Application of liquid chromatography with mass spectrometry combined with photodiode array detection and tandem mass spectrometry for monitoring pesticides in surface waters

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Abstract

Liquid chromatography with photodiode array detection (LC–DAD) and liquid chromatography with mass spectrometry (LC–MS) are two techniques that have been widely used in monitoring pesticides and their degradation products in the environment. However, the application of liquid chromatography with tandem mass spectrometry (LC–MS–MS) for such purposes, once considered too costly, is now gaining considerable ground. In this study, we compare these methods for the multi-residue analysis of pesticides in surface waters collected from the central and southeastern regions of France, and from the St. Lawrence River in Canada. Forty-eight pesticides belonging to eight different classes (triazine, amide, phenylurea, triazole, triazinone, benzimidazole, morpholine, phenoxyalkanoic), along with some of their degradation products, were monitored on a regular basis in the surface waters. For LC–MS, we used the electrospray ionization (ESI) interface in the negative ionization mode on acidic pesticides (phenoxyalkanoic, sulfonylurea), and the atmospheric pressure chemical ionization (APCI) interface in the positive ionization mode on the remaining chemicals. Different extraction techniques were employed, including liquid–liquid extraction with dichloromethane, and solid-phase extraction using C_{18}-bonded silica and graphitized carbon black cartridges. Eleven of the target chemicals (desethylatrazine, desisopropylatrazine, atrazine, simazine, terbuthylazine, metolachlor, carbendazime, bentazone, penconazole, diuron and isoproturon) were detected by LC–MS at concentrations ranging from 20 to 900 ng/l in the surface waters from France, and six pesticides (atrazine, desethylatrazine, desisopropylatrazine, cyanazine, simazine and metolachlor) were detected by LC–MS and LC–MS–MS at concentrations ranging from 3 to 52 ng/l in the samples drawn from the St. Lawrence River. There was good correlation between the LC–DAD and LC–MS techniques for 60 samples. The slope of the curves expressing the relationship between the results obtained with LC–DAD versus those obtained by LC–MS was near 1, with a correlation coefficient (r) of over 0.93. The identification potential of the LC–MS technique, however, was greater than that of the LC–DAD; its mass spectra, mainly reflecting the pseudomolecular ion resulting from a protonation or a deprotonation of the molecule, was rich in information. The LC–MS–MS technique with ion trap detectors, tested against the LC–MS on 10 surface water samples, gave results that correlated well with the LC–MS results, albeit generating mass spectra that yielded far more information about the structure of unknown substances. The sensitivity of the LC–MS–MS was equivalent to the selected ion monitoring (SIM) acquisition mode in LC–MS. The detection limits of the target pesticides ranged from 20 to 100 ng/l for the LC–MS technique (under full scan acquisition), and from 2 to 6 ng/l for LC–MS–MS. These limits were improved by a factor of almost 10 by increasing the sample volume to 10 l. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Liquid chromatography–mass spectrometry; Water analysis; Environmental analysis; Pesticides

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1. Introduction

The monitoring of phytosanitary residue (pesticides, herbicides, fungicides) in water is a highly complex field. There are more than 8500 commercial formulations currently in use, including about 900 active substances listed in the Pesticide manual, and these in turn are the source of several hundred degradation products. To address the issue, European regulations on drinking water quality set a maximum concentration of 0.1 μg/l for individual pesticides and some of their degradation products, and 0.5 μg/l for total pesticides present in a sample.

Determining the degree of ground- and surface-water contamination by these compounds is the fundamental aim of environmental analytical laboratories, especially in France, where the major part of the drinking water supply is obtained from groundwater. Such work requires the use of sensitive multi-residue methods for detecting and identifying the greatest number of compounds possible, with the fewest number of intermediate steps.

To analyze the growing number of modern pesticides and their degradation products, which are generally thermolabile, polar and non-volatile, the analytical methods employing gas chromatography in combination with specific detectors or coupled with mass spectrometry (MS) are not reliable without a time-consuming derivatization step, which itself can generate interferences. Consequently, liquid chromatography (LC) combined with diode array detection (DAD) has been used increasingly in recent years as a complementary method for pesticide analysis. The only UV spectrum used as a means of identification, however, has not proven to be sensitive or selective enough; nor have fluorescent and electrochemical detectors, which provide insufficient information on the compounds detected.

It has been found that the detected substances can only be definitively identified by coupling LC to MS using different interfaces [1,2]. Previously, these couplings were carried out with ''particle beam'' (PB) and ''thermospray'' interfaces, and were of little interest for environmental analyses due to their limited scope of application, their lack of sensitivity and repeatability, and the non-linearity of the calibration curves. The atmospheric pressure ionization (API) systems – APCI (atmospheric pressure chemi-

cal ionization), and ESI (electrospray ionization) – which allow for the soft ionization of a wide range of substances in positive and negative modes, have become the interfaces more generally used, providing sensitive, robust and accurate methods for pesticide analysis in accordance with the requirements of European regulations [3–6].

According to Ref. [7], in the performances of the PB and APCI interfaces, the availability of electron impact (EI) library-searchable spectra for identifying unknown compounds that could be used with PB is one advantage of this interface. Nevertheless, APCI is more sensitive and shows a higher linear range [8].

Several papers have been published demonstrating that the structural information obtained from LC–MS is mostly generated in the mass spectra from protonated molecules or deprotonated molecules, sometimes accompanied by adduct ions resulting from the compound ions combining with the mobile phase [4,5,9]. The degree of confidence in the identification of the compounds can be enhanced by using a single quadrupole combined with collision-induced dissociation (CID), obtained, depending on the LC–MS apparatus, by applying an octapole or an orifice voltage that fragments the molecules in the atmospheric pressure–vacuum interface under soft conditions. Notwithstanding the use of CID, it appears that LC–MS fragmentation is insufficient for the identification of unknown compounds and that tandem mass spectrometry (MS–MS) is of greater interest for this purpose [10–13]. In particular, using ion trap detectors in MS–MS mode allows for the following steps in a sequence of timed events within a single ion trap: ionization of molecules, ion isolation, ion collision with inert gas, and detection of daughter ion fragments. Ions produced after the collision of protonated molecules with helium gas provide reliable information on molecular structure.

The aim of this paper is to present the results obtained on real water samples of different origins by liquid–liquid extraction and by solid-phase extraction (SPE) using C_{18}-bonded silica on groundwater and river water samples, and using SPE with graphitized carbon black cartridges for large volume extraction, followed by LC–MS with APCI and ESI interfaces in the positive ion (PI) and negative ion (NI) modes. For some of the samples, LC–APCI$^+$-

MS–MS is compared with LC–APCI-MS in terms of sensitivity, linearity and identification performance.

2. Experimental

2.1. Chemicals and reagents

The HPLC-grade water was obtained from BDH (UK). Acetonitrile, methanol, acetic acid, and dichloromethane were supplied by Carlo Erba (France). The certified pure products were purchased from CIL Cluzeau Info Labo (France). The purity of all standards was a minimum of 99%.

Pesticide stock solutions of 200 μg/ml were prepared by weighing and dissolving 20 mg of each compound in 100 ml of methanol. These solutions were stored at −20°C and were stable over a period of 6 months.

The analyzed products were:

(1) Neutral pesticides: deisopropylatrazine (DIA), metamitron, deethylatrazine (DEA), carbendazim, simazine, metribuzin, cyanazine, desmetryn, atrazine, secbumeton, methabenzthiazuron, chlortoluron, isoproturon, terbumeton, metazachlor, ametryn, diuron, triadimenol, terbuthylazine, linuron, napropamide, metolachlor, tebuconazole, penconazole, flusilazole, propiconazole, hexaconazole, prochloraz, neburon, fenpropimorph.

(2) Acid pesticides: fluroxypyr, dicamba, bentazone, bromoxynil, 4-chloro-2-methylphenoxyacetic acid (MCPA), 2,4-D, ioxynil, dichlorprop, 2,4,5-T, dinoseb, dinoterb, metsulfuron-methyl.

A mixed intermediate solution containing 10 μg/ml of the pesticides belonging to each analytical group was prepared by diluting the stock solutions in methanol.

Standard mixtures were prepared by appropriate dilution of a mixed stock solution with methanol ranging from 0.025 to 10 μg/ml for LC–MS determinations, and from 0.001 to 1.0 μg/ml for LC–MS–MS determinations.

Among the most frequently detected substances in the real samples, 12 (DIA, DEA, carbendazim, simazine, atrazine, isoproturon, triadimenol, terbuthylazine, tebuconazole, flusilazole, penconazole, propiconazole) were studied by both LC–APCI-MS and LC–APCI-MS–MS in the PI mode.

2.2. Instrumentation and columns

The LC–UV–MS analyses were performed using a Varian (Les Ulis, France) LC system consisting of a 9012 quaternary pump, a 9100 autosampler, a 9065 diode-array UV detector, and a Finnigan SSQ 7000 single quadrupole mass spectrometer equipped with a Finnigan MAT ESI source and an APCI source for mass spectrometry detection (Thermoquest, Les Ulis, France). The LC columns were (i) an LC-ABZ 5 μm, 250 mm×4.6 mm I.D. (Supelco, St. Quentin Fallavier, France) for analyzing neutral pesticides, and (ii) a Kromasil 5 μm, 250×4.6 mm I.D. (Touzart et Matignon, Courtaboeuf, France) for analyzing acid pesticides. The LC–MS system was connected to a digital DEC station 5000/125 computer and run by ICIS software.

For LC–MS–MS, the analyses were performed using a TSP 4000 solvent-delivery system and an LCQ quadrupole ion trap mass spectrometer equipped with an APCI source (Thermoquest) run by EXCALIBUR software. The LC column was a Hypersil ODS 2, 5 μm, 250×4.6 mm I.D. (Thermoquest).

2.3. Analytical conditions and procedure

The analytical conditions for LC–MS have been described by [14]. Briefly, an acetonitrile–water binary gradient of 15% to 60% acetonitrile in 50 min was used on the neutral pesticides determined by LC–APCI-MS, and a methanol–water–acetic acid binary gradient of 1% to 100% methanol in 0.1% acetic acid in 40 min was used on acid pesticides determined by LC–ESI-MS [11].

For LC–MS–MS, the analyses were carried out with an acetonitrile (A) and water (B) linear gradient of 15% to 30% A in 20 min, 30% to 80% A in 20 to 55 min, and 80% to 100% A in 55 to 60 min. The flow-rate of the mobile phase was 1.0 ml/min, and 20 μl of sample or standard solution was injected into the LC system.

2.4. Mass spectrometric analysis

Mass spectrometric analysis was performed by LC–MS in full scan mode from 130 u to 450 u, with ion extractions at a specific m/z in the positive ion

mode when using an APCI source and in the negative ion mode when using an ESI source. An example of a chromatogram in total ion current (TIC) mode on 29 neutral pesticides is shown in Fig. 1.

For LC–APCI[+]-MS–MS, the protonated molecular ions of the target compounds were selected and fragmented with CID helium gas collision in the ion trap. The mass spectra resulting from these fragmentations scanned from m/z 50 to approximately 10 u above the molecular mass of the target compounds. Selection of the ions was scheduled according to the following segments, detailed in Table 1: 0–13.18 min at m/z 174 for DIA; 13.20–17.48 min at m/z 188 for DEA; 17.50–24.00 min at m/z 192 for carbendazim; 24.10–31.10 min at m/z 202 for simazine; 31.20–37.36 min at m/z 216 for atrazine and m/z 207 for isoproturon; 37.40–43.70 min at m/z 296 for triadimenol and m/z 230 for terbuthylazine; 43.80–48.35 min. at m/z 308 for tebuconazole and m/z 316 for flusilazole; and 48.40–62 min at m/z 284 for penconazole and m/z 342 for propiconazole. An example of a chromatogram acquired under these conditions is shown in Fig. 2A and B.

2.5. Sample handling and preparation

Liquid–liquid extraction with dichloromethane was chosen for the majority of the neutral substances studied in 62 ground- and surface-water samples from central and southeastern France [14]. Briefly, it consisted of extracting 1.0 l of each water sample with 60 ml dichloromethane three times, followed by solvent evaporation under a nitrogen stream and a final transfer into 0.5 ml of methanol for LC injections.

The liquid–solid extraction mode was used for the groundwater samples to preconcentrate triazines, phenylureas, carbendazim and degradation products of atrazine and simazine. Between 0.5 to 1.0 l of each sample was extracted on SPE cartridges packed with 1 g of C_{18}-bonded silica (Supelco). Briefly, the solid-phase was first conditioned with 6 ml methanol then with 10 ml deionized water. Extraction of water samples was carried out at a 10 ml/min flow-rate. Following sample extraction the cartridge was aspirated during 10 min to remove residual water. The

compounds were eluted by running twice 2 ml of methanol. After reducing to near dryness under a gentle nitrogen stream, the compounds were transferred into a final volume of 0.5 ml of a mixture of methanol–water (50:50, v/v).

The acid substances were extracted on the same cartridges at pH 2.0 and dissolved in a final volume of 0.5 ml methanol.

The SPE of large volumes of surface water (St. Lawrence River) is described in Ref. [16]. Ten-liter filtered water samples were extracted and preconcentrated on 500 mg of Carbopack B (500–666 μm) graphitized carbon black (Supelco). The final volume was 500 μl of a methanol–water (50:50) mixture.

3. Results and discussion

3.1. Extraction

The extraction methods used in this study were selected according to criteria such as the required quantification limits, the recycling rates of the target substances, the nature and origin of the samples, the physico–chemical properties of the analyzed substances, and the identification requirements. Volumes of 0.5 to 1.0 l were extracted at neutral pH in liquid–liquid mode and at neutral and acid pH in SPE mode for the groundwater and surface river waters. In the case of river waters with a very high flow-rate, pollutants are highly diluted and can represent large mass flows in spite of the low nominal concentrations registered. Therefore a high level of enrichment is required for their detection and an accurate estimation of their mass flows. In this way sample volumes of 10 l were extracted with a previously described SPE mode using the Carbopack B (500–666 μm) carbon graphitized black carbon [16].

The recoveries for each of these methods varied from: (1) 83–93% in liquid–liquid mode, with relative standard deviations (RSDs) of between 2% and 10% [14], (2) 67–98% in SPE mode at neutral pH and 67–100% in SPE mode at acid pH, with RSDs of between 2% and 7% [15] and (3) 60–96% (apart from metribuzin: 7%) in SPE mode on Carbopack B for the 10-l volumes, with RSDs between 3% and 17% [16].
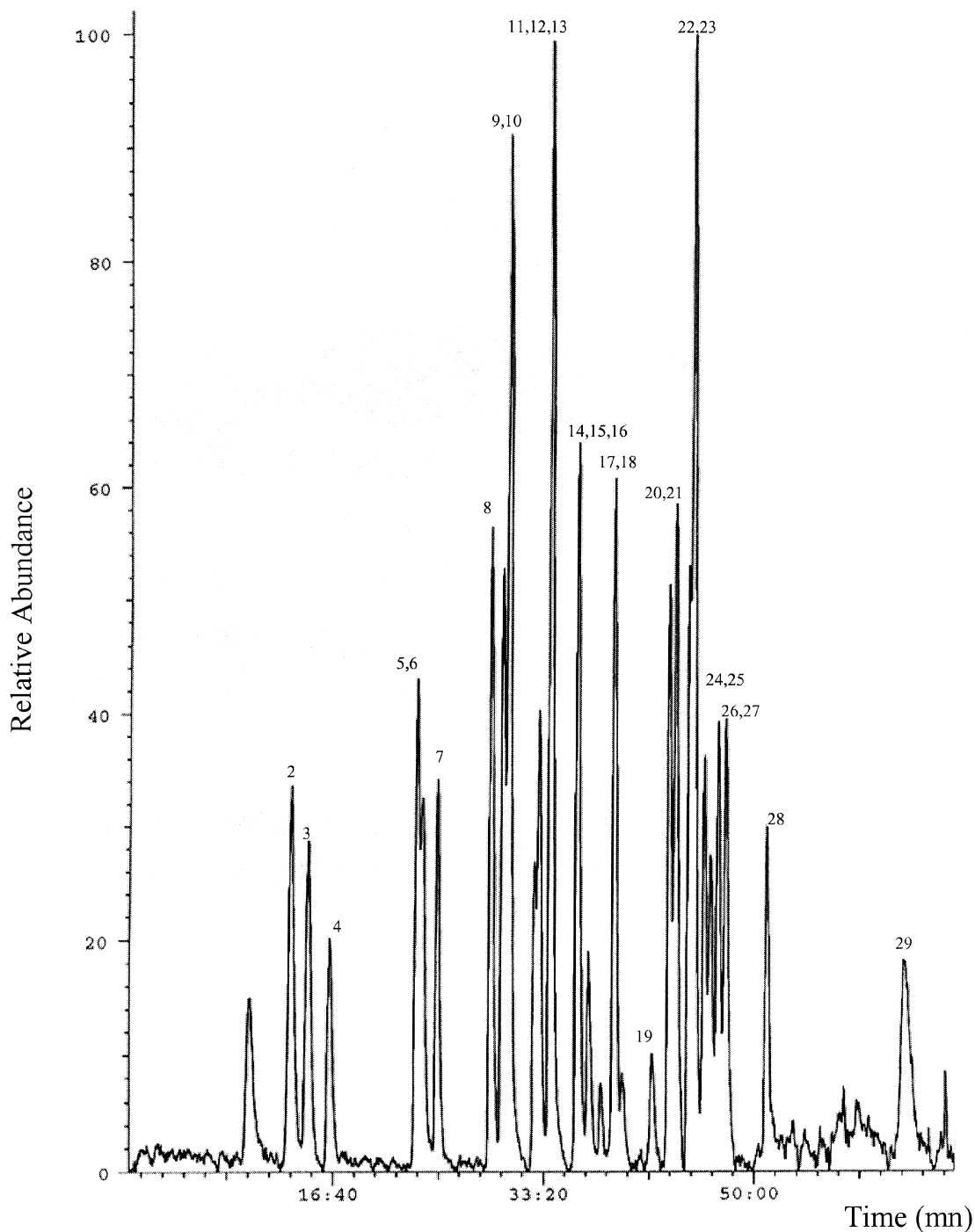
Fig. 1. LC–APCI$^+$-MS chromatogram of 29 neutral pesticides mixture (10 ng/µl of each pesticide in methanol) in TIC mode. Peak assignments: 1=DIA; 2=metamitron; 3=DEA; 4=carbendazim; 5=simazine; 6=metribuzin; 7=cyanazine; 8=desmetryn; 9=atrazine; 10=secbumeton+methabenzthiazuron; 11=chlortoluron; 12=isoproturon; 13=terbumeton+metazachlor; 14=ametryn; 15=diuron; 16=triadimenol (1); 17=terbuthylazine; 18=triadimenol (2); 19=linuron; 20=napropamide; 21=metolachlor; 22=tebuconazole; 23=penconazole+flusilazole; 24+25=propiconazole; 26=hexaconazole; 27=prochloraz; 28=neburon; 29=fenpropimorph. mn=Minutes.

Table 1
General conditions used for LC–APCI-MS–MS analysis

Segment		Protonated ion selected, m/z	Compounds	Daughter ions (% rel. abundance)	MS–MS full scan interval	Retention time (min)
No.	Time (min)					
1	0–13.18	174	DIA	132 (100), 146 (88), 174 (33), 79 (22), 138 (21), 96 (14)	50–184	10.44
2	13.20–17.48	188	DEA	146 (100), 188 (8)	50–198	16.01
3	17.50–24.00	192	Carbendazim	160 (100), 192 (3)	50–202	19.06
4	24.10–31.10	202	Simazine	124 (100), 132 (49), 174 (31), 202 (11), 166 (6), 96 (2)	55–212	27.22
5	31.20–37.36	216	Atrazine	174 (100), 138 (3), 146 (2), 216 (2)	60–226	33.69
		207	Isoproturon	72 (100), 165 (48)	55–217	34.83
6	37.40–43.70	296	Triadimenol	296 (100)	286–306	40.06
		230	Terbuthylazine	174 (100), 230 (9), 214 (4)	60–240	41.02
7	43.80–48.35	308	Tebuconazole	308	298–318	46.02
		316	Flusilazole	210 (100), 288 (73), 187 (9), 316 (3)	85–326	47.05
8	48.40–62.00	284	Penconazole	284 (100)	274–294	49.00
		342	Propiconazole	246 (100), 159 (99), 273 (41), 205 (26), 200 (17), 187 (9), 342 (8)	95–352	49.59

3.2. Optimization of APCI-MS–MS operating conditions

Operating conditions for the APCI source were optimized in infusion mode at a rate of 3 μl/min on the standard mixture of the 12 substances, each at a concentration of 1 mg/l in methanol, the objective being to obtain the maximum population of ions resulting from the protonation of the molecules in the solution. The signal was optimized on the total ion current in MS mode with the following values for

each parameter: vaporizer temperature: 400°C; transfer capillary temperature: 225°C; corona discharge intensity: 5 μA; sheath gas pressure: 0.6 MPa; auxiliary gas flow-rate: 10 l/min.

At the same time, the selection of ions and collision voltages was optimized on the ion trap LCQ mass spectrometer. The selected collision voltages are summarized in Table 2, along with the results obtained on daughter ion scans after the selection of the precursor ions. Due to their non-fragmentation, the full scan range of daughter ions was reduced to
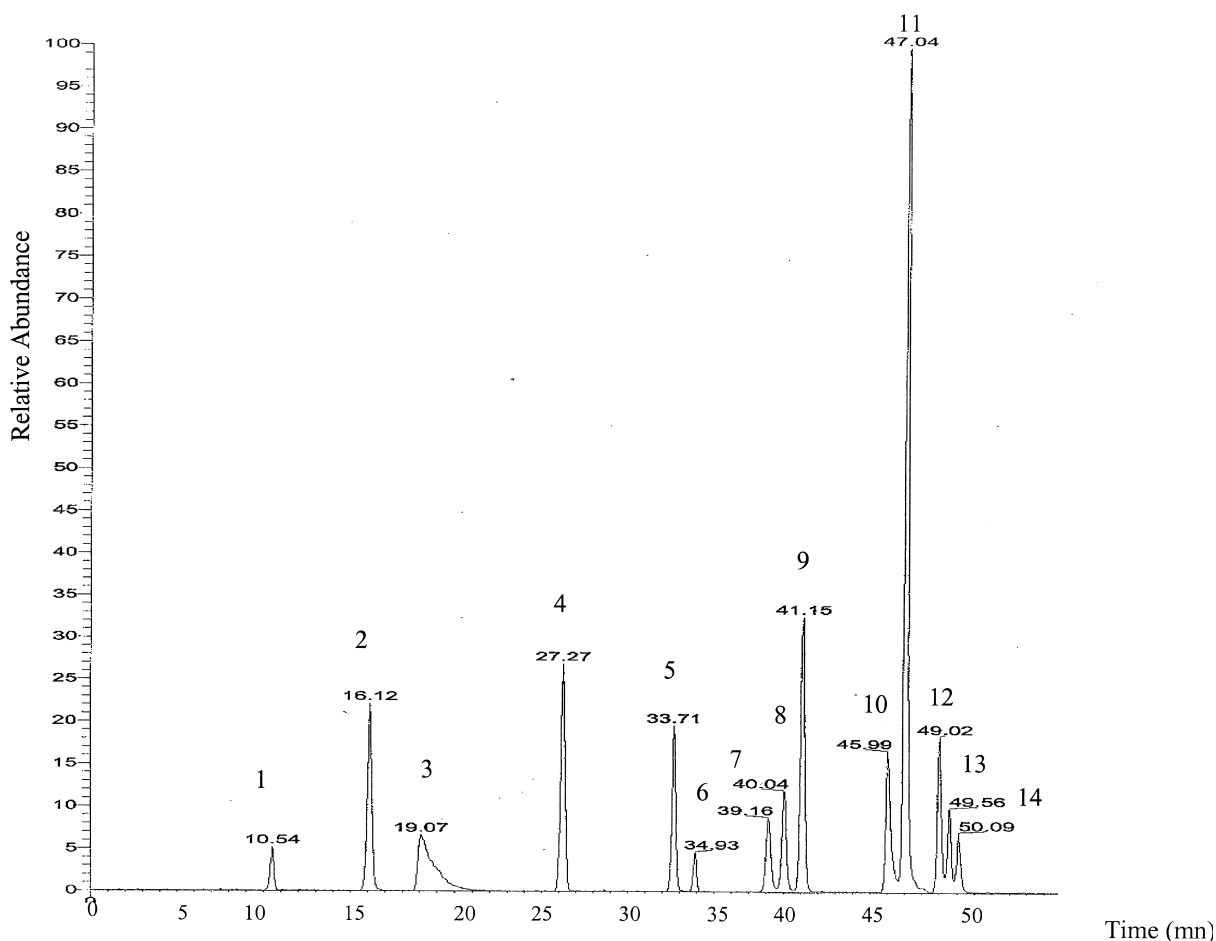

Fig. 2. (a) LC–APCI^{+}-full scan MS–MS chromatogram of a standard solution (1 μg/ml of each compound in methanol) scanning from *m/z* 50 to ca. 10 u above the precursor ions u. Peak assignments: 1=DIA; 2=DEA; 3=carbendazim; 4=simazine; 5=atrazine; 6=isoproturon; 7, 8=triadimenol; 9=terbuthylazine; 10=tebuconazole; 11=flusilazole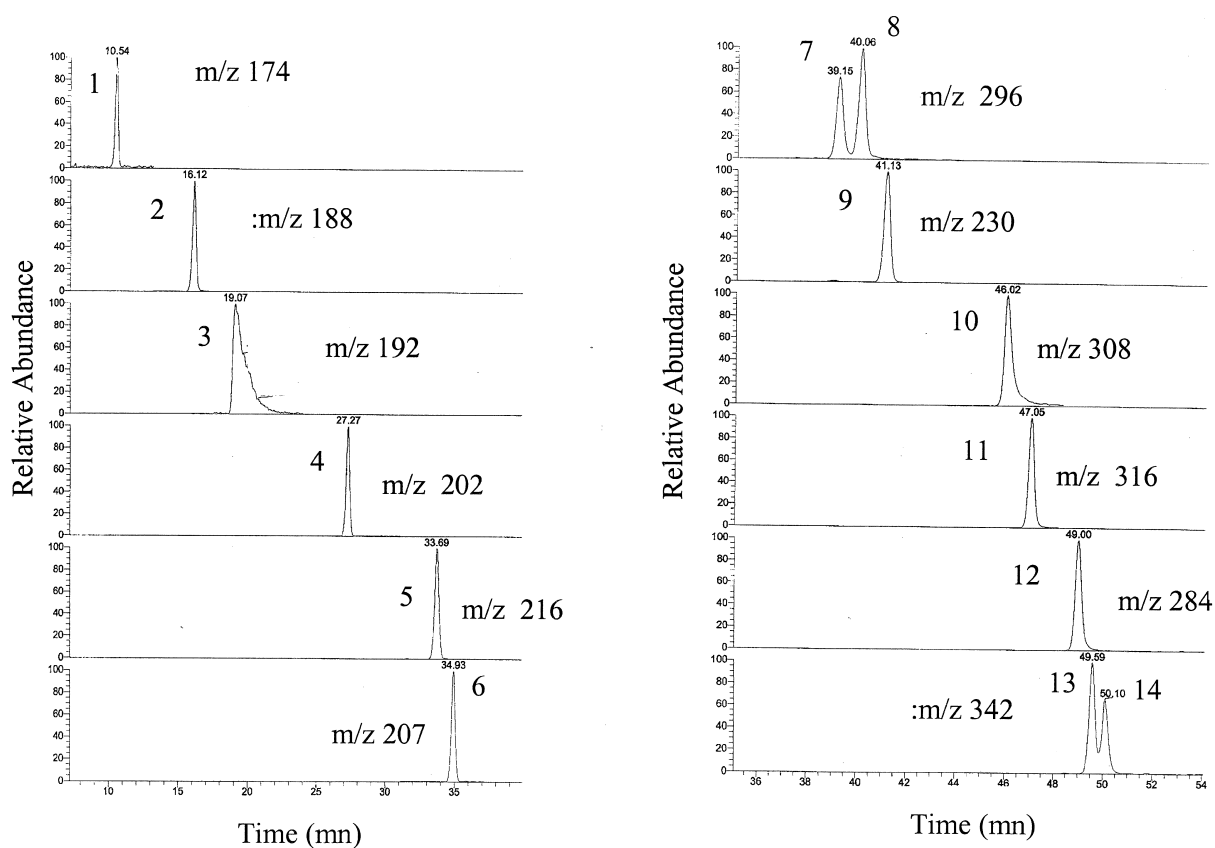; 12=penconazole; 13, 14=propiconazole. (b) LC–APCI+-MS–MS chromatogram of a standard solution (1 μg/ml of each compound in methanol solution), detailed signal on each ion. Peak assignments: 1=DIA; 2=DEA; 3=carbendazim; 4=simazine; 5=atrazine; 6=isoproturon; 7, 8=triadimenol; 9=terbuthylazine; 10=tebuconazole; 11=flusilazole; 12=penconazole; 13, 14=propiconazole.

Fig. 2. (*continued*).

Table 2
APCI–MS–MS data on spectra obtained by collision in ion trap detector used as MS–MS

Compound	Molecular mass	Protonated parent ion	Collision voltage in ion trap detector (V)	Daughter ions (% rel. abundance)
DIA	173	174	0.80	132 (100), 146 (88), 174 (33)
				79 (22), 138 (21), 96 (14)
DEA	187	188	0.80	146 (100), 188 (8)
Carbendazim	191	192	0.75	160 (100), 192 (3)
Simazine	201	202	0.90	124 (100), 132 (49), 174 (31), 202 (11), 166 (6), 96 (2)
Atrazine	215	216	0.90	174 (100), 138 (3), 146 (2), 216 (2)
Isoproturon	206	207	0.80	72 (100), 165 (48)
Triadimenol	295	296	0	296 (100)
Terbuthylazine	229	230	0.75	174 (100), 230 (9), 214 (4)
Tebuconazole	307	308	0	308 (100)
Flusilazole	315	316	0.90	210 (100), 288 (73), 187 (9), 316 (3)
Penconazole	283	284	0	284 (100)
Propiconazole	341	342	0	246 (100), 159 (99), 273 (41), 205 (26), 200 (17), 187 (9), 342 (8)

20 u for triadimenol (286–306), tebuconazole (298–318), and penconazole (274–294).

3.2.1. The MS and MS–MS spectra

The ESI and APCI interfaces used with the LC–MS system mainly generated mass spectra of the protonated molecular ion $(M+H)^+$, for analysis in the APCI positive mode, and of the deprotonated $(M−H)^−$ ion for the acid molecules analyzed in the negative ESI mode, thus agreeing with the observations published in recent articles on the subject [15]. Some compounds analyzed by LC–APCI$^+$-MS (atrazine, terbuthylazine, simazine, carbendazim) undergo partial fragmentation in the soft ionization conditions obtained by applying a 5 V current to the octapole situated at the orifice of the quadrupole. The mechanism of fragmentation under weak collision energy is described in an article detailing the mass spectra of some triazines (atrazine, propazine, terbuthylazine, prometryn) obtained with increasing voltages applied to the octapole downstream of an ESI source [17].

For the analyses of real samples in the present study, a weak current (5 V) was applied to the octapole in the LC–MS configuration in order to obtain information on the structure of the molecules without altering the information on the molecular mass. Repeatability tests on the relative abundance of fragments of the studied substances, using identical experimental conditions, yielded RSDs of between 2 and 12% [14], thus demonstrating that LC–MS associated with CID in the intermediate pressure zone of the APCI interface has great power of identification.

Of the 11 acid substances analyzed with the ESI interface in negative mode, fragmentation was observed in two of them when a 15 V current was applied to the octapole: dicamba (m/z: 175, 219) and fluroxypyr (m/z: 195, 253). The other substances yielded no (or few) fragments under the experimental conditions.

In spite of its high identification potential, the LC–MS technique used in combination with atmospheric pressure interfaces and collision in the octapole above the orifice of the quadrupole has its limitations due to the low rate of ion fragmentation. This is particularly true for the identification of trace amounts of compounds in complex matrices, and in the determination of the structure of unknown substances.

The LCQ ion trap quadrupole spectrometer used in MS–MS mode downstream of the APCI source in positive mode made it possible for structural information to be obtained on the detected molecules by (a) selecting the protonated ions, then (b) inducing a collision of the selected ions in the presence of helium gas under reduced pressure in the trap, and finally (c) analyzing the collision fragments by scanning the m/z ions between 50 and approximately 10 u above the molecular mass of the compound. Ten of the 12 studied compounds show characteristic collision spectra containing at least two fragments, making the identification more reliable. The results are presented in Table 2; three examples of mass spectra are shown in Fig. 3.

3.2.2. Linearity in MS–MS

Calibration curves were established on the 12 substances analyzed at six different concentrations by LC–MS–MS in the selected ion mode, followed by extraction of the signal on one or two of the more abundant daughter ions acquired in full scan mode during the acquisition of the mass spectrum of the selected ion. The m/z values used for the calibration of the system are given in Table 3.

The results show a good linearity of the analytical responses recorded by LC–APCI-MS–MS, with performances quite similar to those of LC–MS in different concentrations ranges for the compounds in a methanol solution (1.25–5 µg/l for the lowest concentrations to 1000 µg/l for the highest concentrations for LC–MS–MS, and 25 µg/l for the lowest concentrations to 10 000 µg/l for the highest concentrations for LC–MS).

Examples of calibration curves for five analytes are shown in Fig. 4.

3.3. Sensitivity and quantification limits

A comparison of the sensitivity of the analytical responses obtained with (a) the LC–APCI-MS system in full scan acquisition mode with extraction of the signal on the specific m/z, and (b) LC–APCI-MS–MS in precursor ion selection mode and the mass spectrum established on this ion, shows a ratio between the response coefficients of the two tech-
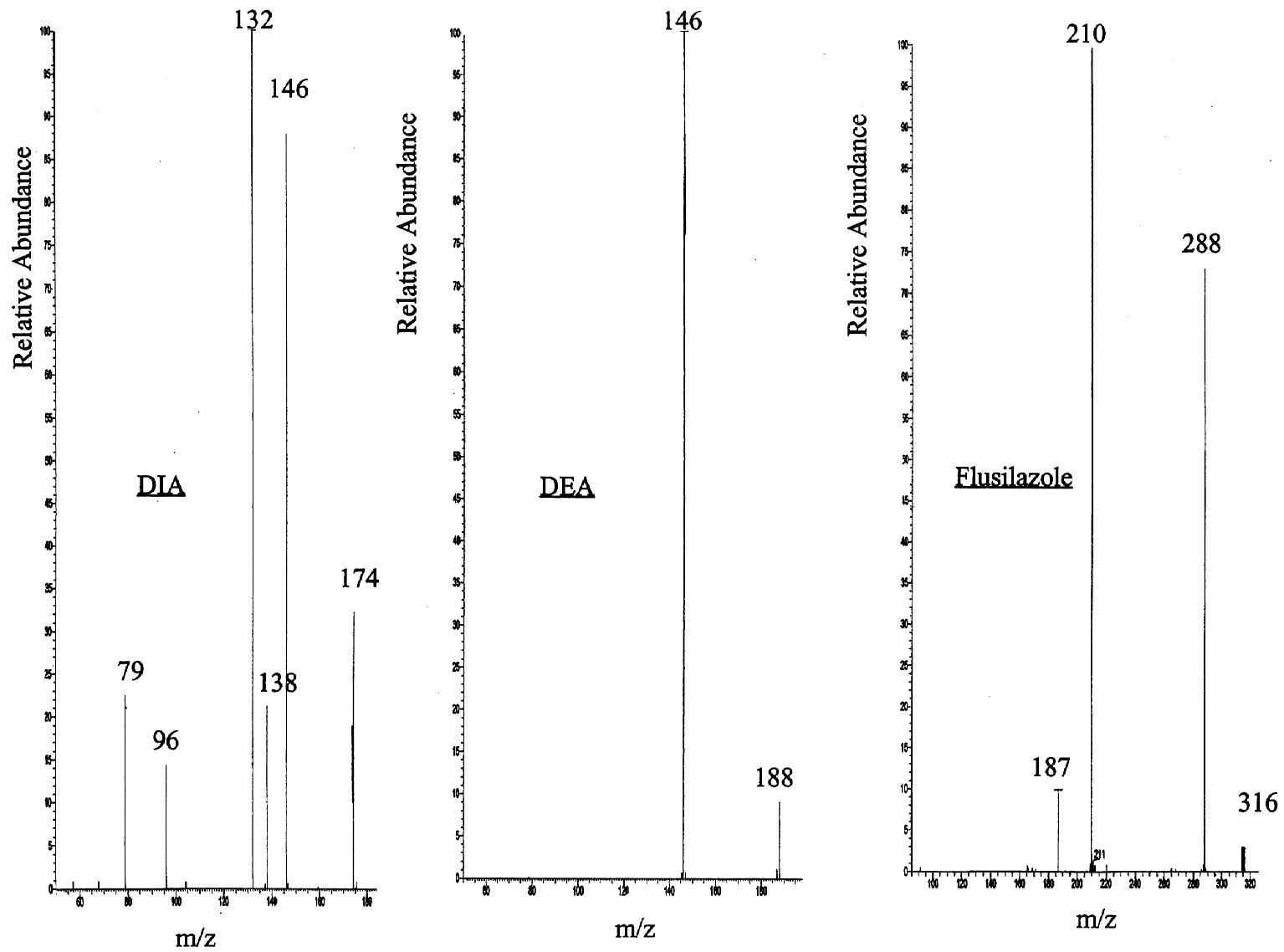
Fig. 3. Examples of full scan product spectra for DIA; DEA; flusilazole (from 50 to ca. 10 u above the precursor ions u).

Table 3
Calibration data obtained with standard solutions of neutral pesticides in methanol ranging 1–1000 µg/l, using LC–APCI-MS–MS in the PI mode[a]

Compound	t_R (min)	Parent ion selected, (m/z)	Daughter ions used for quantification (m/z)	Concentration range studied for linearity (µg/l in injected solution)
(1) Deisopropylatrazine	10.54	174	132+146	5–1000
(2) Desethylatrazine	16.12	188	146	5–1000
(3) Carbendazime	19.07	192	160	5–1000
(4) Simazine	27.27	202	124+132	1.25–1000
(5) Atrazine	33.71	216	174	1.25–1000
(6) Isoproturon	34.93	207	72+165	5–1000
(7) Triadimenol	39.16/40.04	296	296	5–1000
(8) Terbuthylazine	41.16	230	174	1.25–1000
(9) Tebuconazole	45.99	308	308	5–1000
(10) Flusilazole	47.04	316	210+288	1.25–1000
(11) Penconazole	49.02	284	284	5–1000
(12) Propiconazole	49.56/50.09	342	159+245	1.25–1000

[a] Volume injected: 20 µl.

niques, varying between 2 for DIA and 90 for triadimenol.

Flusilazole is the most sensitive of the studied substances in the two modes, whereas DIA is the least sensitive due to the weak ionization of this molecule in APCI. The ratio of the sensitivities between these two compounds is 40.

The quantification limits, defined as the signal producing a ratio with the background noise (S/N) of more than 3, are summarized in Table 4 for both extraction methods used: extraction in liquid–liquid mode on 1 l of sample, and extraction in large-volume SPE mode on 10 l of sample.

The combination of large-volume SPE extraction and LC–APCI-MS–MS makes it possible to attain quantification limits of 0.2–0.5 ng/l instead of 20

Table 4
Limits of detection (LODs) obtained with LC–APCI-MS and LC–APCI-MS–MS for selected pesticides after liquid–liquid extraction of a 1-l sample with dichloromethane or SPE extraction using 1 g C_{18}-bonded silica cartridges and SPE extraction of a 10-l sample on Carbopack B (500–666 µm) graphitized carbon black[a]

Compound	Selected ions	Liquid–liquid extraction and SPE using 1 g C_{18}-bonded silica (volume: 1 l)		SPE large volume extraction (10 l) on Carbopack B	
		LC–MS in full scan mode and ion extraction	LC–MS	LC–MS in full scan mode and ion extraction	LC–MS
DIA[b]	174	20	5	1	0.5
DEA[b]	188	20	3	1	0.3
Carbendazim[b]	192	50	6	n.d.	n.d.
Simazine[b]	202	20	3	0.7	0.3
Atrazine[b]	216	20	2	0.6	0.2
Isoproturon[b]	207	20	7	n.d.	n.d.
Triadimenol	296	100	2	n.d.	n.d.
Terbuthylazine[b]	230	20	2	0.5	0.2
Tebuconazole	308	20	4	n.d.	n.d.
Flusilazole	316	20	1	n.d.	n.d.
Penconazole	284	20	2	n.d.	n.d.
Propiconazole	342	20	5	n.d.	n.d.

[a] n.d.: Non determined.

[b] C_{18}-bonded silica SPE was used for these compounds in groundwater samples and for DIA, DEA, carbendazim analysis in surface water samples.
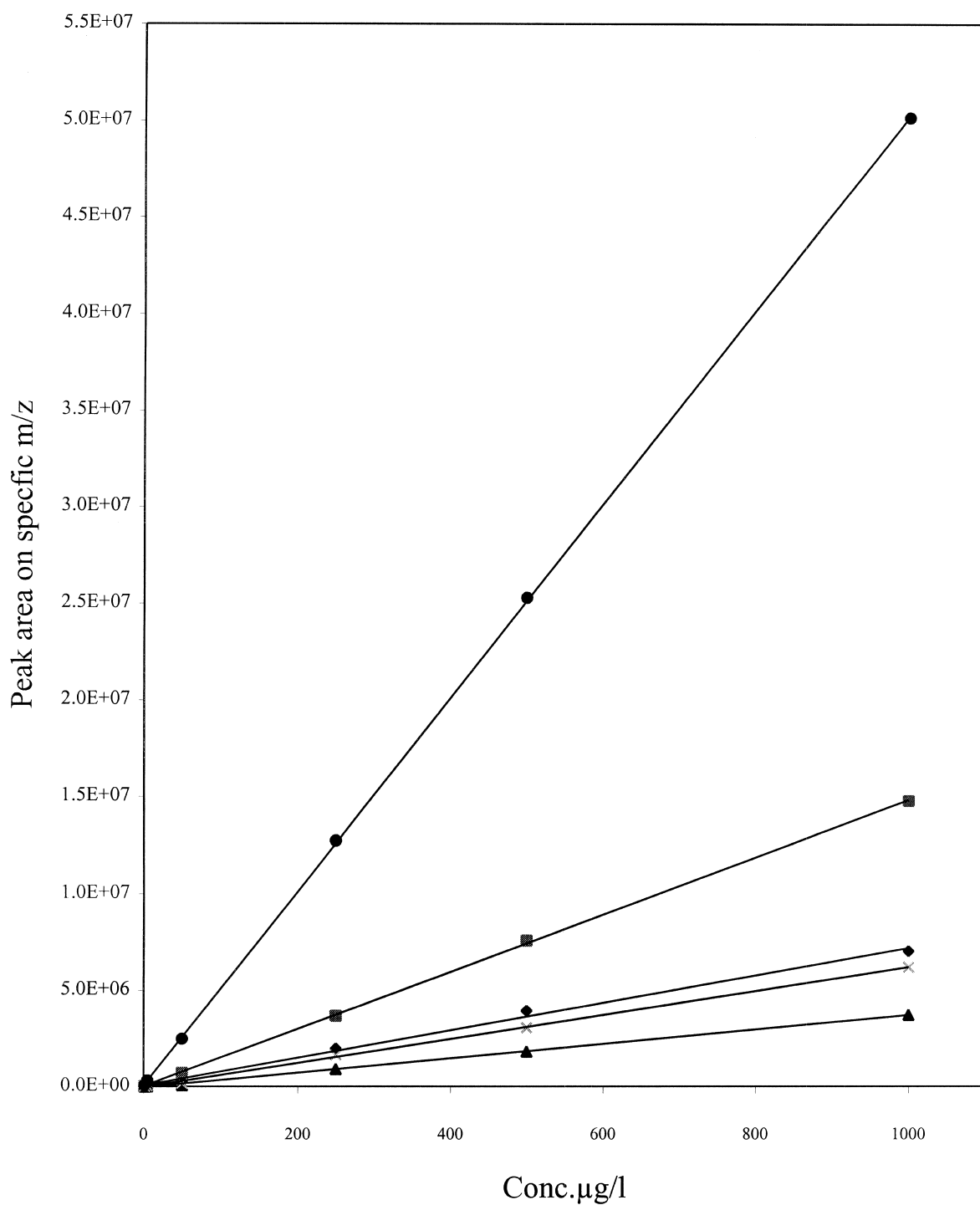
Fig. 4. Calibration curves of five analytes from LC–APCI-full scan MS–MS data. ♦ DEA, ■ triadimenol, ▲ isoproturon, ✕ propiconazole, ● flusilazole.

ng/l in LC–APCI-MS in full scan mode, with ion chromatogram extraction on selected m/z after extraction of a 1-l water sample.

3.3.1. Application to real samples

The LC–MS technique in combination with the DAD applied to real samples with the ESI and APCI interfaces in the negative mode and the positive mode has shown its capacity to detect, identify and quantify a list of 48 neutral and acid substances, including some degradation products (see details in Experimental section above). Sixty ground- and surface-water samples were analyzed after extraction

of 1 l for surface-water samples by the liquid–liquid method and 0.5 l to 1 l for groundwater samples by SPE using C_{18}-bonded silica cartridges; water from the St. Lawrence River was extracted by large-volume SPE (10 l) using Carbopack B graphitized carbon.

The LC–MS results were in keeping with the LC–DAD reference method [18]; the equation linking the LC–MS and LC–DAD values having a slope close to 1 (1.02 for diuron, 1.10 for DEA).

Due to its power of identification and its specificity, the LC–MS application also made it possible to eliminate (a) the spectral interferences in DAD noted in certain samples of atrazine (Fig. 5), and (b)
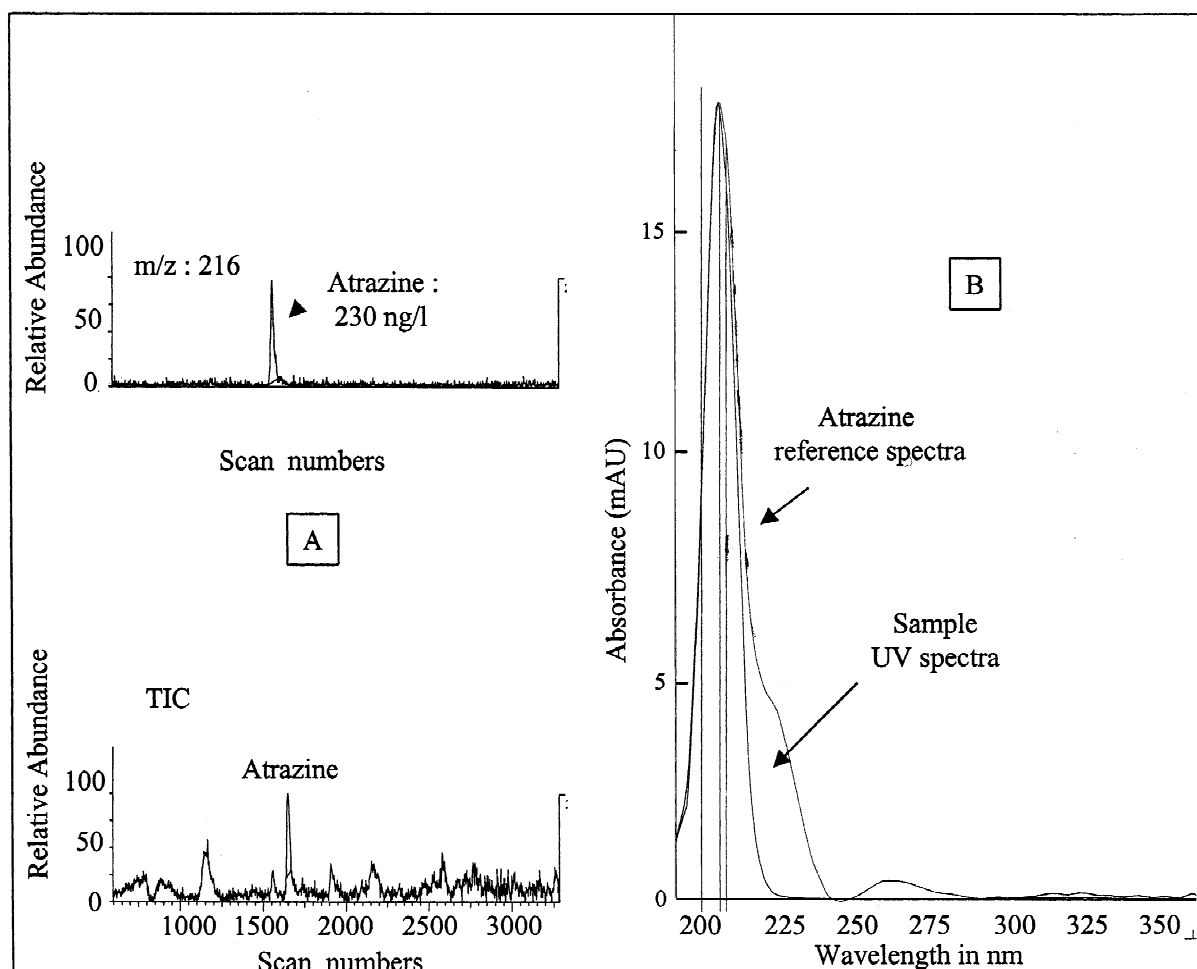
Fig. 5. Example of spectral interferences in DAD for atrazine solved by LC–APCI-MS analysis in the PI mode. The extract into a mixture of methanol–water (50:50, v/v) was obtained after SPE extraction of 1 l of groundwater sample on C_{18}-bonded silica cartridge. (A) LC–MS chromatogram in TIC mode and reconstructed ion chromatogram on m/z 216; (B) sample and reference atrazine UV spectra comparison.

the problems of co-elution of substances encountered and not resolved with DAD. In our application, for example, the trace amounts of isoproturon (95 ng/l) were difficult to quantify in the presence of high concentrations of chlortoluron (5400 ng/l); this problem was solved in LC–APCI-MS by ion extraction on m/z 207 and 213.

Fifteen substances could thus be identified, with concentrations varying between 7 ng/l and 800 ng/l as a result of the information obtained by LC–MS on the molecular mass, enhanced for certain substances by structural information resulting from a fragmentation obtained by CID in the octapole. The most commonly detected molecules were atrazine and its degradation product DEA, followed by simazine and diuron, and sometimes DIA. Terbuthylazine and its degradation product, desethylterbuthylazine, were also detected in some surface waters collected in wine-growing regions. For the acid compounds, only bentazone (m/z 239), dinoterb (m/z 239) and ioxynil (m/z 369) were detected by LC–DAD and LC–ESI^{-}-MS in a sample of well water from a farm.

Fig. 6 gives the results of a comparative study of LC–APCI-MS and LC–APCI-MS–MS applied to six substances detected in 10 real samples (DEA: seven pairs of values; carbendazim: two pairs of values; atrazine: seven pairs of values; simazine: six pairs of values; terbuthylazine: two pairs of values; isoproturon: two pairs of values). The 26 pairs of results are obtained from the three types of extraction contained within the 2–250 ng/l interval and the two techniques (LC–MS and LC–MS–MS). The good agreement of the results obtained by LC–MS (itself correlated with LC–DAD) and LC–MS–MS is shown by the slope value (1.074) of the function expressing the relationship between the two techniques.

An isolated result of a high concentration of atrazine was processed separately so as not to amplify its influence on the evaluation of the correlation results (it being very much higher than the population of the other values). This result was similar for both methods (i.e., 2.55 µg/l for LC–MS and 2.62 µg/l for LC–MS–MS).

Due to the lower quantification limits obtained with LC–MS–MS (Table 4) as a result of acquisition in precursor ion selection mode followed by fragmentation, which produces a higher S/N ratio

that is hardly (if at all) affected by the matrix, LC–MS–MS generates chromatograms and mass spectra that are less influenced by analytical background noise than are those obtained by LC–MS. The richest and most reliable structural information on trace levels obtained from the chromatograms acquired by LC–MS and LC–MS–MS of real samples, after liquid–liquid extraction, liquid–solid extraction and large-volume SPE extraction, respectively, is illustrated in Figs. 7–8 and 9–10. These figures illustrate the greater capacity of LC–MS–MS for identifying trace amounts of the compounds. Thus the two fungicides flusilazole (peak 9, Fig. 8) and tebuconazole (peak 8, Fig. 8) were identified with a low background noise at the 9 ng/l concentration level in a surface water sample using the liquid–liquid extraction method. In one large-volume SPE extract from the St. Lawrence River the full scan product spectrum (Fig. 11) for the m/z 230 precursor ion isolated at the retention time 39.23 (Fig. 10) allowed the identification of propazine. In the same sample, a peak previously detected by LC–MS at the retention time of propazine (Fig. 9) could not be reliably identified due to the high analytical background noise. The mass spectra of the MS–MS technique were decisive in identifying these three compounds in trace amounts by LC–MS–MS. The main advantage of the LC–MS–MS technique compared to the CID LC–MS interface is that there is no uncertainty as to the origin of fragments in the daughter ion spectrum. In the former application, the acquisition mode used for LC–MS–MS was first based on the ion trap detector in the precursor ion selection mode for some monitored compounds, followed by the full scan of the daughter ions. For the screening of unknown samples, it may be more convenient to work in the dependence mode, a method not used here. This mode, full scan MS followed by a full scan of daughter ions, is acquired in case a signal is detected at a predetermined value.

Instead of working off-line with large volumes, an alternative method for the analysis of six triazines and eight phenylureas at the quantification threshold of 10–30 ng/l, using the online preconcentration technique on small volumes (10 ml) and combined with LC–APCI-MS–MS, was described in a recent article [19] demonstrating the high potential of LC–MS–MS.
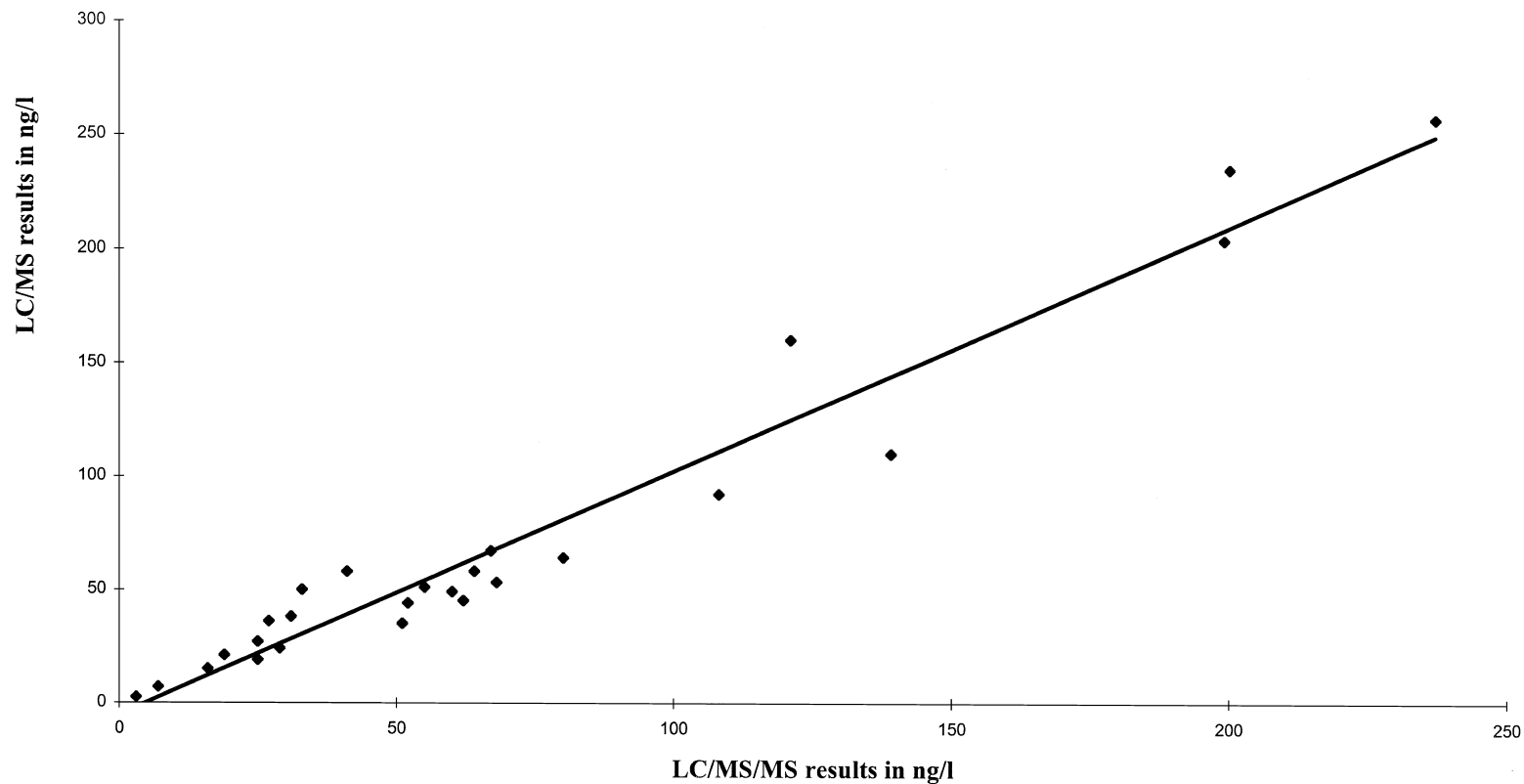
Fig. 6. Results of a comparative study showing a good correlation between LC–APCI-MS and LC–APCI-MS–MS analysis carried out on 10 real water samples including 26 data and five compounds. Extracts were obtained from: liquid–liquid extraction of 1 l for surface waters, enrichment of 1 l by SPE on C_{18}-bonded silica for groundwater, 10-l St. Lawrence River samples enrichment by SPE on Carbopack B (550–666 μm). Compounds studied: DEA, carbendazim, atrazine, simazine, terbuthylazine, isoproturon.
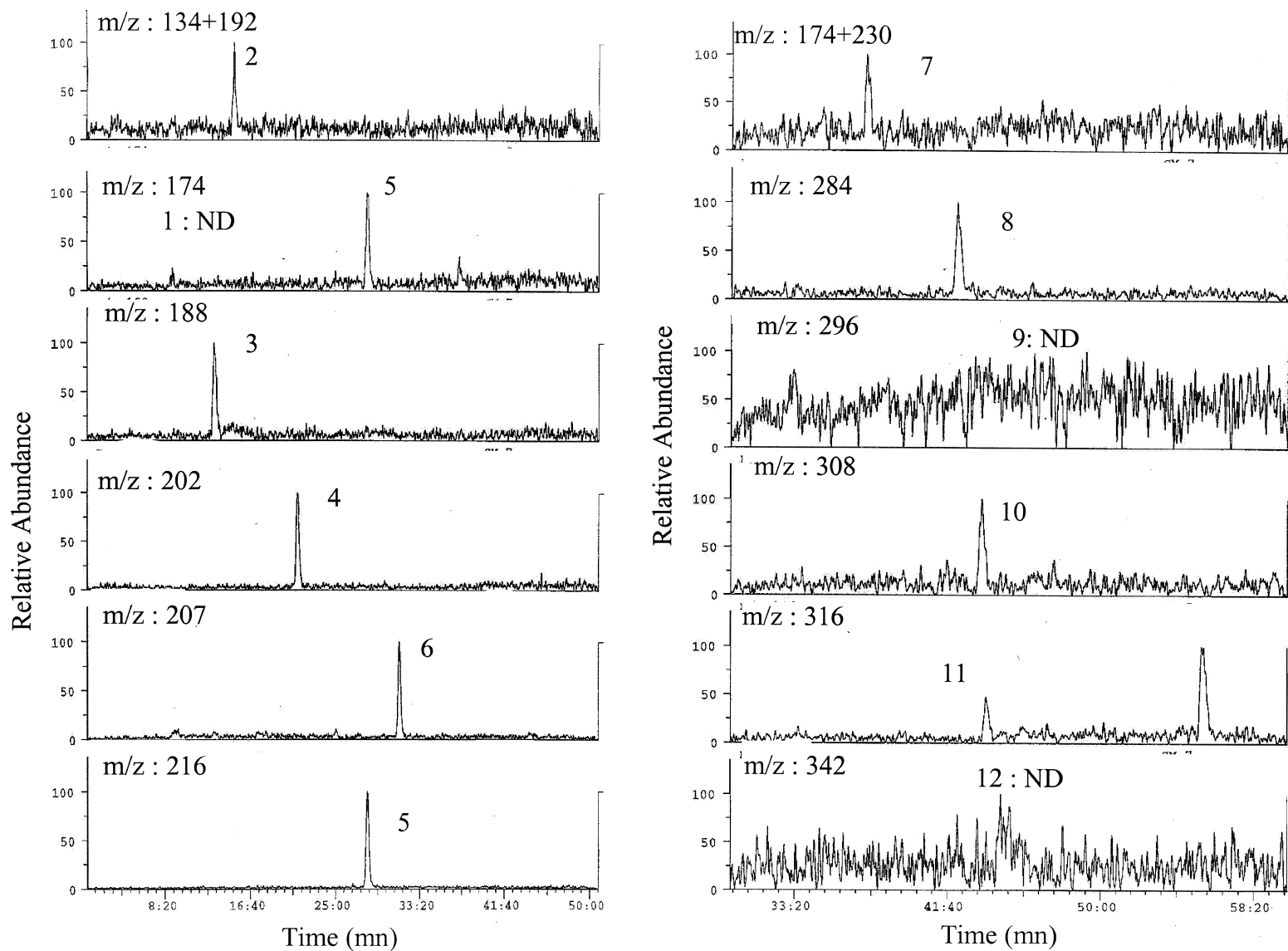
Fig. 7. Reconstructed ion chromatograms obtained from an LC–APCI-MS full scan analysis of a surface water sample after extraction of 1 l by the liquid–liquid method. ND: non detected. Peak assignments (results in ng/l): 1=DIA (ND); 2=carbendazim (36 ng/l); 3=DEA (58 ng/l); 4=simazine (53 ng/l); 5=atrazine (143 ng/l); 6=isoproturon (51 ng/l); 7=terbuthylazine (7 ng/l); 8=penconazole (44 ng/l); 9=triadimenol (ND); 10=tebuconazole (9 ng/l); 11=flusilazole (9 ng/l); 12=propiconazole (ND).
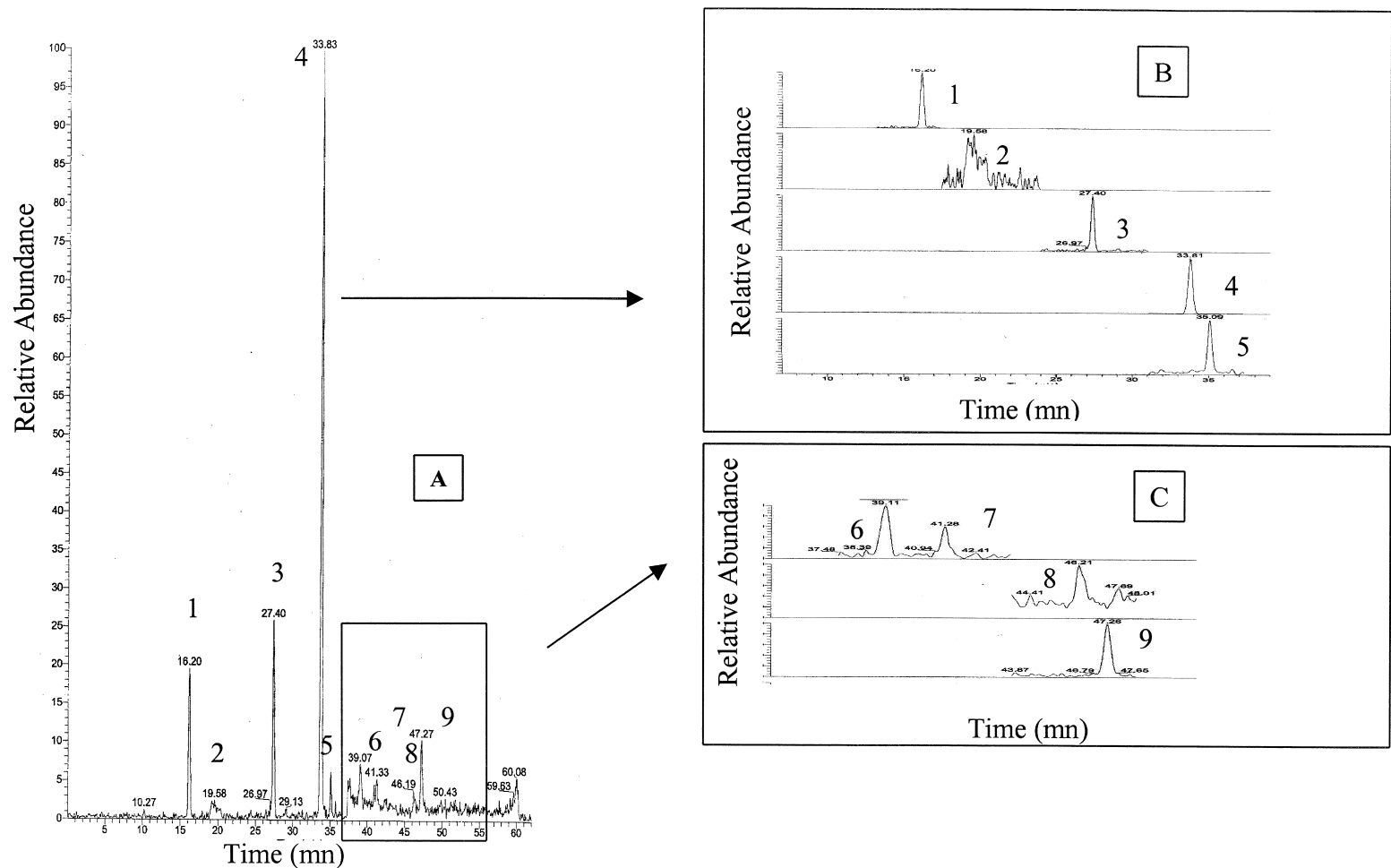
Fig. 8. Chromatograms of an LC–APCI-full scan MS–MS analysis of a surface water sample after liquid–liquid extraction. (A) Chromatogram; (B and C) reconstructed ion chromatograms. Peak assignments (results in ng/l): 1=DEA (58 ng/l); 2=carbendazim (36 ng/l); 3=simazine (53 ng/l); 4=atrazine (143 ng/l); 5=isoproturon (51 ng/l); 6=propazine (n.q.); 7=terbuthylazine (7 ng/l); 8=tebuconazole (9 ng/l); 9=flusilazole (9 ng/l). n.q.=Non quantified.
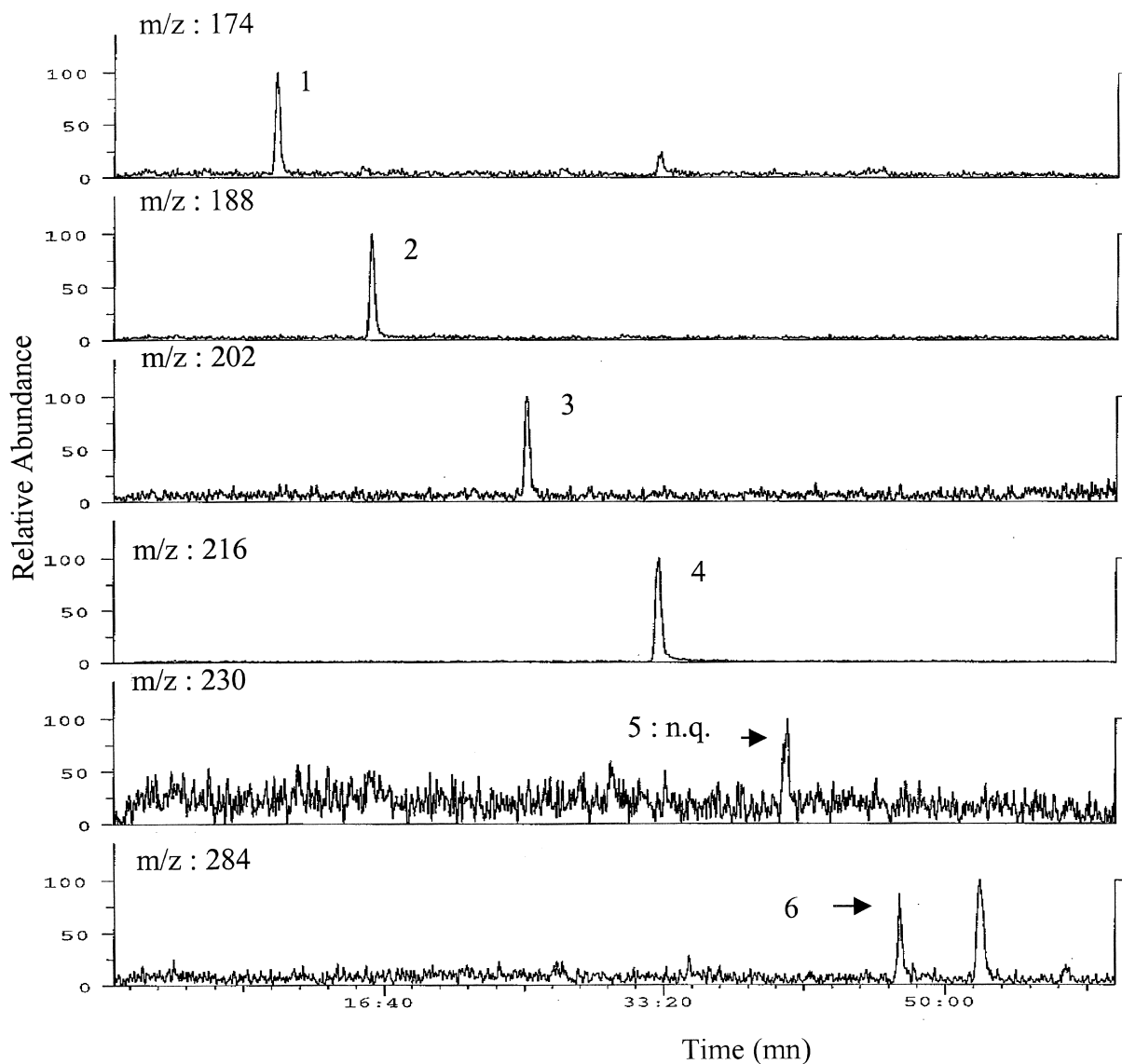
Fig. 9. Reconstructed ion chromatograms from an LC–APCI$^+$-MS full scan analysis of 10-l St. Lawrence River water sample enrichment by SPE on Carbopack B (550-666 μm). n.q.: Non quantified. Peak assignments (results in ng/l): 1=DIA (7 ng/l); 2=DEA (15 ng/l); 3=simazine (2.6 ng/l); 4=atrazine (21 ng/l); 5=propazine (n.q.<1 ng/l); 6=metolachlor (6 ng/l).

4. Conclusions

The quantitative analysis of pesticides in real samples of water using the LC–MS–MS technique with an ion trap spectrometer and the APCI interface correlates well with the quantitative results obtained for the same extracts using LC–MS with CID. LC–

MS results agreed with those obtained with the LC–DAD reference method for samples showing no spectral interference. More sensitive than LC–MS by a factor of 2 to 22, due to a better signal-to-noise ratio based on the mode of selection of a specific ion before fragmentation, LC–MS–MS allows for the multiresidue analysis of pesticides with lower quanti-
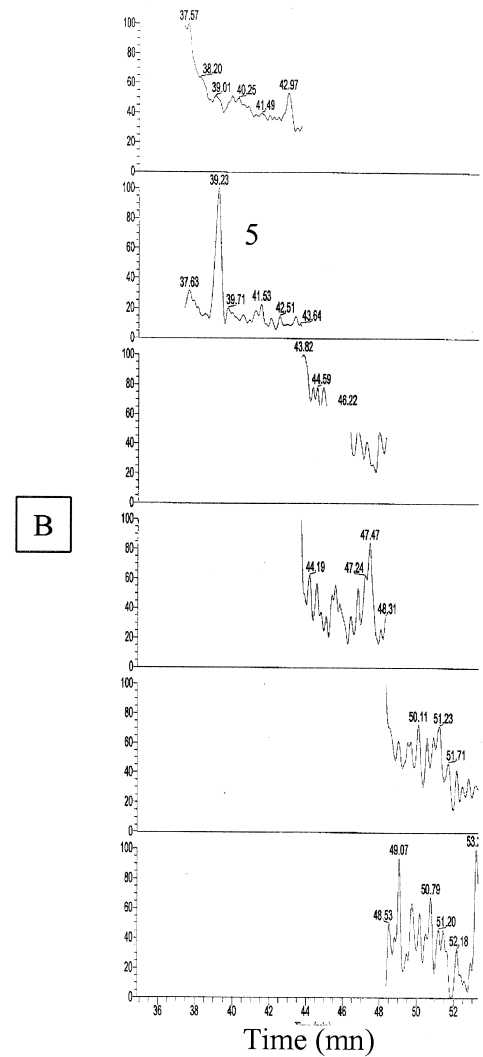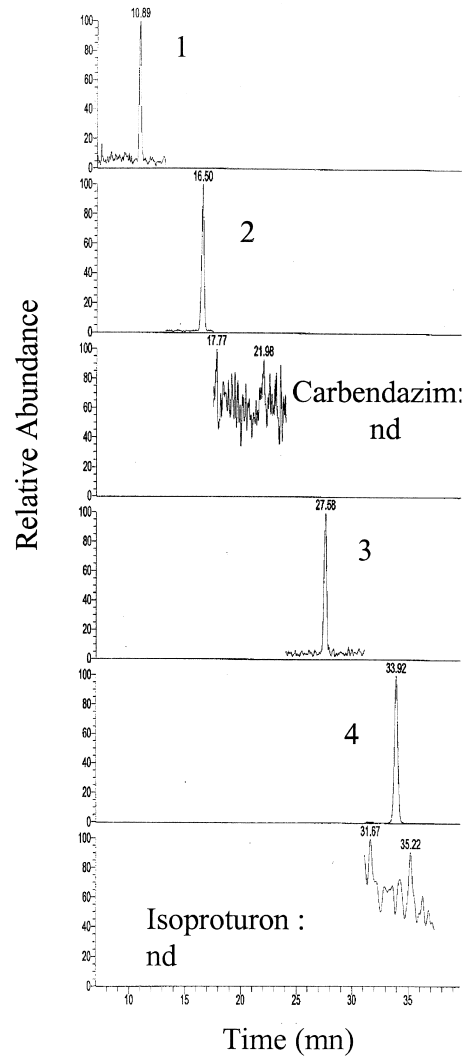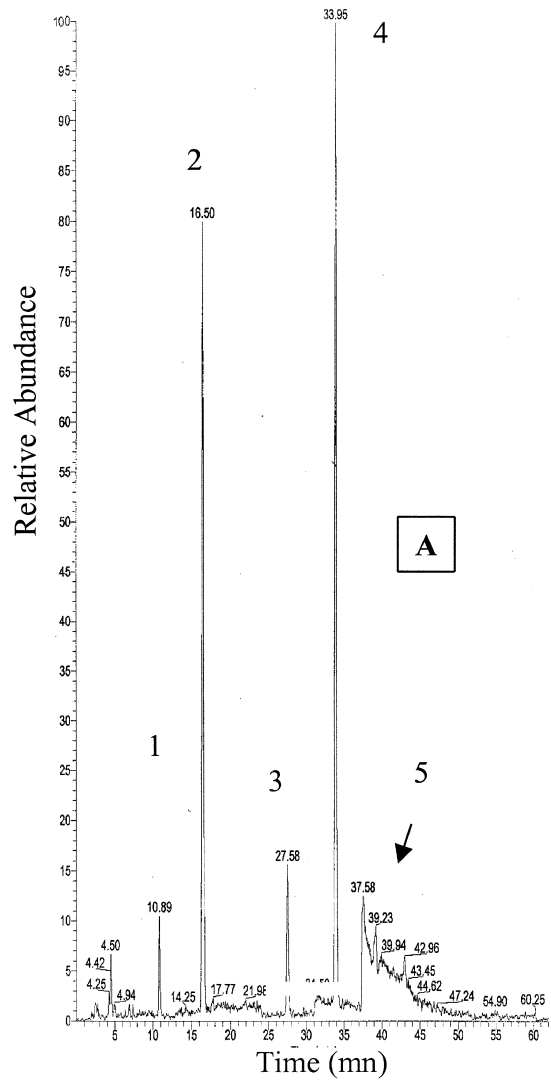
Fig. 10. Chromatogram from an LC–APCI-full scan MS–MS analysis of 10-l St. Lawrence River water enrichment by SPE on Carbopack B (550–666 μm). (A) Chromatogram; (B) reconstructed ion chromatogram. Peak assignments (results in ng/l): 1=DIA (7 ng/l); 2=DEA (15 ng/l); 3=simazine (2.6 ng/l); 4=atrazine (21 ng/l); 5=propazine (n.q.<1 ng/l). n.q.: Non quantified.
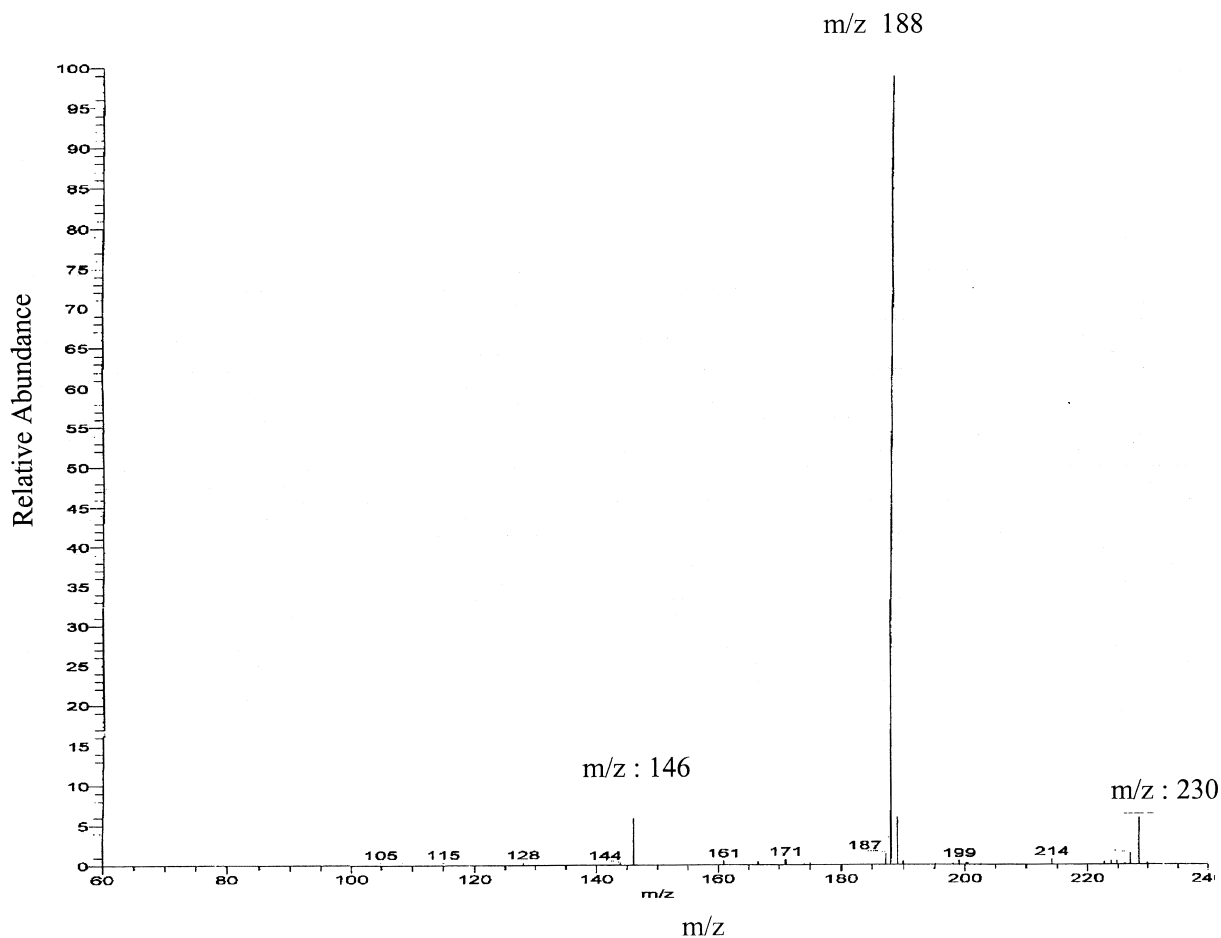
m/z 188

Fig. 11. Confirmation of propazine in a St. Lawrence River water SPE extract by the full scan product spectrum using LC–APCI-MS–MS. Concentration of propazine in this water sample was less than 1 ng/l.

fication limits (reduction by a factor of 4 to 10), and a more reliable identification of the detected and unknown compounds. Reliable structural information can be obtained even at concentrations of a few ng/l.

Acknowledgements

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