Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1054 (2004) 351–357
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Simple and rapid assay for analyzing residues of carbamate insecticides in bovine milk: hot water extraction followed by liquid chromatography–mass spectrometry

Sara Bogialli, Roberta Curini, Antonio Di Corcia*, Aldo Laganà, Manuela Nazzari, Michela Tonci

Dipartimento di Chimica, Università "La Sapienza", Piazza Aldo Moro 5, 00185 Rome, Italy

Available online 18 May 2004

Abstract

A simple, specific and rapid procedure for determining six largely used carbamate insecticides in bovine whole milk is here presented. This method is based on the matrix solid-phase dispersion technique with heated water as extractant followed by liquid chromatography (LC)–mass spectrometry (MS) equipped with a single quadrupole and an electrospray ion source. Target compounds were extracted from milk by water heated at 90 °C. After acidification and filtration, 0.2 mL of the aqueous extract was injected in the LC column. MS data acquisition was performed in the selected ion-monitoring mode, selecting three ions for each target compound. Heated water appeared to be an excellent extractant, since absolute recovery data ranged between 76 and 104% with R.S.D. not larger than 8%. Using butocarboxim (an obsolete carbamate insecticide) as surrogate internal standard, the accuracy of the analysis at three spike levels varied between 85 and 105% with R.S.D. not larger than 9%. On the basis of a signal-to-noise ratio of 10, limits of quantification were estimated to range between 3 ppb (propoxur) and 8 ppb (pirimicarb). The effects of temperature, volume and flow rate of the extractant on the analyte recovery were studied.

Keywords: Milk; Hot water extraction; Food analysis; Solid-phase dispersion; Carbamate insecticides; Pesticides

1. Introduction

The problem of environmental contamination by persistent pesticides evokes major concern due to the presence of their residues in the environment and human tissues. Pesticide residues in food and animal feed are of interest because pesticides enter the human system through direct consumption of contaminated food or through milk, meat, and other products obtained from animals that feed on contaminated feed and fodder.

To ensure the safety of milk for consumer, maximum residue limits for insecticides have been set by several organizations such as Food and Agriculture Organization (FAO) [1], European Union (EU) [2] and US Food and Drug Administration (FDA) [3].

While several methodologies are available for determining organochlorine (OC), organophosphorous (OP) and pyrethroid insecticides [4–6] in milk and milk derivatives, only one method has been proposed for determining carbamate insecticides in milk [7]. The use of carbamates for pest control has increased progressively in recent years, together with the OP insecticides, as alternatives to OCs. Owing to their broad spectrum of biological activity, carbamates can be used as insecticides, miticides, fungicides, nematocides, and molluscicides [8]. Carbamate residues are of concern for food control because some of them have high acute toxicity (e.g. aldicarb and carbofuran exhibit LD_{50} values in the rat of 1 and 8 mg/kg, respectively). Some are suspected carcinogens and mutagens [9]. Such insecticides act as inhibitors of the acetylcholinesterase enzymes, and several adverse effects have been reported [10].

One of the bottlenecks in the analysis of contaminants in milk is the time involved in conventional sample preparation and this can limit the number of samples that can be analyzed. In addition, the amounts of chemicals and toxic solvents present a risk greater than that of the pesticide residues

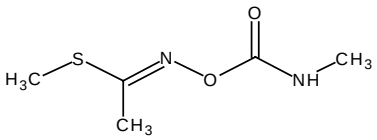
* Corresponding author. Tel.: +39-06-4958251; fax: +39-06-490631.
E-mail address: antonio.dicorcia@uniroma1.it (A. Di Corcia).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.04.014

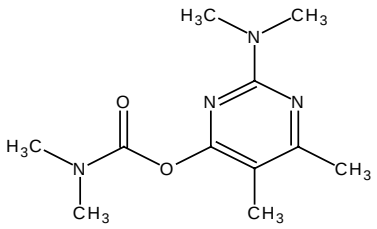
to be determined [11]. These disadvantages show clearly the need for developing rapid, simple and cost-effective techniques that are suitable for routine analysis.

After the pioneering work of Barker et al. [12], many researchers have proposed the so-called matrix solid-phase dispersion (MSPD) technique for extracting xenobiotics from biological matrices [13]. A fine dispersion of the biological matrix onto a solid support such as silica, alumina, diatomaceous earth, C-18-bonded silica and other sorbents, is easily obtained by blending the sample and the sorbent with a mortar and pestle. After blending, this material is packed into a mini-column and analytes are eluted by a suitable extractant. Over classical sample treatment procedures, MSPD offers distinct advantages in that: (i) the analytical protocol is drastically simplified and shortened; (ii) the possibility of emulsion formation is eliminated; (iii) consumption of toxic, flammable and expensive solvents is substantially reduced; and (iv) last but not least, the extraction efficiency of the analytes is enhanced as the entire sample is exposed to the extractant.

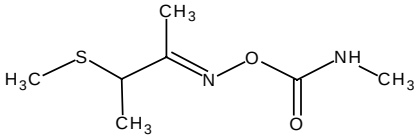
However, the use of even moderate amounts of organic solvents means that problems associated to the use of organic solvents are minimized but not completely removed by MSPD. Moreover, since no organic solvent is capable of selectively extracting target compounds from complex biological matrices, a sample cleanup step is often included in protocols involving analyte extraction by the MSPD technique. Finally, the use of pure organic solvent restricts di-



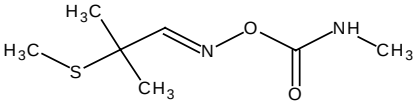
METHOMYL MW 162.2



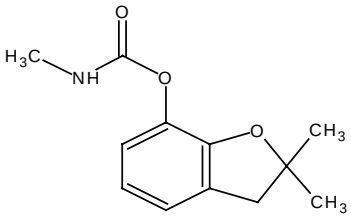
PIRIMICARB MW 238.2



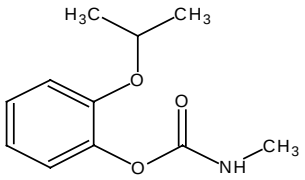
BUTOCARBOXIM (IS) MW 190.2



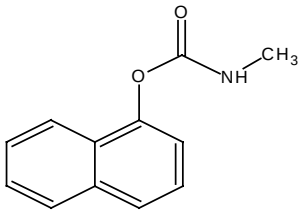
ALDICARB MW 190.2



PROPOXUR MW 209.2



CARBOFURAN MW 221.2



CARBARYL MW 201.2

Fig. 1. Chemical structures and molecular weights of selected carbamate insecticides.

Table 1
Maximum residue limits (ppb) set by the European Union (EU), Food and Agricultural Organization (FAO) and US Food and Drug Administration (FDA) for selected carbamates in milk

	EU	FAO	FDA
Aldicarb	10	10	2
Methomyl	20	20	–
Pirimicarb	–	50	–
Propoxur	50	50	–
Carbofuran	100	50	100
Carbaryl	–	100	300

rect injection of the eluate into a reversed-phase liquid chromatography (LC) column.

An extraction scheme first introduced by Hawthorne et al. [14], which has recently received a considerable interest, involves the use of hot water as an effective extractant for a large number of compounds having a broad spectrum of polarity in solid environmental samples [15–21]. Like CO_2 used in supercritical fluid extraction, water is an environmentally acceptable solvent, it is cost effective and hot water conditions are easily achieved with commercial laboratory equipment. The polarity of water decreases as the temperature is increased. This means that selective extraction of polar and medium-polar compounds can be performed by suitably adjusting the water temperature.

Very recently, we have proposed simple and rapid methods for determining 12 sulfonamide antibacterial in bovine tissues [22,23], milk and eggs [24]. These methods are based on analyte extraction from the matrix dispersed on sand by hot water followed by injection, directly [22] or after little manipulation [23,24], of a large aliquot of the extract on a LC column. Detection of the analytes was performed by a mass spectrometry (MS) system equipped with an ESI ion source and a single quadrupole.

The aim of this work has been that of extending the above analytical strategy to the determination of six carbamate insecticides (Fig. 1) in bovine whole milk at the EU, FAO and FDA regulatory levels (Table 1).

2. Experimental

2.1. Materials

The carbamates (methomyl, pirimicarb, aldicarb, propoxur, carbofuran, carbaryl) and the surrogate internal standard (IS), an obsolete carbamate insecticide (butocarboxim), were obtained from Sigma–Aldrich (Milwaukee, WI). We prepared 1 mg/mL stock solutions of each carbamate by dissolving 10 mg of the pure analytical standards in 10 mL methanol. For recovery studies, a single working composite standard solution was prepared by combining aliquots of each of six individual stock solutions and diluting with methanol to obtain a final concentration of 12 μg/mL. A 20 μg/mL solution of the IS was prepared by diluting the stock solution with methanol. When unused, all the above solutions were stored at 4 °C.

Sand (Crystobalite, 40–200 mesh size) was from Fluka AG, Buchs, Switzerland. Methanol "Plus" of gradient grade was obtained from Carlo Erba, Milan, Italy.

2.2. Samples

Whole pasteurized bovine milk samples used for this study were collected from local markets. The samples used for recovery and sensitivity studies were previously determined to be free of the pesticides considered.

2.3. Extraction apparatus

The design of the homemade extraction apparatus used in this work was very similar to that shown in a previous paper [25], with the exception that nitrogen was bubbled in water to eliminate any trace of dissolved oxygen and the analyte-containing water leaving the extraction cell was collected in a calibrated glass tube instead of a sorbent cartridge. An 8.1 cm × 8.3 mm i.d. stainless steel column was used as extraction cell.

2.4. Sample preparation and extraction

For recovery studies, milk samples were spiked with known variable amounts of carbamates. Under continuous agitation, 15 min were allowed for equilibration at room temperature. Thereafter, 3 mL of milk were taken and poured in a porcelain mortar containing 12 g of sand and the mixture was blended with the pestle for less than 15 min, until an apparently dry material was obtained. This material was then packed into a 16 cm in length extraction cell, taking care to tap the tube to avoid loose packing of the particles. Any void space remaining after packing the solid material was filled with sand. A stainless steel (2 μm pore size) and a polyethylene (20 μm pore size) frits were located, respectively, above and below the packing. The tube was then put into the oven and heated at 90 °C for 5 min. Five milliliters of water was then passed through the cell at 1 mL/min flow rate to extract the analytes and, if present, the surrogate internal standard. The choice of the parameters mentioned above for extracting the analytes resulted from preliminary experiments (see further) showing that this situation offered maximum recovery of the analytes and a restricted number of co-extractives. When experiments were performed to assess the extraction yield by heated water, 500 ng of the IS was added to the extract. To make aqueous extract injectable into the LC column, the pH of the extract was adjusted to 4.6 with 3 mol/L formic acid and then filtered through a regenerated cellulose filter (pore size 0.2 μm, 25 mm diameter, Alltech, Sedriano, Milan, Italy). After filtration, a completely uncolored and transparent solution was obtained. By following the procedure described above, the guard column was replaced with a new one after

Table 2
Time-scheduled multiple-ion selected ion-monitoring conditions for detecting selected carbamates in milk

Compound	Channel mass, *m/z* (relative abundance)	Cone voltage (V)	Retention window (min)
Methomyl	88 (100), 106 (80), **163**[a] (20)	40	0.0–10.7
Pirimicarb	72 (20), 182 (50), **239** (100)	40	10.7–15.6
Butocarboxim	116 (30), 188 (20), *213*[b] (100)	40	15.6–18.7
Aldicarb	89 (60), 116(100), *213* (70)	40	–
Propoxur	111 (40), 168 (100), **210** (60)	35	18.7–21.3
Carbofuran	165 (50), **222** (100) *244* (30)	35	–
Carbaryl	145 (100), 177 (70), **202** (30)	35	21.3–24.0

[a] Protonated ions are reported in boldface.
[b] Sodiated ions are reported in italics.

more about 150 injections of milk extracts. Finally, 0.2 mL of the extracts was injected into the LC column.

2.5. Instrumental analysis

The liquid chromatograph consisted of a Thermoquest pump (model P2000, Manchester, UK), a 250 μL injection loop, and Alltima 5 μm C-18 guard (7.5 mm × 4.6 mm i.d.) and analytical (250 mm × 4.6 mm i.d.) columns (Alltech) thermostated at 35 °C and was interfaced to a Finnigan benchtop single-quadrupole mass spectrometer (model AQA, Thermoquest). Mobile phase component A was 10 mM formic acid in methanol and component B was aqueous 10 mM formic acid. At 1.0 mL/min, the mobile phase gradient profile was as follows (t in min): t_0, A = 25%; t_{25}, A = 75%; t_{26}, A = 100%; t_{29}, A = 100%; t_{31}, A = 25%; t_{39}, A = 25%. Analyte retention times varied ≤0.5% over 2 weeks. A fraction (150 μL) of the column effluent was diverted to an orthogonal ESI source. The characteristic of the AQA instrument is that a constant water spray at a flow rate of 40 μL/min can be applied to the outer upstream side of the sample cone orifice [26] to avoid deposition of salts and other involatile matrix components on the periphery of the ion inlet orifice. When this device was not activated, MS sensitivity diminished during a day of 200 μL injections of milk extracts. The probe temperature was 180 °C and the capillary voltage was 4 kV. Nitrogen was used as drying and nebulizer gases at flow rates of 300 and 50 L/h, respectively. The ESI/MS system was operated in the positive ionization mode. For each analyte, diagnostic fragment ions were obtained by in-source collision-induced dissociation (CID) of the protonated molecule $[M + H]^+$ by suitably adjusting the voltage of the skimmer cone. Ion signals were acquired by the time-scheduled multiple-ion selected ion-monitoring (SIM) mode as reported in Table 2. At least three ions per analyte and up to six ions per retention window were monitored.

3. Results and discussion

3.1. Effect of the temperature on analyte recoveries

As water is heated at high temperatures, its surface tension, viscosity and polarity progressively decrease. Heated water, thus, becomes an efficient medium for extracting from solid matrixes even those organics that are scarcely soluble in water at ambient temperature. On the other hand, a risk inherent to the use of hot water as extractant is that it could decompose those compounds that are thermolabile and/or prone to hydrolytic attack. Therefore, we evaluated the temperature effect on recoveries of the selected pesticides by performing extractions at various temperatures. The aim of this study was also that of finding the minimum extraction temperature able to give good recovery of the analytes and the lowest amount of matrix components that could contaminate the ion source and/or interfere with the rest of the analysis. For this study, a sample of milk was spiked with the analytes and the surrogate internal standard at 100 ppb and a water volume equal to 6 mL that passed through the extraction cell at 1 mL/min flow rate. At each temperature, three extractions were carried out and results are reported in Table 3.

Raising the temperature of the extractant from 50 to 90 °C had the effect of remarkably improving the extraction yield especially of those carbamates having the largest hydrophobic moieties. With the aim of further enhancing the recovery of carbaryl, analyte extraction was also performed with water heated at 110 °C. Under this condition, no improvement of the extraction yield of carbaryl was observed and severe loss of the most thermolabile carbamates, i.e. methomyl, aldicarb and the surrogate internal standard (butocarboxim) were obtained. Thus, an extraction temperature of 90 °C was used for subsequent experiments.

Table 3
Effect of the extraction temperature on the recovery (n = 3) of six carbamate insecticides in milk

	Percentage recovery[a] (R.S.D., %)			
	50 °C	70 °C	90 °C	110 °C
Methomyl	85 (6)	93 (3)	104 (6)	42 (8)
Pirimicarb	74 (5)	87 (6)	89 (6)	92 (5)
Aldicarb	72 (5)	84 (4)	98 (5)	54 (7)
Butocarboxim	75 (4)	82 (5)	95 (5)	60 (7)
Propoxur	71 (5)	84 (6)	96 (4)	84 (8)
Carbofuran	53 (7)	72 (6)	88 (6)	84 (7)
Carbaryl	39 (8)	62 (7)	76 (8)	74 (6)

Spike level: 100 ppb.
[a] Mean values from three experiments.

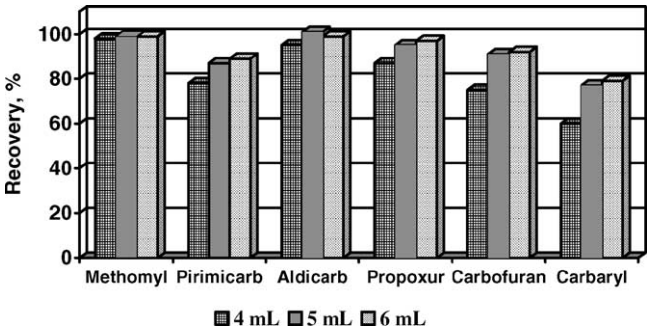


Fig. 2. Effect of the extractant volume on analyte recovery in a bovine milk sample spiked with 100 ppb of selected carbamate insecticides.

3.2. The effect of the extractant volume on analyte recoveries

Besides affecting the extraction yield of the target compounds, the water volume passing through the extraction cell can influence the sensitivity of the method, as this method does not include any concentration step of the extract. For the purpose of finding the minimum volume of water able to extract efficiently the analytes, experiments were performed by spiking a milk sample with the analytes and the surrogate internal standard at 100 ppb level and extracting with increasing water volumes. Experiments were made in triplicate and results are visualized in Fig. 2. As can be seen, extracting with more than 5 mL of water did not increase significantly analyte recovery. Thus, the best compromise between method sensitivity and extraction yield was that of passing through the extraction cell 5 mL of water heated at 90 °C.

3.3. The effect of the extractant flow rate on analyte recoveries

We evaluated the influence that the flow rate at which water passed through the extraction cell on the extraction efficiency. For this experiment, a milk sample spiked with the analytes at 100 ppb level was submitted to the extraction procedure by passing water through the cell at flow rates ranging between 0.5 and 2 mL/min. Results (not shown here) from triplicate experiments at each flow rate selected evidenced that the analyte extraction yield was substantially not dependent on the extractant flow rate. We chose to extract carbamates at a flow rate of 1 mL/min because at 2 mL/min flow rate the extraction cell sometimes clogged.

3.4. Matrix effect

In order to achieve high-throughput determination of analytes in biological matrices, analytical protocols based on LC–ESI/MS with short (3–5 cm) LC columns where analytes are eluted in few minutes are often adopted. However, numerous examples and studies [27–34] have revealed that the yield of protonation (or cationization) of the analytes in the electrosprayed solution can be decreased to a greater or lesser extent by competition effects due to the presence of matrix components. The extent of this unwelcome effect is related to both concentrations and affinities for the proton (or cations) of the co-extracted and co-eluted matrix components. It was shown that ion suppression of the analytes could be minimized or eliminated by adopting selective extraction methods [30,32] and/or efficient chromatographic separation [32]. Initially, recovery studies of carbamates in milk were conducted by chromatographing extracts with a relatively short run time. According to the terminology adopted by Matuszewski et al. [32], this condition will be called “poor separation”. Unsatisfactory low recovery of some of the analytes, i.e. aldicarb, pirimicarb and carbaryl were obtained. In order to ascertain if the apparently poor recovery of the above carbamates was due to partial failure of hot water in extracting the above analytes from animal tissues or to a matrix effect, the experiment was repeated with two substantial modifications. In particular, this experiment was designed as follows: (i) duplicate extractions of six milk samples from different sources; (ii) addition of the carbamate insecticides to the 12 final extracts; (iii) injection of the spiked extracts into the LC apparatus under two different chromatographic conditions obtained by varying the initial concentration of the “strong” solvent, i.e. methanol; and (iv) quantification of the concentrations of the analytes in the milk extracts by comparing their absolute peak areas to those of the same compounds injected from a standard solution. Results reported in Table 4 indicate that the matrix effect was indeed responsible for the apparently low recovery of aldicarb and pirimicarb as their ion signal intensities increased dramatically as the strength of the LC mobile phase was decreased. Under “good separation” conditions, which are those adopted in this work, the matrix effect provoking

Table 4
Effect of the chromatographic conditions on the ion signal intensities of selected carbamate insecticides directly added to a bovine milk extract

	Poor separation[a]		Good separation[b]	
	t_R (min)[c]	Relative peak area[d]	t_R (min)	Relative peak area
Methomyl	7.5	103[e] (9[f])	8.4	102 (7)
Pirimicarb	12.0	30 (5)	14.3	96 (4)
Aldicarb	12.3	42 (6)	17.3	97 (4)
Propoxur	13.0	105 (6)	20.4	94 (6)
Carbofuran	13.0	90 (5)	20.7	89 (4)
Carbaryl	13.5	63 (9)	22.4	78 (7)

Spike level: 100 ppb.

[a] Gradient elution: $t_0 = 25\%$ methanol; $t_5 = 35\%$ methanol; $t_6 = 70\%$ methanol; $t_{12} = 82\%$ methanol; $t_{13} = 100\%$ methanol.

[b] Chromatographic conditions as those reported in Section 2.

[c] Retention time.

[d] Peak area of the analyte injected from a milk extract relative to that of the analyte injected from a standard solution.

[e] Mean values from 12 determinations.

[f] Relative standard deviations (%).

Table 5
Accuracy and precision data on analyzing selected carbamate insecticides in milk at concentrations equal or close to maximum residue limits (MRLs) set by the European Union (EU), the Food and Agriculture Organization (FAO) and US Food and Drug Administration (FDA)

Compound	Percentage accuracy (R.S.D., %)		
	MRL/2[a]	MRL	2MRL
Methomyl	99 (6)	104 (8)	105 (7)
Pirimicarb	91 (5)	95 (4)	95 (6)
Aldicarb	93 (7)	97 (8)	101 (5)
Propoxur	93 (9)	96 (9)	99 (6)
Carbofuran	85 (7)	88 (8)	90 (4)
Carbaryl	93 (5)	94 (8)	98 (6)

[a] MRLs are reported in Table 1. When MRLs differed from institution to institution the lowest values were considered, with the exception of aldicarb whose tolerance level set by FDA are lower than the LOQ of the method.

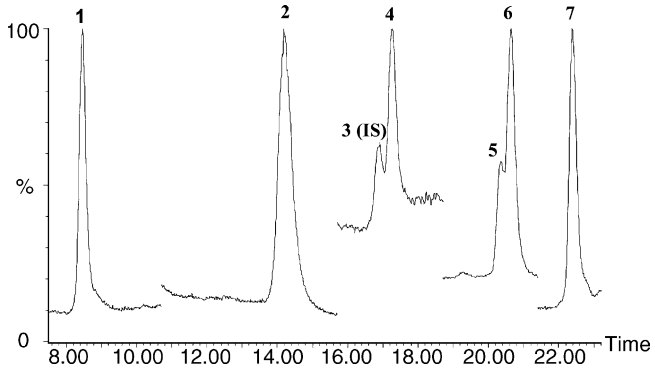


Fig. 3. LC–ESI/MS multiple-ion SIM chromatogram resulting from the analysis of a bovine milk sample spiked with 50 ppb of selected carbamate insecticides. Peak numbering: 1, methomyl; 2, pirimicarb; 3, butocarboxim (IS); 4, aldicarb; 5, propoxur; 6, carbofuran; 7, carbaryl.

severe underestimation of pirimicarb and aldicarb was no more present.

Carbaryl exhibited a different behavior. Decreasing the eluotropic strength of the LC mobile phase resulted in a little increase of the response of the mass detector, this suggesting that carbaryl was still co-eluted with co-extracted unseen endogenous compounds. No significant improvement was observed by further slowing the chromatographic run (data not shown here). By analyzing six milk samples from different sources, the relative standard deviation of the mean concentration of carbaryl were not higher than 7% suggesting that the degree of ion suppression for protonated carbaryl was independent on the particular milk extract analyzed. Therefore, adoption of a carbaryl-fortified control milk extract as reference standard could serve to improve the accuracy of the analysis of this carbamate in incurred samples of milk. This practice is routinely adopted when measuring contaminants in complex biological matrices by LC–ESI/MS.

3.5. Accuracy and precision

Following criteria reported in the EU guidelines [35], this method was validated at three different concentrations corresponding to one-half of the maximum residue limit (MRL) (Table 1), the MRL and two times the MRL. At each analyte concentration, five measurements were performed with the criterion of adding the surrogate internal standard (butocarboxim) *before* analyte extraction. Except for carbaryl, assessment of the analyte concentrations in milk was performed by comparing their relative peak areas to those obtained by injecting standard solutions. Vice versa, measurement of the various concentrations of carbaryl in milk was done by using carbaryl-fortified milk extracts as reference standards. Results are reported in Table 5. The accuracy data varied between 85 and 105% with standard deviations not higher than 9%. Thus, this method meets requirements reported in the EU guidelines [35] indicating that a method can be considered accurate and precise when accuracy data are comprised between 70 and 110% with relative standard deviations not higher than 20%.

3.6. Linear dynamic range

Under the instrumental conditions reported in Section 2, the linear dynamic range of the ESI/MS detector was estimated for all the analytes. Amounts of each analyte varying from 4 to 600 ng and a constant amount of 25 ng of the internal standard were injected from suitably prepared standard solutions into the LC column. At each analyte amount, three replicate measurements were made. Signal against amount-injected curves were then constructed by averaging the peak area resulting from the sum of the signals for parent and fragment ions of each analyte and relating this area to that of the internal standard. Results showed that ion signals of the six carbamates were linearly correlated with injected amounts up to 300 ng, with R^2 ranging between 0.9911 and 0.9999.

3.7. Limits of detection (LODs) and quantification (LOQs)

LOQs of the method were estimated from the SIM LC–MS chromatogram resulting from analyses of 50 ppb of each carbamate in bovine milk (Fig. 3). After extracting the sum of the ion currents of both precursor and fragment ions relative to each analyte, the resulting trace was smoothed twice by applying the mean smoothing method (Mass Lab Software, Thermoquest). Thereafter, the peak height-to-averaged background noise ratio was measured. The background noise estimate was based on the peak-to-peak baseline near the analyte peak. LOQs were then calculated on the basis of a minimal accepted value of the signal-to-noise ratio (S/N) of 10. These data are listed in Table 6. In the same table, LODs of the method are also presented. When using a MS detector, the first condition to be satisfied for ascertaining the presence of a targeted compound is that the precursor ion and at least two product ions produce signals distinguishable from the background ion

Table 6
Limits of detection (LOD) and quantification (LOQ) of the method

Compound	LOD (ppb)	LOQ (ppb)
Methomyl	1 (106)	6
Pirimicarb	5 (72)	8
Aldicarb	3 (116)	5
Propoxur	3 (210)	3
Carbofuran	4 (222)	4
Carbaryl	4 (202)	4

The *m*/*z* values of the ions giving the worst S/N ratio are reported in parentheses.

current. Accordingly, a definition of LOD (S/N = 3) of each analyte was adopted, considering in each case the ion giving the worst S/N. Except for aldicarb at the tolerance level set by FDA (see again Table 1), LOQs of the method are below tolerance levels set by EU, FAO and FDA for residues of the carbamate insecticides considered in bovine milk.

4. Conclusions

This work has again shown that the environmentally friendly and inexpensive water, besides to be an effective extractant for polar and medium-polar contaminants in biological matrices, produces sufficiently clean extracts requiring little manipulation before final analysis by LC–MS. Also, the ESI/MS detector equipped with a single quadrupole, where confirmatory ions are produced by in-source CID, provides specificity similar to that obtained by a much more expensive instrumentation, i.e. tandem MS, and sensitivity sufficient for analyzing carbamate insecticides in milk at regulatory levels, with the exception of aldicarb at the FDA tolerance level. This drawback could be eliminated by using tandem MS in the multi reaction monitoring.

Acknowledgements

This work was supported by Istituto Superiore per la Prevenzione e la Sicurezza del Lavoro (contract number: B/23/DIPIA/00).

References

[1] Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, Pesticides Residues in Food, vol. II, Food and Agriculture Organization and World Health Organization, Rome, 1993.
[2] European Union Council Directive no. 95/39/EC of 7 July 1995, Off. J. Eur. Communities no. L197 of 22 August 1995; European Union Council Directive no. 96/33/EC of 21 May 1996, Off. J. Eur. Communities no. L144 of 18 June 1996; European Union Council Directive no. 94/29/EC of 23 June 1994, Off. J. Eur. Communities no. L189 of 23 July 1994.
[3] Food Quality Protection Act, 3 August 1996, Pub. L. no. 104-170 (1996).
[4] G. Filek, M. Bergamini, W. Lindner, J. Chromatogr. A 712 (1995) 355.
[5] A. Di Muccio, P. Pelosi, D.A. Barbini, T. Generali, A. Ausili, F. Versori, J. Chromatogr. A 765 (1997) 51.
[6] A. Di Muccio, P. Pelosi, I. Camoni, D.A. Barbini, R. Dommarco, T. Generali, A. Ausili, J. Chromatogr. A 754 (1996) 497.
[7] Y. Li, X. Wang, Weisheng Yanjiu 26 (1997) 278.
[8] K.A. Hassal, The Chemistry of Pesticides: Their Metabolism, Mode of Action and Uses in Crop Protection, Macmillan, New York, 1983.
[9] Q.X. Li, B.D. Hammock, J.N. Seiber, J. Agric. Food Chem. 39 (1991) 1537.
[10] G.P. Casale, J.L. Vennerstrom, S. Bavari, T.L. Wang, Immunopharmacol. Immunotoxicol. 15 (1993) 199.
[11] C.M. Torres, Y. Picó, J. Mañes, J. Chromatogr. A 754 (1996) 301.
[12] S.A. Barker, A.R. Long, C.R. Short, J. Chromatogr. 475 (1989) 353.
[13] S.A. Barker, J. Chromatogr. A 885 (2000) 115.
[14] S.B. Hawthorne, Y. Yang, D.J. Miller, Anal. Chem. 66 (1994) 2912.
[15] Y. Yang, S.B. Hawthorne, D.J. Miller, Environ. Sci. Technol. 31 (1997) 430.
[16] J.A. Field, K. Monohan, R. Reed, Anal. Chem. 70 (1998) 1956.
[17] C. Crescenzi, G. D'Ascenzo, A. Di Corcia, M. Nazzari, S. Marchese, R. Samperi, Anal. Chem. 71 (1999) 2157.
[18] A. Di Corcia, A.B. Caracciolo, C. Crescenzi, G. Giuliano, S. Murtas, R. Samperi, Environ. Sci. Technol. 33 (1999) 3271.
[19] Y. Yang, B. Li, Anal. Chem. 71 (1999) 1491.
[20] C. Crescenzi, A. Di Corcia, M. Nazzari, R. Samperi, Anal. Chem. 72 (2000) 3050.
[21] F. Bruno, R. Curini, A. Di Corcia, I. Fochi, M. Nazzari, R. Samperi, Environ. Sci. Technol. 36 (2002) 4156.
[22] S. Bogialli, R. Curini, A. Di Corcia, M. Nazzari, R. Samperi, Anal. Chem. 75 (2003) 1798.
[23] S. Bogialli, R. Curini, A. Di Corcia, M. Nazzari, M. Sergi, Rapid Commun. Mass Spectrom. 17 (2003) 1146.
[24] S. Bogialli, R. Curini, A. Di Corcia, M. Nazzari, M.L. Polci, J. Agric. Food Chem. 51 (2003) 4225.
[25] C. Crescenzi, G. D'Ascenzo, A. Di Corcia, M. Nazzari, S. Marchese, R. Samperi, Anal. Chem. 71 (1999) 2157.
[26] S. Bajic, D.R. Doerge, L. Lu, E.B. Hansen Jr., Rapid Commun. Mass Spectrom. 14 (2000) 156.
[27] P. Kebarle, L. Tang, Anal. Chem. 65 (1993) 972A.
[28] L.G. McLaughlin, J.D. Henion, P.J. Kijak, Biol. Mass Spectrom. 23 (1994) 417.
[29] C. Crescenzi, A. Di Corcia, A. Marcomini, R. Samperi, Anal. Chem. 67 (1995) 1797.
[30] A. Di Corcia, C. Crescenzi, A. Laganà, E. Sebastiani, J. Agric. Food Chem. 44 (1996) 1931.
[31] D. Buhrman, P. Price, P. Rudewicz, J. Am. Mass Spectrom. 7 (1997) 1099.
[32] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 70 (1998) 882.
[33] D.N. Heller, S.B. Clark, H.F. Righter, J. Mass Spectrom. 35 (2000) 39.
[34] S. Riediker, R.H. Stadler, Anal. Chem. 73 (2001) 1614.
[35] Quality Control Procedures for Pesticide Residues Analysis, Guidelines for Residues Monitoring in the European Union, second ed., Document no. SANCO/3103/2000, 1999–2000.