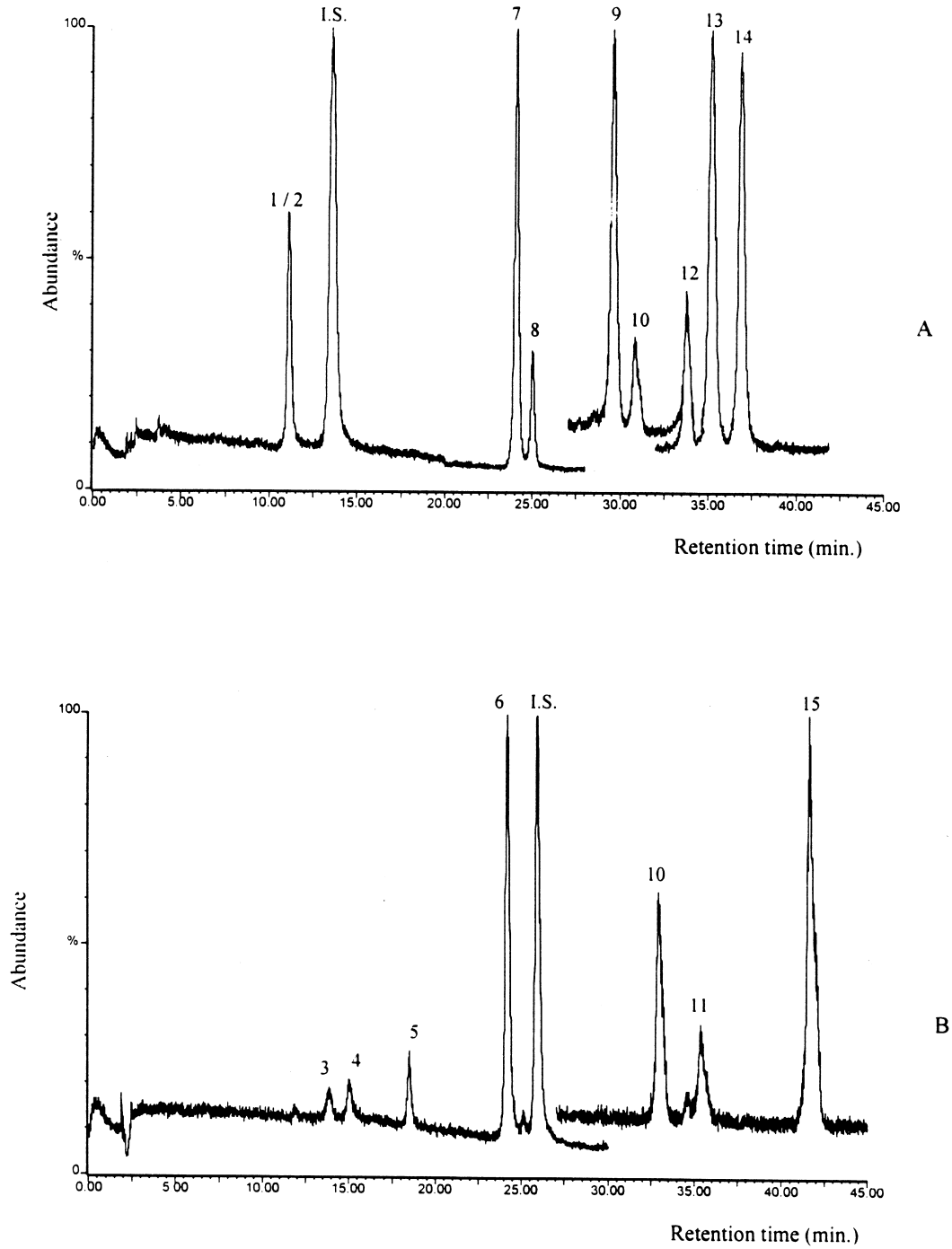

Fig. 2. Chromatogram obtained by on-line SPE–LC–APCI-MS of 200 ml of tap water spiked with 0.04 ng ml^{-1} of pesticides and 0.05 ng ml^{-1} of internal standard (terbuthylazine and dinoterb for PI and NI, respectively) under PI mode and time-scheduled SIM (A) and using NI ionization mode (B). Peaks: (1) bentazone, (2) vamidothion, (3) 4-NP, (4) MCPA, (5) mecoprop, (6) dinoseb, (7) atrazine, (8) isoproturon, (9) ametryn, (10) malathion, (11) fenitrothion, (12) molinate, (13) prometryn, (14) terbutryn and (15) parathion-ethyl.

Table 6
Obtained results for Aquacheck samples by APCI in both positive and negative acquisition modes

Compound	PI		NI	
	Concentration (ng/l)	% Error	Concentration (ng/l)	% Error
Atrazine	0.08	−24.2	n.d.	n.d.
Fenitrothion	n.d.	n.d.	0.02	23.5
Malathion	0.09	27.7	0.05	−23.2
Mecoprop	n.d.	n.d.	0.08	25.4
Parathion-ethyl	n.d.	n.d.	0.02	−23.1

Table 7
Time-scheduled SIM conditions for on-line SPE–PB-MS by EI and CI in both positive and negative ion modes

Compound	EI		PCI		NCI	
	m/z	Acquisition window (min)	*m/z*	Acquisition window (min)	*m/z*	Acquisition window (min)
Bentazone	n.d.	–	n.d.	–	n.d.	–
Vamidothion	87	0–17	146	0–17	272	0–17
4-NP	109	17–21	n.d.	–	n.d.	–
MCPA	n.d.	–	n.d.	–	141	21–26.5
Mecoprop	142	21–26.5	n.d.	–	141	21–26.5
Dinoseb	211	26.5–30	n.d.	–	n.d.	–
Atrazina	200	30–36	216	30–36	214	30–36
Isoproturon	72	30–36	205	30–36	205	30–36
Ametryn	227	36–40.2	228	36–40.2	226	36–40.2
Malathion	173	40.2–42	127	40.2–42	172	40.2–42
Fenitrothion	248	42–44	248	42–44	277	42–44
Molinate	n.d.	–	n.d.	–	n.d.	–
Prometryn	242	44–50	242	44–50	240	44–50
Terbutryn	242	44–50	242	44–50	240	44–50
Parathion-ethyl	262	44–50	262	44–50	291	44–50

[1] n.d.: Not detected under experimental conditions.

oratory program were also analyzed by PB. Due to the low levels present in samples and the limited sensitivity of PB, only atrazine and malathion could be determined and the results obtained are summarized in Table 9. It can be seen that errors are similar to those obtained by APCI and they were below the

Table 8
Calibration data and LODs obtained by PB using EI or CI after the preconcentration of 200 ml of sample

Compound	EI			PCI			NCI		
	r^2	Linearity range ($\mu g\ l^{-1}$)	LOD ($\mu g\ l^{-1}$)	r^2	Linearity range ($\mu g\ l^{-1}$)	LOD ($\mu g\ l^{-1}$)	r^2	Linearity range ($\mu g\ l^{-1}$)	LOD ($\mu g\ l^{-1}$)
Ametryn	0.999	0.1–10	0.05		n.d.		0.999	0.2–10	0.1
Atrazine	0.994	0.1–10	0.05	0.995	0.05–10	0.02		n.d.	
Isoproturon	0.988	0.1–10	0.05	0.991	0.05–10	0.02		n.d.	
Malathion	0.999	0.2–10	0.1		n.d.		0.998	0.05–10	0.02
Fenitrothion	0.994	0.5–10	0.2	0.986	0.2–10	0.1	0.991	0.1–10	0.05
Parathion-ethyl	0.994	0.5–10	0.2	0.981	0.2–10	0.1	0.999	0.1–10	0.05
Prometryn	0.999	0.1–10	0.05	0.990	0.05–10	0.02	0.979	0.1–10	0.05
Terbutryn	0.999	0.1–10	0.05	0.978	0.05–10	0.02	0.993	0.1–10	0.05

maximum accepted error between laboratories given by the AOAC.

Comparing the results obtained by the two interfaces, APCI was shown to be much more sensitive than the PB. The linearity was in general better for APCI than for PB, although for PB better results were obtained than in a previous study [15] where the calibration was carried out with the external standard calibration method.

3.3. Application of the method

3.3.1. APCI

The potential of the presented method was demon-

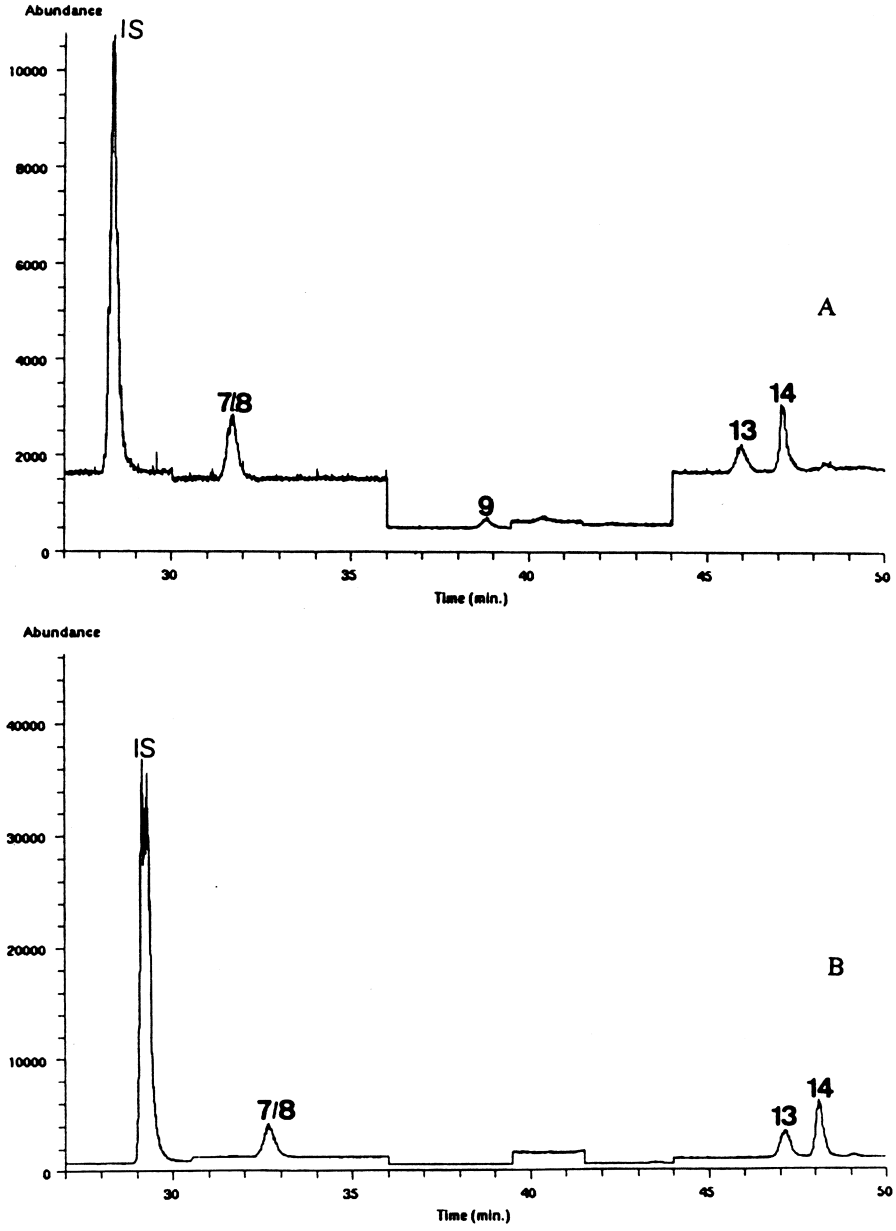

Fig. 3. (*Continued overleaf*)

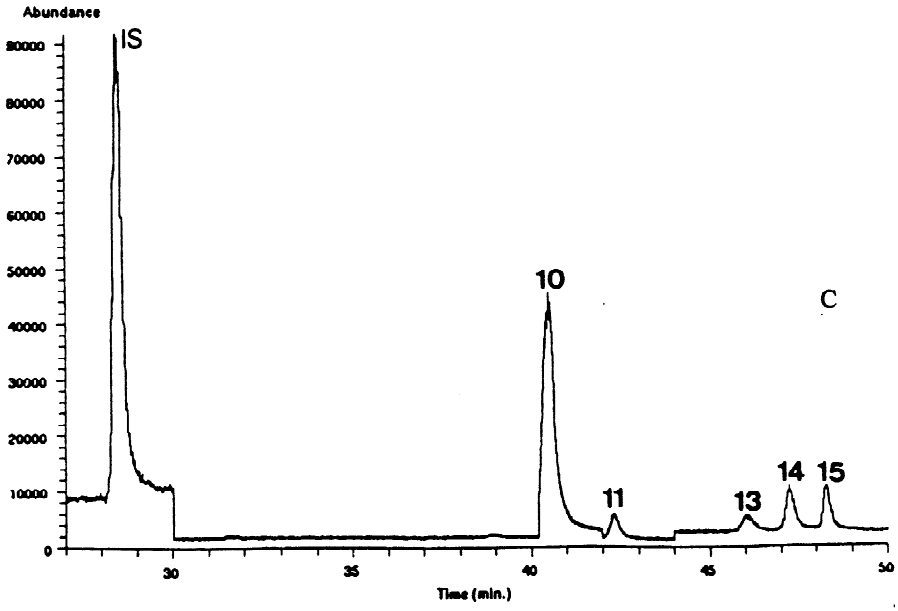

Fig. 3. Selected ion chromatograms obtained by on-line SPE–LC–APCI-MS of 200 ml of tap water spiked with 0.5 ng ml^{-1} of pesticides and 10 ng ml^{-1} of internal standard (fluomethuron) under EI (A), PCI (B) and NCI (C). For compound numbers, see Fig. 2.

strated for an Ebro river water sample. A 200 ml of sample was first analyzed by full-scan acquisition mode. Some peaks appeared in the corresponding chromatogram obtained under PI mode whereas when the NI acquisition was used the chromatogram did not show any peak. Fig. 4 shows the chromatogram obtained for the preconcentration of 200 ml of Ebro river water sample under full-scan and positive acquisition. Two of the peaks were tentatively identified as triazine pesticides, atrazine and simazine. Whereas atrazine was included in the group of pesticides studied, so it could be possible to confirm its presence through the retention time and the corresponding mass spectrum, simazine was not included in that group, so further experiments were necessary in order to verify its presence. A standard solution of simazine was injected using a cone voltage of 20 V to compare the retention time and the mass spectrum with the Ebro river sample. Finally, to unequivocally confirm the two triazine compounds, an extraction voltage of 40 V was used in the analysis of the Ebro river sample to induce more fragmentation and to obtain more structural information. The presence of this compound was confirmed. In Fig. 4 the selected ion chromatograms corresponding to m/z 216 and m/z 202 for atrazine and simazine respectively are also shown. Finally, in order to quantify the atrazine, the SIM acquisition was used. The concentration of this compound was calculated in 0.054 ng ml^{-1}.

3.3.2. PB

The same river Ebro sample that was investigated under APCI was also analyzed by PB in order to

Table 9
Obtained results for Aquacheck samples by APCI in both positive and negative acquisition modes

Compound	EI		PCI		NCI	
	Concentration (ng/l)	% Error	Concentration (ng/l)	% Error	Concentration (ng/l)	% Error
Atrazine	0.09	18.7	0.12	9.1	n.d.	n.d.
Malathion	n.d.	n.d.	n.d.	n.d.	0.06	−11.5

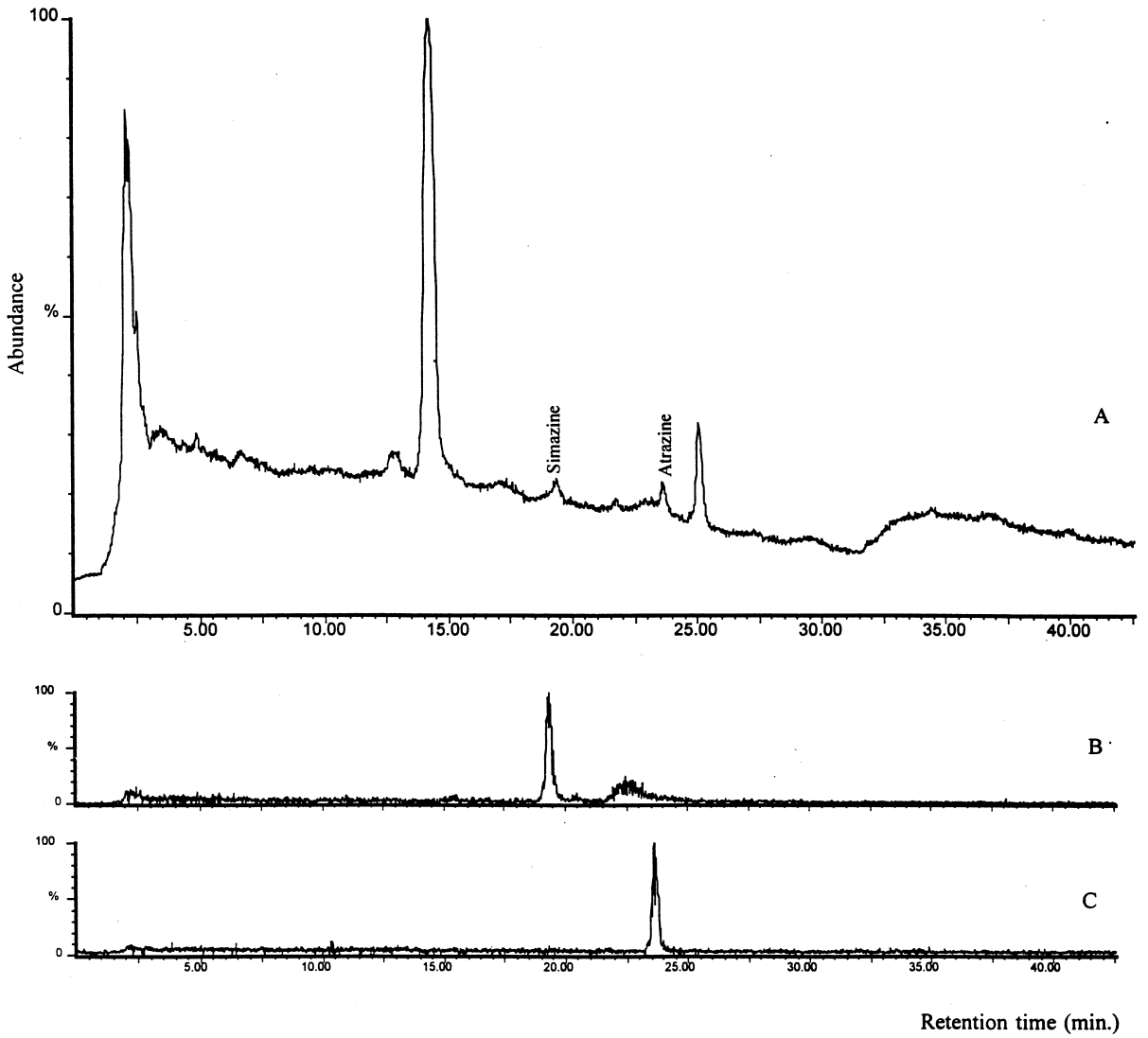

Fig. 4. Chromatogram obtained by APCI in full-scan and positive mode for 200 ml of Ebro river water (A) and extracted ion chromatograms corresponding to simazine (m/z 202) (B) and atrazine (m/z 216) (C).

unequivocally confirm the presence of those pesticides previously reported by the other tested interface, simazine and atrazine. First the analysis by EI and under full-scan acquisition mode was carried out but no peaks appeared in the corresponding chromatogram. The sample was then analyzed by EI and SIM acquisition mode in order to improve the sensitivity but unfortunately no peaks appeared in the corresponding chromatogram. When PCI under SIM acquisition was used a peak at the same retention time as atrazine appeared and through the calibration equation the concentration was calculated in 0.044 ng ml^{-1}. Fig. 5 shows the extracted ion chromatogram (m/z 216) corresponding to the peak assigned to atrazine in the preconcentration of 200 ml of Ebro river water sample under PCI and SIM acquisition mode. For NCI no peaks appeared in the corresponding chromatogram. Simazine was not identified in this sample by this interface probably due to the low concentration.

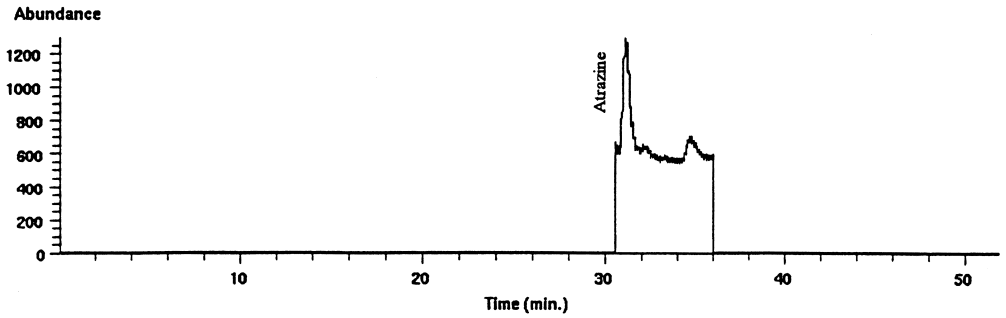

Fig. 5. Extracted ion chromatogram corresponding to atrazine (m/z 216) and obtained under PB-PCI and time-scheduled SIM. The sample is the same as in Fig. 4.

4. Conclusions

HPLC-APCI and PB-MS have been compared for qualitative and quantitative determination of a group of pesticides in environmental waters.

As regards qualitative analysis, in chemical ionization, some compounds gave similar spectra for PB and APCI whereas for some other compounds such as organophosphorous pesticides, different spectra were obtained. With the APCI interface a greater number of compounds may be determined than with the PB interface.

The linearity in both techniques, using the internal standard method was quite good and APCI was more sensitive than PB.

From the results obtained in the interlaboratory exercise it could be pointed that the developed methods are useful for the analysis of herbicides and organophosphorous pesticides at the low levels present in these samples.

Acknowledgements

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References

[1] W.M.A. Niessen, A.P. Tinke, J. Chromatogr. A 703 (1995) 37.
[2] J. Slobodnik, B.L.M. van Baar, U.A.Th. Brinkman, J. Chromatogr. A 703 (1995) 81.
[3] M. Careri, A. Mangia, M. Musci, J. Chromatogr. A 727 (1996) 153.
[4] D.A. Volmer, D. Vollmer, LC·GC 14 (1996) 236.
[5] D. Barceló, G. Durand, V. Bouvot, M. Nielen, Environ. Sci. Technol. 27 (1993) 271.
[6] H. Bagheri, E.R. Brouwer, R.T. Ghijsen, U.A.Th. Brinkman, J. Chromatogr. A 647 (1993) 121.
[7] D. Volmer, K. Levsen, G. Wünsch, J. Chromatogr. A 660 (1994) 231.
[8] S. Lacorte, D. Barceló, J. Chromatogr. A 712 (1995) 103.
[9] C.S. Creaser, J.W. Stygall, Analyst 118 (1993) 1467.
[10] H. Bagheri, J. Slobodnik, R.M. Marcé, R.T. Ghijsen, U.A.Th. Brinkman, Chromatographia 37 (1993) 159.
[11] I. Kambhampati, K. Roinestad, T.G. Hartman, J.D. Rosen, E.K. Fukuda, R.L. Lippincott, R.T. Rosen, J. Chromatogr. A 688 (1994) 67.
[12] H. Prosen, L. Zupancic-Kralj, J. Marcel, J. Chromatogr. A 704 (1996) 121.
[13] L. Bonifanti, M. Careri, A. Mangia, P. Manini, M. Maspero, J. Chromatogr. A 728 (1996) 359.
[14] J. Slobodnik, S.J.F. Hoekstra-Oussoren, M.E. Jager, M. Honing, B.L.M. van Baar, U.A.Th. Brinkman, Analyst 121 (1996) 1327.
[15] C. Aguilar, F. Borrull, R.M. Marcé, Chromatographia 43 (1996) 592.
[16] C. Molina, M. Honing, D. Barceló, Anal. Chem. 66 (1994) 4444.
[17] C. Shumate, Trends Anal. Chem. 13 (1994) 104.
[18] S. Chiron, S. Papilloud, W. Haerdi, D. Barceló, Anal. Chem. 67 (1995) 1637.
[19] P.E. Joss, LC·GC Int. 8 (1995) 93.
[20] D.K. Bryant, M.D. Kingswood, A. Belenguer, J. Chromatogr. A 721 (1996) 41.
[21] M.T. Galcerán, E. Moyano, J. Chromatogr. A 731 (1996) 75.
[22] D.A. Volmer, D.L. Vollmer, J.G. Wilkes, LC·GC 14 (1996) 216.
[23] S. Lacorte, D. Barceló, Anal. Chem. 68 (1996) 2464.
[24] A.P. Bruins, Trends Anal. Chem. 13 (1994) 37.

[25] D.M. Garcia, S.K. Huang, W.F. Stansbury, J. Am. Soc. Mass Spectrom. 7 (1996) 59.
[26] K.A. Barnes, J.R. Startin, S.A. Thorpe, S.L. Reynolds, R.J. Fussell, J. Chromatogr. A 712 (1996) 85.
[27] A.C. Hogenboom, J. Slobodnik, J.J. Vreuls, J.A. Rontree, B.L.M. van Baar, W.M.A. Niessen, U.A.Th. Brinkman, Chromatographia 42 (1996) 506.
[28] N.H. Spliid, B. Koppen, J. Chromatogr. A 736 (1996) 105.
[29] J. Slobodnik, A.C. Hogenboom, J.J. Vreuls, J.A. Rontree, B.L.M. van Baar, W.M.A. Niessen, U.A.Th. Brinkman, J. Chromatogr. A 741 (1996) 59.
[30] M.A. Aramendia, I. Garcia, F. Lafont, J.M. Marinas, Rapid Commun. Mass Spectrom. 9 (1995) 503.
[31] M.A. Aramendia, V. Borau, I. Garcia, C. Jiménez, F. Lafont, J.M. Marinas, J. Urbano, Rapid Commun. Mass Spectrom. 10 (1996) 1585.
[32] T.D. Behymer, T.A. Bellar, W.L. Budde, Anal. Chem. 62 (1990) 1686.
[33] T.A. Bellar, T.D. Behymer, W.L. Budde, J. Am. Soc. Mass Spectrom. 1 (1990) 92.
[34] M.J. Incorvia Mattina, J. Chromatogr. 549 (1996) 237.
[35] F.R. Brown, W.M. Draper, Biol. Mass Spectrom. 20 (1991) 515.
[36] J.S. Ho, T.D. Behymer, W.L. Budde, T.A. Bellar, J. Am. Soc. Mass Spectrom. 3 (1992) 662.
[37] D. Barceló, M.C. Hennion, Anal. Chim. Acta 318 (1995) 1.
[38] D. Puig, D. Barceló, I. Silgoner, M. Grasserbauer, J. Mass Spectrom. 31 (1996) 1297.
[39] G. Durand, D. Barceló, Anal. Chim. Acta 243 (1991) 259.
[40] S. Lacorte, C. Molina, D. Barceló, Anal. Chim. Acta 281 (1993) 71.
[41] C. Aguilar, F. Borrull and R.M. Marcé, J. Chromatogr. A, (1997) submitted for publication.
[42] R.J. Mesley, W.D. Pocklington, R.F. Walker, Analyst 116 (1991) 975.
[43] S. Lacorte, D. Barceló, J. Chromatogr. A 725 (1996) 85.