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Journal of Chromatography A, 741 (1996) 59–74

JOURNAL OF CHROMATOGRAPHY A

Trace-level determination of pesticide residues using on-line solid-phase extraction–column liquid chromatography with atmospheric pressure ionization mass spectrometric and tandem mass spectrometric detection

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Received 1 November 1995; revised 31 January 1996; accepted 9 February 1996

Abstract

Column liquid chromatography (LC) with pneumatically assisted electrospray (PA-ESP) or atmospheric pressure chemical ionization (APCI) followed by (tandem) mass spectrometry (MS or MS–MS) was used for the analysis of a test mixture of 17 pesticides. In order to achieve low-ng/l detection limits, solid-phase extraction (SPE) of a 100-ml aqueous sample on a small cartridge packed with a hydrophobic sorbent was used. The LC set-up was coupled on-line to the MS part of the system. The complete analysis was automated by means of a gradient controller and a Prospekt valve switching, solvent selection and cartridge exchange unit. When using SPE–LC with either APCI or PA-ESP, the detection limits of 15 (out of the 17) pesticides in tap water were 0.007–3 μg/l in the full-scan and 0.1–200 ng/l in the SIM mode, with an analysis time of 65 min. Fenchlorphos and bromophos-ethyl could not be detected by either ionization method. APCI full-scan spectra showed much less sodium and acetonitrile/water cluster adducts than PA-ESP spectra. Negative ion (NI) operation was less sensitive for the majority of the compounds tested (73 in total), but several organophosphorus pesticides, nitrophenols and chlorophenols only gave a response in the NI mode. PA-ESP-MS–MS and APCI-MS–MS gave similar product-ion spectra from protonated molecules; an MS–MS library was built for more than 60 pesticides and their degradation products, at constant settings of collision gas pressure (argon, 2.0×10^{-3} Torr) and collision energy (25 eV). The library was successfully used for searching product-ion spectra from SPE–LC–APCI-MS–MS at low levels (10 ng/l) in tap water and for the identification of atrazine in surface water (estimated concentration 0.25 μg/l).

Keywords: Mass spectrometry; Trace analysis; Tandem mass spectrometry; Pesticides

1. Introduction

The on-going application of pesticides requires frequent monitoring of various environmental matrices; the use of LC–MS for this purpose has been

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PII S0021-9673(96)00139-2

described in several reviews [1,2]. The determination of modern pesticides and their degradation products in real-world samples poses a challenge, because of their polarity and thermolability and because of the low detection limits required. As an example, according to European Union directives individual pesticides in drinking water have to be detected at or below the 0.1 μg/l level [3]. To meet such requirements, an (on-line) trace-enrichment step is generally required, since conventional LC–MS technology is not capable of reaching these low levels [4]. On-line procedures allow large-scale screening and monitoring, particularly because of the possibility of automation. In the past five years, several automated systems were developed which use this on-line approach. Such systems typically handle the preconcentration of analytes from 50–250 ml aqueous samples on a small cartridge packed with a hydrophobic sorbent, subsequent gradient elution of the trapped analytes over an analytical column and detection with an ultraviolet diode array (UV DAD) [5] or mass spectrometric (MS) [6–8] detector. At present, automated SPE–LC–UV DAD systems are routinely used for surface water monitoring in several European countries.

A different approach for analyte preconcentration and separation was described recently [9]. In this alternative method, analytes are preconcentrated from 15-ml samples on the top of a short analytical column (20×4.6 mm I.D.) and subsequently gradient-eluted into an appropriate detector. The combination of such a rapid loading-plus-separation procedure with selective MS or even MS–MS detection appears rather promising. Some results concerning this approach are included in the present study; full details will be published elsewhere [10].

As regards LC–MS interfacing, this currently is generally provided by means of thermospray (TSP) [11], particle beam (PB) [12], atmospheric pressure chemical ionization (APCI) [13–16] or electrospray (ESP) [13–16]. With several pesticides, the latter two atmospheric pressure ionization (API) techniques were reported to be more sensitive than LC–TSP-MS by approximately two orders of magnitude [6,16,17]: detection limits were typically in the low-ng (full-scan) or the picogram (selected ion monitoring; SIM) range [16,18].

In this study we used APCI and pneumatically assisted ESP (PA-ESP) for detection; in the literature, the latter technique is also denoted as high-flow ESP, megaflow ESP or ionspray. Both APCI and PA-ESP facilitate soft ionization with a high ionization efficiency, thus providing molecular-weight information and excellent sensitivity. In addition, structural information can be obtained by means of cone voltage induced fragmentation in the pre-analyzer region [pre-analyzer collision induced dissociation (CID)]. Although the ESP ionization process is not yet fully understood [19], it is known that liquid-phase chemistry plays a major role in the formation of ions. In contrast, the ionization process in APCI involves gas-phase chemistry, typically producing ions by proton transfer reactions. Consequently, APCI is expected to be less dependent on the pre-ionization of analytes in the sample solution and more suitable for the determination of compounds covering a wide polarity range (i.e., including apolar compounds) [16,18]. Both ionization techniques can handle conventional LC flow rates up to 1–1.5 ml/min, although it is advantageous for PA-ESP to be operated at lower flow rates (up to 0.3 ml/min) [17]. As regards this aspect, a post-column split of the LC effluent, prior to PA-ESP, should not pose any problems because the interface is reported to act as a concentration-sensitive device [19,20].

In the present study, the performance of the PA-ESP and APCI interfaces in the determination and identification of various classes of pesticides at or below the 0.1 μg/l level was studied. In addition, suitable conditions for the generation of CID product ion spectra from protonated molecules were established, with the aim of building MS–MS spectral libraries for rapid identification. SPE–LC methods of sample concentration were incorporated into the procedures which were subsequently automated.

2. Experimental

2.1. Materials

2.1.1. Chemicals

All pesticide standards were over 95% pure. They were obtained from several sources [Riedel-de-Haën (Seelze, Germany); Promochem (Germany); Dr.

Ehrenstorfer (Germany); the EPA Repository (Research Triangle Park, NC, USA); the Repository of the Food Inspection Laboratory (Alkmaar, Netherlands)]. HPLC-grade water and methanol were purchased from Rathburn Chemical (Walkerburn, UK). HPLC-grade acetonitrile was supplied by Hipersolv BDH Laboratory Supplies (Poole, UK). Liquid nitrogen (99.998% purity), used as a source of (drying, nebulizing and sheath) nitrogen gas supply and 99.9995% pure argon for MS–MS were supplied by BOC Industrial Gases (Worsley, Manchester, UK). Special precautions were taken to use solvents from the same batch and to always utilize the same glassware in order not to introduce additional sodium and potassium which may form adduct ions, especially in PA-ESP spectra.

2.1.2. Samples

The surface water sample was taken from the Nitra River (Slovakia) and transported to the laboratory in a portable refrigerator kept at 5–10°C. The sample was filtered through a 0.45-μm acetyl-cellulose filter (Schleicher and Schuell, Dassel, Germany) and stored in the dark at 4°C until the time of actual analysis. Prior to analysis, the sample was spiked with two internal standards, propazine and metoxuron, at 1 μg/l.

2.2. Solid-phase extraction

2.2.1. Instrumentation

A Prospekt (Spark Holland, Emmen, Netherlands) was used for automated cartridge exchange, solvent selection, valve switching and sample handling. The solvent delivery unit (SDU) of the Prospekt was equipped with a six-port solvent selection valve, a pulse damper and a single-piston analytical LC pump. 10×2.0 mm I.D. PTFE cartridges were packed with 15–25 μm copolymer PLRP-S (styrene-divinylbenzene) of 100 Å pore size (Polymer Laboratories, Church Stretton, UK); a fresh cartridge was used for each analysis. For details of programming and using the Prospekt, one should consult Ref. [5].

2.2.2. Procedures

Experiments were performed with the set-up shown in Fig. 1. Prior to sample enrichment, the precolumn was conditioned with 4 ml of acetonitrile and 2 ml of HPLC-grade water. Next, 100 ml of

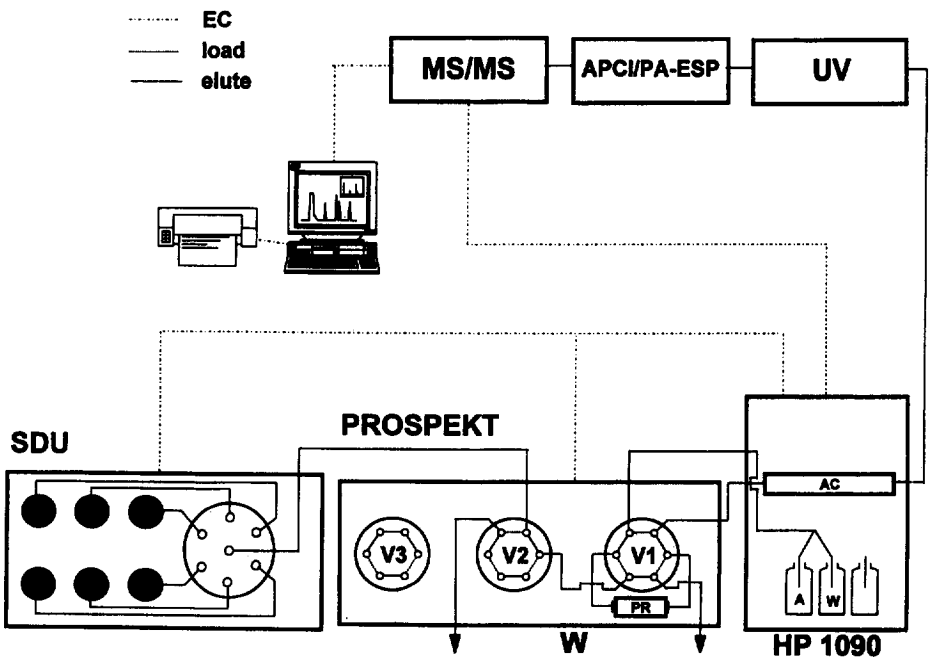

Fig. 1. Schematic representation of the automated on-line SPE–LC–API-MS–MS system. For more details, see text and Refs. [5,8,21].

sample were pumped through the precolumn, the analytes of interest were trapped on the sorbent and, after switching valve V1 to the ELUTE position, the analytes were eluted by the LC gradient onto the analytical column for separation and subsequent API-MS or API-MS–MS detection. The time needed for sample preparation was 30 min.

2.3. Liquid chromatography

HP 1090 and HP 1050 liquid chromatographs (Hewlett-Packard, Waldbronn, Germany), each equipped with an autoinjector, were used for eluent delivery and gradient control. Separations were performed on a 150×4.6 mm I.D. stainless-steel column packed with 3 μm, 100 Å C_{18}-bonded silica (Zorbax, Rockland Technologies, Nuenen, Netherlands). Acetonitrile (A) and water (B) were used at a total flow-rate of 1 ml/min, with a linear gradient from 5% to 95% of A in 30 min, followed by a 5-min isocratic period (95% A). An HP 1050 UV detector (Hewlett-Packard) was positioned between the analytical column and the API interface and operated at 215 nm. The sample preparation and LC gradient elution were combined in one process and automated by means of the Prospekt and gradient-controller time tables.

A post-column splitter, inserted at the UV detector outlet, was used to maintain the flow-rate of the LC effluent entering the PA-ESP interface at 125 μl/min. A microflow syringe pump (Harvard Apparatus 22, South Natick, CA, USA) was used for the infusion of a tuning compound or sample into the PA-ESP interface without separation, at a flow-rate of 25 μl/min.

2.4. Single short column

The LC hardware described above was also used for single short column experiments; the detailed description of the set-up can be found elsewhere [9,10]. Trace enrichment and separation were performed on a 20×4.6 mm I.D. column high-pressure packed with 5-μm C_{18}-bonded silica (Supelcosil LC-18-DB, Supelco, Bornem, Belgium). Prior to separation, the column was conditioned with 6 ml acetonitrile and, next, 6 ml HPLC-grade water (both 1.5 ml/min) and loaded with 15 ml of sample (2 ml/min). During conditioning, the SDU supplied a 1 ml/min flow of acetonitrile–water (50:50, v/v) into the APCI-MS. A flow-rate of 1 ml/min was used during gradient elution (linearly from 100% water to 95% acetonitrile in 15 min). The total analysis time was 30.5 min; all steps of the analysis were automated by means of the Prospekt and gradient controller time tables.

2.5. MS

Mass spectrometry was performed using a VG Quattro II triple quadrupole mass spectrometer (Fisons Instruments, VG Organic Analysis Biotech MS, Altrincham, UK), equipped with standard PA-ESP and APCI LC–MS interfaces. VG MassLynx software (version 2.10), installed on a Digital Pentium PC, was used for system control and data acquisition.

2.5.1. PA-ESP-MS

Mass spectra were acquired in the full-scan mode (m/z 150–420, cycle time 1 s, inter scan time 0.1 s) or by selected ion monitoring (SIM; dwell 0.5 s, span 0.2 Da). The source temperature was maintained at 70°C at an effluent flow-rate of 25 μl/min or at 150°C at 125 μl/min; abundant acetonitrile–water cluster ions appeared in the mass spectra at lower temperatures than the aforementioned optima. The drying and nebulizing nitrogen gas flow-rates were 375 l/h and 15 l/h, respectively. The cone voltage was 35 V with a skimmer offset of 5 V, the capillary voltage was held at 3.5 kV and the counter-current electrode at 1 kV. Similar parameters were used in the NI mode, with reversal of the relevant potentials. The system was tuned daily with a 2 ng/μl solution of gramicidin (mass m/z 571) in acetonitrile–water (50:50, v/v) at an infusion flow-rate of 25 μl/min.

2.5.2. APCI-MS

Unless mentioned otherwise, tuning conditions identical to those of the PA-ESP-MS experiments were maintained throughout the whole study. The APCI source and probe temperatures were 150°C and 500°C, respectively. The sheath gas flow-rate was 25 l/h. The cone voltage was optimized to 20 V with a skimmer lens offset of 5 V, the corona pin was kept at 3.7 kV in the PI and 1.8 kV in the NI mode. Under these conditions, fragmentation and ion intensities

were comparable to those in PA-ESP-MS. The system was tuned daily with an acetonitrile–water (50:50, v/v, 1 ml/min) mixture using the signal of protonated acetonitrile (m/z 42).

2.6. MS–MS

All MS–MS experiments were performed at the same collision chamber pressure (argon) of 2.0×10^{-3} mbar and at a collision energy of 25 eV. The full-scan product-ion spectra were acquired in the time-scheduled product-ion scan mode with the mass range of Q3 ranging from m/z 50 up to 10 amu above the molecular mass of the investigated compound.

2.7. Automation

All parts of the SPE–LC–API-MS system were electronically connected (cf., Fig. 1). The liquid chromatograph was connected to the MS through an electronic contact closure switch enabling the automatic start of MS data acquisition at the pre-programmed time of the LC gradient time table. The Prospekt was connected to the liquid chromatograph by means of the remote cable, thus enabling a start of the liquid chromatograph at the pre-programmed time (after sample loading). In the usual mode of operation, the analysis was performed by a single keystroke on the keyboard of the Prospekt to start a sequence of actions: conditioning of the cartridge, enrichment of 100-ml sample, switching the cartridge on-line with the analytical column and start of the LC gradient and MS data acquisition.

As pointed out earlier [5,21] it is possible to install a special software package for control of the Prospekt from the keyboard of the data system and instrument control computer and, thus, to implement features of the so-called fully automated system where all components are synchronized; details of this procedure will be reported elsewhere.

3. Results and discussion

3.1. General strategy

The aim of the present study was detection of pesticides at levels below 0.1 μg/l and the simultaneous acquisition of structural information for their identification. The general strategy for the analysis of real-world samples consisted of analyte enrichment by trapping, on-line elution onto an analytical column and API-MS detection under full-scan conditions. Data evaluation yielded a selection of m/z values to be subjected to MS–MS in a second run. In that run, time-scheduled product-ion API-MS–MS of selected protonated molecules was performed. The product-ion spectra were then interpreted and/or searched in the MS–MS library for tentative identification of the parent compound. Finally, the SIM mode can be used for the quantification of the identified analytes down to the low-ng/l level. Moreover, the MS–MS experiment can be used for the direct targeting of protonated molecules of suspected pollutants.

3.2. On-line SPE–LC-API with MS and MS–MS detection

3.2.1. Trace enrichment

The full-scan detection limits of the tested pesticides in direct-injection, i.e., loop injection, LC–API-MS usually are in the low-mg/l range, with rather large variations between the different classes of compounds. The limited sensitivity has to be improved by injecting the equivalent of, typically, some 100 ml of an aqueous sample, because pesticides and their degradation products are present at sub-μg/l levels. This can be achieved by means of on-line SPE: when using conventional-size SPE cartridges, ca. 10×3 mm I.D., a sample volume of 100–200 ml can be used for many medium- and non-polar analytes without the risk of breakthrough [5]. Admittedly, losses may start to occur for more polar compounds (e.g., oxamyl, dimethoate, aldicarb).

3.3. Comparison of APCI and PA-ESP

3.3.1. Positive ions

In a search for the "best" interface, the responses of 17 selected compounds, representing six different classes of pesticides (see Table 1), were studied by direct on-column injections. Fig. 2 gives a typical representation of the system performance: all 17 analytes are responding well in the UV trace (Fig. 2A), but amounts of even up to 250 ng (SPE

Table 1
Detection limits (signal-to-noise ratio 3) of 17 pesticides obtained from SPE–LC–PA-ESP-MS and SPE–LC–APCI-MS in full-scan, SIM and MS–MS modes, using 100-ml samples

No.	Compound	SPE–LC–PA-ESP-MS		SPE–LC–APCI-MS			Class
		Full-scan	SIM	Full-scan	SIM	MS–MS	
		(ng/l)	(ng/l)	(ng/l)	(ng/l)	(ng/l)	
1	Oxamyl	500	3	500	3	n.m.	Carbamates
2	Dimethoate	30	1	20	1	7	Organophosphates
3	Aldicarb	100	2	25	2	n.m.	Carbamates
4	Monuron	40	2[a]	10	0.6	3	Phenylureas
5	Propoxur	30	0.5	30	1.5	n.m.	Carbamates
6	Diuron	300	2[a]	20	0.6	8	Phenylureas
7	Propazine	8	0.1	7	0.4	1	Triazines
8	Terbutylazine	10	0.1	8	0.4	1	Triazines
9	Fenamiphos	10	0.2	10	0.8	1	Organophosphates
10	Alachlor	50	3	60	6.0	30	Anilides
11	Neburon	40	2	20	1.5	4	Phenylureas
12	Fenthion	n.d.	n.m.	3000	n.m.	n.m.	Organophosphates
13	Coumaphos	500	20	350	15	75	Organophosphates
14	Fenchlorphos	n.d.	n.m.	n.d.	n.m.	n.m.	Organophosphates
15	Chlorpyriphos	n.d.	n.d.	2500	150	n.m.	Organophosphates
16	Trifluralin	n.d.	n.d.	3000	200	n.m.	Nitrophenols
17	Bromophos-ethyl	n.d.	n.m.	n.d.	n.m.	n.m.	Organophosphates

n.m., not monitored; n.d., not detected at highest concentration analyzed (see text).
[a] SIM detection limits obtained in the NI mode.

experiments; 2.5 μg/l, 100-ml spiked tap water) are already close to or below the APCI (Fig. 2B) detection limits for fenthion, coumaphos, chlorpyriphos and trifluralin (Table 1). The response of PA-ESP-MS (Fig. 2C) is generally less good than that of APCI-MS, the differences being most pronounced for the phenylureas monuron and diuron, the carbamate aldicarb and the compounds eluting after more than 24 min. With the exception of coumaphos, the latter category of compounds could not be detected by PA-ESP.

On the basis of the data for the twelve test compounds which display satisfactory analyte detectability, one can conclude that the detection limits with APCI-MS and PA-ESP-MS are rather similar (Table 1). The APCI and PA-ESP interfaces showed approximately the same results with limits of detection of 0.1–50 ng in the full-scan mode for 12 (out of the 17) compounds. The organophosphorus pesticides fenchlorphos and bromophos-ethyl were not detected by either interface. In both modes of MS operation used, the detection limits span a range of one to two orders of magnitude. In order to compare the performance of the two interfaces under identical conditions, in the present study no additives (volatile acids) were added to the mobile phase. However, it should be kept in mind that addition of e.g. 0.05–0.1% of trifluoroacetic acid or formic acid may be favourable for the detection of some compounds, especially in PA-ESP-MS.

3.3.2. *Information content of PI spectra*

Significant differences were observed in the spectra obtained with the two interfaces, as is shown in Table 2 (PA-ESP) and Table 3 (APCI). PA-ESP-MS spectra show prominent protonated molecules for most of the detectable analytes. In addition, they often exhibit sodium adduct ions and, occasionally, low-abundant potassium adduct ions, sodium-plus-solvent adduct ions, e.g., $[M+Na+acetonitrile]^+$, sodium adducts with two molecules of analyte (e.g., m/z 403 for aldicarb) as well as some fragments of these ions. The major fragment of alachlor (m/z 238) is related to the loss of methanol from the protonated molecule; propoxur and dimethoate give $[M+H-CH_3CHCH_2]^+$ and $[M+H-CH_3NH_2]^+$ as the base peak, respectively.

In APCI-MS most of the detectable compounds

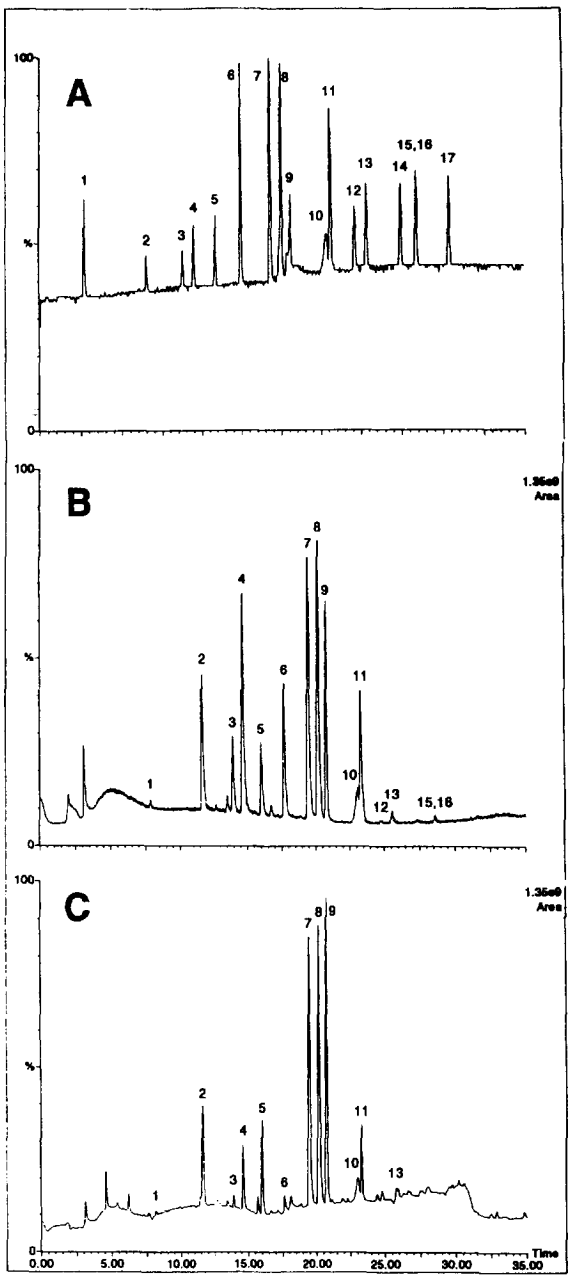

Fig. 2. Chromatograms of the mixture of 17 pesticides obtained by (A) LC–UV, (B) on-line SPE–LC–APCI-MS and (C) SPE–LC–PA-ESP-MS. Injected amount in (A) is 125 ng of each pesticide; the UV detector was kept at 215 nm. Enriched volume in (B) and (C) is 100 ml tap water; concentration of each analyte is 2.5 μg/l, MS is operated in the full-scan mode, scan range m/z 150–420. Peaks: 1=oxamyl; 2=dimethoate; 3=aldicarb; 4=monuron; 5=propoxur; 6=diuron; 7=propazine; 8=terbutylazine; 9=fenamiphos; 10=alachlor; 11=neburon; 12=fenthion; 13=coumaphos; 14=fenchlorphos; 15=chlorpyriphos; 16=trifluralin; 17=bromophos-ethyl. For other conditions, see text.

show prominent $[M+H]^+$ ions. Aldicarb and oxamyl give $[M+Na-CH_3NCO]^+$ and $[M+H-CH_3NCO]^+$ as the base peak, and oxamyl is the only test compound to produce $[M+Na]^+$, $[M+K]^+$ and $[M+Na+acetonitrile]^+$ adducts. For the rest, apart from $[M+H+acetonitrile]^+$, adduct ions are virtually absent. The ions observed for trifluralin cannot easily be related to the structure of the compound, but the signals are clearly discernible at the trifluralin retention time.

The general conclusion is that, since many of the fragment ions in PA-ESP-MS originate from adducts, the interpretation of APCI-MS spectra is often more straightforward.

3.3.3. Negative ions

The NI operation in LC–MS is frequently utilized because of its selectivity and excellent sensitivity with compounds preferentially ionized by electron capture or proton abstraction. Using PA-ESP-MS, for most of the 17 test compounds the sensitivity of NI was found to be inferior to that of PI operation. NI performed better for two (from the three) phenylureas tested and for coumaphos. The NI response of the third phenylurea, neburon, was comparable in the PI and NI mode. With monuron and diuron, the advantage of an up to 4-fold higher response was used by switching to the NI mode within selected time windows of the time-scheduled SIM runs, in order to monitor the $[M-H]^-$ ions (cf. Fig. 4 below).

The NI-APCI signal intensities of the majority of the test analytes were 1–2 orders of magnitude lower than in PI-APCI (Fig. 3). From among a total of 73 pesticides and pesticide degradation products investigated in this part of our study, a limited number, i.e., several of the late-eluting analytes such as fenchlorphos, chlorpyriphos, trifluralin and bromophos-ethyl, and several chlorophenols (pentachlorophenol, 3,4,5-trichlorophenol) and nitrophenols (2-nitrophenol, 2,6-dinitrophenol, dinoterb), gave appreciable responses in the NI mode only. This suggests that methods can be developed for target compounds or for selected classes of compounds, e.g., for chloro- and nitrophenols, based on the use of the NI mode (with proper pH adjustment to prevent undue peak broadening in the LC part of the system). An example of such a specific target analysis for phenoxy acetic acids was published recently [6].

Table 2
Major ions in spectra of 17 pesticides obtained using SPE–LC–PA-ESP-MS

Compound	MW	$[M+H]^+$	[a] $[M+Na]^+$	Fragments	[a] $[M+Na+AcN]^+$
Oxamyl	219	220 (5)	242 (100)	177 (1)	283 (2)
Dimethoate	229	230 (10)	252 (8)	157 (5), 171 (58), 199 (100)	
Aldicarb	190		213 (100)	156 (4)	254 (10)
Monuron	198	199 (100)	221 (1)		
Propoxur	209	210 (7)	232 (21)	153 (19), 168 (100), 209 (2)	273 (7)
Diuron	232	233 (100)	255 (12)	156 (8), 177 (3), 197 (5), 218 (10)	296 (7)
Propazine	229	230 (100)		188 (17)	
Terbutylazine	229	230 (100)		174 (86)	
Fenamiphos	303	304 (100)	326 (31)	217 (3), 234 (7), 245 (1), 262 (10), 276 (13)	367 (19)
Alachlor	269	270 (41)	292 (6)	162 (41), 238 (100)	333 (3)
Neburon	274	275 (100)	297 (1)		
Fenthion	278	n.d.			
Coumaphos	362	363 (100)	385 (21)		
Chlorpyriphos	349	n.d.			
Trifluralin	335	n.d.			

Sample volume 100 ml, spiked with 2.5 μg/l of each analyte; scan range *m/z* 150–420. MW, monoisotopic molecular weight; AcN, acetonitrile. For other conditions, see text. Numbers in parentheses show relative abundance of the base peak in spectrum.
[a] Relative intensities are strongly dependent on amount of sodium ions in solvents and glassware and may vary with time.

3.3.4. Information content of NI spectra

As regards the spectra of some of the analytes showing relatively high signal intensities in the NI modes, the NI-PA-ESP spectra of the phenylureas (and also the triazines and fenamiphos) showed $[M-H]^-$ as a pronounced peak with little further fragmentation, whereas spectra of the other compounds were dominated by various fragment or adduct ions and did not provide any molecular-mass information. Nine compounds did not give any signal at all.

NI-APCI provided spectra with the specific fragment ion, $[M-H]^-$ for almost all compounds. As

Table 3
Major ions in spectra of 17 pesticides obtained using SPE–LC–APCI-MS

Compound	MW	$[M+H]^+$	Fragments	$[M+H+AcN]^+$
Oxamyl	219	220 (4)	163 (100), 204 (2)	
Dimethoate	229	230 (37)	171 (9), 199 (100)	
Aldicarb	190		157 (100), 175 (17)	
Monuron	198	199 (100)		240 (1)
Propoxur	209	210 (9)	153 (22), 168 (100), 209 (6)	
Diuron	232	233 (100)		274 (4)
Propazine	229	230 (100)	188 (1)	271 (5)
Terbutylazine	229	230 (100)	174 (12)	271 (5)
Fenamiphos	303	304 (100)	262 (4), 276 (3)	345 (12)
Alachlor	269	270 (20)	162 (46), 192 (7), 204 (8), 220 (4), 226 (5), 238 (100)	
Neburon	274	275 (100)		316 (4)
Fenthion	278	279 (100)		
Coumaphos	362	363 (100)	177 (41), 211 (4), 329 (7)	404 (12)
Chlorpyriphos	349	350 (100)	322 (13)	
Trifluralin	335		229 (100), 260 (42), 302 (91)	

Sample volume 100 ml, spiked with 2.5 μg/l of each analyte; scan range *m/z* 150–420. MW, monoisotopic molecular weight; AcN, acetonitrile. For other conditions, see text. Numbers in brackets give relative abundance of the base peak in spectrum.

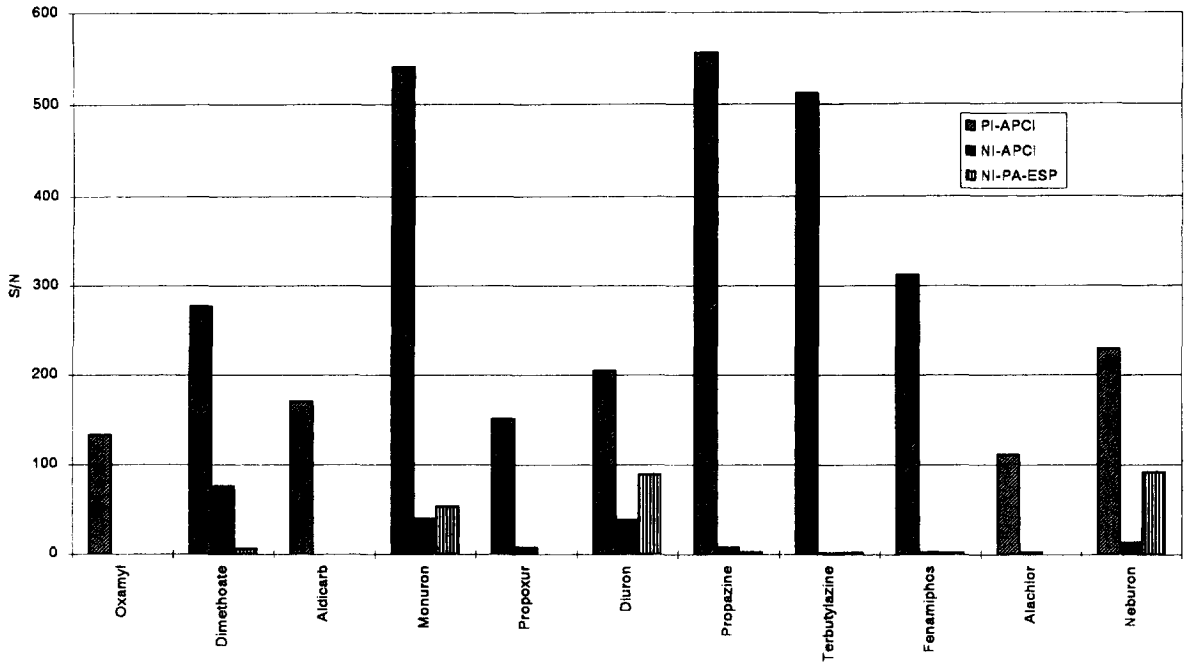

Fig. 3. Comparison of *S/N* values of selected 11 pesticides obtained with APCI-MS in positive and negative ion mode and PA-ESP in negative ion mode. Injected amounts, 125 ng of each pesticide; peaks were quantified at the base peak values from their full-scan spectra. For other conditions, see text.

regards the spectra of fenchlorphos and bromophos-ethyl which could not be detected in the PI mode, the former gives a base peak of $[M-(CH_3O)_2PO]^-$ (*m/z* 211) associated with the formation of the stable phenylsulphate ion through the rearrangement of sulphur; the latter has its base peak of $[M-(CH_3CH_2O)_2PS]^-$ at (*m/z* 239). Both base peaks exhibit characteristic isotopic patterns of all halogens in the molecule which provides additional information. NI-mode spectra of several well responding compounds are shown in Table 4.

It is obvious from the above that the practicality of the NI-based API-MS techniques for trace-level identification purposes is rather limited. It has been stated [13,18] that the use of different sheath gases with both API interfaces or a change in the solution

Table 4
Major ions in spectra of selected pesticides obtained using APCI-MS in NI mode

Compound	MW	$[M-H]^-$	Fragments
Monuron	198	197 (100)	
Diuron	232	231 (100)	
Coumaphos	362	361 (13)	333 (10), 225 (100), 209 (26), 169 (37)
Fenchlorphos	321		211 (100), 305 (18), 271 (4), 235 (3), 195 (14), 161 (3)
Bromophos-ethyl	392		239 (100), 365 (30), 331 (13), 257 (74), 212 (70), 169 (83)
2-Nitrophenol	139	138 (100)	122 (16), 108 (13)
Pentachlorophenol[a]	264	265 (9)	231 (100)

Amount of each analyte, 125 ng; scan range, *m/z* 150–420; MW, monoisotopic molecular weight; numbers in parentheses give relative abundance of the base peak in spectrum. For other conditions, see text.
[a]Values indicate the most abundant peak in the isotopic cluster.

chemistry for PA-ESP may drastically change the observations in the NI mode; however, such adjustments to the experimental set-up are beyond the scope of the present investigations and, probably, of most routine-orientated studies.

Detection limits obtained with APCI and PA-ESP interfaces are rather close to each other for the majority of compounds (Table 1) and, therefore, both can be used for the sensitive detection of pesticides in water samples. However, a major disadvantage of the PA-ESP interface is the formation of alkali metal adduct ions; it was observed that ion intensities are strongly dependent on the amount of sodium or potassium in the experiment (glassware, solvents) and, as regards the solvent adducts, on the source temperature. As an example, the relative intensities of dimethoate and propoxur sodium adduct ions, reported as 8% and 21% of the base peak in Table 2, respectively, were observed to change within a one-week period to 100% and 77%, respectively. This indicates that both identification and quantification of analytes in real-world samples using the PA-ESP-MS can be seriously hampered.

In conclusion, LC–APCI-MS in the PI mode is the preferred method of analysis for the determination of pesticides in water.

3.3.5. MS–MS

A frequently quoted advantage of the API interfaces is the possibility of inducing additional fragmentation by means of pre-analyzer CID. This form of CID is based on changing the potential difference between the sample cone and the first skimmer, thus increasing the frequency and energy of collision of analyte and drying gas molecules [13]. However, the use of pre-analyzer CID for the detection of unknowns requires full-scan operation, whereas the detection at sub-μg/l levels requires SIM operation and, thus, implies target compound analysis. In MS–MS, the excellent sensitivity of SIM can be combined with obtaining characteristic product-ion spectra.

A typical example of the performance of SPE–LC–APCI-MS in the time-scheduled-SIM mode is shown in Fig. 4A. Ten analytes from the mixture of 17 pesticides were detected in 100 ml of tap water at the 10 ng/l level. In the next run, the mixture was analyzed in the time-scheduled product-ion scanning MS–MS mode with eight channels set to select the observed $[M+H]^+$ ions and to monitor the product-ions. The response in the MS–MS mode was comparable to that in the time-scheduled-SIM experiment for propazine, terbutylazine and fenamiphos and 3–12-fold lower for dimethoate, monuron, diuron and neburon (Table 1); alachlor and coumaphos could not be detected at this low concentration (Fig. 4B). No memory effects were observed either in the SIM or in the MS–MS runs. It should be noted that with SIM the base peaks of the individual analytes were monitored, which are not necessarily the protonated molecules subjected to MS–MS (cf., Table 3). Consequently, with some analytes the less intense ions of the protonated molecules will have less abundant product-ion spectra/signals. All detected compounds provided structurally informative product-ion spectra. A typical comparison of a spectrum obtained at the 10 ng/l level with that from a 125-ng loop injection is given in Fig. 5 for terbutylazine. Despite several background peaks in the 10 ng/l spectrum, at least five characteristic fragments can be discerned which can be used for structure assignment. In SPE–LC–PA-ESP-MS–MS, product-ion spectra identical with those observed in the APCI mode, and comparable MS–MS detection limits were obtained (data not shown).

In conclusion, MS–MS operation of the system is superior to (i) full-scan operation, viz., in terms of sensitivity and (ii) SIM operation, viz., in terms of structural information obtained. This does not mean that the other two modes should not be used; full-scan spectra still are the only means to obtain the first information on unknowns and SIM performs excellently in target analysis.

3.4. MS–MS library

A major problem for many LC–MS users is the interpretation of mass spectral information. This is even more pressing when pre-analyzer CID or MS–MS is used. In a triple-quad MS–MS product-ion experiment, effectively only two parameters, collision gas pressure and collision energy, are optimized in order to obtain the product-ion spectra. To our opinion, the product-ion spectrum of the protonated molecule is the most informative for the identifica-

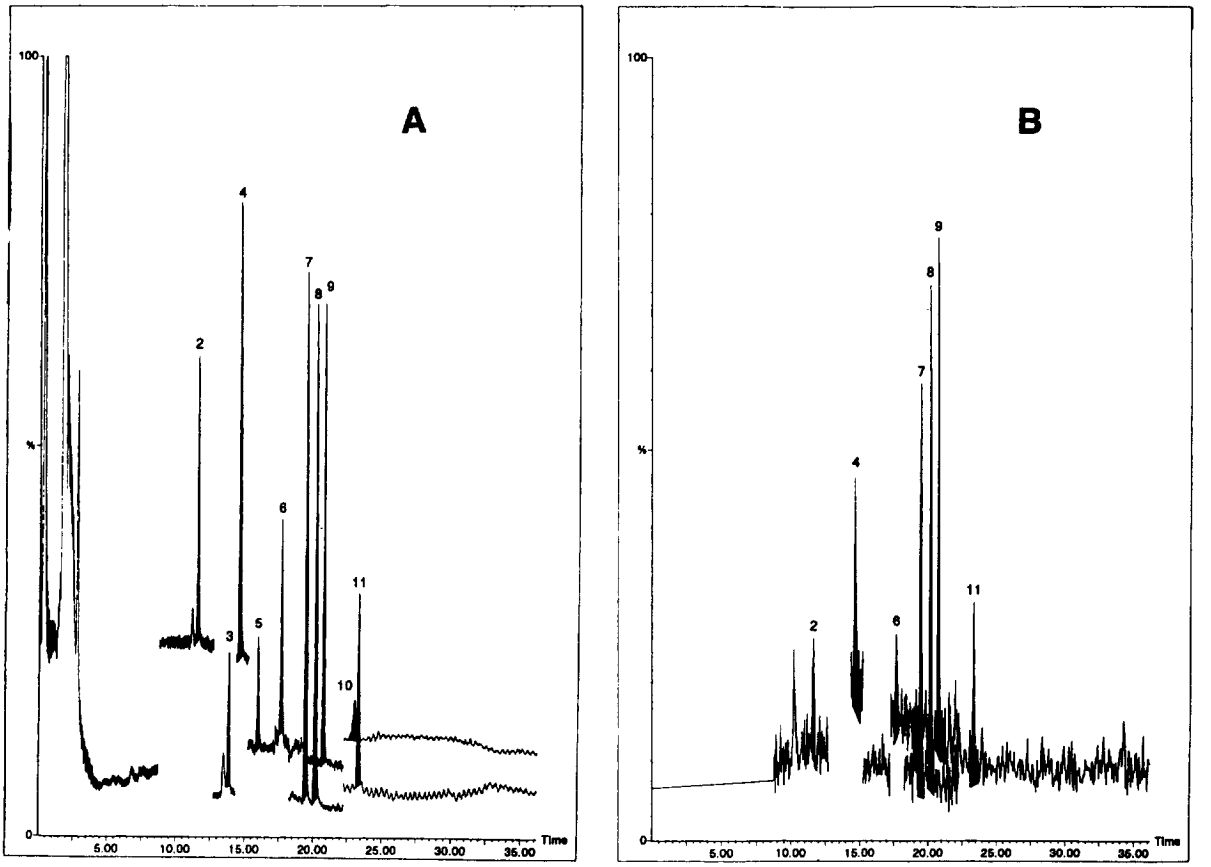

Fig. 4. Chromatograms of 100 ml tap water spiked with the mixture of 17 pesticides, obtained by (A) SPE–LC–APCI-MS in time-scheduled-SIM (monuron and diuron monitored in NI mode) and (B) SPE–LC–APCI-MS–MS time-scheduled product-ion scanning mode. Concentration of each analyte is 10 ng/l (for numbers, see Table 1).

tion of unknowns. Therefore, this was the only MS–MS mode used in the present study.

Following the general strategy outlined above, an MS–MS library of product-ion spectra of protonated molecules of the compounds investigated was compiled, in order to speed up the identification of analytes detected in real-world samples. Since analytical separations with short-column LC–APCI-MS took only 15 min, this method was applied for the injection of 73 pesticides and pesticide degradation products (7 mixtures containing 125 ng of each compound), first in the PI full-scan mode and, next, with $[M+H]^+$ ions selected as precursor ions, in the MS–MS product-ion mode.

When the pressure of the collision gas and the collision energy were kept at the same values (see Section 2) the spectra recorded by means of APCI-MS–MS and PA-ESP-MS–MS were similar for all compounds. This is shown in Fig. 6 for representatives of four pesticide classes. Since product-ion spectra are relatively "clean", even low-intensity ions are relevant for structure elucidation. Only exceptionally, e.g., for diuron, the MS–MS spectrum exhibited less than five characteristic fragments. In general, the main differences in the spectra are the slightly varying abundances of individual ions. This observation is confirmed when comparing low-energy CID product-ion MS–MS spectra published in the literature. For example, the triple-quad product-ion spectra of the $[M+H]^+$ ion of dimethoate reported in the past nine years using five different instruments are almost identical. The five major

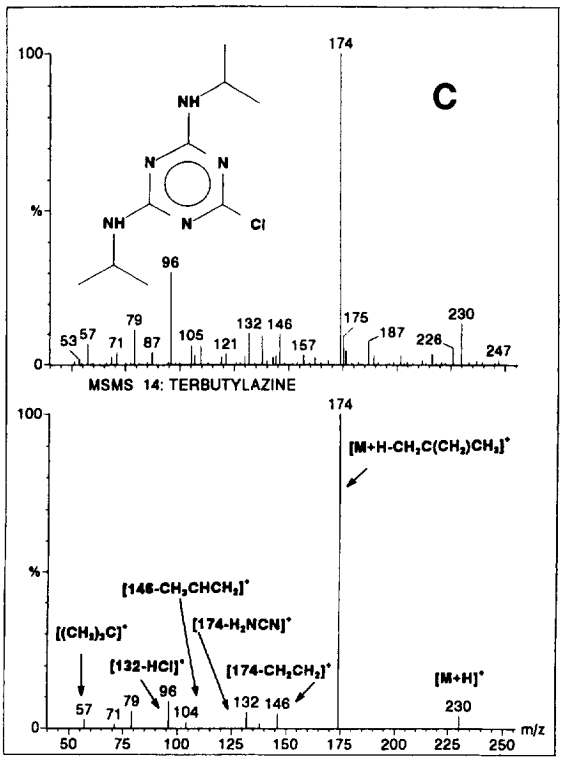

Fig. 5. Comparison of product-ion (MS–MS) spectrum of terbutylazine (peak No. 8) at 10 ng/l level and MS–MS spectrum of standard compound obtained from 125 ng loop injection. For other conditions, see text.

peaks reported are present in each spectrum despite the fact that the protonated molecules were obtained by means of LC–APCI-MS–MS, GC–PCI-MS–MS, LC–PA-ESP-MS–MS, LC and FIA-TSP-MS–MS (Table 5). The major difference again is in the relative ion intensities. These variations are more obvious than in the example above since each author worked with different settings of the collision gas pressure and collision energy. Most authors used argon as a collision gas, with pressures ranging from 1×10^{-3} to 6×10^{-3} Torr; enhanced fragmentation was observed by Betowski et al. [25] who used nitrogen as a collision gas. The collision energy was between 14 and 50 eV (in some papers only the collision offset voltage, 10–30 V, is mentioned). These considerations, together with our observations for a large number of compounds, indicate that fragmentation of molecules under low-energy MS–MS conditions is regulated more by the nature of the analyte than by the operating conditions and that comparable spectra can be obtained independent of the way of generation of the ions.

Our MS–MS library was successfully used for the identification of standard compounds at low concentration levels (cf., Fig. 4 and Fig. 5). The construction of an MS–MS $[M+H]^+$ product-ion library is of course limited by the ability of an ionization technique to generate protonated molecules. Product ions of fragments or adduct ions certainly may bring valuable information as well but their incorporation into MS–MS libraries seems too specific for practical use. In general, the structural information obtained from product-ion spectra is in many instances comparable to that from EI spectra.

3.4.1. Application to real-world samples

The potential of the methods presented above for the analysis of real-world samples was demonstrated for a surface water sample from the river Nitra (Slovakia). A 100-ml sample, spiked with metoxuron and propazine (1 μg/l each) as internal standards, was first screened by full-scan SPE–LC–APCI-MS and, subsequently, the signals attributed to $[M+H]^+$ ions of unknowns were monitored in a second run, using SPE–LC–APCI-MS–MS to obtain product-ion spectra. Both experiments were performed using the automated set-up and run unattended.

At least ten unknown compounds were detected in the sample, with NI analysis of the sample (no data shown) not bringing any additional information. As an example, the product-ion spectrum of the compound eluting at 15.5 min, shown in Fig. 7, was searched in our MS–MS library and identified as atrazine; the concentration was calculated to be ca. 0.25 μg/l, as derived from the loop injection of a standard solution of atrazine. The presence of atrazine in the sample was confirmed by SPE–GC–MS. As regards the other unknowns, their structural elucidation is still being attempted.

4. Conclusions

Coupling API-MS and API-MS–MS on-line to SPE–LC procedures can be used for the detection and identification of low ng/l levels of pesticides in water samples. The whole analytical procedure is

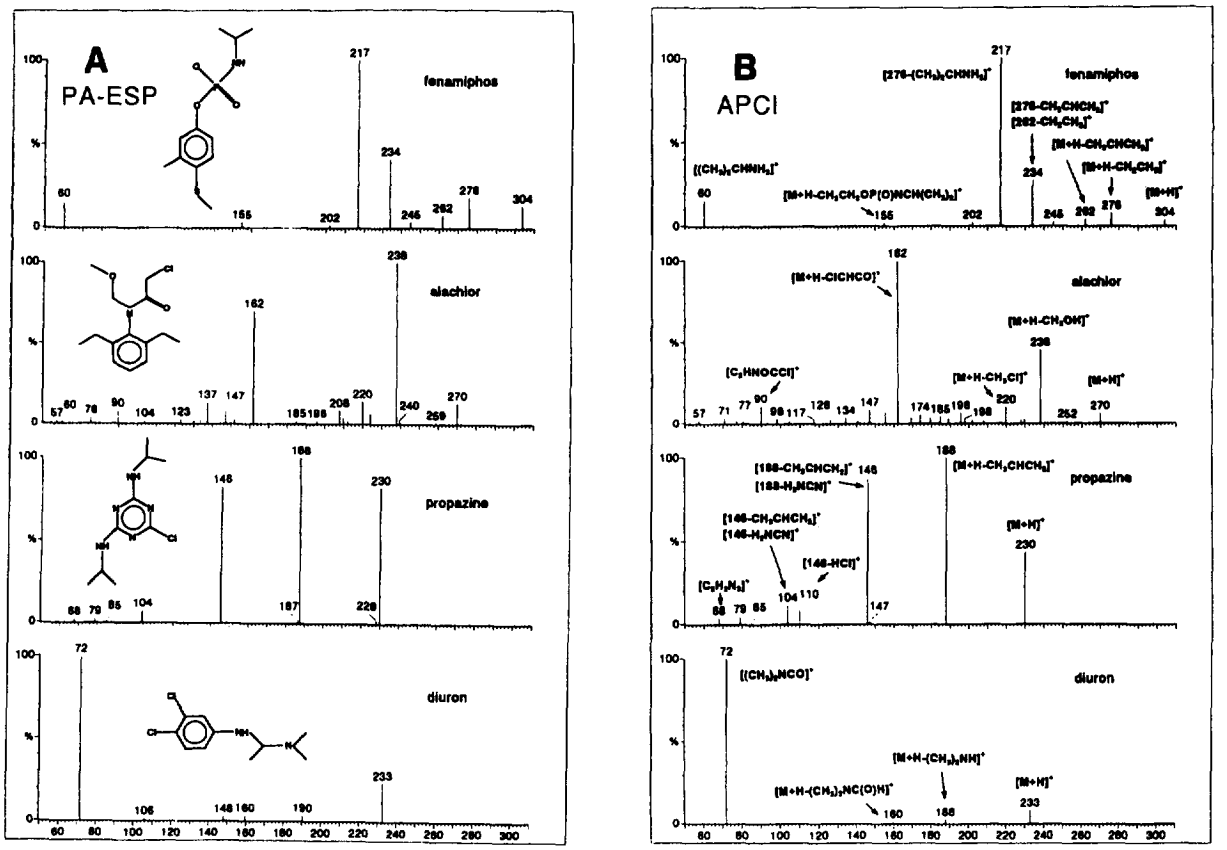

Fig. 6. Product-ion spectra obtained from protonated molecules of fenamiphos, alachlor, propazine and diuron in (A) APCI-MS–MS and (B) PA-ESP-MS–MS. The APCI experiment was performed with injections of 125 ng of each compound onto a single short column. Temperature of the ion source, 150°C; LC eluent flow-rate, 1 ml/min. The PA-ESP-MS–MS spectra were obtained at a flow-rate of 25 μl/min; temperature of the ion source, 70°C. For other conditions, see text.

automated by means of the Prospekt, an automated valve switching, cartridge exchange and solvent selection unit. Target analytes can be determined directly in the SIM or MS–MS mode of operation; unknown compounds can be identified in two subsequent runs, viz., a full-scan and an MS–MS product-ion run. Method detection limits obtained with the two interfaces used, APCI and PA-ESP, for a test mixture of 17 pesticides (trace enrichment of 100 ml of sample) were similar, viz., 0.007–3 μg/l in the full-scan and 0.1–200 ng/l in the SIM mode; two organophosphorus pesticides could not be detected. Product-ion MS–MS spectra could be obtained at concentration levels which were up to 10-fold higher than those required for SIM.

The RSD values of retention times recorded within a one-week period were typically less than 1%; the RSDs of peak areas varied between 1–20%. No technical problem was observed during the whole study; this supports our earlier conclusions that SPE–LC–MS is a robust procedure [5,7,8,21].

The mass spectra provided in APCI-MS and PA-ESP-MS were rather different. Spectra obtained by both techniques in the PI mode are typically dominated by protonated molecules and, occasionally, by structure-related fragments or sodium adducts as the base peak. In general, APCI produces less sodium and solvent cluster adducts and, therefore, the interpretation of spectra is more straightforward than in PA-ESP. In addition, in PA-ESP a split had to be introduced in front of the LC–MS interface in order to allow the use of LC eluent flows commonly used

Table 5
APCI-MS–MS and literature MS–MS data on dimethoate (MW 229[a]) product-ion spectra

APCI-MS–MS	PA-ESP-MS–MS[b]	TSP-MS–MS [c]	TSP-MS–MS[d]	GC–PCI-MS–MS[e]	Proposed identification
230 (2)	230 (40)	230 (10)	230 (n.i.)	230 (32)	$[M+H]^+$
199 (14)	199 (20)	199 (35)	199 (5)	199 (100)	$[M+H-CH_3NH_2]^+$
171 (31)	171 (10)	171 (70)	171 (15)	171 (27)	$[M+H-CH_3NHC(O)H]^+$
157 (13)			157 (22)		$[M+H-(CH_3O)_2PSO]^+$
143 (5)			143 (11)		$[M+H-CH_3NHC(O)CH_2OH]^+$
125 (100)	125 (100)	125 (100)	125 (100)	125 (51)	$[M+H-CH_3NHC(O)CH_2SH]^+$
88 (38)	88 (30)	88 (70)	88 (20)	88 (34)	$[M+H-(CH_3O)_2PS_2]^+$
			79 (10)		
	47 (10)		47 (5)		
	42 (10)		42 (8)		

[a] Monoisotopic molecular mass.
[b] PA-ESP-MS–MS (VG Quattro II tandem quadrupole MS), collision energy 18–45 eV, collision cell pressure (argon) 6×10^{-3} mbar [23].
[c] TSP-MS–MS (Finnigan MAT TSQ 70 triple quad MS); first quadrupole operating as high-pass filter (only masses equal to or above m/z 70 could enter collision cell); data obtained at collision offset of −20 V, collision cell pressure (argon) 1.5×10^{-3} Torr [24].
[d] TSP-MS–MS (Finnigan MAT TSQ 45 triple quad MS; collision energy 20 eV, collision gas pressure (nitrogen) 1.0×10^{-3} Torr; n.i. not indicated [25].
[e] GC–PCI-MS–MS (Finnigan MAT TSQ 45 triple quad GC–MS–MS-DS); methane as reagent gas; collision energy 20 eV, collision gas pressure (argon) 2.0×10^{-3} Torr [22].

in conventional-size LC. A flow rate of up to 1 ml/min can be used with APCI, while PA-ESP had its optimum at 100–300 μl/min. As was to be expected, for both interfaces NI operation generally performed worse than PI operation. However, several chlorophenols, nitrophenols, phenylureas and higher halogenated organophosphorus pesticides showed better detectability in the NI mode. Therefore, the NI mode can be recommended for the analysis of specific (classes of) compounds, as shown in this paper for time-scheduled-SIM of phenylureas with PA-ESP-MS.

A library of MS–MS product-ion spectra from protonated molecules was built for more than 60 pesticides and their degradation products, using constant settings of the collision gas pressure (argon, 2.0×10^{-3} Torr) and collision energy (25 eV). For the compounds tested, the pattern of the spectra is nearly independent of the way of parent ion formation (PA-ESP vs. APCI). The vast majority of the analytes tested shows the presence of at least five structurally related ions in the MS–MS spectrum which is usually considered sufficient for unambiguous identification of an analyte according to US-EPA procedures [26]. The library was successfully used for searches of product-ion spectra obtained from low levels (down to 10 ng/l) of pesticides spiked in tap water and for the identification of a pollutant in surface water. In other words, the directives of the EU Drinking Water Guideline for the control of pesticide residues in drinking water [3] can be met.

The present results indicate that SPE–LC–API-MS is ready to be used on a routine basis. As an illustration: the system can be installed in a central environmental laboratory [21,27] and aqueous samples or SPE cartridges, loaded with 100–250-ml samples, can be transported from monitoring stations or remote sampling sites for the confirmation of pollutants detected by early-warning systems or for the identification of unknowns. Current research deals with the evaluation of a promising alternative, short-column API-MS which still requires proper optimization, as well as with validation.

Acknowledgments

The authors would like to acknowledge Fisons VG, Organic Analysis Biotech (Altrincham, UK) for providing the excellent facilities of their laboratory, Mr. M. van Oosterhout (Fisons VG, Benelux) for his help in organizing the study and Mr. Jan Brands (Supelco, Bornem, Belgium) and Zorbax (Rockland Technologies, Nuenen, Netherlands) for supplying analytical columns. Registry No. Alachlor, 15972-60-8; aldicarb, 116-06-3; atrazine, 1912-24-9;

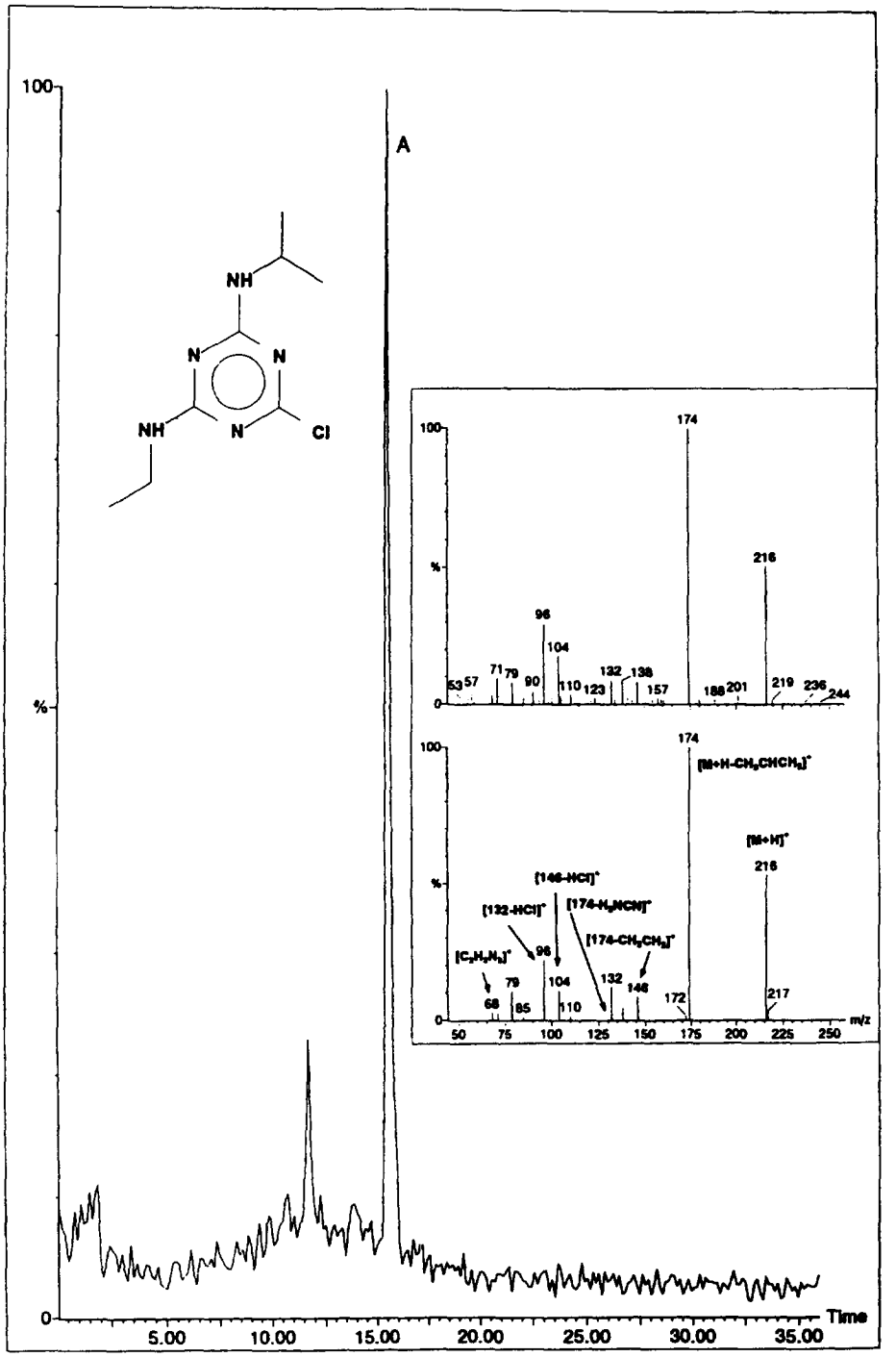

Fig. 7. Chromatogram of 100 ml Nitra river (Slovakia) water sample obtained by SPE–LC–APCI-MS–MS of one of the simultaneously monitored parent ions, m/z 216. The insert is the result of an MS–MS library search for unknown pollutant A (retention time, 15.5 min) elucidated as atrazine. For other conditions, see text.

bromophos-ethyl, 4824-78-6; chlorpyriphos, n.r.; coumaphos, 56-72-4; dimethoate, 60-51-5; diuron, 330-54-1; fenamiphos, 22224-92-6; fenchlorphos, 299-84-3; fenthion, 55-38-7; monuron, 150-68-5; neburon, 555-37-3; oxamyl, 23135-22-0; propazine, 139-40-2; propoxur, 114-26-1; terbutylazine, 5915-41-3; trifluralin, 1582-09-8. n.r., compound not found in CAS registry.

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