JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

A Simple and Rapid Assay for Analyzing Residues of Carbamate Insecticides in Vegetables and Fruits: Hot Water Extraction Followed by Liquid Chromatography-Mass Spectrometry

SARA BOGIALLI, ROBERTA CURINI, ANTONIO DI CORCIA,* MANUELA NAZZARI, AND DAVIDE TAMBURRO

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

A simple, specific, and rapid analytical method for determining seven largely used carbamate insecticides in tomato, spinach, lettuce, zucchini, pear, and apple 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 the vegetal matrixes by water heated at 50 °C. After acidification and filtration, 0.25 mL of any 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 because recovery data ranged between 76 (carbaryl in spinach) and 99% (pirimicarb in spinach), with RSDs not larger than 10%. Using trimethacarb (an obsolete carbamate insecticide) as a surrogate internal standard, the accuracy of the analysis varied between 84 and 110%, with RSDs not larger than 9%. On the basis of a signal-to-noise ratio of 10, limits of quantification were estimated to range between 2 (pirimicarb) and 10 ppb (oxamyl) and were not influenced by the type of matrix. When trying to fractionate analytes by using a short chromatographic run time, marked weakening of the ion signals for oxamyl, methomyl, and aldicarb were observed. This effect was traced to polar endogenous co-extractives eluted in the first part of the chromatographic run that interfered with gas-phase ion formation for carbamates. Adopting more selective chromatographic conditions eliminated this effect.

INTRODUCTION

N-Methylcarbamate (NMC) insecticides are widly used chemicals for crop protection. The reason for this is that they proved to have a high insect toxicity but a generally low toxicity toward warm-blooded species. In addition, carbamates are much less persistent that organochlorine pesticides and produce fewer toxic degradation products. Nevertheless, because carbamates are inhibitors of acetylcholinesterase, they are considered toxic for the environment and for human beings. This has compelled the introduction in the EU of regulations stating that the most toxic carbamates cannot be present in fruits and vegetables at levels higher than 50 ng/g.

Monitoring programs for pesticide residues in fruits and vegetables routinely use liquid chromatography (LC) to determine NMCs, because many of these compounds lack the thermal stability necessary for gas chromatography (GC) determination. Moye et al. (*1*) were the first to adapt an LC/fluorogenic labeling technique to the detection of NMCs. Their technique involved a reversed-phase separation followed by a postcolumn base hydrolysis that liberated methylamine, which further reacted with *o*-phthalaldehyde to form a highly fluorescent isoindole. Later, Krause (*2*) used this technique to develop a method for monitoring NMCs in fruits and vegetables. With further modifications (*3*), this method is at present largely used in regulatory agencies. However, this method suffers from some drawbacks and limitations. First, it makes use of time-consuming cleanup procedures. Second, co-extracted substances having native fluorescence may interfere with the analysis.

Public health agencies in many countries rely on detection by mass spectrometry (MS) for unambiguous confirmation of antibiotics in foodstuff. The Commission Decision 93/256/EEC states that "Methods based only on chromatographic analysis without the use of molecular spectrometric detection are not suitable for use as confirmatory methods". Research in new methodologies in MS, notably LC-MS, has greatly benefited from the international need of protecting food quality and now can serve to fulfill the goals initially sought by such a technique, which is monitoring non volatile, thermally labile, and polar target compounds with the specificity and sensitivity similar to GC-MS. In the past 20 years, a large variety of interfaces have been developed to make the high vacuum of the mass analyzer compatible with the large amounts of liquids coming out from the LC column. At present, only the electrospray (ESI) and

* To whom correspondence should be addressed. Fax: +39-06-490631. E-Mail: antonio.dicorcia@uniroma1.it.

10.1021/jf035195j CCC: $27.50
Published on Web 01/21/2004

atmospheric pressure chemical ionization ion sources are commercially available and have been employed for developing methods for determining carbamate residues in fruits and vegetables (*4*−*10*). Despite mass spectrometry's capability to confirm the identity of target analytes, protocols for extracting NMCs from vegetables still required relatively complex labor-intensive cleanups because nonselective organic solvents were used.

After the pioneering work of Barker and his colleagues (*11*), many researchers have successfully adopted the so-called matrix solid-phase dispersion (MSPD) technique for extracting xenobiotics, particularly drugs, from biological matrixes (*12*). 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. The abrasive action of the sorbent during blending has been demonstrated to disrupt the gross architecture of the matrix (*12*), so that a tight and quasi-homogeneous layer of the matrix components is formed on the sorbent surface. Over classical sample treatment procedures, MSPD offers distinct advantages in that (1) the analytical protocol is drastically simplified and shortened; (2) the possibility of emulsion formation is eliminated; (3) consumption of toxic, flammable, and expensive solvents is substantially reduced; and (4), last but not least, the extraction efficiency of the analytes is enhanced as the entire sample is exposed to the extractant.

With the exception of a work proposing water at ambient temperature for extracting the highly hydrophilic aminoglycoside antibiotics from bovine kidney (*13*), all other methods based on MSPD have used moderate amounts of organic solvents as extractants. This means that problems associated with the use of organic solvents are minimized but not completely removed by MSPD. Moreover, because no organic solvent is capable of selectively extracting target compounds from complex biological matrixes, a sample cleanup step is often included in protocols involving analyte extraction by the MSPD technique. Finally, the use of pure organic solvent restricts direct injection of the eluate into a reversed-phase LC column.

Very recently, we have proposed rapid methods for determining 12 sulfonamide antibacterial in bovine and fish tissues (*14*, *15*), milk, and eggs (*16*). These methods are based on analyte extraction from the matrix dispersed on sand by hot water followed by injection, directly (*14*) or after little manipulation (*15*, *16*), of a large aliquot of the extract on an LC column. Detection of the analytes was performed by an 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 seven NMCs (**Figure 1**) in six different vegetal matrixes, at the European Union (EU), Food and Agriculture Organization (FAO) and United States Food and Drug Administration (FDA) regulatory levels (**Table 1**).

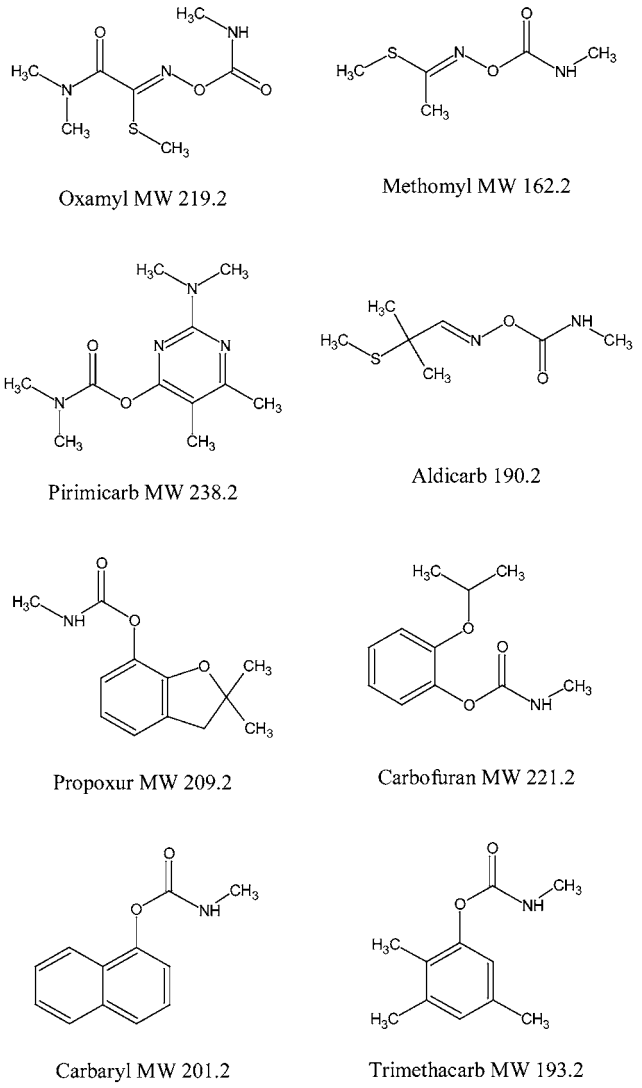

Figure 1. Chemical structures and molecular weights of selected carbamate insecticides.

MATERIALS AND METHODS

Materials. The carbamates (oxamyl, methomyl, pirimicarb, aldicarb, propoxur, carbofuran, and carbaryl) and the surrogate internal standard (IS), an obsolete carbamate insecticide (trimethacarb), 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 of methanol. For recovery studies, a single working composite standard solution was prepared by combining aliquots of each of seven individual stock solutions and diluting with water/methanol (75:25, v/v) to obtain a final concentration of 4 μg/mL. A 30-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, Milano, Italy.

Samples. Fruits and vegetables used for this study were collected from local markets. Various combinations of samples were chosen from a list of crops: lettuce, tomato, zucchini, spinach, apple, and pear. The samples used for recovery, and sensitivity studies were previously determined to be free of the pesticides considered.

Extraction Apparatus. The design of the homemade extraction apparatus used in this work was very similar to that shown in a previous paper (*17*), 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.

Sample Preparation and Extraction. Vegetal samples were finely diced with scissors or knife. For evaluating the extraction yield, 2 g of each sample was put in a porcelain mortar and spiked with variable volumes of the partially aqueous working standard solution, taking care of uniformly spreading it on the sample. For assessing the accuracy and precision of the method, vice versa, the surrogate internal standard (trimethacarb) was added together with the analytes. After deposition of the compounds on the matrix, 1 h was allowed for equilibration, storing the mortar at 4 °C. Thereafter, 6 g of sand was added to the

Table 1. Maximum Residue Limits (ppm) Set by the European Union[a], Food and Agricultural Organization,[b] and United States Food and Drug Administration[c] for Selected Carbamates in Different Vegetal Matrixes

	matrix					
compound	lettuce	zucchini	spinach	tomato	apple	pear
oxamyl				2[b,c]	2[b,c]	2[c]
methomyl	2[a], 5[b,c]	0.05[a], 0.2[c]	2[a], 5[b], 6[c]	0.5[a], 1[b,c]	0.2[a], 2[b], 1[c]	0.2[a], 2[b], 4[c]
pirimicarb	1[b]		1[b]	1[b]	1[b]	1[b]
aldicarb	0.05[a]	0.05[a]	0.05[a]	0.05[a]	0.05[a]	0.05[a]
propoxur	0.05[a], 0.5[b]	0.05[a]	0.05[a], 2[b]	0.05[a,b]	0.05[a], 3[b]	0.05[a], 3[b]
carbofuran	0.1[a]	0.1[a]	0.1[a]	0.1[a,b]	0.1[a]	0.1[a]
carbaryl	3[a], 10[b,c]		1[a], 10[b], 12[c]	1[a], 5[b], 10[c]	3[a], 5[b], 10[c]	3[a], 5[b], 10[c]

mortar, and the mixture was blended with the pestle for less than 15 min, until an apparently homogeneous and dry material was obtained. This material was then packed into the 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 above and below the packing, respectively. The tube was then put into the oven and heated at 50 °C for 5 min. Three 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 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, 300 ng of the IS was added to the extract. Depending on the nature of the vegetal sample, extracts appeared more or less opaque, with pH ranging between 4.5 and 6.4. To make aqueous extracts injectable into the LC column, their pH was adjusted to 3−3.3 with 3 mol/L formic acid and then filtered through a regenerated cellulose filter (pore size 0.2-μm, 25-mm d, Alltech, Sedriano, Milan, Italy). After filtration, a completely transparent final extract was obtained. By following the procedure described above, the guard column was replaced with a new one after more than about 150 injections of vegetal extracts. Finally, 250 μL of the extracts was injected into the LC column.

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- × 4.6-mm i.d.) and analytical (250- × 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, $A = 5\%$; t_{17}, $A = 35\%$; t_{18}, $A = 55\%$; t_{28}, $A = 75\%$; t_{29}, $A = 100\%$; t_{32}, $A = 100\%$; t_{34}, $A = 5\%$; t_{42}, $A = 5\%$. Analyte retention times varied ≤0.5% over two weeks. Column effluent (150 μL) 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 (*18*) 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 250-μL injections of vegetal 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 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 3 ions per analyte and up to 9 ions per retention window were monitored.

Quantitation. Absolute recoveries of the analytes added to fruits and vegetables at any given concentration were assessed by measuring peak areas resulting from the sum of the interference-free ion current profiles of parent and fragment ions, normalizing them to the summed peak areas of the IS, which was added *after* extraction, and comparing these ratios to those obtained by injecting a standard solution. Accuracy and precision data were obtained in a similar way, with the exception that the IS was added *before* analyte extraction. The mass spectrometry data handling system used was the "Mass Lab" software from Thermoquest.

Table 2. Time-Scheduled Multiple-Ions Selected Ion Monitoring Conditions for Detecting Carbamates in Vegetal Matrixes

compound	channel mass, *m/z* (relative abundance)	cone voltage, *V*	retention window, min
oxamyl	72 (80), 122 (50), *242*[a] (100)	40	0.0–16.8
methomyl	88 (100), 106 (80), **163**[b] (20)	40	16.8–22.0
pirimicarb	72 (20), 182 (50), **239** (100)	40	22.0–26
aldicarb	89 (60), 116(100), *213* (70)	40	22.0–26
propoxur	111 (40), 168 (100), **210** (60)	35	26–28.5
carbofuran	165 (50), **222** (100) *244* (30)	35	26–28.5
carbaryl	145 (100), 177 (70), **202** (30)	35	26–28.5
trimethacarb	137 (100), **194** (60) *216* (30)	35	28.5–31.0

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

RESULTS AND DISCUSSION

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, we selected a lettuce sample spiked with the analytes and the surrogate internal standard at 100 ppb and a water volume equal to 4 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 25 to 50 °C had the effect of remarkably improving the extraction yield especially of those carbamates having the largest hydrophobic moieties. By further raising the extraction temperature to 75 °C, the amounts of the analytes and the surrogate internal standard removed from the lettuce sample did not significantly increase. Further increasing the extraction temperature at 100 °C had the effect of decomposing the most thermolabile carbamates (i.e., oxamyl, methomyl, and aldicarb). Compared

Table 3. Effect of the Extraction Temperature on the Recovery (n = 3) of Seven *N*-Methylcarbamates in a Lettuce Sample[a]

	25 deg C	50 deg C	75 deg C	100 deg C
	% recovery (% RSD)			
oxamyl	77 (6)	82 (5)	84 (6)	34 (10)
methomyl	85 (6)	92 (6)	93 (7)	39 (9)
pirimicarb	82 (5)	93 (6)	95 (6)	91 (5)
aldicarb	64 (6)	79 (7)	76 (7)	57 (7)
propoxur	73 (4)	92 (5)	93 (5)	81 (7)
carbofuran	67 (7)	83 (5)	83 (6)	87 (7)
carbaryl	61 (8)	84 (7)	86 (8)	71 (6)
trimethacarb	58 (9)	86 (7)	87 (5)	82 (7)

[a] Spike level, 100 ppb; extractant volume, 4 mL; extractant flow-rate, 1 mL/min.

to the extract obtained at 75 °C, that one obtained at 50 °C appeared to contain lesser amounts of matrix components. Thus, this extraction temperature was used for subsequent experiments.

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 each of the matrixes considered with the analytes and the surrogate internal standard at 100 ppb level and extracting. Aliquots (1 mL) of the aqueous extract coming out from the cell were collected and analyzed. For each matrix, experiments were performed in duplicate. For the sake of clarity, only the results of the experiments performed with spinach and pear samples are visualized in **Figure 2**. As can be seen, NMC extractability by heated water depended somewhat on the matrix, as some crops required larger volumes for complete recovery. Maybe, interaction forces acting between the analytes and some of the non extracted matrix components vary in intensity by changing the nature of the vegetal matrix. Anyway, 3 mL of heated water sufficed to extract the analytes from any matrix considered and they were used in subsequent experiments.

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 lettuce 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, especially when extracting analytes from pear and apple samples.

Extraction Efficiency. According to the parameters reported in the Experimental section, we evaluated the ability of water to extract carbamates from the various matrix considered. This study was conducted by spiking the various vegetal matrixes considered with the analytes at 100 ppb level. Absolute recoveries were estimated by adding the internal standard (trimethacarb) *after* extraction and *before* extract filtration. For each matrix, four experiments were performed and results are shown in **Table 4**. The extraction yields varied from 76 to 99% with relative standard deviations not higher than 10%.

Accuracy and Precision. Following criteria reported in the EU guidelines (*19*), this method was validated at three different

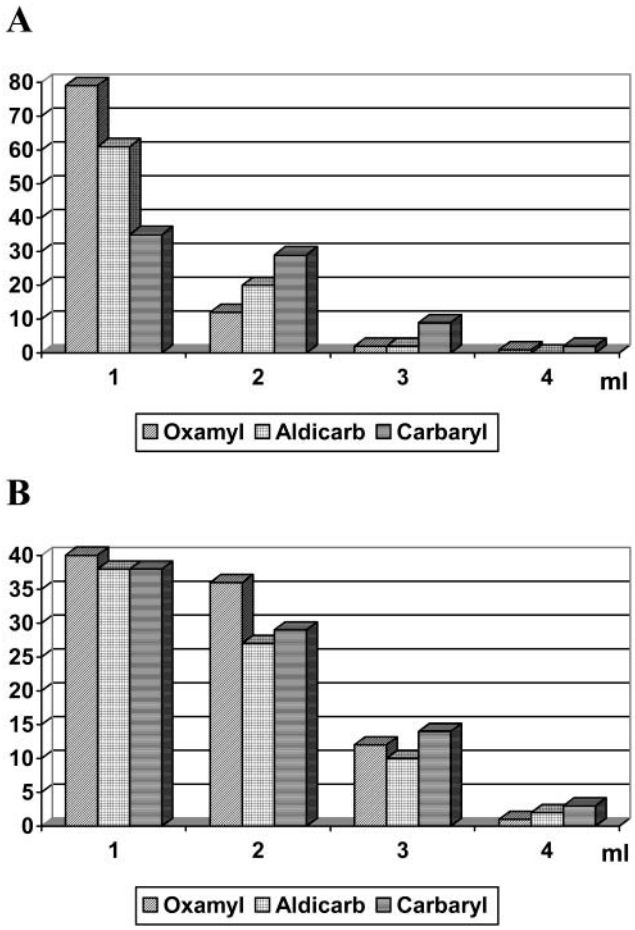

Figure 2. Effect of the extractant volume on analyte recovery in (A) spinach and (B) pear samples.

Table 4. Recovery (%) of the *N*-Methylcarbamate Insecticides Extracted (n = 4) from Vegetal Matrixes[a]

	matrix					
compound	lettuce	spinach	zucchini	tomato	apple	pear
oxamyl	84 (8)[b]	92 (5)	97 (7)	85 (7)	81 (5)	90 (3)
methomyl	89 (6)	96 (6)	97 (5)	83 (7)	85 (7)	97 (6)
pirimicarb	88 (5)	99 (7)	93 (2)	92 (5)	83 (6)	83 (4)
aldicarb	77 (6)	81 (9)	84 (8)	78 (8)	79 (6)	77 (9)
propoxur	88 (7)	92 (6)	96 (6)	85 (7)	93 (7)	95 (8)
carbofuran	82 (7)	93 (5)	95 (6)	90 (4)	93 (4)	93 (10)
carbaryl	79 (6)	76 (7)	85 (7)	82 (5)	83 (5)	79 (8)

[a] Spike level, 100 ppb. [b] Relative standard deviations are reported in parentheses.

concentrations corresponding to one-half of the MRL (see **Table 1**), the MRL and two times the MRL. When the injected amount of an analyte was out of the linear dynamic range of the mass detector (see below), a suitable lower volume of the final extract was re-injected in the LC column. At each analyte concentration and for any matrix considered, four measurements were performed with the criterion of adding the surrogate internal standard (trimethacarb) *before* analyte extraction. For the sake of conciseness, **Table 5** reports typical results obtained by analyzing spiked samples of tomato and apple. To check that the extraction efficiency of each insecticide in each type of vegetable was not dependent on the analyte concentration, mean accuracy data were compared among them by using the one-way ANOVA (analysis of variance) test at the $P = 0.05$ significance level. In any case, the calculated $F_{2,9}$ values (not

Table 5. Accuracy and Precision Data on Analyzing *N*-Methylcarbamate Insecticides in Two Selected Vegetal Matrixes at Concentrations Close to Maximum Residue Limits Set by the European Union, the Food and Agriculture Organization and the United States Food and Drug Administration

	tomato			apple		
compound	MRL/2[a]	MRL	2 MRL	MRL/2	MRL	2 MRL
	accuracy, % (RSD, %)					
oxamyl	104 (7)	100 (9)	99 (7)	98 (6)	94 (7)	93 (8)
methomyl	101 (5)	103 (5)	98 (8)	98 (8)	103 (8)	103 (9)
pirimicarb	109 (6)	110 (5)	106 (4)	98 (7)	103 (6)	100 (8)
aldicarb	98 (7)	93 (8)	96 (5)	92 (9)	88 (7)	90 (5)
propoxur	104 (9)	99 (9)	104 (6)	110 (6)	105 (5)	107 (6)
carbofuran	110 (6)	108 (4)	106 (7)	105 (5)	107 (4)	109 (3)
carbaryl	96 (4)	95 (7)	99 (6)	96 (7)	95 (7)	100 (8)

[a] MRLs are reported in **Table 1**. When MRLs differed from institution to institution, the lowest values were considered.

shown here) were lower than the critical value (4.256), showing that the extraction method was not influenced by the concentration of the analyte in a particular vegetal matrix. The accuracy data varied between 84 and 110% with standard deviations not higher than 9%. Thus, this method meets requirements reported in the EU guidelines (*19*), 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%.

Linear Dynamic Range. Under the instrumental conditions reported in Materials and Methods, the linear dynamic range of the ESI/MS detector was estimated for all the analytes. Amounts of each analyte varying from 4 to 1200 ng, and a constant amount of 125 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 seven NMCs were linearly correlated with injected amounts up to 300 ng, with R^2 ranging between 0.9911 and 0.9999.

Limits of Detection and Quantification of the Method. LOQs of the method were estimated from the LC-MS SIM chromatograms resulting from analyses of 25 ppb of each carbamate. We observed little effect of crop on the respective LOQ, all of which were ≤10 ppb. As an example, **Figure 3** shows a typical mass chromatogram for a spinach sample. After extracting the sum of the ion currents of both parent and fragment ions relative to each analyte that were free from interferences for coeluted endogenous compounds, the resulting trace was two-times smoothed by applying the mean smoothing method (Mass Lab software, Thermoquest). **Figure 4** shows ion current profiles of parent and daughter ions relative to 25 ppb of propoxur in the spinach sample. 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 an MS detector, the first condition to be satisfied for ascertaining the presence of a targeted compound is that all its related ions currents produce signals distinguishable from the background ion current. Accordingly, a definition of LOD (S/N 3) of each analyte was adopted considering that ion giving the worst S/N. This method detected and quantified NMCs at a few ppb in selected crops.

Matrix Effect. Suppression of analyte ionization in the electrosprayed solution by competitive ionization of eluting matrix components is well recognized. The extent of the suppression is related to both concentrations and affinities for the proton (or cations) of the co-extracted and coeluted matrix

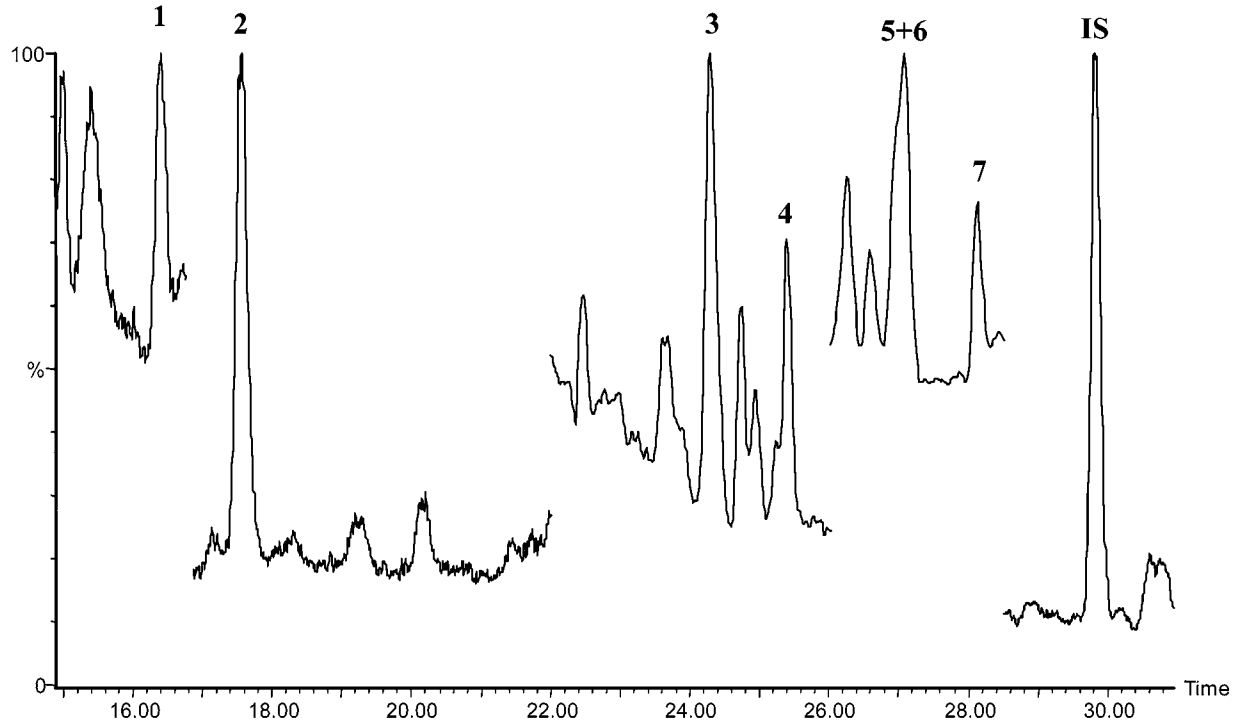

Figure 3. LC-ESI/MS multiple-ions SIM chromatogram resulting from the analysis of a spinach sample spiked with 25 ppb of carbamates. Peak numbering: 1, oxamyl; 2, methomyl; 3, pirimicarb; 4, aldicarb; 5, propoxur; 6, carbofuran; 7, carbaryl; IS, Trimethacarb.

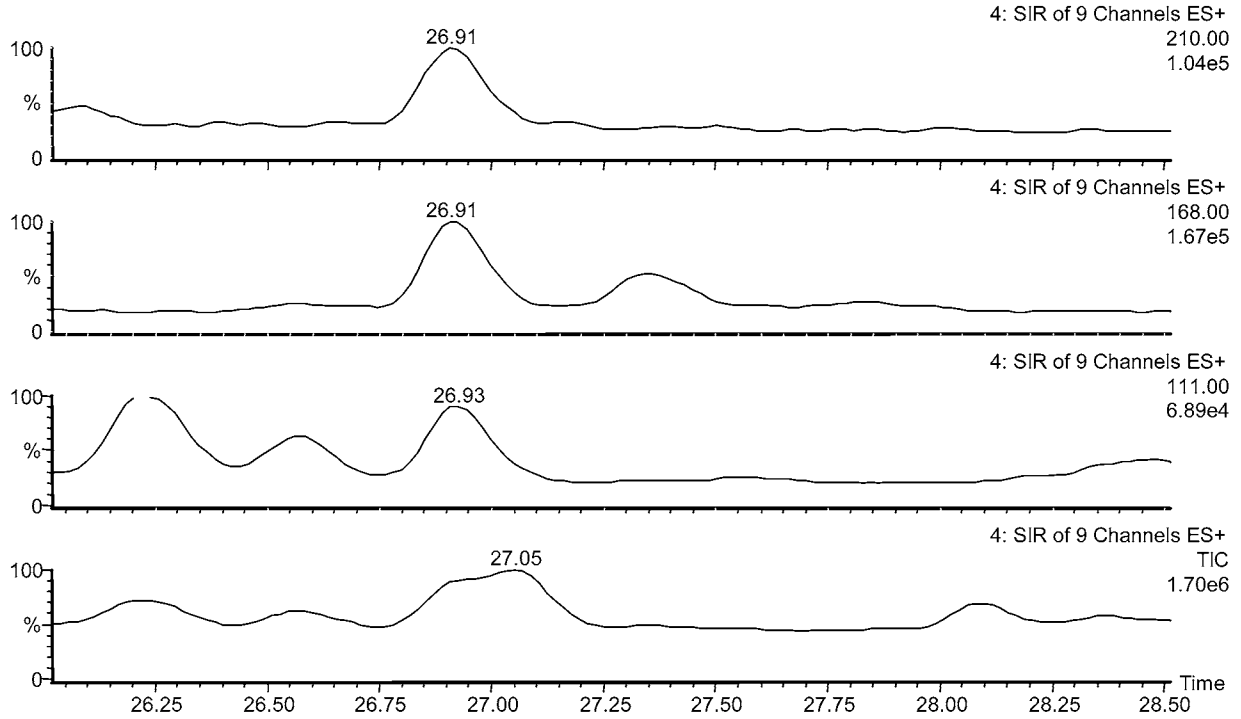

Figure 4. Single and sum of the ion current profiles relative to product ions of propoxur resulting from analysis of a spinach sample spiked with 25 ppb of carbamates.

Table 6. Limits of Detection (LOD) and Quantification (LOQ) of the Method

compound	LOD, ppb	LOQ, ppb
oxamyl	5 (242)[a]	10
methomyl	4 (163)	5
pirimicarb	2 (239)	2
aldicarb	2 (213)	4
propoxur	7 (210)	8
carbofuran	2 (222)	3
carbaryl	2 (202)	4

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

components. With complex biological matrixes and fractionating analytes by reversed-phase LC, some authors (*14*, *15*, *20*, *21*) observed that severe ion signal suppressions of the analytes occurred mainly in the first part of the chromatographic run. This suggests that an abundant fraction of co-extracted "unseen" endogenous compounds are polar in nature. It was shown that the matrix effect could be minimized or eliminated at all by adopting more selective extraction methods (*20*, *22*) and/or "good" chromatographic separation (*14*, *20*). Increasing the retention time of the analyte by decreasing the strong solvent percentage should enhance the capability of the column of separating the analyte from scarcely retained endogenous compounds. To optimize the method in terms of speed of the analysis, we investigated the extent of the matrix effect by varying the eluotropic strength of the mobile phase. For this purpose, we added, *post-extraction*, the analytes and the surrogate internal standard to final extracts of the six vegetal matrixes considered. Adding the analytes *after* extraction had the purpose of estimating weakening of the ion signals caused exclusively by matrix effects. In particular, this experiment was designed as follows: (1) duplicate extractions of the vegetal samples, (2) addition of the seven NMCs at 100 ppb level to the twelve final extracts, (3) alternated injections of the spiked extracts and a working standard solution into the LC-ESI/MS

Table 7. Effect of the Chromatographic Conditions on the Ion Signal Intensities of Carbamate Insecticides Directly Added to Three Selected Vegetal Extracts[a]

	matrix					
	zucchini		pear		spinach	
	SCR[b]	FCR[c]	SCR	FCR	SCR	FCR
	relative ion signal intensity[d]					
oxamyl	0.96	0.66	0.93	0.73	0.97	0.80
methomyl	1.06	0.79	0.97	0.75	1.01	0.65
pirimicarb	0.99	0.95	0.95	0.96	0.97	0.95
aldicarb	1.02	0.72	1.03	0.81	1.03	0.85
propoxur	1.00	0.98	0.98	0.99	0.99	1.01
carbofuran	1.05	0.99	1.02	1.04	0.97	0.98
carbaryl	1.03	0.97	1.01	0.98	0.96	1.00

[a] Spike level, 100 ppb. [b] SCR, slow chromatographic run (see the Materials and Methods). [c] FCR, fast chromatographic run. Gradient elution: t_0, $A = 25\%$; t_6, $A = 35\%$; t_7, $A = 70\%$; t_{13}, $A = 82\%$; t_{14}, $A = 100\%$. [d] For any analyte, the ion signal intensities were calculated by comparing their peak areas to those obtained by injecting a reference standard under the same chromatographic conditions.

apparatus under two different chromatographic conditions (see footnote of **Table 7**); (4) quantification of the concentrations of the analytes in any extract by comparing their absolute peak areas to those of the same compounds injected from a standard solution. For the sake of conciseness, representative results concerning NMCs in zucchini, pear, and spinach extracts are reported in **Table 7**. Significant signal weakening of oxamyl, methomyl, and aldicarb in all the matrixes considered took place when trying to decrease the analysis time by eluting them with a short chromatographic run. This finding supports the hypothesis that abundant amounts of co-extracted undetected endogenous compounds, being polar in nature, are eluted in the first part of the chromatogram and interfere with the process of formation of the $[M + H]^+$ ions and/or the ion evaporation process for those analytes that are coeluted with the co-extractives. The fact that pirimicarb ionization was not sup-

pressed by the matrix, even though the analyte eluted between methomyl and aldicarb suggested that, as a weak base pirimicarb has a relatively greater tendency for protonation.

This work has shown that an environmentally friendly and inexpensive solvent, such as water, can be successfully used for extracting contaminants from vegetal matrixes. Compared to other confirmatory methods quoted in the literature, our method is much simpler and faster. Confirmation of the presence of one particular carbamate insecticide in vegetable or fruits could be accomplished in <1 h upon sample receipt, after suitable adjustment of chromatographic conditions.

LITERATURE CITED

(1) Moye, H. A.; Scherer, S. J.; St. John, P. A. Dynamic fluorogenic labeling of pesticides for HPLC: detection of *N*-methylcarbamates with *o*-phthaladehyde. *Anal. Lett.* **1977**, *10*, 1049−1073.

(2) Krause, R. T. Liquid chromatographic determination of n-methylcarbamate insecticides and metabolites in crops. *J. AOAC Int.***1985**, *68*, 726−733.

(3) Liu, C. H.; Mattern, G. C.; Yu, X.; Rosen, R. T.; Rosen, J. D. Multiresidue determination of nonvolatile and thermally labile pesticides in fruits and vegetables by thermospray liquid chromatography/mass spectrometry. *J. Agric. Food Chem.* **1991**, *39*, 718−723.

(4) Pleasance, S.; Anacleto, J. F.; Bailey, M. R.; North, D. H. An evaluation of atmospheric pressure ionization techniques for the analysis of *N*-methylcarbamate pesticides by liquid chromatography mass spectrometry. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 378−397.

(5) Newsome, W. H.; Lau, B. P.-Y.; Ducharme, D.; Lewis, D. Comparison of liquid chromatography-atmospheric pressure chemical ionization/mass spectrometry and liquid chromatography-postcolumn fluorometry for determination of carbamates in food. *J. AOAC Int.* **1995**, *78*, 1312−1316.

(6) Di Corcia, A.; Crescenzi, C.; Laganà, A.; Sebastiani, E. Evaluation of a method based on liquid chromatography/electrospray/mass spectrometry for analyzing carbamate insecticides in fruits and vegetables. *J. Agric. Food Chem.* **1996**, *44*, 1930−1938.

(7) Lacassie, E.; Dreyfuss, M.-F.; Daguet, J. L.; Vignaud, M.; Marquet, P.; Lachâtre, G. Liquid chromatography-electrospray mass spectrometry multi-residue determination of pesticides in apples and pears. *J. Chromatogr*. **1999**, *830*, 135−143.

(8) Fernández, M.; Picó, Y.; Mañes, J. Determination of carbamate residues in fruits and vegetables by matrix solid-phase dispersion and liquid chromatography−mass spectrometry. *J. Chromatogr*. **2000**, *871*, 43−56.

(9) Taylor, M. J.; Huntwer, K.; Hunter, K. B.; Lindsay, D.; Le Bouhellec, S. Multi-residue method for rapid screening and confirmation of pesticides in crude extracts of fruits and vegetables using isocratic liquid chromatography with electrospray tandem mass spectrometry. *J. Chromatogr*. **2002**, *982*, 225−236.

(10) Goto, T.; Ito, Y.; Oka, H.; Saito, I.; Matsumoto, H.; Nakazawa, H. Simple and rapid determination of *N*-methylcarbamate pesticides in citrus fruits by electrospray ionization tandem mass spectrometry. *Anal. Chim. Acta* **2003**, *487*, 201−209.

(11) Barker, S. A.; Long, A. R.; Short, C. R. Isolation of drug residues from tissues by solid-phase dispersion. *J. Chromatogr. A* **1989**, *475*, 353−361.

(12) Barker, S. A. Matrix solid-phase dispersion. *J. Chromatogr. A* **2000**, *885*, 115−127.

(13) McLaughlin, L. G.; Henion, J. D.; Kijak, P. J. Multiresidue confirmation of aminoglycoside antibiotics in bovine kidney by ion spray high-performance liquid chromatography/tandem mass spectrometry. *Biol. Mass Spectrom.* **1994**, *23*, 417−429.

(14) Bogialli, S.; Curini, R.; Di Corcia, A.; Nazzari, M.; Samperi, R.A LC-MS assay for analyzing sulfonamide antibacterials in cattle and fish muscle tissues. *Anal. Chem.* **2003**, *75*, 1798−1804.

(15) Bogialli, S.; Curini, R.; Di Corcia, A.; Nazzari, M.; Sergi, M. Confirmatory analysis of sulfonamide antibacterials in bovine liver and kidney: extraction with hot water and liquid chromatography coupled to a single- or triple-quadrupole mass spectrometer. *Rapid Comm. Mass Spectrom.* **2003**, *17*, 1146−1156.

(16) Bogialli, S.; Curini, R.; Di Corcia, A.; Nazzari, M.; Polci, M. L. Rapid confirmatory assay for determining 12 sulfonamide antimicrobials in milk and eggs by matrix solid-phase dipersion and liquid chromatography−mass spectrometry. *J. Agric. Food Chem.* **2003**, *51*, 4225−4232.

(17) Crescenzi, C.; D'Ascenzo, G.; Di Corcia, A.; Nazzari, M.; Marchese, S.; Samperi, R. Multiresidue herbicide analysis in soil: subcritical water extraction with an on-line sorbent trap. *Anal. Chem.* **1999**, *71*, 2157−2163.

(18) Bajic, S.; Doerge, D. R.; Lu, L.; Hansen, E. B., Jr. Analysis of erythromycin by liquid chromatography/mass spectrometry using involatile mobile phases with a novel atmospheric pressure ionization source. *Rapid Comm. Mass Spectrom.* **2000**, *14*, 156−160.

(19) *Quality control procedures for pesticide residues analysis*. Guidelines for Residues Monitoring in the European Union, 2nd ed., Document No. SANCO/3103/2000, 1999/2000.

(20) Matuszewski, B. K.; Constanzer, M. L.; Chavez-Eng, C. M. Matrix effect in quantitative LC/MS/MS analyses of biological fluids: a method for determination of finasteride in human plasma at picogram per mL concentrations. *Anal. Chem.* **1998**, *70*, 882−889.

(21) Riediker, S.; Stadler, R. H. Simultaneous determination of five β-lactam antibiotics in bovine milk using liquid chromatography coupled with electrospray ionization tandem mass spectrometry. *Anal. Chem.* **2001**, *73*, 1614−1621.

(22) Buhrman, D. L.; Price, P. I.; Rudewicz, P. J. Quantitation of SR 27417 in human plasma using electrospray liquid chromatography-tandem mass spectrometry: a study of ion suppression. *J. Am. Mass Spectrom.* **1996**, *7*, 1099−1105.

Received for review October 15, 2003. Revised manuscript received December 2, 2003. Accepted December 10, 2003.

JF035195J