Evaluation of a Method Based on Liquid Chromatography/ Electrospray/Mass Spectrometry for Analyzing Carbamate Insecticides in Fruits and Vegetables

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The feasibility of using reversed-phase liquid chromatography/mass spectrometry (LC/MS) with an electrospray (ES) interface for measuring traces of *N*-methylcarbamate insecticides in 10 different types of fruits and vegetables was evaluated. Twelve carbamates added to vegetable materials were extracted with methanol by the aid of a homogenizer. After filtration, an aliquot of the homogenizate equivalent to 5 g of the vegetable material was suitably diluted with water and passed through a 1-g Carbograph 1 extraction cartridge. Carbamates were eluted by passing through the cartridge 6 mL of a CH_2Cl_2/CH_3OH (80:20 v/v) mixture. After eluate concentration down to 100 μL, 5 μL of the final extact was injected into the LC column. Recovery of the analytes was better than 80%, irrespective of the type of vegetable matrix to which the analytes were added. Replacement of CH_3OH with CH_3CN as organic modifier resulted in a significant decrease of the ion signal for carbamates. The same occurred by adding moderate amounts of HCOOH or NaCl to the LC mobile phase. Varying the skimmer cone voltage produced characteristic fragment ions without significant sensitivity loss. The presence in the electrosprayed solution of a lot of vegetable constituents did not interfere significantly with the ionization process of carbamates. The analysis of a tomato extract spiked with carbamates at the individual level of 5 ng/g of vegetable performed by selected ion monitoring showed that the limit of detection for these analytes could be set at a few hundreds of picograms per gram of vegetable or fruit. Over 1 working day of heavy use of the ES/MS intrumentation, the ion signal intensities for carbamates were found to be unaffected by the rate of contamination of the ES source by vegetable constituents.

Keywords: *Carbamates; vegetables; LC/MS; electrospray*

Carbamate insecticides are chemicals widespreadly used for crop protection. Some of them have high toxicity levels. This has impelled the introduction in many European countries of regulation stating that the most toxic carbamates cannot be present in fruits and vegetables at levels higher than 20−50 ng/g of vegetable. This severe limitation coupled with the complexities of the matrices in which carbamates may occur has urged researchers to develop sensitive and specific analytical procedures for determining these target compounds. Owing to thermolability of carbamates, traditional GC/MS methods are not of use, unless derivatization procedures are included (Ting and Kho, 1991). Based on the pioneering work by Moye et al. (1977), various sensitive and selective liquid chromatography (LC) methods involving postcolumn reaction to form fluorescent derivatives for simultaneously determining *N*-methylcarbamates in water and vegetables have been recently proposed (de Kok et al., 1987; Page and French, 1992). A specific multiresidue method making use of LC/thermospray/MS with selected ion monitoring to determine pesticides, including carbamates, in fruits and vegetables has been evaluated (Liu et al., 1991). Limits of detection of the method for carbamates were found to be between 250 and 1000 ng/g, and thus this method is not sensitive enough for detecting some carbamates at the levels required by the regulation cited above.

The electrospray (ES) ionization source has become in recent years the best arrangement for coupling LC to MS not only for large molecules, such as peptides and proteins, but also for relatively small molecules, such as pesticides (Doerge and Bajic, 1992; Molina et al., 1994; Crescenzi et al., 1995a; Chiron et al., 1995) and surfactants (Popenoe et al., 1994; Crescenzi et al., 1995b), of environmental interest. Pleasance et al. (1992) evaluated extensively the feasibility of using an ES source for the mass spectral analysis of carbamates, and they succeeded in determining 0.1 μg/g levels of three carbamates in green pepper by LC/ES/MS. More recently, the same technique coupled with on-line immunoaffinity chromatography has been effectively employed for determining low levels of a carbamate insecticide, namely carbofuran, in a crude potato extract (Rule et al., 1994). Similarly to thermospray, ES is a technique able to produce ions at atmospheric pressure but without the need for high temperatures that could decompose labile compounds. The most accepted model of gas-phase ion production involves the formation of charged small droplets formation by an electrical field, which are more and more shrunken by heat transfer and repeated coulombic explosion until the radius of curvature of the daughter droplets becomes so small that field-assisted ion "evaporation" competes with further droplet disintegration. In this situation, even

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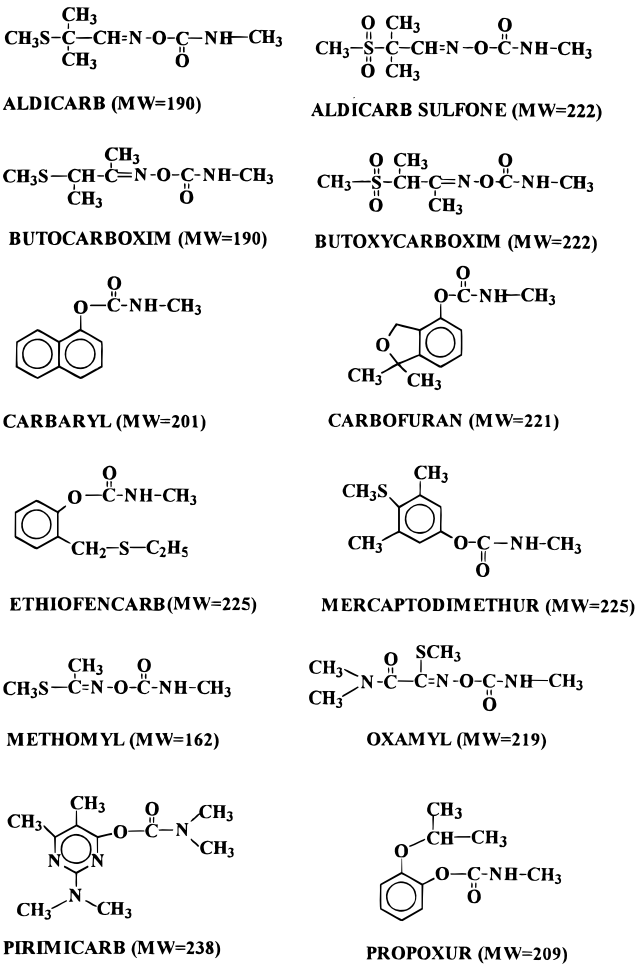

Figure 1. Structures and common names of carbamates considered in this study.

nonionic organic species can be electrically charged in the gaseous phase, provided they are able to form stable adducts or complexes with inorganic ions. Moreover, although the electrospray process is able to generate gas-phase ions in a gentle way, structural information on separated analytes can be easily achieved by collision-induced dissociation (CID) with a suitable adjustment of the electrical field existing in the desolvation, intermediate-pressure chamber located between the ionization source and the mass analyzer region. By this expedient, Voyksner (1994) showed that a single-mass analyzer can provide structural information very similar to that obtained by the much more expensive tandem MS−MS technique.

The purpose of this work has been that of evaluating the feasibility of using the LC/ES/MS technique for routinely monitoring low levels of carbamate insecticides in fruits and vegetables.

EXPERIMENTAL PROCEDURES

Reagents and Chemicals. The 12 carbamates considered in this study were from Riedel-de-Haën, Seelze, Germany. Their structures and common names are shown in Figure 1. Individual standard solutions were prepared by dissolving 100 mg of each carbamate in 100 mL of methanol. A composite working standard solution was prepared by mixing 0.2 mL of each solution and diluting to 10 mL with methanol (20 ng/μL).

Methanol "Plus" and acetonitrile "Plus" of LC grade were from Carlo Erba, Milan, Italy. For LC, distilled water used was further purified by passing it through a Milli-Q RG apparatus (Millipore Intertech, Marlborough, MA). All other solvents were of reagent grade (Carlo Erba) and were used as supplied.

Carbograph 1, a well-known example of graphitized carbon black, was kindly supplied by Alltech, Deerfield, IL, while the other materials for preparing extraction cartridges were from Supelco, Bellefonte, PA. The preparation and pretreatment of the 1-g reversible extraction cartridge were carried out as previously reported (Di Corcia et al., 1994). With respect to this work, the design of the cartridge was slightly modified in that the Teflon piston had a Luer tip and was inserted into the syringe-type cartridge after water extraction and washings (Crescenzi et al., 1996).

The trap was fitted into a side-arm filtering flask, and liquids were forced to pass through the cartridge by vacuum from a water pump.

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, grape, endive, spinach, orange, potato, apple, peach, and sugar beet. The samples used for recovery and sensitivity studies were previously determined to be free of the pesticides considered.

Procedure. For recovery studies, freshly produced carbamate-free samples of fruits and vegetables of 10 different types were washed and air-dried. Samples were first chopped into small pieces and mixed well with a Hobart 8185 food chopper. Then, 20 g of any chopped sample was spiked with carbamates at two different concentration levels, that is 20 and 200 ng/L, by adding them from the methanolic standard solution. Next, each spiked sample was transferred into a centrifuge tube. Forty milliliters of methanol was added, and the mixture was homogenized by means of homogenizer Kinematica CH-6010 (Kriens-Luzern, Switzerland) for 5 min and then filtered through a 1.5-μm pore size Whatman GF/C glass fiber pads (Maidstone, U.K.), followed by two 4-mL methanol rinses of the glassware. The volume of the methanolic solution was adjusted to 50 mL with water, and 12.5 mL of this solution was then taken and diluted to 250 mL with water. This solution was forced to pass through the Carbograph 1 cartridge at the maximum flow rate allowed by the extraction apparatus (ca. 60 mL/min). After the passage of the sample, the cartridge was washed with 7 mL of distilled water. Water remaining in the cartridge was partially removed by drawing room air through it by vacuum for 1 min. Another fraction of water was removed by slowly passing through the cartridge 0.9 mL of methanol. The pump was disconnected and a Teflon piston was forced to enter the syringe-like cartridge until it reached the upper frit. Then, the cartridge was turned upside down and a small glass reservoir was attached to it. A 1.4 cm i.d. glass vial with conical bottom was placed below the cartridge, and carbamates were back-eluted by passage of 1 mL of methanol followed by 6 mL of dichloromethane/methanol (80:20 v/v).

The eluate was concentrated to a small volume (~100 μL) in a water bath at 40 °C under a nitrogen stream. After the exact volume was measured, 5 μL of the final extract was injected into the LC apparatus.

LC/ES/MS Analysis. Liquid chromatography was carried out with a Varian (Walnut Creek, CA) Model 9010 equipped with a Rheodyne Model 7125 injector having a 10-L loop. The analytes were chromatographed on an "Alltima" 25 cm × 4.6 mm i.d. column filled with 5-m C_{18} reversed-phase packing (Alltech). For fractionating the analytes, phase A was a methanol/acetonitrile mixture (85:15 v/v) and phase B was water. The initial composition of the mobile phase was 14% A that was first increased linearly to 35% within 16 min and then to 84% within 20 min. The flow rate of the mobile phase was 1 mL/min; 40 μL of the column effluent was diverted to the ES source, while the rest of the effluent was delivered to an UV detector set at 220-nm wavelength. A Fisons VG "Platform" bench top mass spectrometer (Fisons Instruments/VG BioTech, Milano, Italy) equipped with a pneumatically assisted electrospray LC/MS interface and a single quadrupole was used for detecting and quantifying target compounds in the LC column effluent. This was introduced into the ES interface through a 40-cm length of 75-m-diameter PEEK

Table 1. Time-Scheduled SIM Conditions for Monitoring 12 Carbamate Insecticides in Fruit and Vegetable Samples (Skimmer Cone Voltage = 20 V; Dwell Time = 0.2 s; Span = 1)

compd	channel mass, *m/z*	retention window, min
butoxycarboxim	245.5	10−13.5
aldicarb sulfone	245.5	
oxamyl	242.5	
methomyl	128.5 + 163.5 + 185.5	13.5−16.5
butocarboxim	213.5	26.5−29.5
aldicarb	213.5	
propoxur	233	30−32
carbofuran	245	
carbaryl	224.5	32−35
ethiofencarb	248.5	
pirimicarb	239.5	
mercaptodimethur	248.5	36−39

capillary tube. The MS was operated in the positive-ion mode by applying to the capillary a voltage of 3.8 kV. Full-scan LC/MS chromatograms and mass spectra were obtained by scanning from *m/z* 70 to 250 with 2-s scan, after the skimmer cone voltage was set at 30 V, unless otherwise specified. Time-scheduled, selected ion monitoring (SIM) LC/MS was performed by following the procedure reported in Table 1. The source temperature was maintained at 70 °C. Ions were generated using highly pure nitrogen as drying and nebulizing gases. The optimum flow rates of the drying and nebulizing gas were found to be 200 L/h and 13 mL/min, respectively.

On a daily basis and with the system beyond the sample cone still under vacuum, the sample cone was cleaned with use of a methanol-imbibed paper, while the counter electrode was cleaned with a concentrated nitric acid/water (50:50 v/v) mixture and 10-min sonication. Thereafter, it was extensively washed with distilled water, followed by acetone and methanol. By the same procedure, the sample cone was cleaned on a weekly basis. All of the cleaning procedure required no more than 25 min.

The concentrations of carbamates in a spiked vegetable sample were calculated by measuring peak areas from extracted ion-current profiles and comparing them with those obtained from standard solutions. These were prepared by dissolving known and appropriate volumes of the working standard solution in the eluant phase used for eluting carbamates from the Carbograph 1 cartridge and then following the same procedure as that reported above.

The mass spectrometry data handling system used was the "Mass Lynx" software from Fisons Instruments.

RESULTS AND DISCUSSION

Recovery Studies. Recovery experiments were conducted as reported under Experimental Procedures, and the resulting data are shown in Table 2. These figures resulted from averaging recovery data obtained by analyzing in duplicate the 10 samples of different origins spiked at two different concentrations. Recovery exceeded 80% for all analytes investigated, with relative standard deviations ranging between 5 and 12%.

Ion Signal Optimization. With the view of optimizing the sensitivity of the ES/MS detector, the dependence of the ion signal intensities for carbamates upon the composition of the LC mobile phase was evaluated. For this purpose, 100 ng each of six selected carbamates was separately introduced into the ES source by the flow injection technique. The composition of the liquid stream carrying analytes into the ES source was varied by changing the nature of the organic solvent mixed with water, namely methanol and acetonitrile. For each carbamate, ion signal intensities were measured by extracting from the TIC chromatogram the summed ion current profiles for four of the most abundant ions, among parent and fragment ions. Mean values from five determinations are shown in Table 3. When CH_3OH was replaced with CH_3CN, a significant ion signal weakening was observed. This fact might be accounted for by considering that, compared to methanol, acetonitrile is more basic in nature and thus it can compete with solutes for formation of adducts with inorganic cations.

Table 2. Percentage Recovery of 12 Carbamates Added to 10 Different Types of Crops at Two Concentration Levels

compd	recovery,[a] % (range) at 20 μg/kg	recovery,[a] % (range) at 200 μg/kg
butoxycarboxim	87 (83−93)	87 (84−93)
aldicarb sulfone	94 (91−102)	94 (92−100)
oxamyl	93 (92−102)	94 (93−101)
methomyl	92 (88−98)	92 (88−99)
butocarboxim	90 (88−100)	92 (91−99)
aldicarb	89 (83−94)	92 (88−98)
propoxur	93 (89−98)	93 (89−97)
carbofuran	91 (89−96)	93 (90−100)
carbaryl	95 (91−98)	96 (93−98)
ethiofencarb	94 (90−97)	96 (94−98)
pirimicarb	94 (88−95)	93 (90−95)
mercaptodimethur	93 (88−95)	94 (89−97)

[a] Mean values obtained from duplicate measurements for each of the 10 vegetable and fruit samples of different types.

Table 3. Effect of the Composition of the Electrosprayed Solution on the Ion Signal Intensities for Six Selected Carbamates

compd	ion signal, arbitrary units: CH_3OH/H_2O (50:50 v/v)	ion signal, arbitrary units: CH_3CN/H_2O (50:50 v/v)
aldicarb sulfone	1600 ± 130[a]	1100 ± 110
carbofuran	1800 ± 160	1250 ± 100
ethiofencarb	1650 ± 130	1000 ± 110
oxamyl	1450 ± 140	770 ± 90
pirimicarb	2100 ± 180	1500 ± 120
propoxur	1450 ± 140	1250 ± 130

[a] Mean values and standard deviations from five determinations.

Spectra for carbamates displayed major peaks for MH^+ and MNa^+ ions, the latter being generally more abundant than the former ones. In contrast to results obtained by Pleasance et al. (1992), the addition of 0.1% HCOOH to the LC mobile phase failed to suppress production of Na^+ adduct ions. Probably, methanol used by us as organic modifier of the LC mobile phase contained a higher level of Na^+ ion impurities than that used by the above authors.

From an analytical point of view, we were interested to know if an increment of the concentration of either H^+ or Na^+ ions in the LC effluent was able to improve the yield of transition of ions from the liquid to the gas phase. This experiment was performed by adding increasing amounts of either HCOOH or NaCl to a water/methanol mixture and measuring variations of the total ion currents produced by fragment and parent ions relative to each of six selected carbamates obtained by flow injection analysis. Mean values from five measurements are reported in Table 4. The increase of the Na^+ ion concentration in the electrosprayed solution had the effect of lowering the ion signal strength. This tendency was much more pronounced when the H^+ ion concentration was increased. These results suggested that trace amounts of cations originally present in the electrosprayed solution sufficed to give a virtually complete formation of charged liquid droplets in the electrosprayed solution from which

Table 4. Effect of Increasing the Concentration of both H^+ and Na^+ Ions in the Electrosprayed Solution (H_2O/CH_3OH, 50:50 v/v) on the Ion Signal Intensities for Six Selected Carbamates

	ion signal, arbitrary units				
compd	no addition	HCOOH, 0.1 mM	HCOOH, 1 mM	NaCl, 0.1 mM	NaCl, 0.3 mM
aldicarb sulfone	1500 ± 140[a]	900 ± 80	550 ± 160	1300 ± 120	950 ± 90
carbofuran	1700 ± 160	1100 ± 100	700 ± 80	1550 ± 140	1050 ± 90
ethiofencarb	1450 ± 120	800 ± 70	550 ± 60	1300 ± 120	900 ± 80
oxamyl	1350 ± 120	800 ± 80	650 ± 60	1100 ± 100	800 ± 70
pirimicarb	2000 ± 170	1800 ± 160	1600 ± 150	1700 ± 160	1400 ± 130
propoxur	1500 ± 140	900 ± 90	650 ± 70	1400 ± 150	1100 ± 100

[a] Mean values and standard deviations from five determinations.

Table 5. Variation of the Ion Signal Intensities and of the Relative Abundance of Daughter and Parent Ions for 12 Carbamates by Varying the Voltage Applied to the Skimmer Cone

	signal/noise [*m/z* (relative abundance, %)] at skimmer cone voltage of		
compd	20 V	30 V	40 V
butoxycarboxim[a]	18 [107 (25), 166 (10), 223 (40), 245 (100)]	20 [107 (50), 130 (60), 166 (30), 245 (100)]	16 [107 (40),130 (100), 166 (30), 245 (40)]
aldicarb sulfone	18 [148 (10), 166 (10), 223 (20), 245 (100)]	19 [86 (50), 148 (50), 166 (50), 245 (100)]	17 [76 (50), 86 (100), 166 (40), 245 (50)]
oxamyl	16 [72 (100), 90 (30), 242 (70)]	16 [72 (100), 242 (20)]	16 [72 (100)]
methomyl	13 [88 (100), 106 (60), 163 (50), 185 (70)]	11 [88 (100), 106 (30), 128 (40), 185 (40)]	11 [73 (40), 88 (100), 106 (50), 128 (30)]
butocarboxim	13 [75 (80), 116 (10), 191 (10), 213 (100)]	15 [75 (100), 116 (10), 213 (20)]	14 [75 (100), 213 (10)]
aldicarb	13 [89 (30), 116 (100), 213 (70)]	11 [89 (100), 116 (30), 213 (30)]	12 [89 (100), 116 (20), 213 (20)]
propoxur	16 [153 (20), 168 (50), 210 (30), 232 (100)]	19 [111 (100), 168 (50), 232 (20)]	18 [93 (30), 111 (100), 168 (10)]
carbofuran	25 [222 (10), 244 (100)]	22 [123 (50), 165 (100), 222 (10), 244 (20)]	23 [123 (60), 165 (100), 222 (10), 244 (20)]
carbaryl	14 [145 (80), 202 (40), 224 (100)]	17 [145 (100), 202 (10), 224 (50)]	16 [145 (100)]
ethiofencarb	14 [107 (10), 164 (10), 226 (10), 248 (100)]	13 [107 (100), 164 (10), 248 (30)]	15 [107 (100), 164 (10), 248 (10)]
pirimicarb	31 [182 (10), 239 (100)]	37 [72 (80), 182 (70), 239 (100)]	40 [72 (100), 182 (60)]
mercaptodimethur	18 [169 (50), 226 (40), 248 (100)]	19 [169 (100), 226 (30), 248 (90)]	17 [123 (70), 169 (100), 248 (10)]

adduct ions will evaporate. Moreover, the decrease of the ion signal as the electrolyte concentration in the mobile phase was increased may be traced to mutual repulsion of highly charged droplets with a droplet spread in regions of the ES interface where evaporated ions are lost (Raffaelli and Bruins, 1991). The loss of sensitivity could also be the consequence of a decrease of the yield of gas-phase ions from droplets having high electrolyte concentration (Ikonomu et al., 1991).

Sensitivity and Specificity. By the LC-ES/MS technique, the correspondence of retention time and molecular weight could provide sufficient specificity for identification of a target compound in a complex matrix, yet legal criteria for testing the presence of contaminants in various matrices usually accept spectra displaying the molecular ion species plus characteristic ion fragments. As mentioned above, the ES/MS system provides fragment ions to be obtained by CID reactions. With our instrumentation, molecular ion decomposition can be achieved by increasing the voltage between sample and skimmer cones in the desolvation chamber. The effects of varying the skimmer cone voltage on both the response of the MS detector and the production of diagnostic ions were investigated. This experiment was conducted by injecting into the LC column 5 ng each of the 12 carbamates considered from the composite working standard solution. The analytes were separated following chromatographic conditions reported under Experimental Procedures. At any skimmer cone voltage and for each carbamate, the S/N ratio was calculated by measuring the peak height from the total ion current (TIC) chromatogram against the average background noise. Measurements were made in triplicate. The calculated S/N values together with the four most abundant fragment and parent ions displayed in spectra from each carbamate are shown in Table 5. Ions having relative abundances less than 10% were not considered.

As expected on the basis that *N*-methylcarbamate insecticides are labile compounds, decomposition of these compounds occurred even at low skimmer cone voltage with generation of various characteristic fragment ions. As an example, at 20 V of cone voltage, the base peak in the spectrum from oxamyl was a charged fragment at *m/z* 72 relative to cationized *N*,*N*-dimethyl isocyanate. Increasing the cone voltage to 40 V completely converted the H^+ adduct ions of oxamyl entering the desolvation/fragmentation chamber to the above fragment. According to a previous study (Pleasance et al., 1992), the common feature of MH^+ ions from carbamates was a ready loss of methyl isocyanate (−57 Da), with the exception of aldicarb and butocarboxim. Aldicarb and, to a lesser extent, its isomer butocarboxim were inclined to lose by collision methylcarbamic acid (−75 Da). Among the carbamates considered, methomyl was the only one capable of releasing methyl isocyanate from both MH^+ and MNa^+ adduct ions.

Interestingly, the CID process was able to produce different fragmentations of both aldicarb sulfone−butoxycarboxim and aldicarb−butocarboxim isomeric pairs. As an example, the CID process of aldicarb sulfone at low collision energy produced an ion at *m/z* 148 due to the loss of methylcarbamic acid. At higher collision energies, the preferential degradation route of aldicarb sulfone was that of producing an ion at *m/z*

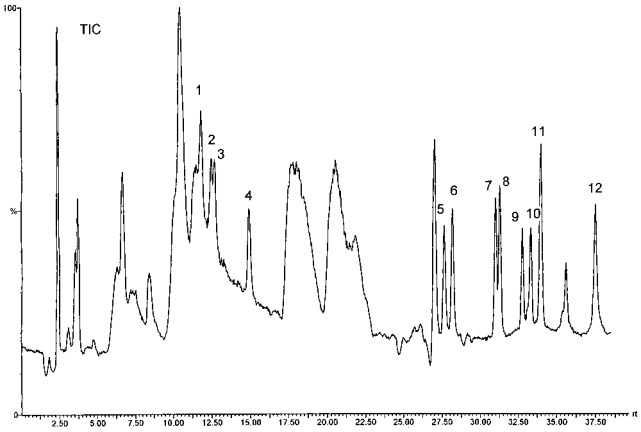

Figure 2. TIC chromatogram obtained by analyzing a spinach sample amended with 12 carbamates at the individual level of 200 ng/g: 1, butoxycarboxim; 2, aldicarb sulfone; 3, oxamyl; 4, methomyl; 5, butocarboxim; 6, aldicarb; 7, propoxur; 8, carbofuran; 9, carbaryl; 10, ethiofencarb; 11, pirimicarb; 12, mercaptodimethur.

86, which was previously assigned to the $[(CH_3)_2CCHNO + H]^+$ fragment ion (Trehy et al., 1984). Protonated butoxycarboxim followed a different degradation pathway leading presumably to generation of both the $[CH_3SO_2CH{=}CH_2 + H]^+$ (m/z 107) and $[CH_3CHCH{=}NOCONHCH_3 + H]^+$ (m/z 130) fragment ions.

The decomposition pathways of aromatic carbamates led generally to formation of stable phenol derivative H^+ adducts with the exception of ethiofencarb, from which protonated benzoxethane was generated (m/z 107). At a low collision energy, propoxur lost propene (−42 Da) or methyl isocyanate. These two species were simultaneously lost by propoxur by increasing the cone voltage from 20 to 30 V, leading to generation of the H^+ adduct of catechol (m/z 111). By gradually increasing the cone voltage, carbofuran first exhibited cleavage of the carbamate moiety and then breakage of the furan ring with release of propene. Spectra from carbaryl at various cone voltages displayed only one single fragment ion due to a phenol derivative H^+ adduct. The favorite CID process of ethiofencarb was that of losing simultaneously methyl isocyanate and ethanethiol (−62 Da). A gradual increase of the collision energy provoked first the loss of methyl isocyanate and then the release of $CH_2{=}S$ (46 Da) from mercaptodimethur.

It was reported (Pleasance et al., 1992) that CID spectra of carbamates by ES in a single-analyzer instrument could be acquired at the expense of sensitivity. Vice versa, other authors (Banks et al., 1994; Voyskner and Pack, 1991) did not note a significant loss of the total ion signals over a wide range of the voltage applied to the skimmer cone. Data reported in Table 5 show that the CID process of carbamate adduct ions could be varied with negligible fluctuations of the sensitivity. This result can be explained by considering that the expected improvement of the ion-focusing effect with increasing skimmer cone voltage (Duffin, 1992) was counterbalanced by the gradual fruitless disappearance of MNa^+ ions, from which only Na^+ ions were generated.

In terms of sensitivity and specificity, the best operative conditions were those of setting the skimmer cone voltage at 30 V and scanning the quadrupole from m/z 70 to 250 with a 2-s scan. Under these conditions, well-defined TIC chromatographic profiles were obtained for the 12 carbamates present in vegetables at the individual level of 200 ng/g, as exemplified in Figure 2. Moreover, full-scan background-subtracted mass spectra

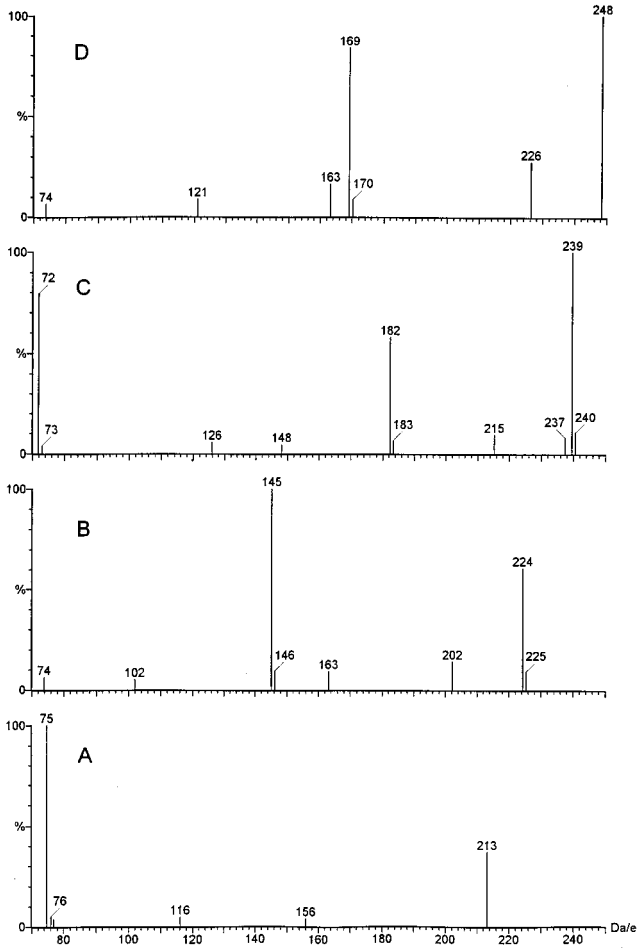

Figure 3. Full-scan background-subtracted spectra taken from the average of the peaks for butocarboxim (A), carbaryl (B), pirimicarb (C), and mercaptodimethur (D) acquired by on-column injection of 5 ng each of the analytes.

from on-column injection of 5 ng each of carbamates displayed all major peaks for parent and daughter ions and minimal interference from background ions, as shown in Figure 3 for selected analytes.

By acquiring in full-scan mode, the limit of sensitivity (S/N = 3) of the method ranged between 0.24 ng (pirimicarb) and 1.6 ng (butocarboxim) per gram of vegetable. These limits were calculated by on-column injection of 5 ng each of the analytes, extracting from the TIC chromatogram the sum of the ion chromatographic profiles of the most abundant parent and fragment ions related to each analyte, and measuring the resulting peak heights against average background noise. Figure 4 shows extracted ion chromatographic profiles for some selected analytes obtained by analyzing an orange extract spiked with the analytes at the level of 40 ng/g.

As the response of the ES/MS detector is related to the analyte concentration in the mobile phase, the limits of detection reported above could be significantly decreased by adopting narrower LC columns. However, in our experience and as already extensively discussed elsewhere (Hopfgartner et al., 1993), the use of small-bore LC columns does not offer practical advantages, since lower extract volumes of actual samples are to be injected into these columns to avoid severe overloading effects and retention time variations of the early-eluting analytes.

In terms of sensitivity, the potential of the ES/MS instrumentation used was fully exploited by extracting

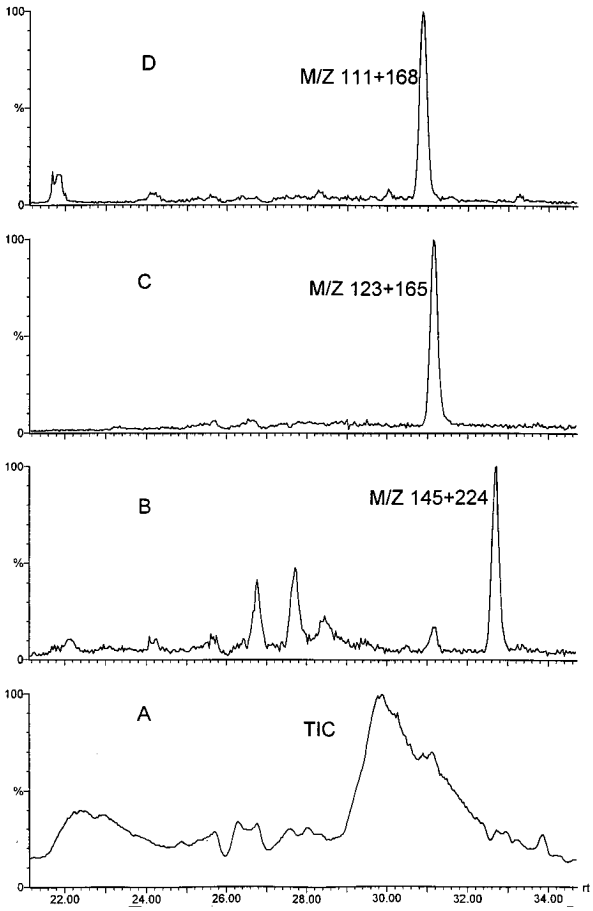

Figure 4. Partial view of a TIC LC/MS chromatogram (A) and extracted ion chromatographic profiles relative to carbaryl (B), carbofuran (C), and propoxur (D) obtained by analyzing an orange extract spiked with carbamates at the individual level of 40 ng/g.

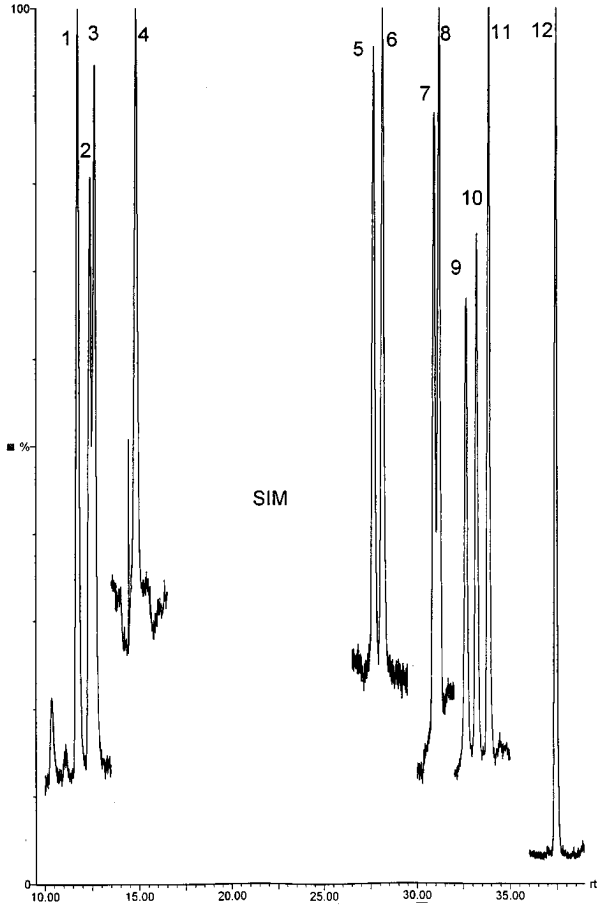

Figure 5. Time-scheduled, SIM LC/MS chromatogram obtained by analyzing a tomato extract amended with carbamates at the individual level of 5 ng/g. Peak numbering is the same as in Figure 2.

a tomato sample fortified with the analytes at the individual level of 5 ng/g and analyzing the final extract by a time-scheduled, selected ion monitoring (SIM) scheme. The resulting chromatogram is shown in Figure 5. By this option, the limit of sensitivity of the method was estimated to be between 0.04 ng/g (pirimicarb) and 0.4 ng/g (butocarboxim).

Linear Dynamic Range. Under the conditions reported under Experimental Procedures, the linear dynamic range of the ES/MS detector for two selected carbamates was estimated. This set of measurements was performed by injecting into the LC column known and variable amounts of carbaryl and carbofuran. For each amount injected, measurements were made in triplicate. The average peak areas of each set of injections were plotted against the amount injected, and the resulting plot (Figure 6) indicated that a fairly good linear response could be obtained over less than 2 orders of magnitude. This finding is in accordance with other studies (Raffaelli and Bruins, 1991; Keberle and Tang, 1993).

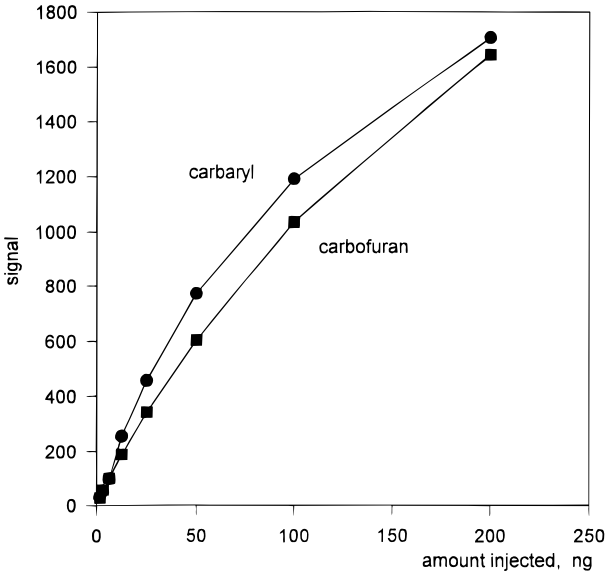

Figure 6. Signal vs amounts of two selected carbamates injected into the LC column.

Accuracy. On the basis of the results obtained by flow injection experiments (see above), the LC separation of the 12 carbamates under study was initially afforded by using methanol as organic modifier. Under this condition, a suitable aliquot of a lettuce extract spiked with the 12 carbamates at the individual level of 200 ng/g was analyzed. On extracting the ion current profile at m/z 245−247 relative to the butoxycarboxim and aldicarb sulfone isomeric pair, we realized that an anomalously low peak at the expected retention time for butoxycarboxim was produced (Figure 7A). The TIC chromatogram showed that butoxycarboxim was coeluted with a large amount of an endogeneous component of the lettuce capable of forming both MH^+ (m/z 205) and MNa^+ (m/z 227) adducts. To a greater or lesser extent, this substance was observed to be present in every extract of the various fruits and vegetables

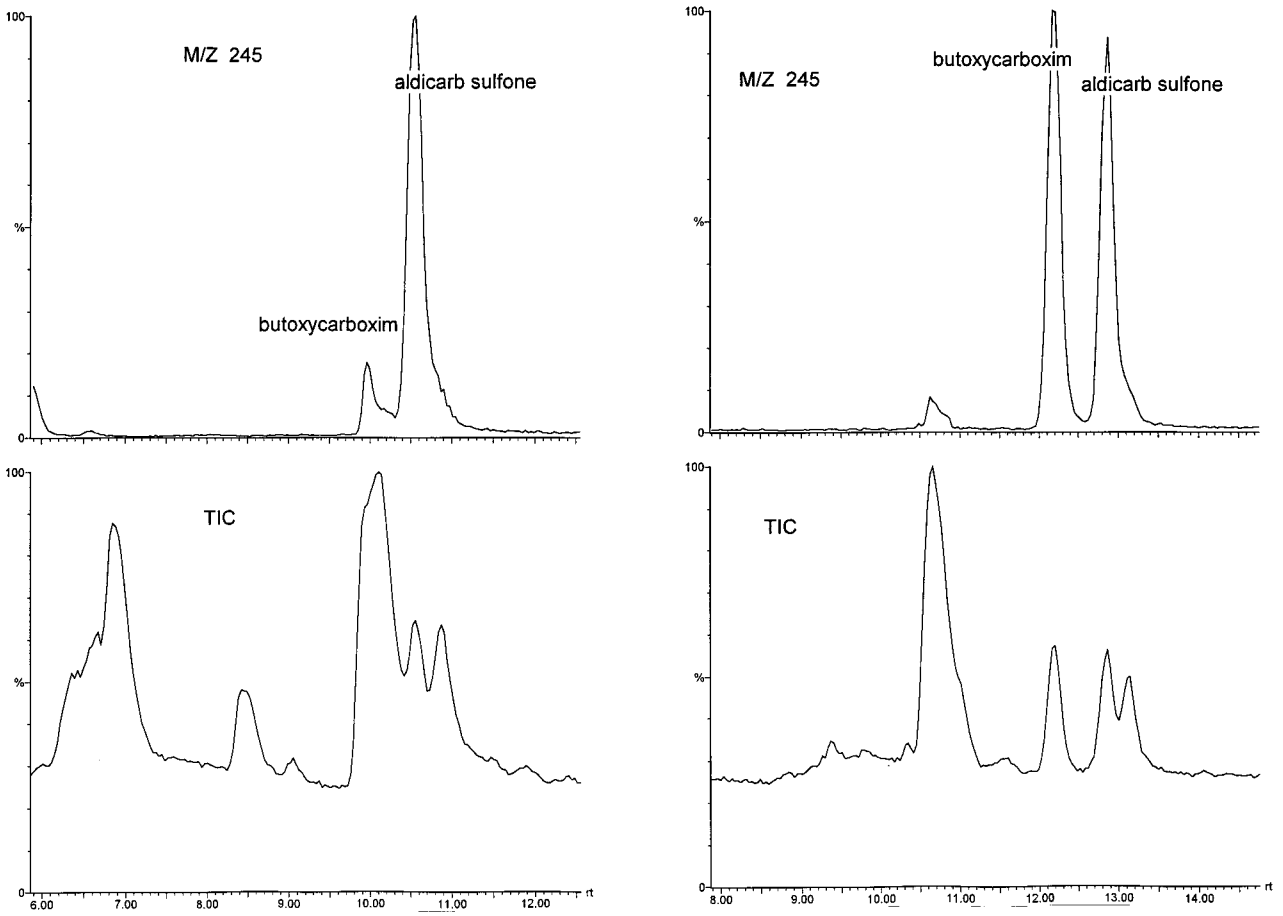

Figure 7. Partial view of the TIC chromatogram and selected-ion current profile (*m*/*z* 245) obtained by analyzing a lettuce extract containing carbamates at the level of 200 ng/g. (A, left) The LC mobile-phase was a water/methanol mixture, the composition of which was linearly varied from 15% to 84% methanol after 32 min. (B, right) Chromatographic conditions are reported under Experimental Procedures.

considered. The weak ion signal for butoxycarboxim suggested that the lettuce constituent interfered with the ionization process of butoxycarboxim. A similar adverse effect was observed by other authors analyzing carbofuran in a crude potato extract (Rule et al., 1994). This problem was circumvented simply by modifying the chromatographic conditions with the addition of 15% acetonitrile to methanol (Figure 7B). By this modification, butoxycarboxim was eluted well after the lettuce component with the result that the ion signal for the analyte was restored. It has to be noted that the presence of acetonitrile in the mobile phase provoked some decrease of the ion signal intensities for all of the analytes, in accordance with the results reported above. These results demonstrate that, when using LC/ES/MS instrumentation, optimization of the chromatographic parameters can become as important as optimization of the spectrometric ones.

Figures 8 shows both LC/UV 220 nm and TIC LC/MS chromatographic traces obtained, respectively, by analyzing an apple sample spiked with carbamates at the individual level of 200 ng/g. Especially when fruit extracts were analyzed, the UV trace indicated that enormous amounts of naturally occurring materials were coeluted with some of the carbamates. Even by increasing the upper limit of the mass scan range up to 700, these endogeneous compounds appeared to be unable to form significant amounts of charged species. However, the possibility that the presence of abundant amounts of these vegetable constituents in charged liquid droplets could interfere with the ionization process of the analytes by complex mechanisms was taken into consideration. For this purpose, samples of each of the 10 vegetables and fruits considered were submitted to the extraction procedure and, just before the relative extracts were injected into the LC column, they were spiked with known volumes of a solution containing the 12 carbamates under study. This solution was standardized by analyzing it in triplicate, with the ES source uncontaminated by vegetable components.

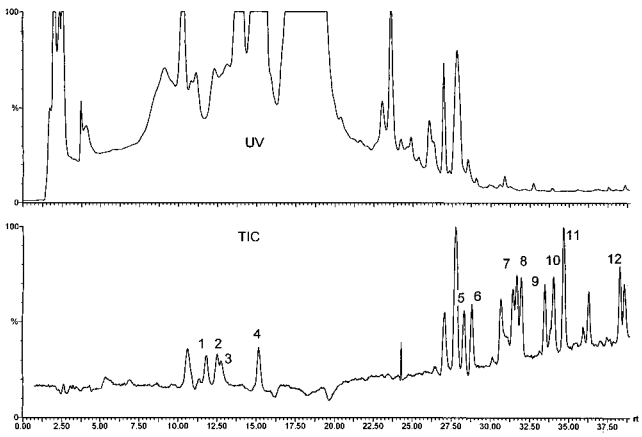

Figure 8. TIC and UV-220 nm chromatogram obtained by analyzing an apple extract amended with carbamates at the individual level of 200 ng/g. Peak numbering is the same as in Figure 2.

After measuring for each carbamate the resulting 10 ion signals from extracted chromatograms, they were

Table 6. Matrix Effect on the Accuracy of the Determination of Carbamates in Fruits and Vegetables by the ES/MS System

	analytical data				
compd	X^a	SD[b]	RSD[c]	d	$\|t\|^e$
butoxycarboxim	442	49	11	477 ± 46	2.1
aldicarb sulfone	408	46	11	437 ± 40	1.9
oxamyl	409	30	7.5	428 ± 29	1.9
methomyl	275	27	9.8	293 ± 23	2.0
butocarboxim	452	48	9.4	483 ± 42	1.9
aldicarb	492	57	12	462 ± 48	1.6
propoxur	536	61	11	565 ± 53	1.4
carbofuran	526	59	11	560 ± 56	1.7
carbaryl	347	29	8.4	333 ± 20	1.4
ethiofencarb	384	25	6.5	367 ± 21	2.0
pirimicarb	822	78	9.4	847 ± 71	1.0
mercaptodimethur	384	20	5.2	374 ± 16	1.8

[a] X = mean values of ion signals obtained by analyzing final extracts of 10 samples of different types spiked with known amounts carbamates. [b] SD = standard deviation. [c] RSD = relative standard deviation. [d] $\mu \pm$ SD = mean values and standard deviations of ion signals calculated by injecting three times known amounts of 12 carbamates. [e] $t(0.05) = 2.3$.

averaged and compared with those obtained from the standard solution by applying the t test ($n = 9$, $P = 0.05$) (Table 6). As can be read, the calculated t values were lower than the critical value, showing that the constituents of fruits and vegetables did not affect significantly the ionization process of carbamates.

Robustness Test. The sample preparation procedure followed by us is relatively rapid, as it does not involve any cleanup step after solvent extraction of carbamates from vegetable materials. The role played by the Carbograph 1 extraction cartridge is simply that of isolating the analytes from water released by the vegetable samples. On repeated analysis of crude vegetable extracts, the ES source could be, however, contaminated to such a point that ion signals for the analytes become irreproducible over time, thus making analyte quantification unreliable. The feasibility of using the ES/MS as a detector for routine determination of carbamates in vegetables was evaluated over 1 day of heavy use of the instrumentation. This investigation was performed by injecting into the LC column at regular intervals 10 μL of a final extract of a lettuce sample spiked with five selected analytes at the level of 200 ng/g. It has to be pointed out that the extract volume injected was 2 times larger than that suggested under Experimental Procedures for analyzing carbamates in vegetables and fruits. For each compound, the ion signal strength was assessed by measuring peak areas produced by the MS detector. The resulting data showed that ion signals did not show any definite tendency to decreasing over 1 working day. Relative standard deviations from averaged peak areas for each component of the standard solution ranged between 7.1 and 7.9%. These figures were in fair agreement with those obtained by analyzing in triplicate the solution containing the five carbamates, with the ES ion source still uncontaminated by vegetable materials. Thus, it appeared that a moderate contamination of the ES source did not affect the correct quantification of the analytes. Anyway, on a daily basis and with the system beyond the sampling cone still under vacuum, the ion source was cleaned as reported under Experimental Procedures.

In conclusion, LC/ES/MS coupled to a simple sample preparation, as that described in this work, appears to be a valuable technique that could be used in the near future for routinely monitoring pesticide residues in vegetable foodstuffs.

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