Available online at www.sciencedirect.com
SCIENCE DIRECT®
Talanta 65 (2005) 603–612
Talanta
www.elsevier.com/locate/talanta

Short communication

Identification of phenyl-*N*-methylcarbamates and their transformation products in Tunisian surface water by solid-phase extraction liquid chromatography–tandem mass spectrometry

Latifa Latrous El Atrache[a,*], Sadok Sabbah[a], Jean Pierre Morizur[b]

[a] *Laboratoire de Chimie Analytique et Electrochimie, Département de Chimie, Faculté des Sciences de Tunis, Campus Universitaire, 1060 Tunis, Tunisia*
[b] *Laboratoire Analyse et Environnement, Bâtiment des Sciences, Université d'Évry val d'Essonne, Évry-Paris*

Received 17 March 2004; received in revised form 15 June 2004; accepted 30 June 2004
Available online 15 September 2004

Abstract

Liquid chromatography-pneumatically assisted electrospray mass spectrometry with both negative and positive ionization has been used for the determination of carbamates pesticides and their transformation products in Tunisian surface water. Eight pesticides and four of their hydrolysis products were covered in this study.

Optimization of electrospray inlet conditions is described as well as results from investigations of the linearity of the detector response. Conditions for tandem mass spectrometry (MS–MS) detection of characteristic daughter ions formed by collision induced dissociation (CID) of the parent ion are described. Detection limits using MS in the selected ion monitoring (SIM) mode were generally in the order of 0.5 $\mu g\,L^{-1}$ or below. A principle of analysis is proposed based on triple quadrupole MS as a method for quantitative determination followed by verification of positive findings by CID–MS–MS. Application of the method for detecting carbamates residues in surface water is demonstrated.

Keywords: *N*-Methylcarbamates; Liquid chromatography–mass spectrometry; Water analysis

1. Introduction

The importance of the presence of carbamates and their transformation products (TPs) in water must also be considered. These TPs are formed as a consequence of several processes such as hydrolysis, biodegradation, oxidation, photolysis, biotransformation, etc [1–4]. These processes lead to compounds more toxic than the parent pesticides [1] and more persistent in the environment. To analyze carbamates and their hydrolysis products liquid chromatography (LC) has been applied routinely [5–7].

As regards detection, it has been found that substances can only be definitively identified by coupling LC to mass spectrometry (MS) using different interfaces [8,9]. Previously, these couplings were carried out with particle beam (PB) and thermospray (TS) interfaces and were of little interest for environmental analysis because of their limited scope of application, their lack of sensitivity and repeatability and non-linearity of the calibration curves. However, during the last few years, atmospheric pressure ionization (API) techniques, high flow pneumatically assisted electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) have become the more popular interfacing techniques. Both of these API techniques are soft ionization methods that predominantly give rise to the protonated $(M + H)^+$ or deprotonated $(M - H)^-$ molecular ions in positive and negative mode, respectively, and adduct ions resulting from the compound ions combining with the mobile phase [10–12]. The degree of confidence in the identification of the

* Corresponding author. Fax: +216 71 885 008.
E-mail address: latrous@wowmail.com (L.L.E. Atrache).

0039-9140/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2004.06.039

compounds can be enhanced using a quadrupole combined with collision-induced dissociation (CID), obtained, depending on the LC–MS apparatus, by applying a declustring potential (DP) 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 [13–16]. In particular using triple quadrupole detectors in MS–MS allows the following steps: ionization of molecules, ion isolation, ion collision with inert gas and detection of daughter ion fragments. Ions produced after the collision of molecules provide reliable information on molecular structure.

We had previously successfully analyzed phenyl-*N*-methylcarbamates insecticides and their transformation products in environmental waters using LC–UV [7]. We also briefly reported the applicability of ES–MS for the determination of carbamates in positive mode ion [7] but, to our knowledge, the application of this technique for the detection of both the carbamates and their hydrolysis products has not been published.

The aim of this paper was to further investigate the possibility of using LC–ES–MS in the positive ion and negative ion modes for the determination of carbamates and their hydrolysis products in surface water of different origins. Furthermore, another object of this study was to establish suitable conditions for performing MS–MS analysis using CID of molecular ion.

2. Experimental

2.1. Chemicals and materials

The HPLC grade-water was obtained by purification of demineralized water in a Milli-Q system (Millipore, Elix). Methanol, gradient grade, and acetonitrile, LC grade, were purchased from LAB SCAN (Ireland). The certified pure products were purchased from Supelco. The purity of all standards was a minimum of 99%.

Standard stock solutions of 1000 $\mu g\,mL^{-1}$ were prepared by weighing and dissolving 20 mg of each compound in 20 mL of methanol. These solutions were stored at 4 °C. The analyzed products were

- pesticides carbamates: aminocarb, baygon, carbofuran, carbaryl, methiocarb, baycarb, landrin and zectran;
- hydrolysis products: α-naphtol, 2-isopropoxyphenol, 2,3,5-trimethylphenol, 2,2-dimethyl-2-dihydrobenzofuran-7-ol.

Standard mixtures were prepared by appropriate dilution of a mixed stock solution with methanol ranging from 0.005 to 1 $\mu g\,mL^{-1}$.

Table 1
Important mass spectral fragments and their relative abundances (*R*%) obtained by LC–ES–MS at fragmentor voltage of 20 V

Compound	M_w	Positive mode *m/z* and tentative ions	*R*%	Negative mode *m/z* and tentative ions	*R*%
Aminocarb	208	209.3 $[M + H]^+$	10		
		152.2 $[M + H–CH_3NCO]^+$	45		
Baygon	209	232.2 $[M + Na]^+$	100		
		210.3 $[M + H]^+$	35		
		153.3 $[M + H–CH_3NCO]^+$	10		
Carbofuran	221	244.2 $[M + Na]^+$	100		
		222.1 $[M + H]^+$	100		
		165.2 $[M + H–CH_3NCO]^+$	16		
Carbaryl	201	224.1 $[M + Na]^+$	100		
		145 $[M + H–CH_3NCO]^+$	37		
		202.1 $[M + H]^+$	26		
Landrin	193	216.2 $[M + Na]^+$	100		
		194.3 $[M + H]^+$	47		
		137.3 $[M + H–CH_3NCO]^+$	27		
		211.3 $[M + NH_4]^+$	24		
Baycarb	207	230.1 $[M + Na]^+$	100		
		208.2 $[M + H]^+$	40		
		225.3 $[M + H–CH_3NCO]^+$	21		
Methiocarb	225	248.1 $[M + NH_4]^+$	100		
		226.1$[M + H]^+$	45		
		169.2 $[M + H–CH_3NCO]^+$	30		
Zectran	222	223 $[M + H]^+$	100		
		245.1$[M + Na]^+$	37		
		166.1 $[M + H–CH_3NCO]^+$	4		
α-Naphtol	144			143 $[M–H]^-$	100
Benzofuranol	163			135 $[M–H]^-$	100
2-Isopropoxyphenol	152			163 $[M–H]^-$	100
2,3,5-Trimethylphenol	136			151.1 $[M–H]^-$	100

Silica-based sorbents with octadécyle functional group Bond Elut Jr. C18 were acquired from Varian.

2.2. Apparatus

The LC–MS analyses were performed using a Perkin Elmer LC system consisting of a 200 quaternary pump, a Rehodyne injection valve (model 7125) and PESciex API 2000 triple quadrupole mass spectrometer equipped with ESI ionization source.

The HPLC column was Xterra, 250 × 4.6 mm i.d., 5 μm particulate size.

The LC–MS system was connected to analyst station for recording chromatograms.

2.3. Chromatographic conditions

An acetonitrile–water binary gradient of 20–100% acetonitrile in 40 min was used. The flow rate of the mobile phase was 1 mL min^{-1}. The column effluent was split, allowing only 100 μL to enter the mass spectrometer.

2.4. Mass spectrometric analysis

The ES–MS interface was operated in positive mode under the conditions of 325 °C gas temperature, 30 psi drying gas pressure, 35 psi nebulizer gas pressure, 70 psi additional gas pressure and 5000 V of capillary voltage. The experimental conditions of the ES in negative mode were the same used for the positive mode. Full-scan LC–MS chromatograms were obtained by scanning from *m*/*z* 50 to 400 amu. Time scheduled SIM of the most abundant ion of each compound was used for quantification. All MS–MS experiments were performed using N_2 as the collision gas at a collision cell pressure of 2 mTorr and with collision energy ranging between 5 and 20 eV. Mass analysis was performed in MS–MS product ion mode with the first quadrupole locked on the *m*/*z* value corresponding to the molecule ion of the target compound, with the second quadrupole known as the collision cell either locked on a characteristic product ion *m*/*z* or scanning from *m*/*z* 50 to ca.50 amu above the molecular mass of the target compound (product ion scan) and the third quadrupole that filters the product ions and allows ions of certain mass-to-charge ratio to pass through the detector.

2.5. Sample preparation

Solid-phase extraction was used to preconcentrate selected compound from water. Each sample (100 mL) [7] was extracted on the SPE cartridges packed with 1 g of C18 bonded silica, the solid phase was first conditioned with 6 mL of methanol, then with 7 mL of deionized water. Following sample extraction, the solutes were eluted with 5 mL of methanol. The eluent was collected in a graduated tube and concentrated, under stream of nitrogen with Kuderna-Danish to 1 mL.

3. Results and discussion

3.1. Optimization of LC–MS parameters

To optimize LC–MS conditions different parameters influencing mass spectra were checked in both positive and negative mode for ES sources. The drying and nebuliser nitrogen flow rates, the vaporizer and drying temperatures did not drastically improve the sensitivity, the optimum working conditions were those reported in Sections 2–4.

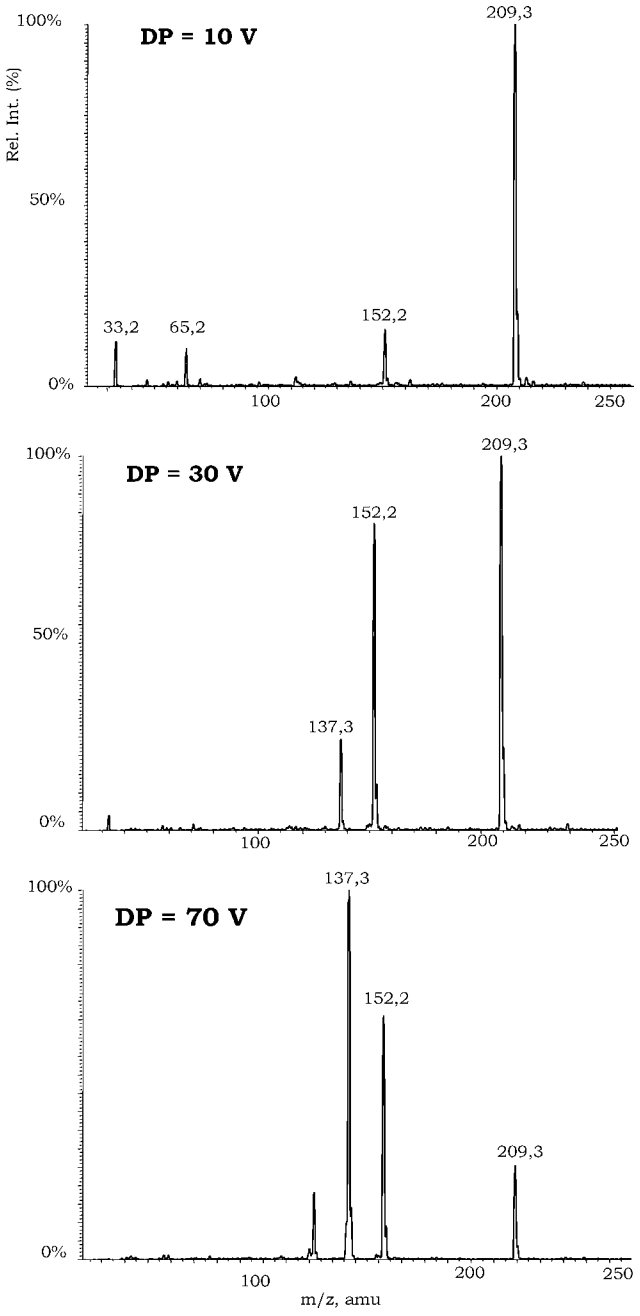

Fig. 1. Variation of the abundance (%) for some fragment ions of aminocarb vs. the extraction voltage.

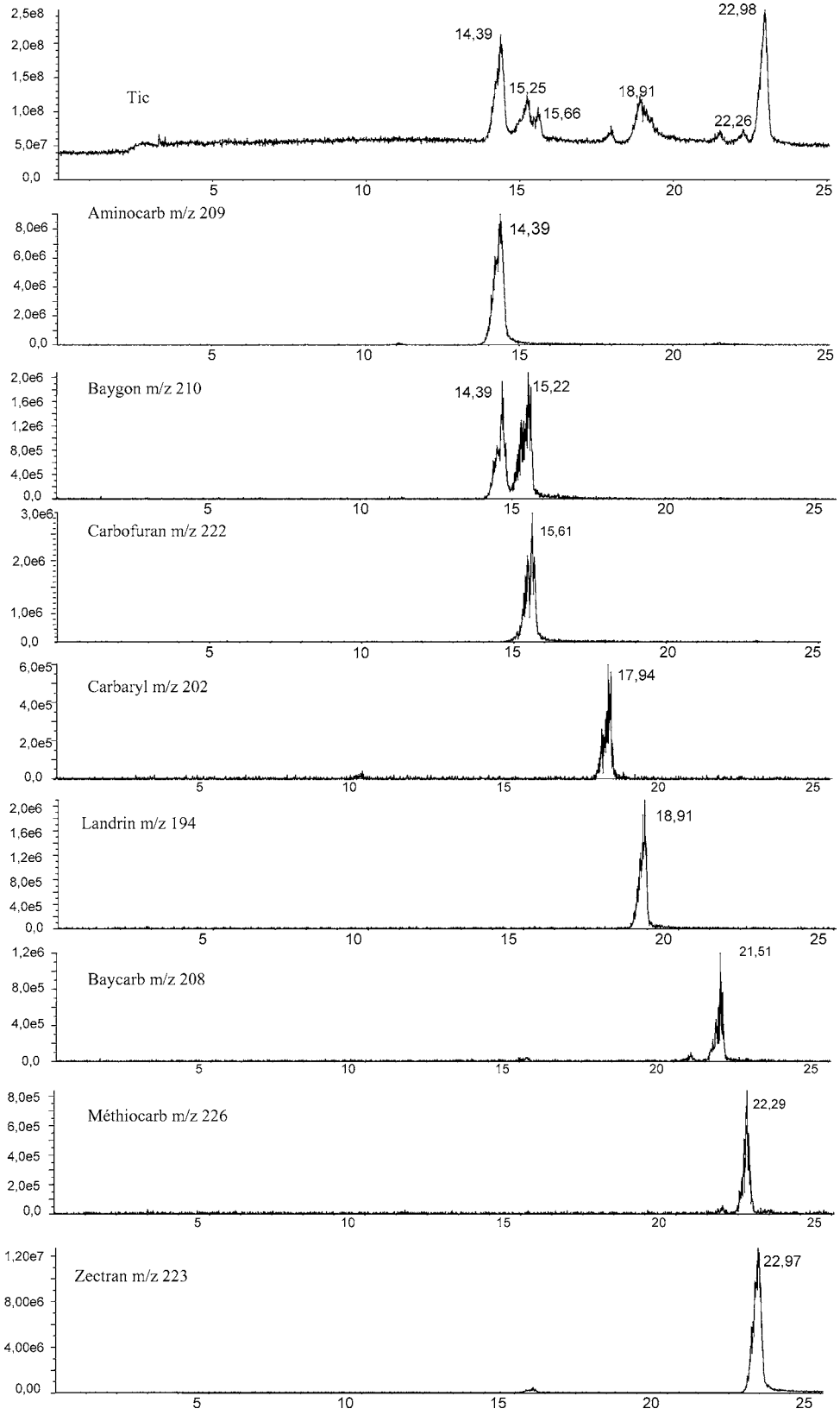

Fig. 2. Extracted ion chromatograms in positive mode corresponding to the selected pesticides of a standard mixture solution.

The fragmentor voltage or declustering potential was varied from 10 to 70 V in order to find the maximum response using the LC–MS conditions. In positive mode, the fragmentor voltage of 20 V provided molecular mass information through the quasi-molecular ions $[M + H]^+$, caused little fragmentation and the sensitivity was the highest for all the compounds. The main ions obtained and their tentative assignations are shown in Table 1. Mass spectra fragments followed the general patterns previously indicated in the literature [17–20]. The obtained mass spectra of carbamates show a pattern with the protonated molecular ion $[M + H]^+$ with the fragment ion $[M + H–CH_3NCO]^+$. Molecular adduct ions such as ammonium, sodium, potassium were also reported.

The effect of modifying the fragmentor voltage in the production of diagnostic ions is illustrated in (Fig. 1) for aminocarb in ES positive.

At 10 V, there are two main ions corresponding to mass 209.3 and 152 increasing the voltage to 30 V, a pick at *m/z* 137 appears. At 70 V the base peak in the spectrum is the fragment ion at mass 137, so aminocarb is converted to this fragment ion.

For TPs, Es in negative mode was used. In fact, the production of positive or negative ions depends considerably on the acidity of the analyte in question [21,22]. The formation of ions $[M - H]^-$ was observed for most of the TPs.

The system sensitivity was fully optimized using selected ion monitoring (SIM). It can be deduced that positive mode is required for carbamates detection, however negative mode could be a useful tool for TPs detection. Extracted ion chromatograms corresponding to characteristic *m/z* of the selected pesticides of a standard mixture are shown in (Fig. 2). In Fig. 3, a chromatogram is also shown for LC–ES–MS in negative mode analysis of the four standard TPs mixture. The quantification was carried out using the proposed SIM program in order to obtain lower detection limits. LC with ES provided a linear response from amount injected in the range of 0.005–1 $\mu g\,mL^{-1}$ with a good correlation coefficient (r^2 between 0.9872 and 0.9995). Detection limits (LODs), ob-

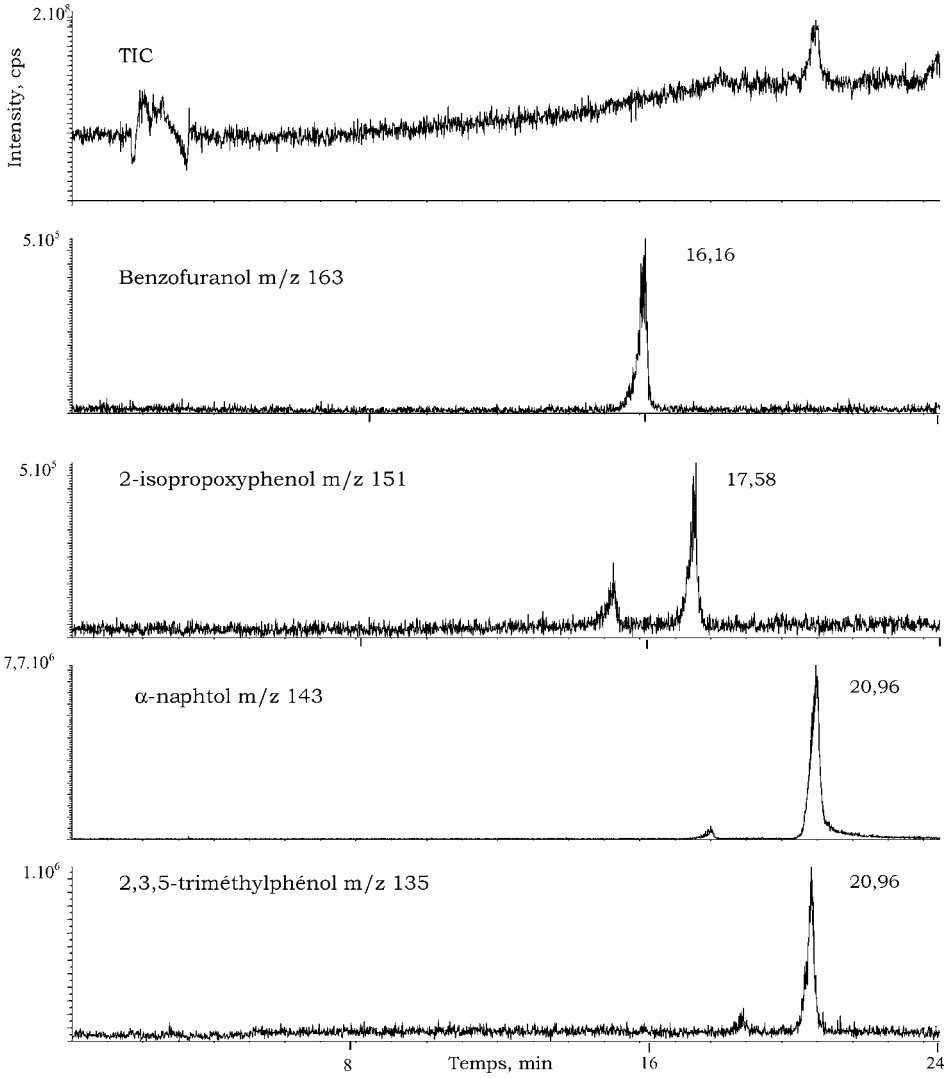

Fig. 3. Extracted ion chromatograms in negative mode corresponding to the selected hydrolysis product of a standard mixture solution.

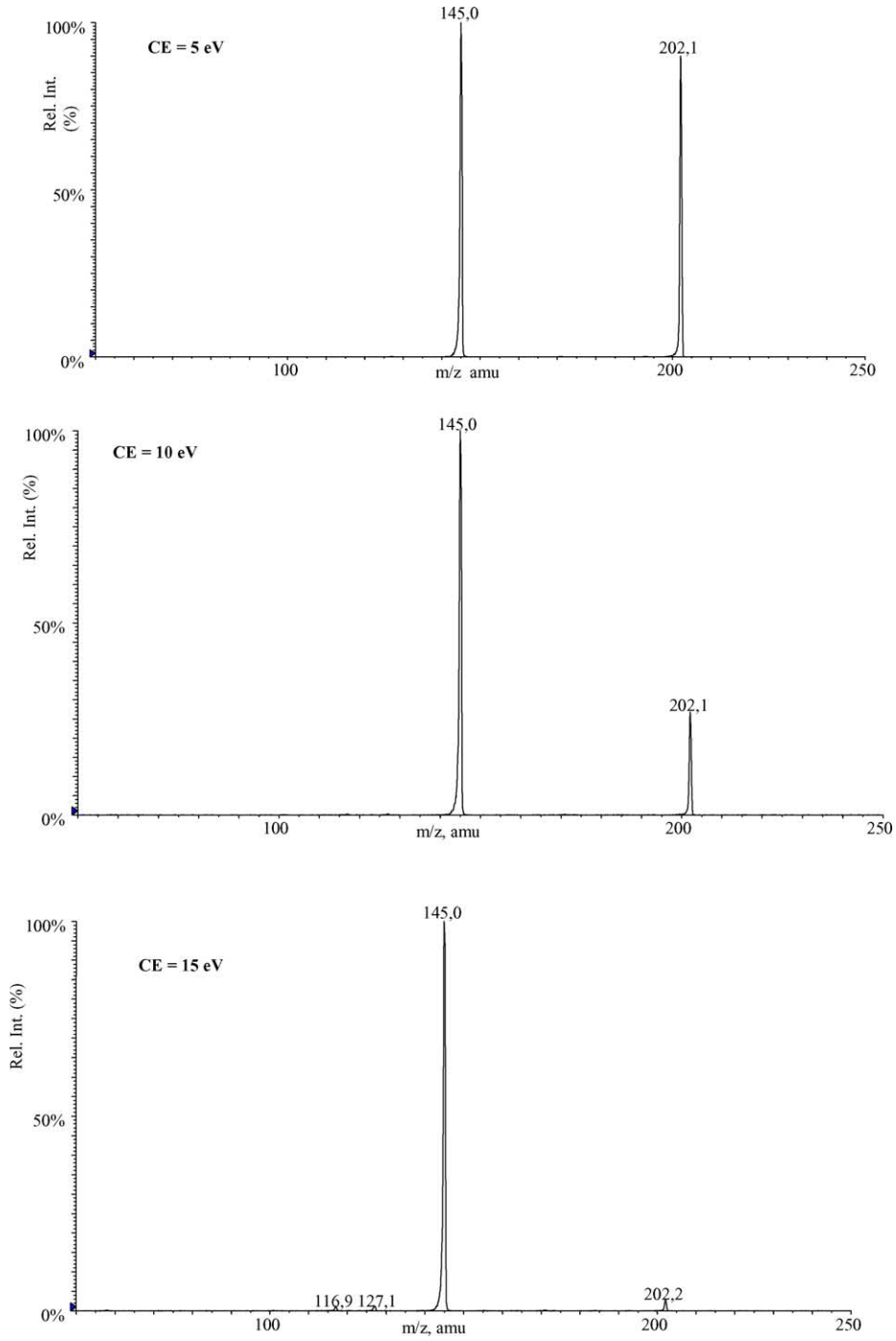

Fig. 4. The effect of increasing the collision energy of carbaryl.

tained by direct injection of the standard mixture and calculated with a signal to noise ratio of three in SIM mode, were between 0.1 and 0.5 $\mu g\,mL^{-1}$ (Table 2).

3.2. Conditions for CID MS–MS

MS–MS using CID is a means of obtaining structurally related spectral information from the initially formed parent ion. We have investigated the possibilities of using this technique to improve the probability of correct identification of carbamates and their hydrolysis products analyzed by ESI–MS. The extent of the fragmentation of the initially formed parent ion depends on the collision energy and the collision gas pressure in the collision cell between the first quadrupole and the collision cell. We have collected daughter ion spectra for the 12 solutes at collision

energies ranging between 5 and 20 eV. A typical example of the effect of increasing the collision energy is shown in (Fig. 4), where an increased degree of fragmentation of carbaryl parent ion is observed at higher collision energies. Table 3 lists data from daughter ion spectra of the eight carbamates covered in this study. The daughter ion spectra are obtained by injecting a 1 μg mL^{-1} standard solution of each pesticide under flow injection analysis conditions and collecting full scan (50–400 amu) daughter ion spectra.

3.3. Applications to real samples

The potential of the method for the analysis of surface water samples has been demonstrated. In our laboratory, samples of tap and surface water from different sampling sites (river, dam and lagoon) all over Tunis have been collected for pesticides residue analysis. Recovery studies were formed on water samples spiked with 5 and 10 μg L^{-1} of each compound (n = 5). Average recoveries were greater than 73% [7]. The relative standard deviations (R.S.D.) were in the

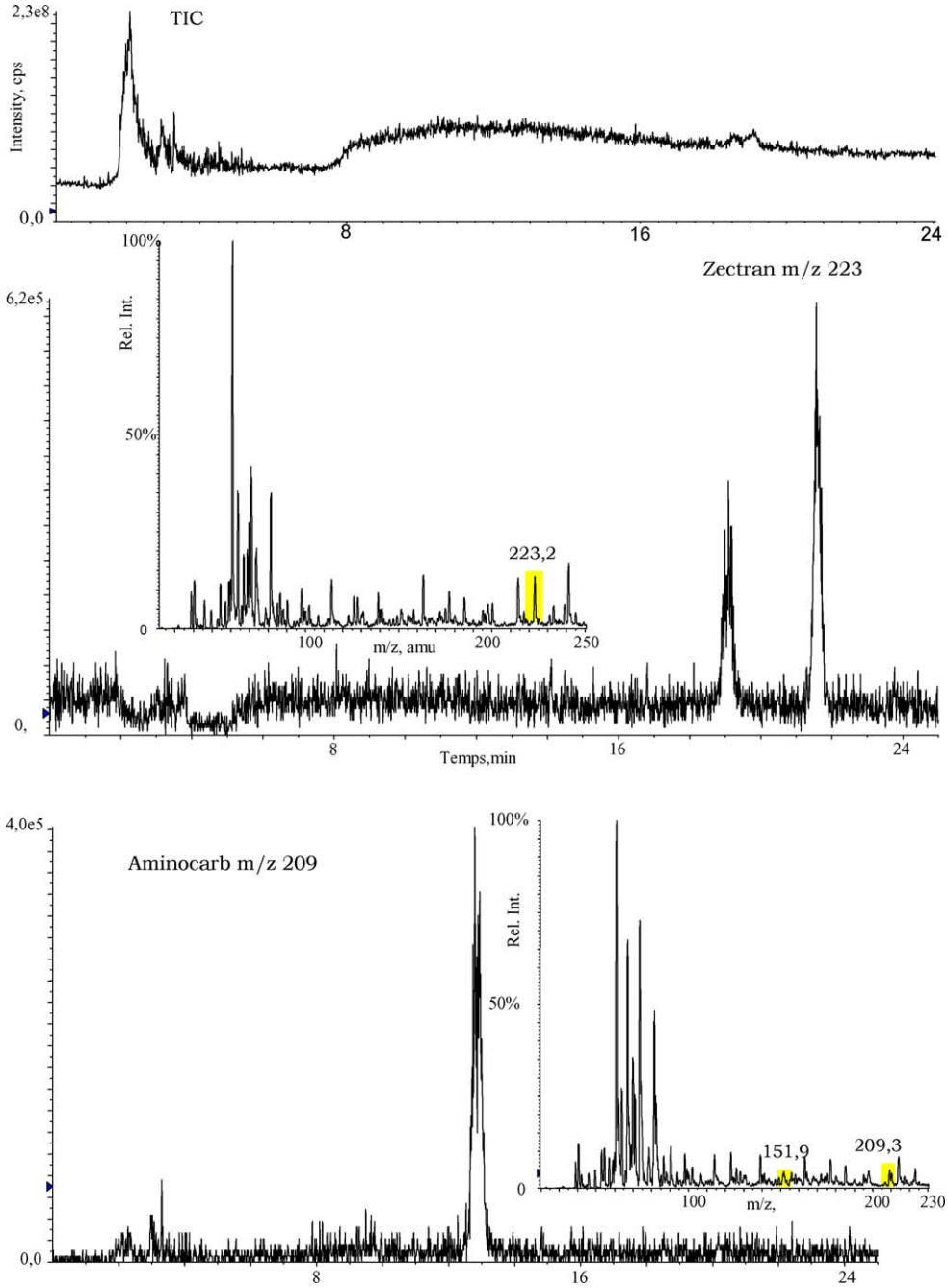

Fig. 5. LC–ESI–MS analysis of dam water sample following preconcentration by solid-phase extraction. Compound identification aminocarb and zectran.

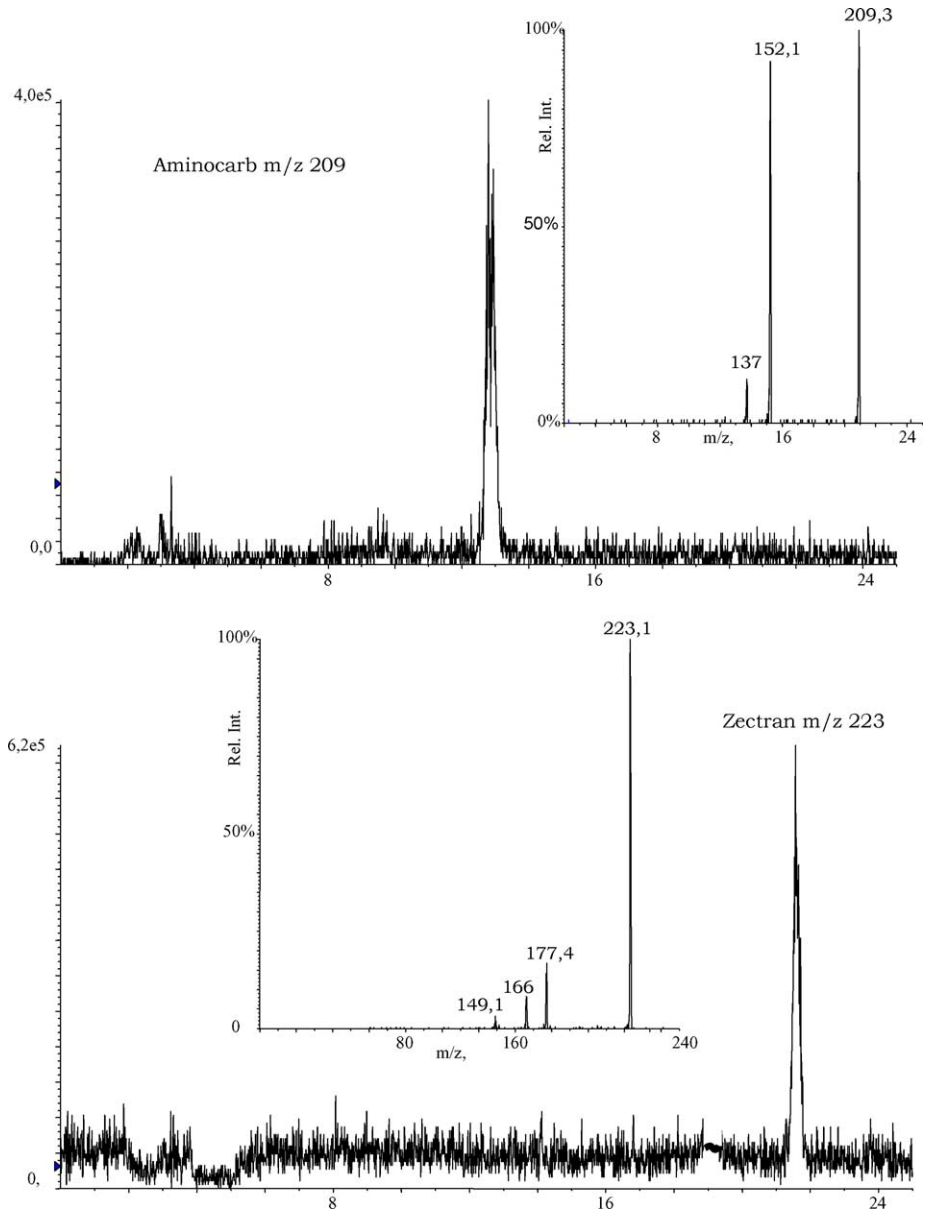

Fig. 6. Confirmation of aminocarb and zectran in dam water SPE extract by the full scan product spectrum using LC–ESI–MS–MS.

Table 2
Calibration data with LC–ESI–MS in SIM mode for the studied pesticides and their hydrolysis products

Compound	$y = ax + b$	R^2	LOD ($\mu g\,L^{-1}$)
Aminocarb	$2 \times 10^9 x + 8 \times 10^7$	0.9974	0.5
Baygon	$8 \times 10^8 x + 4 \times 10^7$	0.9906	0.2
Carbofuran	$4 \times 10^9 x + 5 \times 10^8$	0.9872	0.2
Carbaryl	$2 \times 10^9 x + 10^8$	0.9904	0.1
Landrin	$2 \times 10^9 x + 3 \times 10^8$	0.9958	0.2
Baycarb	$3 \times 10^9 x + 6 \times 10^8$	0.9948	0.5
Methiocarb	$4 \times 10^9 x + 5 \times 10^8$	0.9962	0.2
Zectran	$3 \times 10^9 x + 8 \times 10^8$	0.9943	0.2
α-Naphtol	$8 \times 10^7 x + 4 \times 10^5$	0.9956	0.2
Benzofuranol	$4 \times 10^6 x + 10^4$	0.9955	0.2
2-Isopropoxyphenol	$2 \times 10^6 x + 610^4$	0.9995	0.5
2,3,5-Trimethylphenol	$8 \times 10^5 x + 4 \times 10^3$	0.9956	0.5

range 3–11%. Unspiked samples were first analyzed for the eight carbamates insecticides and four of their TPs using LC with ESI–MS detection in SIM mode. Subsequently positive findings were verified by a second chromatographic analysis using MS–MS. An extract of Sidi Salem dam water was analyzed by SIM (Fig. 5). As can be observed we suspect the presence of aminocarb *m*/*z* 209 and zectran *m*/*z* 223 but the correspondence of the retention time and molecular weight couldn't provide sufficient specificity for identification of target compound since the mass spectra present weak peak intensity. In the second chromatographic analysis (Fig. 6), using MS–MS, these findings were verified by detecting characteristic product ions (*m*/*z* 137 and 152 fragment ions of aminocarb and *m*/*z* 177.4 and 166 fragment ions of zectran). We also confirm the presence of aminocarb *m*/*z* 209, landrin

Table 3
ESI–MS–MS data on product ion spectra for pesticides or pesticide degradation products obtained at different collision energies

Compound	M_W	Parent ion	Collision energy	Daughter ions (relative abundance %)
Aminocarb	208	209	5	209 (100), 152 (10)
			15	209 (91), 152 (100), 137 (20)
			20	209 (22), 152 (100), 137 (50)
Baygon	209	210	5	210 (100), 168 (50), 153 (26)
			10	210 (86), 168 (100), 153 (38), 111 (61)
			15	168 (45), 153 (15), 111 (100)
Carbofuran	221	222	5	222 (100), 165 (15)
			15	222 (55), 165 (100), 123 (18)
			20	222 (12), 165 (100), 123 (57)
Carbaryl	201	202	5	202 (91), 145 (100)
			10	202 (30), 145 (100)
			15	145 (100)
Landrin	193	194	5	194 (100), 137 (60)
			10	194 (50), 137 (100)
			15	137 (100)
Baycarb	207	207	5	208 (100), 152 (17)
			10	208 (100), 152 (51), 95 (56)
			15	208 (15), 152 (30), 95 (100)
Methiocarb	225	226	5	226 (100), 169 (48)
			10	226 (70), 169 (100)
			15	226 (12), 169 (100), 121 (25)
Zectran	222	223	5	223 (100)
			15	223 (100), 166 (60), 151 (10)
			20	223 (40), 166 (100), 153 (35)

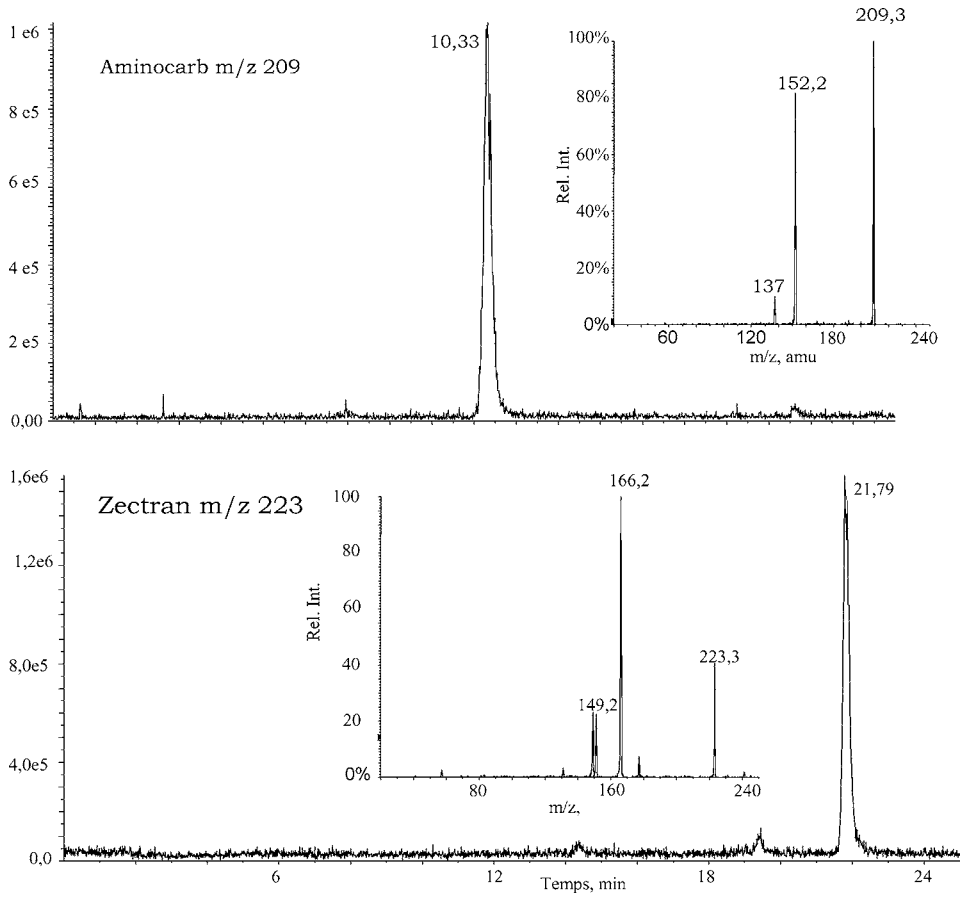

Fig. 7. Confirmation of aminocarb, landrin and zectran in lagoon water SPE extract by the full scan product spectrum using LC–ESI–MS–MS.

m/*z* 194 and zectran *m*/*z* 223 in Bizerte lagoon water sample. The CIDs spectra and daughter ions are shown in (Fig. 7).

4. Conclusion

LC using pneumatically assisted electrospray mass spectrometry detection both in negative and positive ion mode has been shown to be a highly advantageous technique for the identification of carbamates insecticides and their hydrolysis products in surface water. The soft electrospray ionization process results in the formation of the protonated molecular ion $[M + H]^+$of the pesticides and the formation of the deprotonated molecular ion $[M - H]^-$ of the transformation products, which can be determined with maximum sensitivity by SIM mode single MS. This will make the method potentially attractive for target compound analysis, like tap and surface water monitoring studies.

Furthermore, it has been demonstrated that increased confidence in carbamates identification can be obtained by MS–MS based on detection of product ions formed by CID of the initially formed protonated molecular ion. The contamination of Tunisian surface water by carbamates residues such as aminocarb, landrin and zectran was reported.

Acknowledgements

The authors want to thank V. Haldys and B. Desmasyères for excellent laboratory technical assistance.

References

[1] N. de Bertrand, D. Barceló, Anal. Chim. Acta 254 (1991) 235.
[2] M.J. Climent, M.A. Miranda, J. Chromatogr. A 738 (1996) 225.
[3] S. Chiron, J.A. Torres, A. Fernandez-alba, M.F. Alpendurada, D. Barceló, Intern. J. Environ. Anal. Chem. 65 (1996) 37.
[4] C. Hidalgo, J.V. Sancho, F.J. López, F. Hernández, J. Chromatogr. A 823 (1998) 121.
[5] I. Liska, J. Slobodnik, J. Chromatogr. A 733 (1996) 235.
[6] D. Barceló, Analyst 116 (1991) 681.
[7] L.L. El Atrache, S. Sabbah, Can. J. Chem. 81 (2003) 971.
[8] W.M.A. Niessen, A.P. Tinke, J. Chromatogr. A 703 (1995) 37.
[9] M.T. Meyer, M. Thurman, Monitoring pesticides and Metabolites in Surface Water and Ground Water in Spain, American Chemical Society, Washington, DC, 1995.
[10] S. Lacorte, G. Jeanty, J.L. Marty, J. Chromatogr. A 777 (1997) 99.
[11] D. Giraud, A. Ventura, V. Camel, A. Bermond, P. Arpino, J. Chromatogr. A 777 (1997) 115.
[12] C. Molina, P. Grasso, E. Benfenati, D. Barceló, J. Chromatogr. A 737 (1996) 47.
[13] W.M.A. Niessen, J. Chromatogr. A 794 (1998) 407.
[14] S. Lacorte, C. Molina, D. Barceló, J. Chromatogr. A 795 (1998) 13.
[15] B. Koppen, N.H. Spliid, J. Chromatogr. A 803 (1998) 157.
[16] A.C. Hogenboom, R.J.C.A. Steen, W.M.A. Niessen, U.A.Th. Brinkman, Chromatographia 48 (1998) 475.
[17] A. DiCorcia, C. Creszenci, A. Lagana, E. Sebastiana, J. Agri. Food Chem. 44 (19961930).
[18] M. Honing, J. Riu, D. Barceló, B.M.L. van Baar, U.A.Th. Brinkman, J. Chromatogr. A 733 (1996) 283.
[19] J.S. Salau, M. Honing, R. Tauler, D. Barceló, J. Chromatogr. A 795 (1998) 3.
[20] L. Lacassie, M.D. Dreyfuss, J.L. Daguet, M. Vignaud, P. Marquet, G. Lachâtre, J. Chromatogr. A 830 (1999) 135.
[21] H. Itoh, S. Kawasaki, J. Tadano, J. Chromatogr. A 754 (1996) 61.
[22] C. Aguilar, I. Ferrer, F. Borrull, R.M. Marcé, D. Barceló, J. Chromatogr. A 794 (1998) 147.