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Determination of ten carbamate pesticides in aquatic and sediment samples by liquid chromatography–ionspray and thermospray mass spectrometry

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Abstract

Ten carbamate pesticides which exhibit large differences in polarity were determined simultaneously in various environmental samples, using both column liquid chromatography (LC)–thermospray (TSP) mass spectrometry (MS) and LC–ionspray (ISP) MS. For sample clean-up, column chromatography with three stationary phases, neutral aluminium oxide, Florisil and aminopropyl-bonded modified silica, were tested. The aminopropyl stationary phase showed the best results, with acetone–dichloromethane (25:75) as eluent; analyte recoveries were 76–100% for all compounds with a relative standard deviation of 2–8%. In the ISP mass spectra of eight of the ten carbamates, the sodium adduct ion, $[M + Na]^+$, was the base peak, while the protonated molecule, $[M + H]^+$, was the most abundant ion with carbendazim and aminocarb. An eluent flow-rate between 100 and 300 μl/min was found to be optimum, with optimized nebulizer and drying gas flow-rates of 350 and 15 l/h, respectively. A capillary voltage of 3.5 kV resulted in the largest total ion current. The optimum extraction voltage with regard to analyte detectability and confirmation purposes was between 15 and 35 V. The detection limits obtained with the LC–ISP-MS system were typically in the 10–60 pg range (10 μl of 10 μg/l standard solutions), which is 10–150-fold better than obtained with LC–TSP-MS (selected-ion monitoring mode used in both instances). Large-volume injections of tap water spiked with selected carbamates at a level of 0.1 μg/l illustrated the potential of LC–ISP-MS with respect to analyte detectability. Furthermore, carbofuran was identified at a concentration level of ca. 5 μg/l in water samples from the Ebro delta. LC–TSP-MS of oxamyl and methomyl in a sediment sample containing a high percentage of organic matter was adversely affected by the presence of co-extractives. This problem did not occur with LC–ISP-MS.

Keywords: Environmental analysis; Water analysis; Sediments; Liquid chromatography–mass spectrometry; Sample preparation; Pesticides; Carbamates

1. Introduction

The need to obtain structural information, low limits of detection (LODs) and good reproducibility of data is of general importance for the determination and confirmation of pollutants in environmental samples. For the determination and confirmation of carbamates, various types of LC–MS interfaces, e.g., thermospray (TSP) [1–

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7], particle beam (PB) [8,9], heated pneumatic nebulizer (HN) with atmospheric pressure chemical ionization (APCI) [7,10–12] and electrospray (ESP) [7,13–15] interfaces have been used.

The use of the TSP interface for carbamate analysis provides structural information, although at the expense of sensitivity [1]. In all ionization modes, comparable mass spectra could be obtained from different types of TSP interfaces, including the interface used in this work, under standardized parameter settings [16]. Unfortunately, thermal degradation in the vaporizer was observed for some carbamates, e.g., methiocarb. Although this adversely affects the reproducibility of the ion signal (with R.S.D.s typically larger than 15%), it was recently used for carbamate quantification [17], which is indeed possible provided that all parameters are carefully adjusted and controlled.

The PB interface allows recording of electron ionization (EI) and of solvent-independent chemical ionization (CI) mass spectra and, thus, the structural confirmation of compounds of interest. Unfortunately, this is often accompanied by thermal dissociation of the carbamates, and LODs are relatively high [18]. In general, PB gives better precision than TSP, particularly at high concentration levels, while TSP is the more sensitive technique (for chlorinated herbicides) [19].

Recently developed carbamate LC–MS analyses involve the combination of an atmospheric pressure ionization (API) source with an HN interface, in APCI [12]. Although no clear evidence was found for thermally induced dissociation of the carbamates tested, it was suggested that the thermolabile compounds can undergo this type of degradation prior to ionization in the HN system [7].

The introduction of the ESP interface, which is combined with an API source, offers new possibilities for the determination of thermolabile and polar compounds [20]. Most applications employing ESP deal with large biomolecules [21,22], and environmental analysis has not received much attention as yet [23]. A major disadvantage of ESP is that low LC eluent flow-rates, typically below 10 μl/min, have to be used, which means that with conventional LC columns postcolumn splitting of the eluent stream must be applied. Modifications to ESP have been made to eliminate this problem: pneumatically [24], thermally [25,26] and ultrasonically [27,28] assisted nebulization have been reported. Although eluent flow-rates up to 100 μl/min can then be accommodated, the best sensitivity was still obtained in the low flow-rate range, typically 10 μl/min [29]. Recently, a pneumatically assisted electrospray [ionspray (ISP)] interface was developed and used for the determination of various types of compounds, using flow-rates up to 2 ml/min [23,30].

As regards carbamate pesticides, the main advantages of the ISP interface technique is the "cold" nebulisation of the column eluent. In addition, there is the possibility of collisionally induced fragmentation by the application of an extraction cone voltage, which can help to confirm compound identity [7,14,31–33]. The latter advantage holds for all API techniques. In an extensive comparative study on the performance of two types of API interfaces (ISP and APCI) and the TSP and PB interfaces, both API-related interfaces were found to provide structural information and good sensitivity [7].

In this study, a recently developed ISP interface [24,30] was optimized and used for the determination of ten selected carbamate pesticides in spiked tap water, surface water from the Ebro delta (Spain) and two types of spiked sediment. The performance was compared with that of a TSP interface.

2. Experimental

2.1. Chemicals

Carbendazim and carbofuran (both 99%) were purchased from Riedel-de Haën (Seelze, Hannover, Germany). Oxamyl, methomyl, aldicarb, propoxur, aminocarb, pirimicarb, promecarb and barban were obtained from Promochem (Wesel, Germany). Water (for chromatography; J.T.

Baker, Deventer, Netherlands), gradient-grade methanol (J.T. Baker), residue analysis-grade acetone (Merck, Darmstadt, Germany), and residue analysis-grade dichloromethane (Merck) were used to prepare eluent and for extraction. Ammonium acetate (98%, Merck) was dissolved in water and added postcolumn in the TSP experiments. Florisil, aminopropyl-bonded silica and neutral aluminium oxide (Merck) were used as sorbents in the clean-up columns. The columns were protected against water by adding a 1-cm layer of anhydrous sodium sulphate (Merck) on top of the sorbent.

2.2. Liquid chromatography

LC separations were performed on a 12.5 cm × 3.0 mm I.D. Merck column packed with 3-μm particles coated with a LiChrospher 60 RP-select B base-deactivated phase. Eluent flow-rates of 0.6 (TSP) and 0.3 ml/min (ISP) were delivered by a HP 1090 Series 1 solvent-delivery system (Hewlett-Packard, Waldbronn, Germany) and a Waters (Cincinnati, OH, USA) Model 616 pump system, which contained four pumps and was coupled to a Model 600S pump controller. For LC–TSP-MS, a linear gradient from 20% to 70% methanol in water was used; the initial conditions were held for 1 min; the gradient was completed in 31 min. A 175 m*M* ammonium acetate solution in water was added postcolumn with a Knauer (Bad Homburg, Germany) Model 64 pump at a flow-rate of 0.4 ml/min; this resulted in a total flow-rate of 1.0 ml/min and a final ammonium acetate concentration of 70 m*M* in the effluent entering the TSP interface. For LC–ISP-MS, methanol–water (40:60) was used for the initial 9 min, with a subsequent linear gradient to 85% methanol over 23 min. For the recovery studies with the various sorbents, an HP 1090 Series 1 (Hewlett-Packard) LC system equipped with an UV diode-array detector was used. For the determination of the linearity in the concentration range of the LC–ISP-MS system, 10 μl of standard solutions [methanol–water (40:60)] containing 5, 10, 50, 100, 400, 800 or 1000 μg/l of the analytes were injected. The separation conditions were the same as those used before.

2.3. MS detection

The LC–TSP-MS experiments were performed on an HP 5988A quadrupole mass spectrometer coupled to a HP 59970C data system (Hewlett Packard, Palo Alto, CA, USA). Typical temperatures of the vaporizer, tip and ion source were 135, 208 and 250°C, respectively. Because of the application of gradient elution, a vaporizer temperature programme had to be used. LC–ISP-MS analyses were performed on a VG Platform instrument (VG Biospec, Manchester, UK) equipped with a megaflow ISP interface with a coaxial flow-probe. Calibration of the ISP system was performed with sodium–methanol cluster ions, $[Na(CH_3OH)_n]^+$, as reported before for this type of interface [34].

With both systems, time-scheduled selected-ion monitoring (SIM) of the most abundant ion of each compound was used for quantification. Optimum performance was achieved with 350 l/h drying gas, 15 l/h nebulizer gas, 300 μl/min eluent flow-rate, an ion source temperature of 125°C and a cone extraction voltage of 20 V. Tuning of the ISP interface prior to quantification was performed with methanol–water (50:50) containing methomyl, promecarb and thiodicarb each at a concentration of 1 μg/ml.

2.4. Sample pretreatment

Water samples collected from canals in the Ebro delta in Spain were prefiltered over 0.45-μm PTFE fibre-glass filters (Millipore, Bedford, MA, USA) to eliminate particulate matter. Prior to extraction the C_{18}-bonded silica disks of 47 mm diameter and 0.5 mm thickness, which contain 500 mg of the bonded phase (J.T. Baker) [36], were washed with 10 ml of methanol under vacuum, then 10 ml of acetonitrile (after drying). Subsequently (taking care that the disks did not become dry), 30 ml water (for chromatography) and then 2 l of Ebro river water sample were passed through the disks. The extraction time was vacuum-adjusted to 1 h. After drying the

disks, the pesticides were recovered by two extractions with 10 ml of acetonitrile. After careful evaporation of the acetonitrile, the samples were dissolved in 500 μl of methanol.

Two types of sediment sample from the Ebro delta were used in the study. Sediment type P contains 31% clay, 57% silt and 12% sand and has an organic matter content of 3.3 mass-%; sediment type T contains 8% clay, 28% silt and 64% sand with 2.5 mass-% of organic matter. Before extraction and clean-up, the sediment samples were sieved through 125-μm sieves and freeze-dried. Next, the samples were spiked with standard carbamate solutions in methanol, to yield levels of 50 and 500 μg/kg. Subsequently, 10-g aliquots were Soxhlet extracted for 16 h with double-thickness cellulose extraction thimbles (80 mm × 22 mm I.D.) (Whatman, Maidstone, UK) with acetone–dichloromethane (50:50) [36]. Prior to extraction, the Soxhlet system and the thimbles were cleaned for 14 h by refluxing with methanol. The extracts were concentrated nearly to dryness in a rotary evaporator operating at 35°C; evaporation to dryness was then achieved under a nitrogen flow.

The Soxhlet extract was dissolved in 500 μl of *n*-hexane (for residue analysis). This solution was applied to the top of a 15-cm long glass column containing ca. 2 g of aminopropyl-bonded silica (purified by Soxhlet extraction for 14 h) and eluted with 20 ml of acetone–dichloromethane (25:75). After collection, the eluate was evaporated nearly to dryness in a rotary evaporator and to complete dryness under a nitrogen flow. Subsequently, the sample was dissolved in 1 ml of methanol–water (20:80). For LC analysis of the sediment and Ebro delta water samples, 25 μl were injected.

3. Results and discussion

Ten carbamates which cover the entire polarity range were analysed by LC–MS using both a TSP and a novel ISP interface. The selected carbamates were methomyl, oxamyl and aldicarb (oxime N-methylcarbamates), carbofuran, propoxur, aminocarb and promecarb (aryl N-methylcarbamates), carbendazim [methyl N-(2-benzimidazole)carbamate], pirimicarb (an N,N-dimethylcarbamate) and barban (an N-phenylcarbamate). The performance of the ISP interface was optimized and compared with that of the TSP interface, then LC–ISP-MS and LC–TSP-MS were used for the determination of the compounds in spiked and non-spiked aquatic and sediment samples.

3.1. Optimization of the ISP interface

The influence of various parameters, such as the nebulizing gas flow-rate, the capillary voltage applied, the LC eluent flow-rate and the percentage of organic modifier, has been discussed by Ikonomou et al. [40] for both ESP and ISP. They observed that the formation of the spray in ISP is mainly dependent upon the nebulizing gas flow-rate and is less sensitive to the potential difference between the capillary tip and the counter electrode [40]. Obviously, both the nebulizer and drying gas flow-rate and, to a lesser extent, the capillary voltage, are important for the evaporation of the solvent droplets and the stability of the spray. Therefore, in the present study the nebulizing and drying gas flow-rates were optimized by monitoring the ion signal of the sodium adduct ions, $[M + Na]^+$, of methomyl, promecarb and pirimicarb at LC eluent flow-rates of 40, 200 and 600 μl/min. The LC eluent was methanol–water (50:50).

Maximum signal intensities of the $[M + Na]^+$ ions of the three compounds were found for a nebulizer gas flow-rate of 15 l/h (the maximum applicable flow-rate) at an LC eluent flow-rate of 40 μl/min. To check whether the maximum of the nebulizer flow-rate (15 l/h) is a limiting factor with regard to the eluent flow-rate, the ion currents of the $[M + Na]^+$ ions of methomyl, pirimicarb, promecarb and thiodicarb were measured at four different eluent flow-rates, applying optimum settings for all other parameters. As can be seen from Fig. 1, with methomyl as an example, a flow-rate of ca. 160 μl/min gives the maximum sodium adduct ion intensity. Hence the nebulizer flow-rate is a limiting factor with

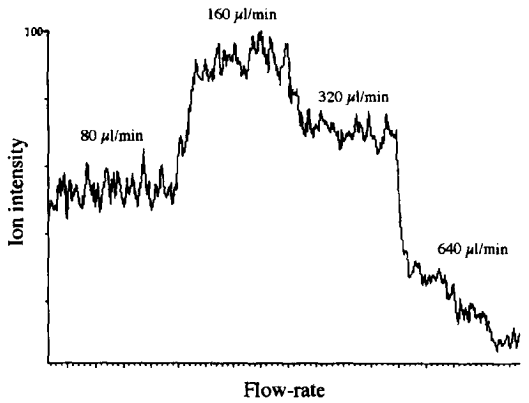

Fig. 1. Ion current of the sodium adduct ion, with m/z 185, of methomyl using full-scan (m/z 50–350) acquisition and LC eluent [methanol–water (50:50) containing 1 g/ml of the analyte] flow-rates of 80, 160, 320 and 640 l/min, with a nebulization flow-rate of 15 l/h.

regard to the optimum eluent flow-rate, because in an earlier report [30] maximum sensitivity was obtained at an LC eluent flow-rate of 1.0 ml/min.

An explanation of the differences may be the different designs of the interfaces and ion sources. Varying the drying gas flow-rate from 200 to 400 l/h, at an eluent flow-rate of 200 μl/min and a nebulizer flow-rate of 15 l/h, resulted in no significant change in ion abundances. A drying gas flow-rate of 350 l/h was found to be optimum. The flow-rates applied in all further experiments were 300 μl/min for the LC eluent (because a 3 mm I.D. analytical column was used) and 15 and 350 l/h for the nebulizer and drying gas, respectively. Further, it was observed that the source temperature could be changed from 75 to 150°C without any notable effect on the signal intensity of the $[M + Na]^+$ ions of the three test compounds. The source temperature was arbitrarily set at 125°C in all further experiments.

At an eluent flow-rate of 300 μl/min, a capillary voltage of 3.5 kV was found to be optimum, although it seemed to be less effective for nebulizing the eluent in ISP than in conventional electrospray (ESP) experiments [37]. Without the use of additives in a methanol–water eluent, solvent adduct ions with the alkali metal additives, $[(CH_3OH)_n + Na]^+$, with $n = 1$–8 (m/z 55, 87, 119, 151, 183, 215, 247 and 279) are observed in the ISP mass spectra at low capillary voltages [37,38]. Increasing this voltage or decreasing the distance between the capillary tip and the counter electrode (which obviously increases the electric field strength at the tip of the capillary) causes the formation of protonated solvent clusters, $[(CH_3OH)_n + H]^+$, with $n = 1$–7 (m/z 33, 65, 97, 129, 161, 193 and 225), which is explained by electrical gas discharges in the ion source [38]. This is illustrated in Fig. 2, where the mass spectra of the eluent stream are depicted for the distances x mm (i.e., the minimum distance), $x + 4$ mm and $x + 8$ mm between the capillary tip and the counter electrode.

Increasing the distance results in a decrease in the intensity of the $[(CH_3OH)_n + H]^+$ ions with m/z 33 ($n = 1$), 65 ($n = 2$), 97 ($n = 3$), 129 ($n = 4$), etc. The intensity of the $[(CH_3OH)_n + Na]^+$ ions remains the same when the distance is changed. In all further experiments, mass spectra were searched from m/z 10 to 200 for the presence of protonated solvent clusters. If present, the distance was increased until no protonated methanol cluster ions were observed.

The cone extraction voltage is known to influence the sampling efficiency in API sources [7,14]. Increasing this voltage will increase the total ion current and, thus, the sensitivity. In addition, for various type of analytes, increase of this voltage has been shown to induce a fragmentation comparable to that observed in collisionally induced dissociation (CID) mass spectra [7,14]. For example, for aldicarb, increasing of the extraction voltage from 10 to 70 V resulted in the appearance of the fragment ions with m/z 116, $[M + H - CH_3NHCOOH]^+$, and m/z 89, $[M + H - CH_3NHCOOH - HCN]^+$ [14]. In our experiments, it was found that for the more basic aminocarb the protonated molecule with m/z 209 was formed at a voltage of 25 V. For this compound, increasing the extraction voltage resulted in the formation of the specific fragment ion $[M + H - CH_3NCO]^+$ with m/z 152. Fig. 3 shows the ISP mass spectra of aldicarb recorded at extraction voltages of 10 and 45 V.

For propoxur, an increase in the extraction voltage from 10 to 30 V generated the appear-

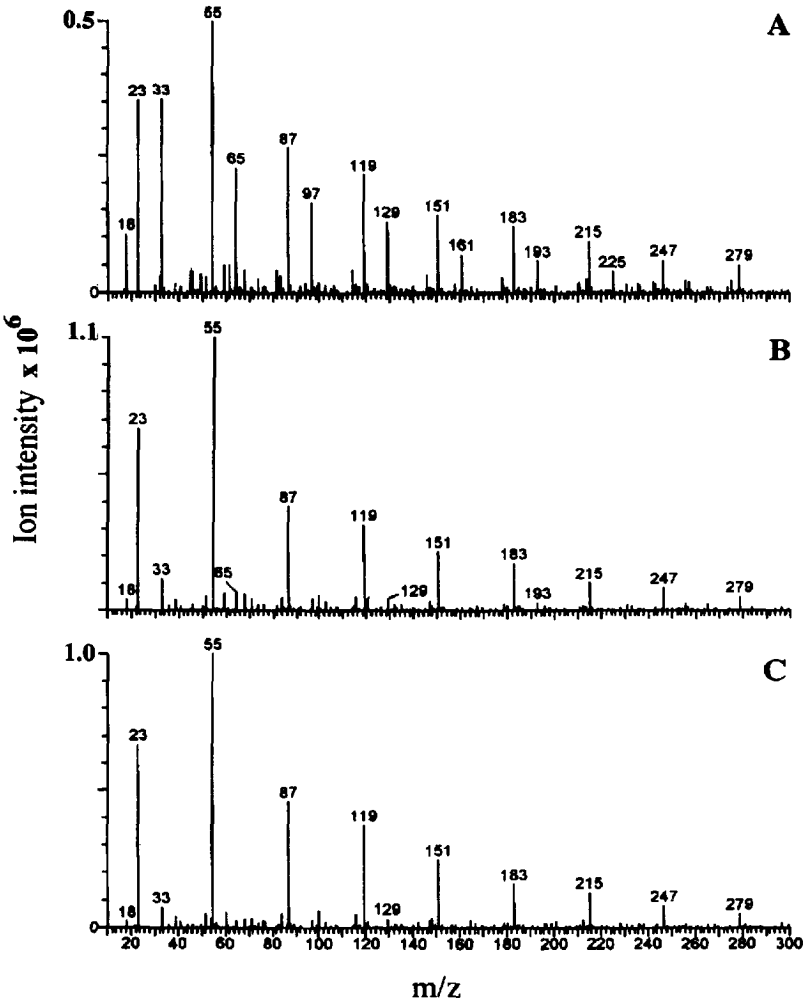

Fig. 2. Dependence of the intensity of the protonated methanol cluster ions, $[(CH_3OH) + H]^+$, with m/z 33, 65, 97, 129, 161, 193 and 225, from a methanol–water (50:50) effluent at a flow-rate of 0.3 ml/min on the distance between the capillary tip (at 3.5 kV) and the counter electrode. (A) The minimum possible distance, x mm; (B) $x + 4$ mm; (C) $x + 8$ mm.

ance of the fragment ion $[M + H - C_3H_6]^+$ with m/z 168. A further increase resulted in the formation of a fragment ion with m/z 111, $[M + H - C_3H_6 - CH_3NCO]^+$. Unfortunately, a further increase in the extraction voltage above 50 V caused a decrease in the total ion current for all compounds, and thus reduced the sensitivity. This is illustrated for aldicarb in Fig. 4, which depicts the abundances of the fragment ions with m/z 116 and 89, at different extraction voltages. In other words, when high extraction voltages are needed for identification purposes, the sensitivity is decreased. Owing to this effect, the best sensitivity is obtained within a limited range of extraction voltages.

The type and percentage of organic modifier influence the analyte detectability with all types of ESP and ISP interfaces [21,37,38]. The type of organic modifier was shown to be particularly important for the carbamate carbofuran when using an ISP-MS system coupled to a 1 mm I.D. LC column [21]. Postcolumn solvent addition to the LC eluent (water–acetonitrile containing 5 m*M* ammonium acetate) was used to increase the

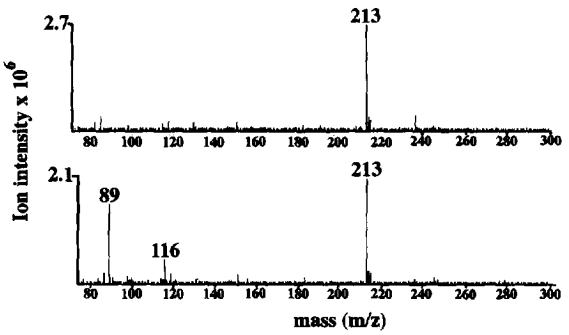

Fig. 3. Full-scan (m/z 50–300) ISP mass spectra of aminocarb at extraction voltages of (A) 25 and (B) 45 V and a capillary voltage of 3.5 kV. Analyte: 0.5 μg/ml dissolved in methanol–water (50:50), with a flow-rate of 0.3 ml/min.

LC eluent flow-rate from 50 to 500 μl/min prior to entry into the ISP-MS system. Changing the postcolumn solvent from water–acetonitrile (60:40) to pure acetonitrile resulted in a 94% decrease in the ion intensity of carbofuran. In contrast, using pure methanol as postcolumn solvent resulted in a threefold increase in the ion intensity.

This large difference in results is probably due to the higher protic nature of methanol [21]. Therefore, methanol was used as the organic modifier in the present LC–ISP-MS studies. With regard to analyte detectability, the percentage of modifier is also of importance. In our experiments, reducing the percentage of methanol from 100 to 50% resulted in a twofold decrease in the intensity of the sodium adduct ions for all carbamates. Simultaneously, a strong increase in the abundance of the protonated molecule was observed for the more basic carbamates (pirimicarb, aminocarb and carbendazim), but not for the other carbamates. Unfortunately, owing to the limited range of eluent flow-rates typically required for a 3 mm I.D. column, postcolumn addition of a substantial additional flow of pure methanol could not be used to increase the ion signal and obtain optimum sensitivity.

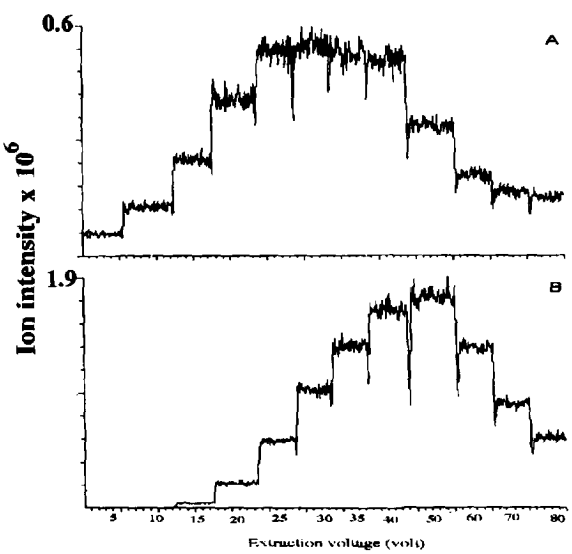

Fig. 4. Intensity of the fragment ions at (A) m/z 116 and (B) m/z 89 from aldicarb under full-scan (m/z 50–300) conditions, at extraction voltages of 5 to 80 V and a capillary voltage of 3.5 kV. Analyte: 1 μg/ml dissolved in methanol–water (50:50), with a flow-rate of 0.3 ml/min.

3.2. Performance of LC–ISP-MS and LC–TSP-MS

The ISP-MS and TSP-MS systems were tuned using a 1 μg/ml solution of methomyl, promecarb and thiodicarb in methanol–water (50:50), which offers optimum mass spectrometric conditions for the carbamates; the sodium adduct ions (except for carbendazim and aminocarb) and the protonated molecules were monitored in LC–ISP-MS and LC–TSP-MS, respectively. The ISP mass spectrum of pirimicarb contains both the sodium adduct ion and the protonated molecule, with the former ion as the base peak. Therefore, for this compound both ions were monitored. In the ISP experiments no acid or ammonium acetate was added to the eluent, to prevent the formation of protonated molecules of the other seven carbamates.

The LODs ($S/N = 5$–10) in LC–ISP-MS were determined from the chromatographic peaks acquired under SIM conditions, using 10-μl injections of standard solutions containing between 5 and 1000 μg/l of the ten carbamates; they are presented in Table 1. In LC–TSP-MS, 25 μl of standard solutions containing 50–10 000 μg/l of the ten carbamates were injected, and the protonated molecules, $[M + H]^+$, or the ammonium

Table 1
Limit of detection (LOD) of carbamates in LC–TSP-MS and LC–ISP-MS, using time-scheduled SIM, at an extraction and a capillary voltage of 20 and 3500 V, respectively

Compound	Type of ion	m/z	t_R (min)	LOD (pg)	
				TSP	ISP
Oxamyl	$[M + Na]^+$	242	4.1		15
	$[M + NH_3]^+$	237	5.9	2500	
Methomyl	$[M + Na]^+$	185	5.0		25
	$[M + NH_3]^+$	180	6.8	2500	
Carbendazim	$[M + H]^+$	192	13.2		30
	$[M + H]^+$	192	15.3	900	
Aldicarb	$[M + Na]^+$	213	13.2		15
	$[M + NH_3]^+$	208	15.0	1000	
Propoxur	$[M + Na]^+$	232	19.0		15
	$[M + NH_3]^+$	227	18.8	1200	
Carbofuran	$[M + Na]^+$	244	19.7		15
	$[M + NH_3]^+$	239	19.5	900	
Aminocarb	$[M + H]^+$	209	20.1		25
	$[M + H]^+$	209	18.8	500	
Pirimicarb	$[M + H]^+$	239	23.2		60
	$[M + Na]^+$	261	23.2		10
	$[M + H]^+$	239	22.8	500	
Promecarb	$[M + Na]^+$	230	28.5		5
	$[M + NH_3]^+$	225	28.9	600	
Barban	$[M + Na]^+$	280	29.8		8000
	$[M + NH_3]^+$	275	30.1	900	

LC was performed on a 12.5 cm × 3 mm I.D. column containing 3-μm C_8-deactivated silica, using a methanol–water gradient as LC eluent.

adduct ions, $[M + NH_4]^+$, were monitored. Using time-scheduled SIM, LODs of the carbamates with LC–TSP-MS were between 0.5 and 2.5 ng (Table 1), which agrees with data reported before (between 1 and 2 ng) [43]. The LODs obtained with LC–ISP-MS were lower by a factor of 10–150 for all carbamates except barban [7,23]. For barban the LOD in LC–ISP-MS is 8 ng, compared with 0.9 ng in LC–TSP-MS. Possibly for this compound, sodium adduct formation in LC–ISP-MS is less favoured under the present experimental conditions. Fig. 5 shows the calibration graphs for four carbamates using LC–ISP-MS over the concentration range 0.01–1.0 μg/ml. As can be seen, linearity was lost above an analyte concentration of ca. 0.4 μg/ml; this holds true for all the carbamates.

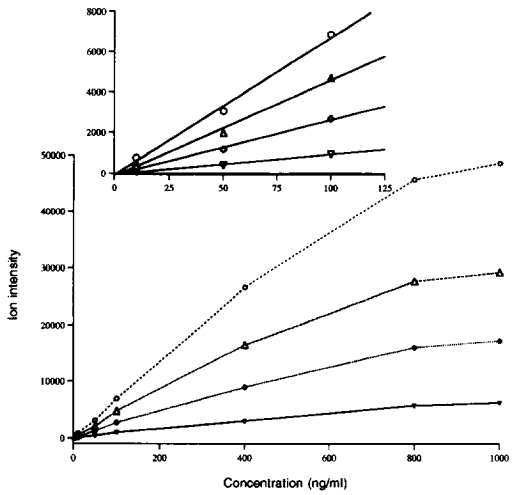

Fig. 5. Calibration graphs for (○) promecarb, (△) pirimicarb, (◇) aldicarb and (▽) aminocarb obtained from 10-μl injections of standard solutions containing 10, 50, 100, 400, 800 and 1000 μg/l, on to a 12.5 cm × 3 mm I.D. LC column, using time-scheduled SIM LC–ISP-MS and an LC eluent flow-rate of 0.3 ml/min.

This observation is consistent with data presented before, which showed that in general linearity in ESP ionization methods is lost above an analyte concentration of 10^{-5} M [29]. Relevant calibration data for all compounds except barban are listed in Table 2. The calibration graphs for the ten carbamates recorded with

Table 2
Calibration data from five standard solutions (5–400 μg/l) containing nine carbamates using LC–ISP-MS, injecting 10-μl samples on to a 12.5 cm × 3 mm I.D. LC column and using time-scheduled SIM

Analyte	Calibration equation[a]	R^2
Oxamyl	$y = 59074x + 362$	0.9988
Methomyl	$y = 41399x - 13$	0.9996
Carbendazim	$y = 4241x + 63$	0.9945
Aldicarb	$y = 22179x + 117$	0.9987
Propoxur	$y = 28709x + 65$	0.9995
Aminocarb	$y = 7278x + 53$	0.9973
Carbofuran	$y = 27254x + 101$	0.9995
Pirimicarb	$y = 41064x + 130$	0.9993
Promecarb	$y = 66531x + 18$	0.9998

[a] y = Signal intensity; x = concentration.

LC–TSP-MS were linear over the concentration range 0.2–10.0 μg/ml, which is in agreement with earlier data from our group [39,40].

The repeatability of the LC–ISP-MS procedure was studied by repeatedly injecting a standard solution containing 80 ng/ml of each of the test compounds. The ion signal intensities of all carbamates were found to display R.S.D. values of 2–10% ($n = 5$). This is distinctly better than earlier results obtained for LC–TSP-MS (R.S.D.s ca. 20% [17]).

3.3. Applications

Because of the low LODs obtained in LC–ISP-MS, 500 μl of a tap water sample, spiked at a level of 0.1 μg/l, were injected directly on to the LC column. The time-scheduled SIM chromatogram of oxamyl, methomyl, propoxur, carbofuran, pirimicarb and promecarb is shown in Fig. 6. The retention times of the compounds were ca. 2 min longer than when a 10-μl injection was used. This is probably due to the large injection volume. Surprisingly, carbendazim and aminocarb were not retained when using this large-volume injection. However, if the carbamates were injected in methanol–water (50:50) rather than in pure water, aminocarb could also be detected, viz., at a retention time of 21 min, i.e. between propoxur and carbofuran. Strong changes in the retention time of carbendazim, caused by increasing the percentage of organic modifier in the sample solution or the injection volume, have been reported before [41]. Even though this phenomenon certainly requires further study, the present result with an LOD at or below 0.1 μg/l for seven analytes, using a 500-μl injection volume only, can be considered good.

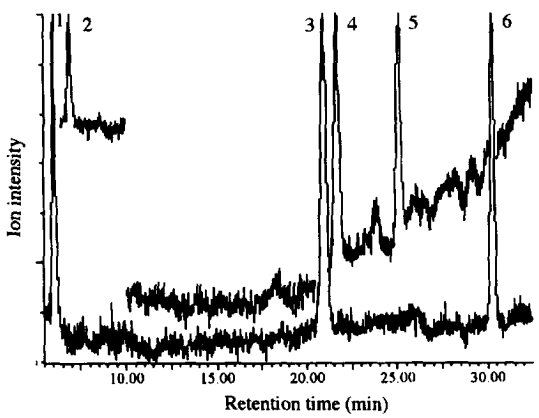

Fig. 6. Time-scheduled SIM LC–ISP-MS chromatogram of tap water, spiked with (1) oxamyl, (2) methomyl, (3) propoxur, (4) carbofuran, (5) pirimicarb and (6) promecarb at 0.1 μg/l, utilising a 500-μl injection on to a 12.5 cm × 3 mm I.D. LC column. The ion chromatograms depicted are from the ions at (1) m/z 242 and (2) m/z 185 monitored from 0 to 10.0 min, (3) m/z 232 and (4) m/z 244 monitored from 10.0 to 23.5 min, (5) m/z 261 monitored from 23.5 to 27.5 min and (6) m/z 230 monitored from 27.5 to 35 min.

The possibility of providing structural information at low concentration levels in LC–ISP-MS is illustrated in Fig. 7. Here the ion-extracted chromatogram of the ion at m/z 244 (after full-scan acquisition over the range m/z 100–300) and the mass spectrum of the peak at a retention time of 20 min of a raw water sample from the Ebro delta that had been concentrated 4000-fold (for sample pretreatment, see Experimental) are shown. The mass spectrum of the peak, recorded at an extraction voltage of 60 V, allowed the identification of carbofuran; quantification showed the concentration level to be ca. 5 μg/l, or about 1 ng/l in the initial water sample.

For the determination of carbamates in sediment samples, clean-up procedures are generally needed after Soxhlet extraction, to separate interfering co-extractives from the analytes. Although the conditions for Soxhlet extraction in our study were the same as those reported before [36], the further sample clean-up procedure was optimized, using column chromatography with Florisil, neutral aluminium oxide and aminopropyl-bonded silica as the sorbents. With alumina and Florisil, and with acetone–dichloromethane mixtures as eluents, low recoveries of less than 10% were invariably found for carbofuran, carbendazim and aldicarb. Good recoveries of 85–100% were obtained for all other carbamates [Florisil with acetone–dichloromethane (40:60) and alumina with acetone–dichloromethane (60:40)]. On the other hand, with aminopropyl-bonded silica and acetone–dichloromethane (25:75) as eluent, good recoveries

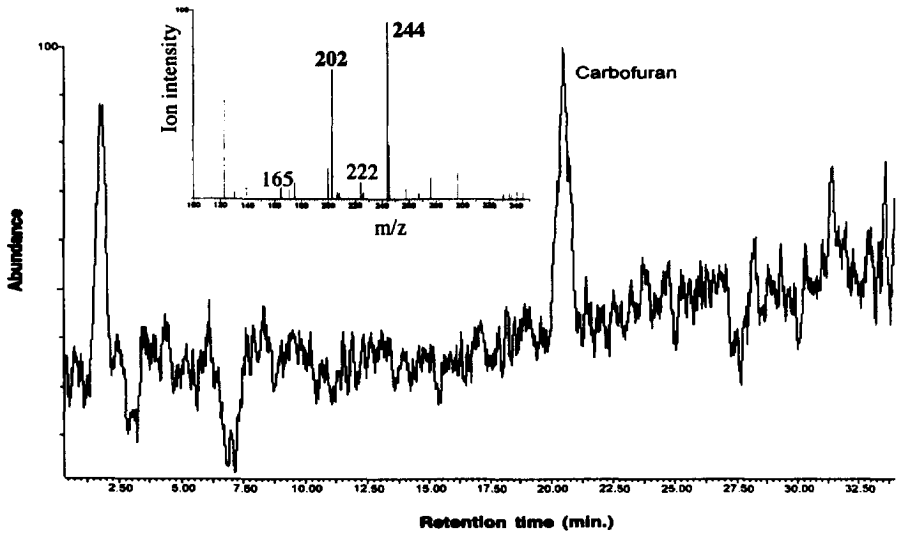

Fig. 7. LC–ISP-MS ion chromatogram of the ion at m/z 244 obtained after full-scan (m/z 50–300) acquisition and injecting 25 μl of a water sample (after 4000-fold preconcentration) from the Ebro river on to a 12.5 cm × 3 mm I.D. LC column. LC eluent flow-rate, 0.3 ml/min; extraction voltage, 60 V. The mass spectrum of the peak at a retention time of 20 min, shown as an inset, could be attributed to carbofuran ($[M + Na]^+$, m/z 244).

of between 76 and 100% (R.S.D. 2–10%; $n = 4$) were found for all carbamates tested (see Table 3).

In view of these results, the aminopropyl-bonded column was used in all further studies.

Table 3
Recoveries for 1 ml of dichloromethane containing 1 μg/ml of the carbamates using aminopropyl-bonded silica as sorbent and 20 ml of (A) 5:95, (B) 10:90, (C) 25:75 and (D) 50:50 acetone–dichloromethane mixtures for elution and an LC–diode-array detection system for quantification

Compound	Recovery (%)[a]			
	A	B	C	D
Oxamyl	89 (3)	86 (4)	92 (4)	86 (1)
Methomyl	95 (1)	97 (2)	102 (5)	105 (3)
Carbendazim	<5	70 (6)	87 (15)	57 (47)
Aldicarb	92 (8)	61 (8)	76 (8)	59 (11)
Propoxur	93 (4)	91 (3)	93 (8)	90 (1)
Aminocarb	94 (11)	96 (2)	97 (3)	94 (3)
Carbofuran	93 (3)	78 (8)	81 (3)	66 (10)
Pirimicarb	94 (4)	99 (3)	97 (4)	96 (1)
Promecarb	95 (3)	100 (3)	98 (3)	101 (4)
Barban	79 (6)	79 (6)	87 (1)	80 (1)

[a] Mean values with R.S.D. (%) in parentheses ($n = 4$).

Early-eluting co-extractives (which still are present in the samples after the clean-up procedure) can strongly influence the LC separation of the carbamates and the LODs when sediment samples have to be analysed. Therefore, two sediment samples which contained distinctly different percentages of organic matter were analysed using LC–TSP-MS with time-scheduled SIM. In sediment type T (2.5% organic matter), oxamyl and methomyl could be detected and quantified by mean of LC–TSP-MS with 90–100% recovery (R.S.D. 10–15%; $n = 5$) at both the 500 and 50 μg/kg spiking levels. Aldicarb was detected with a recovery of 79% at 500 μg/kg, but was lost at a concentration of 50 μg/kg. In sediment type P (3.3% organic matter), methomyl and aldicarb could not be detected at all and the recovery of barban and oxamyl was distinctly less than 50%. Carbendazim, propoxur, aminocarb, carbofuran, pirimicarb and promecarb could be detected and quantified in both sediment types by means of LC–TSP-MS with acceptable recoveries (60–120%) (R.S.D. 4–17%, $n = 5$) at both the 500 and 50 μg/kg spiking levels.

With LC–ISP-MS, on the other hand, no such problems were encountered, as illustrated in Fig.

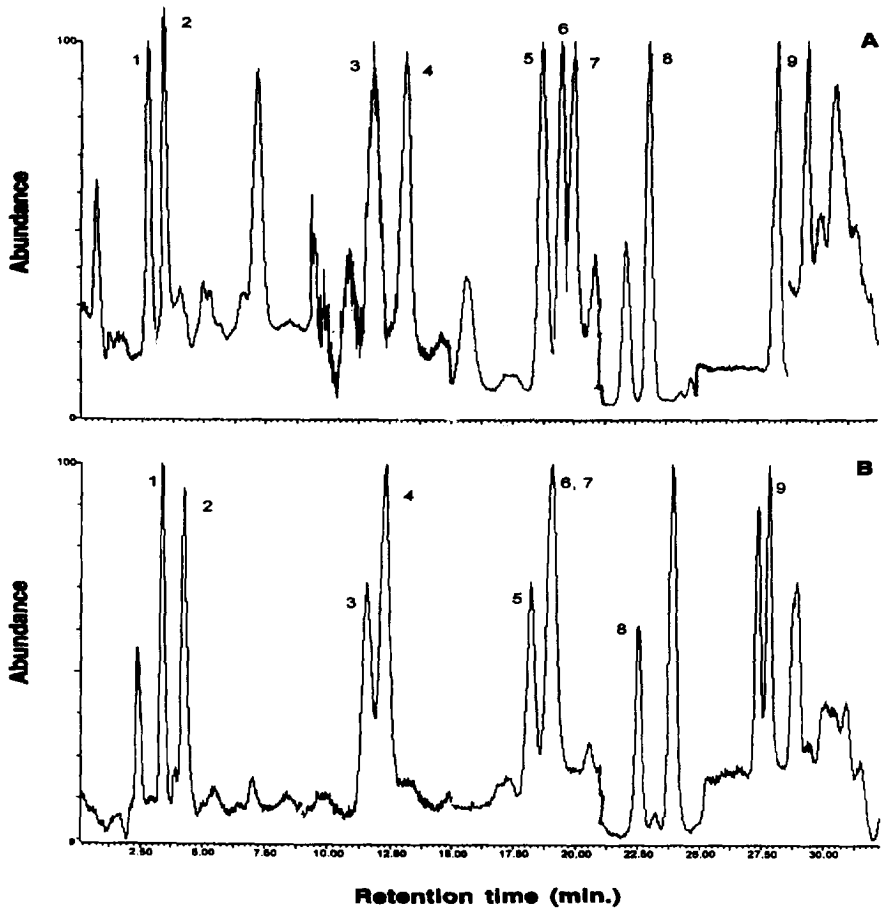

Fig. 8. Time-scheduled SIM LC–ISP-MS chromatograms of (A) sediment P and (B) sediment T, spiked with (1) oxamyl, (2) methomyl, (3) aldicarb, (4) carbendazim, (5) propoxur, (6) aminocarb, (7) carbofuran, (8) pirimicarb and (9) promecarb each at a concentration of 50 μg/kg. LC: 25 μl of sediment extract (after extraction and clean-up) injected on to a 12.5 cm × 3 mm I.D. LC column with methanol–water gradient elution at a flow-rate of 0.3 ml/min.

8. Here, the time-scheduled SIM LC–ISP-MS chromatograms of all carbamates (except barban) are shown for type T and type P sediments which had been spiked at the 50 μg/kg level. Obviously, the system featuring the novel ISP interface provides better selectivity and sensitivity.

4. Conclusions

The use of LC–ISP-MS for the determination of nine carbamates of widely varying polarity yields LODs between 10 and 50 pg when the SIM mode is used. This is a substantially better result than can be obtained with LC–TSP-MS, where SIM scan-model LODs are typically between 0.5 and 5 ng. The repeatability of the ion signal intensities is also much better with the ISP-based system.

With this technique, application of direct large-volume injections of about 0.5 ml permits the determination of carbamates at levels below those of EU drinking water directives (0.1 μg/l for individual pesticides). In addition, recording full-scan mass spectra with LC–ISP-MS allows the detection and identification of the carbamates at the 1–5 μg/l alert/alarm level in sur-

face water. Finally, it is interesting that the presence of co-extractives does not seriously interfere with the determination of polar carbamates in sediment samples down to the 50 μg/kg level. In summary, LC–ISP-MS has a high potential in trace-level environmental analyses.

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