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Considerations on ultra trace analysis of carbamates in water samples

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Abstract

A new routine method for the ultra trace analysis of carbamates in water samples is presented, using solid-phase extraction followed by high-performance liquid chromatography coupled to atmospheric pressure electrospray ionisation mass spectrometry (SPE–LC–ESI-MS). Instrumental conditions of LC–ESI-MS in the selected ion monitoring (SIM) mode, showed excellent linear response for the six *N*-methyl carbamates studied (aldicarb, carbaryl, carbofuran, methomyl, oxamyl and pirimicarb) in the range from 1 to 50 μg/l and a precision having a relative standard deviation below 7.8% was achieved. Instrumental limits of detection of 0.10 μg/l were found for these carbamates, with the exception of methomyl for which 0.50 μg/l was measured. The SPE assays were shown to be easy, fast, very sensitive, requiring a low volume (50 ml) of water sample. For laboratory-spiked water samples having 0.03 and 0.30 μg/l of individual *N*-methyl carbamates, higher selectivities were achieved in cartridges having octadecylsilica, polystyrene–divinylbenzene and *N*-vinylpyrrolidane–divinylbenzene as solid phases, for which reasonable average recoveries were obtained. Ten replicates using octadecylsilica SPE cartridges, showed average recoveries between 73.7 and 92.6% with a relative standard deviation lower than 14.7%. The present methodology evidences good robustness, accuracy and precision for monitoring of *N*-methyl carbamates in water samples, and is shown to be a suitable alternative to replace the currently dedicated analytical systems. The limits of detection for the analysis of *N*-methyl carbamates in water samples reached in the present methodology (0.5 to 3 ng/l), clearly cover the maximum concentration admissible for pesticides, established by the European Union directive on water quality.

Keywords: Water analysis; Trace analysis; Solid-phase extraction; Carbamates; Pesticides

1. Introduction

N-Methylcarbamates are a class of compounds derived from carbamic acid and some derivatives are intensively used as insecticides in agriculture because of their broad spectrum of activity, which are considered to have toxicological effects in the environment, as well as in human beings, since they are inhibitors of acetylcholinesterase [1].

Pesticide residues used to arouse a great deal of

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public concern, especially in drinking water, where an efficient monitoring plays an important task. The US Environmental Protection Agency (EPA) on drinking water regulations requires one to monitor 45 unregulated substances, including carbamates, but maximum contaminant levels have not yet established. On the other hand, the European Union directive on drinking water quality (98/83/CE) established a maximum permissible concentration for pesticides of 0.1 μg/l for individual and 0.5 μg/l total. Due to this legal limit, methods that can easily screen ultra-trace carbamates in water samples are needed.

Currently, methods to monitor *N*-methyl carbamates in several types of water matrices usually require sample enrichment, namely liquid–liquid extraction or solid-phase extraction (SPE), followed by high-performance liquid chromatography (HPLC), since these compounds are somewhat polar and thermally unstable for the traditional gas chromatographic methods. UV–Vis detection and post-column reaction–derivatization followed by fluorescence detection (hydrolysis with 0.05 *M* NaOH at 95 °C, reaction with *o*-phthalaldehyde and 2-mercaptoethanol) are widely used [2,3]. For instance, the US EPA method 8318 for the determination of *N*-methyl carbamates from water matrices, recommends such dedicated analytical systems, where a limit of detection (LOD) ranging from 1.7 to 9.4 μg/l is reported [4].

Nowadays, mass spectrometry (MS) is becoming the detection system of choice for liquid chromatography (LC), due to its versatility, providing high selectivity and spectral evidence of individual solutes [5–7]. LC–MS has been widely accepted as the advantageous technique for the analysis of *N*-methyl carbamates in water matrices, which is more rugged and versatile without derivatization. Atmospheric pressure sources such as electrospray ionisation (ESI), present several advantages as the samples are ionised directly in the liquid phase at a quasi-ambient temperature, minimising the degradation of thermally labile compounds [8]. Moreover, the major attraction of ESI applied to water analysis is the low LOD usually found.

In the last few years, several applications on the analysis of carbamates had been proposed using LC–MS analytical systems [9,10], but the LODs reported do not cover the European Union guideline on water quality.

The aim of the present work is to combine the selectivity and sensitivity of the SPE–LC–ESI-MS procedure, as an alternative routine method for the ultra trace analysis of *N*-methyl carbamates in water samples.

2. Experimental

2.1. Reagents, standards and samples

N-Methyl carbamates (aldicarb, carbaryl, carbofuran, methomyl, oxamyl and pirimicarb) were supplied from Riedel-de Haën, having purities higher than 99.5%. Fig. 1 shows the chemical structures of the six carbamates studied.

Stock solutions of individual carbamates having a concentration of 100 mg/l were prepared in methanol and stored at 4 °C. Six standard calibration solutions of mixing carbamates, ranging from 1 to 50 μg/l each, were prepared daily in deionised water by appropriate dilution of aliquots of the stock solutions.

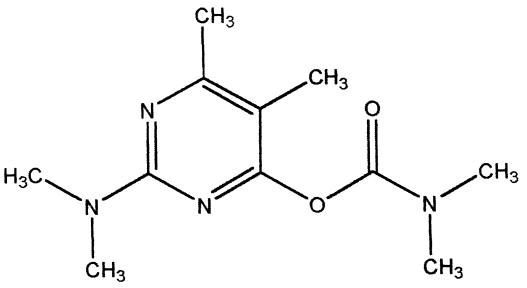
Laboratory-spiked water samples were prepared in 50 ml of deionised and drinking water by appropriate dilution of aliquots of the stock solutions to concentrations at 0.03 and 0.30 μg/l of individual carbamates.

For the SPE assays, a manifold (Agilent Technologies) and the following cartridges were used: Zorbax (Agilent Technologies—C_{18}; 3 ml, 500 mg), LC_{18} (Supelco—C_{18}; 3 ml, 250 mg), Envicarb (Supelco; 3 ml, 250 mg), Oasis HLB (Waters; 6 ml, 200 mg), Envi (IST; 3 ml, 100 mg) and Bond Elut Envi (Varian; 3 ml, 500 mg).

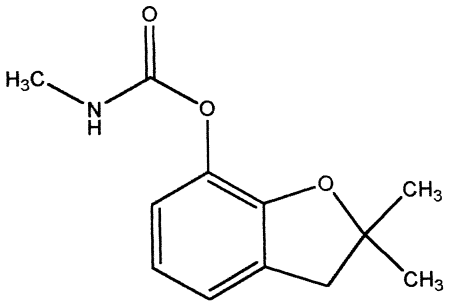
HPLC-grade methanol, acetonitrile, ammonium acetate and deionised water were obtained from Merck. Drinking water samples were obtained from Kortrijk (Belgium).

2.2. Sample preparation

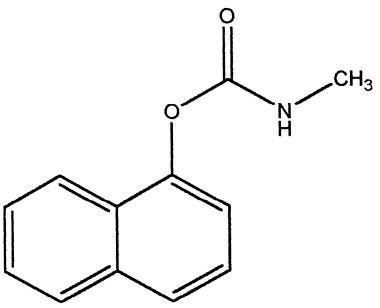
For the SPE assays of the laboratory-spiked water samples, each cartridge was conditioned with 3 ml of methanol–acetonitrile (50:50, v/v), 3 ml of methanol and two times with 3 ml of deionised water, then



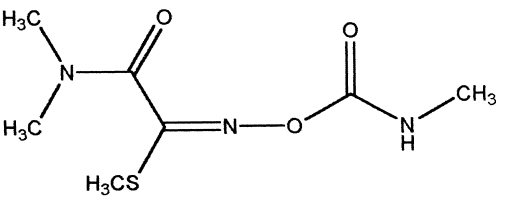
Pirimicarb



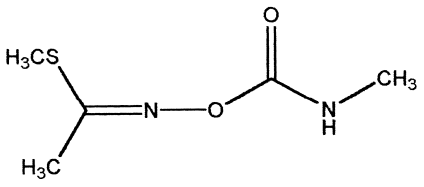
Carbofuran



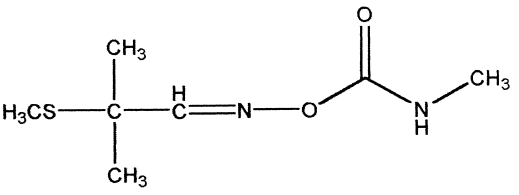
Carbaryl



Oxamyl



Methomyl



Aldicarb

Fig. 1. Structures and common names of the *N*-methyl carbamates studied.

slowly aspirated (−0.2 bar). After loading the spiked water samples (−0.4 bar), the column was washed twice with 3 ml of deionised water, followed by vacuum drying for 2 min. Subsequently, the elution took place with three volumes of 1 ml of methanol–acetonitrile (50:50, v/v), followed by evaporation to dryness under a gentle nitrogen stream. The dry residues obtained from the SPE assays were re-dissolved in 200 μl of deionised water and after agitation by vortex were analysed by LC–ESI-MS.

For blank assays the same procedure as above was employed for each type of cartridge, using drinking and deionised water instead of spiked samples.

2.3. Instrumentation

The analyses were carried out on a benchtop Agilent 1100 series LC–MS SL single quadrupole instrument (Agilent Technologies). An Inertsil ODS3 column, 100 mm×2.1 mm, 5 μm particle size (Zorbax Eclips XDB C_{18}, Agilent Technologies) was used. The mobile phase consisted of 10% (v/v) ammonium acetate (10 m*M*) aqueous solution in methanol (solvent A) and 10% (v/v) methanol in ammonium acetate (10 m*M*) aqueous solution (solvent B) and the gradient applied was: 0–10 min: 10–90% B, 10–20 min: 90% B isocratic. The flow-rate was 0.25 ml/min, the analyses were performed at 25 °C and the injection volume was 50 μl having a draw speed of 200 μl/min. Atmospheric pressure ESI was carried out in the positive mode. In the full-SCAN mode the parameters were as follows: mass range from 100 to 500 u; fragmentor voltage 20–100 V; gain 1; N_2 drying gas flow-rate: 12 l/min at 350 °C; nebulizer pressure: 35 p.s.i.g; quadrupole temperature: 100 °C; capillary voltage: 4000 V (1 p.s.i.=6894.76 Pa).

Flow injection analyses (10 μl) were performed on individual carbamate solutions (10 mg/l) in order to obtained the spectrum data, from which ions were careful chosen for analysis in the selected ion monitoring (SIM) mode, using the parameter described above. Direct injection (100 μl) of laboratory-spiked drinking water samples (0.3 μg/l) without sample preparation was performed with gain at 5.

3. Results and discussion

3.1. Optimisation of the instrumental conditions

Six *N*-methyl carbamates, aldicarb, carbaryl, carbofuran, methomyl, oxamyl and pirimicarb, were selected as model compounds for the present study. The carbamates were separated on a 100 mm×2.1 mm I.D. reversed-phase (RP) column using a methanol–water gradient, which showed good resolution and suitable analytical time (<15 min) under the optimised chromatographic conditions.

In order to survey the best spectral data in function of the collision-induced dissociation (CID or fragmentor) voltage, flow injection analysis was performed on individual standards (10 mg/l), at 20, 40, 60, 80 and 100 V. In the positive mode, the fragmentor voltage at 60 V provided molecular mass information through the base peak, where the abundance was highest for almost all compounds studied. In the negative mode, carbamates are not detected at acceptable levels, according to previous work in the literature [1].

ESI is a soft ionisation technique that produces a large number of molecular-related ions. The full-SCAN mode provides protonated, ammoniated or sodiated species in almost all carbamate spectra, for which $[M+H]^+$ ions are the base peaks for carbofuran, methomyl and pirimicarb at *m*/*z* 222, 163 and 239, respectively. For aldicarb, carbaryl and oxamyl the base peaks observed are the ions at *m*/*z* 116, 219 and 237, respectively. The explanation for such species is the breakage of the N–O bond with loss of $CH_3NHCOOH$ and NH_3 for aldicarb and the formation of ammoniated adducts ($[M+NH_4]^+$) both for carbaryl and oxamyl, according to several authors [1,7,8].

Ammonium acetate was added to the mobile phase (10 m*M*) in order to stabilise the cationic species formation. Other mobile phases containing formic and acetic acids, induce a solvent-assisted hydrolysis in the liquid phase prior to analysis, resulting in a much more complex spectral pattern for all carbamates studied.

For quantitative purposes, the abundance of the base peaks observed in the spectral data of individual carbamates were used to performed a six-point calibration plots in the SIM mode, using standard mixtures having 1, 2, 5, 10, 25 and 50 μg/l of each compound. Nevertheless, for carbaryl and oxamyl calibration plots, a better linearity was achieved with $[M+H]^+$ and $[M+Na+2\times H_2O]^+$ species [7], respectively, from ions at *m*/*z* 202 and 278. As can be seen in Fig. 2, such ions present relative abundances above 80%, due the higher chemical stability observed in relation to the base peaks.

For six replicates using the external standard method, linear responses of the peak areas vs.

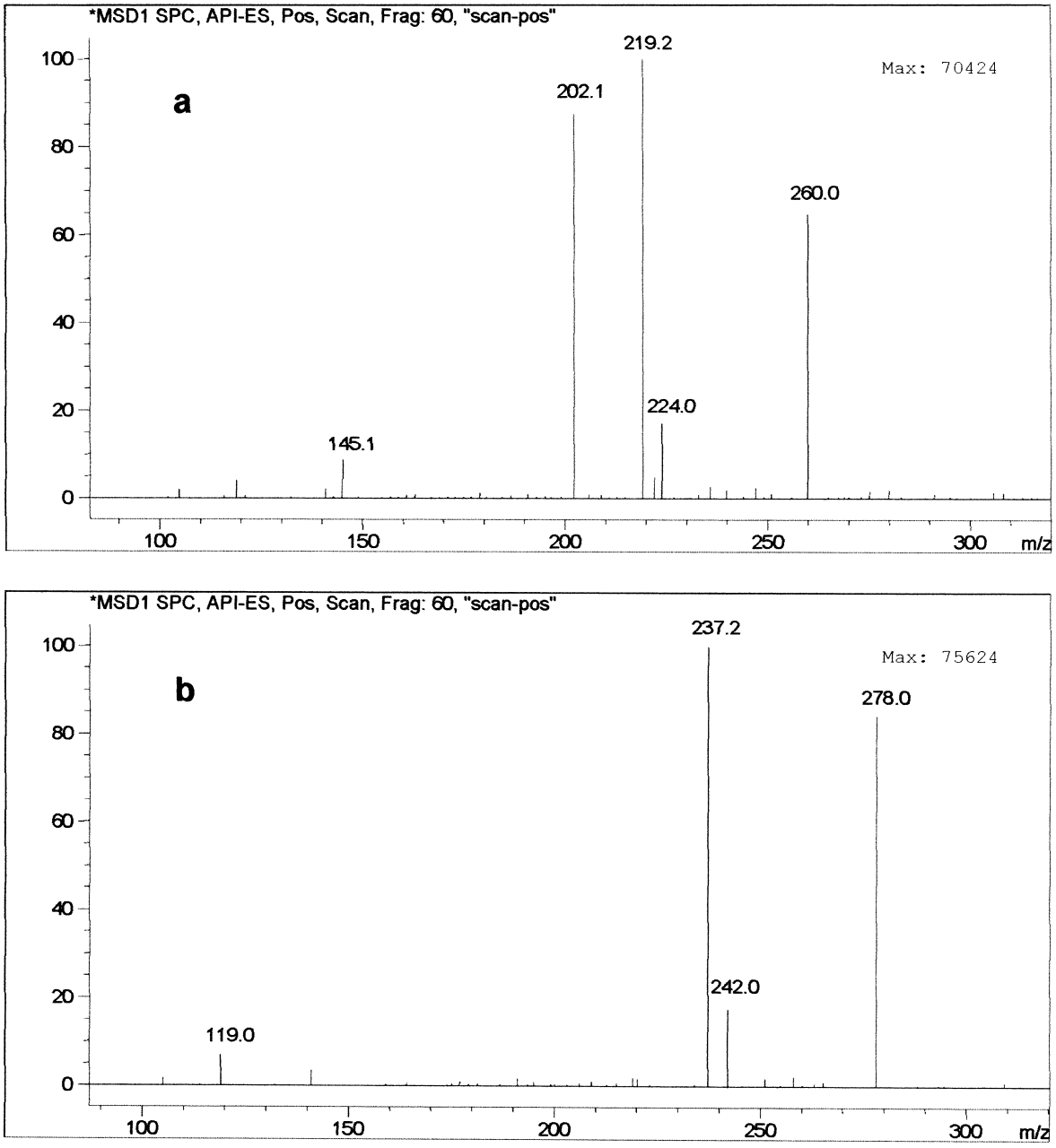


Fig. 2. Relative abundances of the mass spectra of carbaryl (a) and oxamyl (b) obtained by LC–ESI-MS in the flow injection analysis mode, under the experimental conditions used.

standard calibration mixtures of carbamates ranging from 1 to 50 μg/l, were obtained with excellent correlation coefficients. Moreover, the carbamate standard solutions used during 7 days allowed substantial stability without significant degradation. Table 1 shows the molecular mass (M_r) of each carbamate studied, the target ions selected at 60 V and the respective assignation of each species, as

Table 1
Molecular mass (M_r), assignation of the target ions used in the calibration plots by LC–ESI-MS in the SIM mode, correlation coefficients and instrumental LODs achieved for the six carbamates studied

Compound	M_r	Ion (m/z)	Assignation (species)	r^2 (1–50 μg/l)[c]	LOD (μg/l)[d]
Aldicarb	190	116[a]	$[M\text{-}CH_3NHCOOH\text{-}NH_3+H]^+$	0.9996	0.10
Carbaryl	201	202[b]	$[M+H]^+$	0.9946	0.10
Carbofuran	221	222[a]	$[M+H]^+$	0.9992	0.10
Methomyl	162	163[a]	$[M+H]^+$	0.9999	0.50
Oxamyl	219	278[b]	$[M+Na+2\times H_2O]^+$	0.9907	0.10
Pirimicarb	238	239[a]	$[M+H]^+$	0.9990	0.10

[a] Base peak.
[b] Relative abundance higher than 80%.
[c] SIM mode, $n=6$.
[d] $S/N=3$.

well as the correspondent correlation coefficients obtained from the regression plots.

To evaluate the instrumental precision, six replicates of a standard mixture of carbamates at a 10 μg/l level of each, were carried out in the SIM mode. Repeatability studies allowed a suitable relative standard deviation (RSD) below 7.8% (carbofuran). The instrumental sensitivity was also checked through the LOD, obtained by the injection of standard mixtures of carbamates in the SIM mode and calculated with a signal-to-noise (S/N) ratio of 3. In the present study, an instrumental LOD of 0.10 μg/l for all carbamates was found with the exception of methomyl, for which 0.50 μg/l was measured (Table 1). Thus, LODs ranging from 5 to 25 pg on-column were achieved for the six carbamates studied under the optimised instrumental conditions.

3.2. Ultra trace analysis of laboratory-spiked water samples

Considering the instrumental LODs measured, it is possible to analyse the six carbamates without sample enrichment. Direct analysis of a laboratory-spiked drinking water sample at 0.3 μg/l of individual carbamates, was performed in order to evaluate the LC–MS instrumental sensitivity (100 μl injection; gain at 5) in the SIM mode to real water matrices. Although good sensitivities were reached with an $S/N>5$ (methomyl), a sample enrichment procedure for ultra-trace analysis of carbamates in water samples is a must to decrease the LODs, in order to reach the European Union directive on water quality. By using an enrichment factor of 250 (from 50 to 0.2 ml), LODs in water samples can decrease easily to the low-ng/l level.

Analysis of laboratory-spiked water samples having individual concentrations of carbamates at 0.03 and 0.30 μg/l, were screened using different types of SPE cartridges for enrichment followed by LC–ESI-MS in the SIM mode. In order to evaluate the best selectivity, solid phases such as octadecylsilica (Agilent-Zorbax, Supelco-LC_{18}), polystyrene–divinylbenzene (Varian-Bond Elut Envi), carbon graphitized (Supelco-Envicarb), *N*-vinylpyrrolidane–divinylbenzene (Waters-Oasis HLB) and hydroxylated polystyrene–divinylbenzene copolymer (IST-Envi) were tested.

Preliminary studies, indicated acceptable average recoveries for the cartridges tested, as can be seen in Table 2 for duplicate assays under the optimised experimental conditions. Nevertheless, octadecylsilica, polystyrene–divinylbenzene and *N*-vinylpyrrolidane–divinylbenzene solid phases, shows the best effective average recoveries (50.7–126.1%) for the six carbamates studied, with the exception for aldicarb. Although carbon graphitized cartridges presented a reasonable average recovery for aldicarb, they show unacceptable values for pirimicarb and carbofuran. The hydroxylated polystyrene–divinylbenzene copolymer cartridges also show unacceptable recoveries for pirimicarb.

In blank assays, no interference compounds were observed from the cartridges tested, showing substantial selectivity for the ultra-trace analysis of carbamates in spiked water samples. Fig. 3 exem-

Table 2
Average recoveries of two assays using several SPE cartridges followed by LC–ESI-MS in the SIM mode for the six carbamates from laboratory-spiked water samples at the 0.03 and 0.30 μg/l levels

$n=2$	Recovery (%)					
	Agilent (Zorbax)	Supelco (LC_{18})	Supelco (Envicarb)	IST (Envi)	Waters (Oasis HLB)	Varian (Bond Elut Envi)
Aldicarb	76.3	26.3	86.8	47.4	50.0	50.0
	50.7	*61.3*	*55.6*	*25.4*	*56.3*	*33.8*
Carbaryl	91.9	68.3	83.1	59.5	74.6	73.6
	68.3	*83.5*	*79.6*	*65.5*	*78.8*	*82.5*
Carbofuran	122.0	101.1	20.0	77.8	87.8	95.6
	75.8	*82.0*	*1.4*	*66.9*	*79.2*	*88.8*
Methomyl	118.2	72.7	86.6	42.0	123.9	126.1
	75.0	*92.0*	*92.7*	*84.7*	*92.0*	*93.2*
Oxamyl	97.5	77.8	82.8	41.1	66.4	90.3
	84.6	*85.0*	*76.3*	*77.8*	*78.5*	*89.6*
Pirimicarb	99.1	99.7	13.8	25.1	71.7	76.3
	71.3	*85.0*	*1.4*	*20.0*	*75.3*	*80.8*

Values in italic are obtained from the 0.30 μg/l level.

plifies the analysis of the carbamates from a laboratory-spiked water sample after SPE enrichment (Zorbax) followed by LC–ESI-MS in the SIM mode. Six discrete mass fragmentograms of individual carbamates could be observed at 0.03 μg/l levels, showing that such methodology presents substantial sensitivity and selectivity.

As no certified reference materials is available to

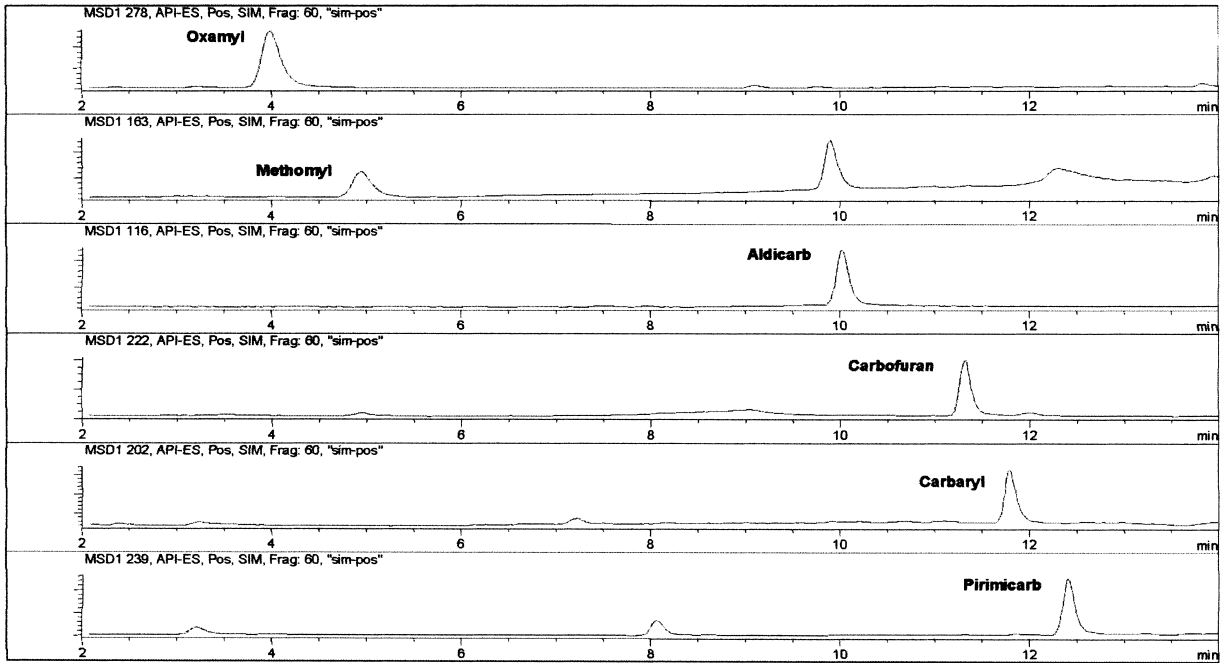


Fig. 3. LC–ESI-MS in the the SIM mode showing the mass fragmentograms of the six carbamates from a laboratory-spiked water sample at the 0.03 μg/l level, after SPE enrichment (Zorbax).

Table 3
Average recoveries and relative standard deviation (RSD) of 10 assays using SPE enrichment (Zorbax), followed by LC–ESI-MS in the SIM mode for the six carbamates from laboratory-spiked water samples at the 0.03 μg/l level

$n=10$	Recovery (%)	RSD (%)
Aldicarb	73.7	14.1
Carbaryl	88.4	14.7
Carbofuran	85.3	13.8
Methomyl	92.6	13.4
Oxamyl	86.5	11.1
Pirimicarb	92.3	11.2

evaluate the accuracy of the present methodology, 10 replicates of laboratory-spiked water samples having the six carbamates at the 0.03 μg/l level were screened, using a octadecylsilica SPE enrichment (Zorbax) followed by LC–ESI-MS in the SIM mode. Table 3 shows very good recoveries between 73.7 and 92.6% for the six carbamates studied with an RSD lower than 14.7% (carbaryl).

In short, the SPE–LC–ESI-MS procedure could reach LODs for the six carbamates in water samples in the range from 0.5 to 3 ng/l, which can easily decrease to lower values by tuning both sample or injection volumes.

4. Conclusions

In the present work, the optimisation of ultra trace analysis of six *N*-methyl carbamates (aldicarb, carbaryl, carbofuran, methomyl, oxamyl and pirimicarb) in water samples was performed using SPE–LC–ESI-MS.

LC–ESI-MS optimisation in the SIM mode, showed an excellent linear response for the six carbamates in the range from 1 to 50 μg/l, a precision having a RSD below 7.8% and instrumental LODs of 0.10 μg/l were found with the exception of methomyl, for which 0.50 μg/l was measured.

The SPE assays were shown to be easy, fast, very sensitive and a low volume (50 ml) of water sample is required. For laboratory-spiked water samples at 0.03 and 0.30 μg/l levels, reasonable average recoveries were obtained, especially using cartridges of octadecylsilica, polystyrene–divinylbenzene and *N*-vinylpyrrolidane–divinylbenzene as solid phases. Ten replicates on octadecylsilica SPE cartridges at the 0.03 μg/l level, allowed average recoveries between 73.7 and 92.6% with an RSD lower than 14.7%.

The present methodology showed analytical performance and seems to be a suitable routine alternative to the currently dedicated analytical systems for carbamate analysis from water matrices, in particular the non-specific post-column derivatization using fluorescence detection. To the best of our knowledge, the LODs for water samples in this work (0.5–3 ng/l) are the lowest that have ever been reported for the ultra trace analysis of carbamates, which clearly covers the maximum concentration admissible for pesticides established by the European Union directive on water quality.

Acknowledgements

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