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Determination of carbamate residues in fruits and vegetables by matrix solid-phase dispersion and liquid chromatography–mass spectrometry

M. Fernández, Y. Picó*, J. Mañes

Laboratori de Bromatologia i Toxicologia, Facultat de Farmàcia, Universitat de València, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, València, Spain

Abstract

Thirteen carbamates were analysed in orange, grape, onion and tomatoes by matrix solid-phase dispersion (MSPD) followed by liquid chromatography–mass spectrometry (LC–MS). Electrospray (ES) and atmospheric pressure chemical ionisation (APCI) were compared and both gave similar results in terms of sensitivity and structural information because at 20 V fragmentor voltages the fragmentation is minimal. The efficiency of different solid-phases (C_{18}, C_8, cyano, amine and phenyl) for the MSPD was compared. Mean recoveries using C_8 varied from 64 to 106% with relative standard deviations of 5–15% in the concentration range of 0.01–10 mg kg^{-1}. Matrix constituents did not interfere significantly with the ionisation process of carbamates. The limits of detection were typically in the 0.001–0.01 mg kg^{-1} range, which were between 10 and 100 times lower than the maximum residue levels (MRLs) established by the European Union (EU). The method was applied to residue detection in fruit and vegetable samples taken from Valencian markets, in which carbamates were detected at low concentrations.

Keywords: Fruits; Vegetables; Matrix solid-phase dispersion.; Carbamates; Pesticides

1. Introduction

Carbamate pesticides have become increasingly important in recent years, due to their broad spectrum of activity, relatively rapid disappearance and generally low mammalian toxicity, but since they are inhibitors of acethylcholinesterase, they are considered toxic for the environment and for human beings [1]. The detection of their residues in food has aroused a great deal of public concern because carbamates are used in households and in agriculture on a large number of crops [2]. Analysis involves a number of stages such as extraction, removal of interfering substances from the extract and determination [3–9]. Recently, special attention has been given to the extraction of very small samples (a few grams) and to selective detection techniques.

Matrix solid-phase dispersion (MSPD) has been proven to be a good alternative to liquid–liquid extraction of carbamates and other pesticides because of its simplicity and robustness [21–25]. It avoids the general drawbacks, such as the use of large amounts of solvent, the occurrence of troublesome emulsions with certain fruit and vegetables, and the slowness associated with liquid–liquid extraction. However, MSPD has only been adapted to LC

*Corresponding author. Fax: +34-96-386-4954.
E-mail address: pico@uv.es (Y. Picó)

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determination in a few applications, due to the difficulty in obtaining, from the matrix, clean extracts free of interferences that are compatible with the use of universal LC detectors, such as UV or DAD [21,26]. No references to the use of MSPD for the determination of pesticide residues employing more selective LC detectors such as fluorescence or mass spectrometry appear to exist.

In this way, liquid chromatography with mass spectrometry detection (LC–MS) has been widely accepted as the preferred technique for the identification and quantification of carbamates and other polar and thermally labile compounds [10,11]. The reason is that the progress in LC–MS, in recent years, has remarkably improved the possibility of identification and/or confirmation of the compounds at very low concentration [10,12–15]. The development of atmospheric pressure ionisation (API) systems offers new opportunities. API includes a group of interfaces, commonly called electrospray (ES), ionspray (IS) and atmospheric pressure chemical ionisation (APCI) [10,16–20]. The disadvantage of the original ES interface was the difficulty of achieving low flow-rates (around 20 μl) for conventional LC [10,16]. Nowadays, ES can be performed at higher flow-rates (typically between 300–500 μl min^{-1}), by directing a gas flow into the effluent stream (designated 'pneumatically assisted ES', ionspray, IS, or simply electrospray, ES). The situation improved with the APCI, which can be operated at a flow-rate up to 2 ml min^{-1}. However, ES is not prone to thermal degradation as the sample is ionised directly in the liquid phase at quasi-ambient temperature, thus leaving intact fragile pesticides [18–20]. The main limitation of APCI is that pesticides can undergo thermal degradation in comparison with ES, where no heat is applied [10,16,19].

This paper focuses on the suitability of MSPD using LC–MS for the determination of carbamate residues in fruit and vegetables. The aims of this study were: (1) to compare the most common LC–MS ionisation techniques, ES and APCI, in both positive and negative mode; (2) to show the aptitude of MSPD for extracting of carbamates in fruit and vegetables and determining them by LC–MS; and (3) to apply the selected method to real samples taken from Valencian markets.

2. Experimental

2.1. Chemical and materials

Carbamates (Carbaryl, Carbofuran, Diethofencarb, Ethiofencarb, Fenobucarb, Fenoxycarb, Isoprocarb, Methiocarb, Metholcarb, Oxamyl, Pirimicarb, Propoxur and Thiobencarb) were supplied by Aldrich (Madrid, Spain). The chemical structures and molecular weights of all the carbamates studied are shown in Fig. 1. Standard stock solutions were prepared in methanol and contained each carbamate at a concentration of 100 μg ml^{-1} and were stored in glass-stopper bottles at 4°C. Standard working solutions of various concentrations were prepared daily by appropriate dilution of aliquots of the stock in methanol.

HPLC-grade methanol, acetonitrile and dichloromethane were purchased from Baker (Deventer, The Netherlands). Deionized water of 18 MΩ cm resistivity was obtained from a Milli-Q water purification system. Acetic acid and sodium chloride were from Panreac (Montcada i Reixac, Spain). All the solvents were passed through a 0.45 μm cellulose filter from Scharlau (Barcelona, Spain) before use.

Silica based sorbents with octadecyl, octyl, cyano, amine and phenyl functional groups (particle diameter in the range of 45–55 μm) were acquired from Análisis Vínicos (Tomelloso, Spain). Silica (particle diameter in the range of 40–60 μm) from Scharlau was used without deactivated.

2.2. Sample preparation

The samples analyzed, (orange, onion, grape and tomatoes), were obtained from a local market. All the samples were taken in accordance with the guidelines of the European Union (EU) Directive 79/700/CEE [27]; that is, as far as possible the sample was taken at various places distributed throughout the lot (size ca. 50 kg). The sample weighed at least 1 kg and consisted of at least 10 individual fruit or vegetables.

A representative portion of sample (200 g of whole fruit or vegetable) was chopped and homogenized in a Bapitaurus food chopper (Taurus, Berlin, Germany). Then, 0.5 g portions were weighed and placed into a mortar.

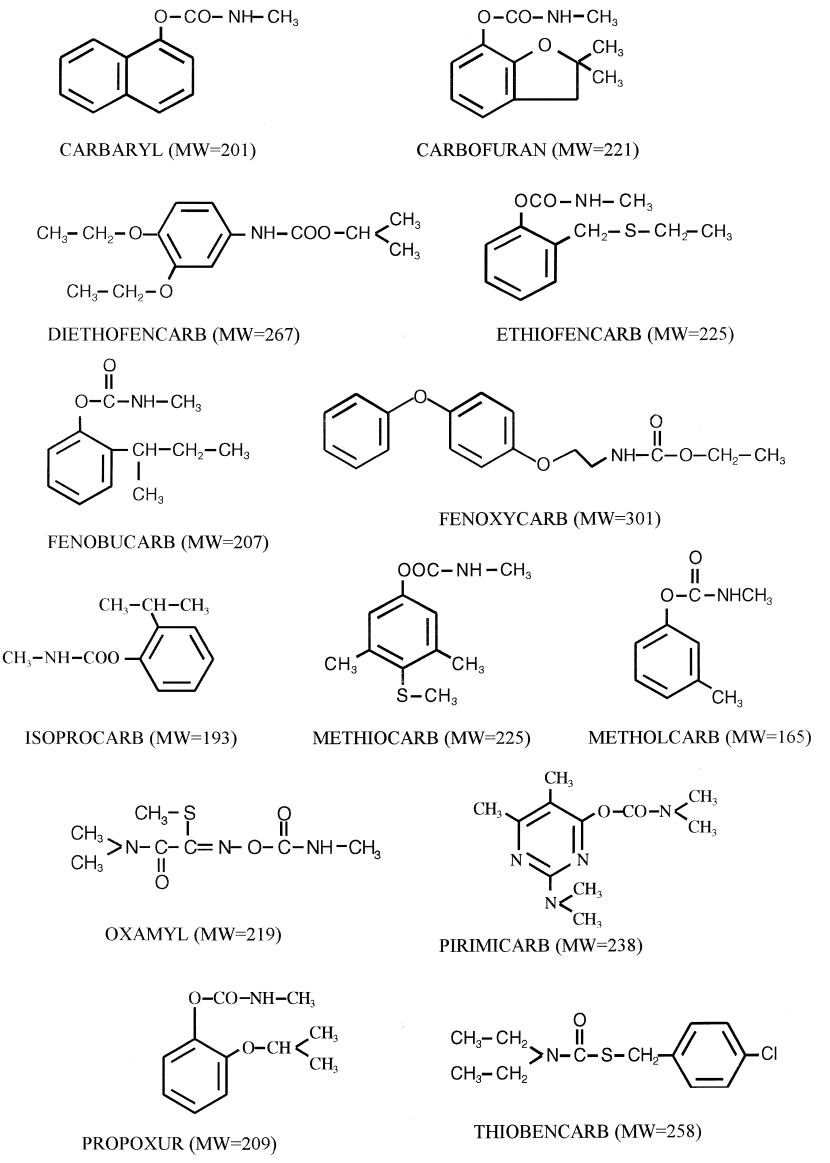

Fig. 1. Structures, common names and molecular weight of carbamates studied.

For the preparation of fortified samples, volumes between 50 and 100 μl of the standard working solutions were added to 0.5 g of sample. They were then allowed to stand at room temperature for 3 h. Samples were spiked with carbamates at four concentration levels: 0.01, 0.1, 1 and 10 mg kg^{-1}.

The 0.5 g portions placed into a glass mortar (50 ml capacity) were gently blended with 0.5 g of the material to be tested (C_{18}, C_8, phenyl, cyano or amine) for 5 min using a pestle, to obtain a homogeneous mixture. This mixture was was introduced into a 100×9 mm I.D. glass column. Ten ml of a dichloromethane–acetonitrile (60:40, v/v) mixture was added to the column and the sample was allowed to elute dropwise by applying a slight vacuum. The eluent was collected in a graduated

conical tube (15 ml) and concentrated, under stream of nitrogen, to 0.5 ml. Five μl of the final extract was injected into the LC apparatus.

An additional purification step, that consisted of fill the glass column with 0.5 g of silica before adding the homogenized mixture, so that the eluent of the MSPD are simultaneously purified in a single process, was tested.

2.3. Chromatographic conditions

A Hewlett-Packard (Palo Alto, CA, USA) HP-1100 Series LC–MSD system equipped with a binary solvent pump, an autosampler, and a MSD coupled with an analytical work station was used. The MSD consisted of a standard API source that can be configured as APCI or ES. A Spherisorb C_8 (150×4.6 mm I.D., 3 μm) stainless steel column and a guard column LiChrosorb RP-8 (10×4.6 mm, 5 μm) both from Supelco (Madrid, Spain). The solvents examined as mobile phases were methanol, acetonitrile and water, using different gradients methanol–water or acetonitrile–water. Water addition of increasing amounts of either CH_3COOH or NaCl was also tested.

The gradient selected for LC–APCI-MS at the flow-rate of 1 ml min^{-1} was methanol 50%, isocratic for 5 min, that was first increased linearly to 60% methanol in 5 min, held at 60% for 5 min and then increased to 90% in 5 min, and held at 90% for 7 min. Return to the initial conditions was carried out in 10 min.

Typical operating conditions of the APCI interface in positive mode were as follows: vaporized temperature, 325 °C; nebulizer gas, nitrogen at a pressure of 4.1 bar; drying gas, also nitrogen, at a flow-rate of 4 l min^{-1} and temperature of 350 °C; capillary voltage, 4000 V; and corona current 4 μA. The experimental conditions of the APCI in negative mode were the same as those used for the positive mode but in this case the corona current was 25 μA.

The analytical separation for the LC–ES-MS was performed using the following gradient: methanol 50%, isocratic for 15 min, then increased to 70% in 5 min, held 5 min, then increased to 90% in 5 min, and held at 90% for 5 min. The flow-rate of the mobile phase was maintained at 0.5 ml min^{-1}.

The ES–MS interface was operated in positive mode under the conditions of 350 °C gas temperature, 13.0 l min^{-1} drying gas flow, 30 p.s.i. nebulizer gas pressure and 4000 V of capillary voltage. The experimental conditions of the ES in negative mode were the same used for the positive mode but, in this case, the capillary voltage was 3500 V.

Full-scan LC–MS chromatograms were obtained by scanning from m/z 100 to 310. Time-scheduled selected-ion monitoring (SIM) of the most abundant ion of each compound was used for quantification.

3. Results and discussion

3.1. Optimization of HPLC–MS parameters

Thirteen carbamates were separated using a methanol–water gradient. When methanol was replaced with acetonitrile, the chromatographic resolution of the peaks was better, but a drastic ion signal weakening and a rapid contamination of the corona needle were observed in APCI. A possible explanation of this is that compared with methanol, acetonitrile is not an ionizable solvent [10].

To optimise the LC–MS conditions different parameters influencing mass spectra were checked in both positive and negative modes for APCI and ES sources. The drying and nebuliser nitrogen flow-rates, the vaporiser and drying temperatures, and the corona and cone potentials were varied in flow injection analysis (FIA) experiments with the analytes (5 μl of a 10 mg l^{-1} individual standard solutions). Although the modification of these parameters did not drastically improve the sensitivity, the optimum working conditions were those reported in Section 2.3.

For the evaluation of the spectral information, FIA was also used, and mass spectra were obtained in full scan mode. The fragmentor voltage was varied from 10 to 120 V in order to find the maximum response using the optimum LC–MS conditions. In positive mode, for both APCI and ES sources, the fragmentor voltage of 20 V provided molecular mass information through the quasi-molecular ions $[M+H]^+$, caused little fragmentation and the sensitivity was the highest for all the compounds. The main ions obtained and their tentative assignation are shown in Table 1. Mass spectra fragments followed the gener-

Table 1
Important mass spectral fragments and their relative abundances (R%) obtained by FIA-LC–APCI-MS and FIA-LC–ES-MS at fragmentor voltages of 20 V in positive mode and 40 V in negative mode

Compound	M_w	APCI				ES	
		Positive mode		Negative mode		Positive mode	
		M/z and tentative ions	R%	M/z and tentative ions	R%	M/z and tentative ions	R%
Carbaryl	201	202 $[M+H]^+$	100	143 $[M-H-CH_3NCO]^-$	100	202 $[M+H]^+$	95
		234 $[M+H+CH_3OH]^+$	13			145 $[M+H-CH_3NCO]^+$	100
						224 $[M+Na]^+$	75
Carbofuran	221	222 $[M+H]^+$	100	163 $[M-H-CH_3NCO]^-$	100	222 $[M+H]^+$	100
						244 $[M+Na]^+$	20
Diethofencarb	267	268 $[M+H]^+$	100	266 $[M-H]^-$	100	268 $[M+H]^+$	100
		182 $[M+H-(CH_3)_2CH_2NCO]^+$	22			290 $[M+Na]^+$	20
Ethiofencarb	225	226 $[M+H]^+$	100	167 $[M-H-CH_3NCO]^-$	100	226 $[M+H]^+$	100
						248 $[M+Na]^+$	62
						107 $[M-CH_3CH_2S-CH_3NCO]^+$	50
						164 $[M-CH_3CH_2S]^+$	50
Fenobucarb	207	208 $[M+H]^+$	100	149 $[M-H-CH_3NCO]^-$	100	208 $[M+H]^+$	100
						226 $[M+NH_4]^+$	25
Fenoxycarb	301	302 $[M+H]^+$	100	185 $[M-H-(CH_2)_3CH_3NCO_2]^-$	100	302 $[M+H]^+$	100
		230 $[M+H-(CH_3)_2NCO]^+$	28			324 $[M+Na]^+$	41
Isoprocarb	193	194 $[M+H]^+$	100	135 $[M-H-CH_3NCO]^-$	100	194 $[M+H]^+$	100
						216 $[M+Na]^+$	15
Methiocarb	225	226 $[M+H]^+$	100	167 $[M-H-CH_3NCO]^-$	100	226 $[M+H]^+$	100
				152 $[M-H-CH_3NCO\text{-}CH_3]^-$	60	248 $[M+Na]^+$	22
Metholcarb	165	166 $[M+H]^+$	100	107 $[M-H-CH_3NCO]^-$	100	166 $[M+H]^+$	100
						188 $[M+Na]^+$	19
Oxamyl	219	163 $[M+H-CH_3NCO]^+$	100	161 $[M-H-CH_3NCO]^-$	100	242 $[M+Na]^+$	100
				147 $[M-(CH_3)NCO]^-$	40	258 $[M+K]^+$	40
						237 $[M+NH_4]^+$	27
						251 $[M+CH_3OH]^+$	17
Pirimicarb	238	239 $[M+H]^+$	100			239 $[M+H]^+$	100
		261 $[M+Na]^+$	29				
Propoxur	209	210 $[M+H]^+$	100	151 $[M-H-CH_3NCO]^-$	100	210 $[M+H]^+$	100
						168 $[M+H-CH_3CH=CH_2]^+$	33
						153 $[M+H-CH_3NCO]^+$	20
Thiobencarb	257	258 $[M+H]^+$	100	132 $[M-CH_2C_6H_4Cl]^-$	100	258 $[M+H]^+$	100

al patterns previously indicated in the literature [10,13,15,16].

The effect of modifying the fragmentor voltage in the production of diagnostic ions is illustrated in Fig. 2 for carbaryl in ES positive. At 20 V, there are three main ions corresponding to $[M+Na]^+=224$, $[M+H]^+=202$ and $[M+H-CH_3NCO]^+=145$. Increasing the voltage to 40 V the base peak in the spectrum from carbaryl is the fragment ion with m/z 145, and the second peak is the quasi-molecular ion at m/z 202. At 60 V fragmentor carbaryl is completely converted to the fragment ion with m/z 145.

On the basis that *N*-methylcarbamate insecticides are labile compounds, some of them suffered decomposition, even at low fragmentor voltage. As an example, at 20 V, the base peak in the APCI-positive spectrum from oxamyl was a charged fragment m/z 163 relative to loss of methylisocyanate.

ES in positive mode produces both $[M+H]^+$ and $[M+Na]^+$ molecular adduct ions without the use of additives in the methanol water eluent, whereas APCI in positive mode only produces the molecular adduct $[M+H]^+$, as listed in Table 1. Formation of sodium adduct ions has extensively been reported in both ES [10] and APCI [18,19]. The presence of sodium ions has been attributed as an impurity in methanol solution employed as the mobile phase, or as sodium in the metal tubing.

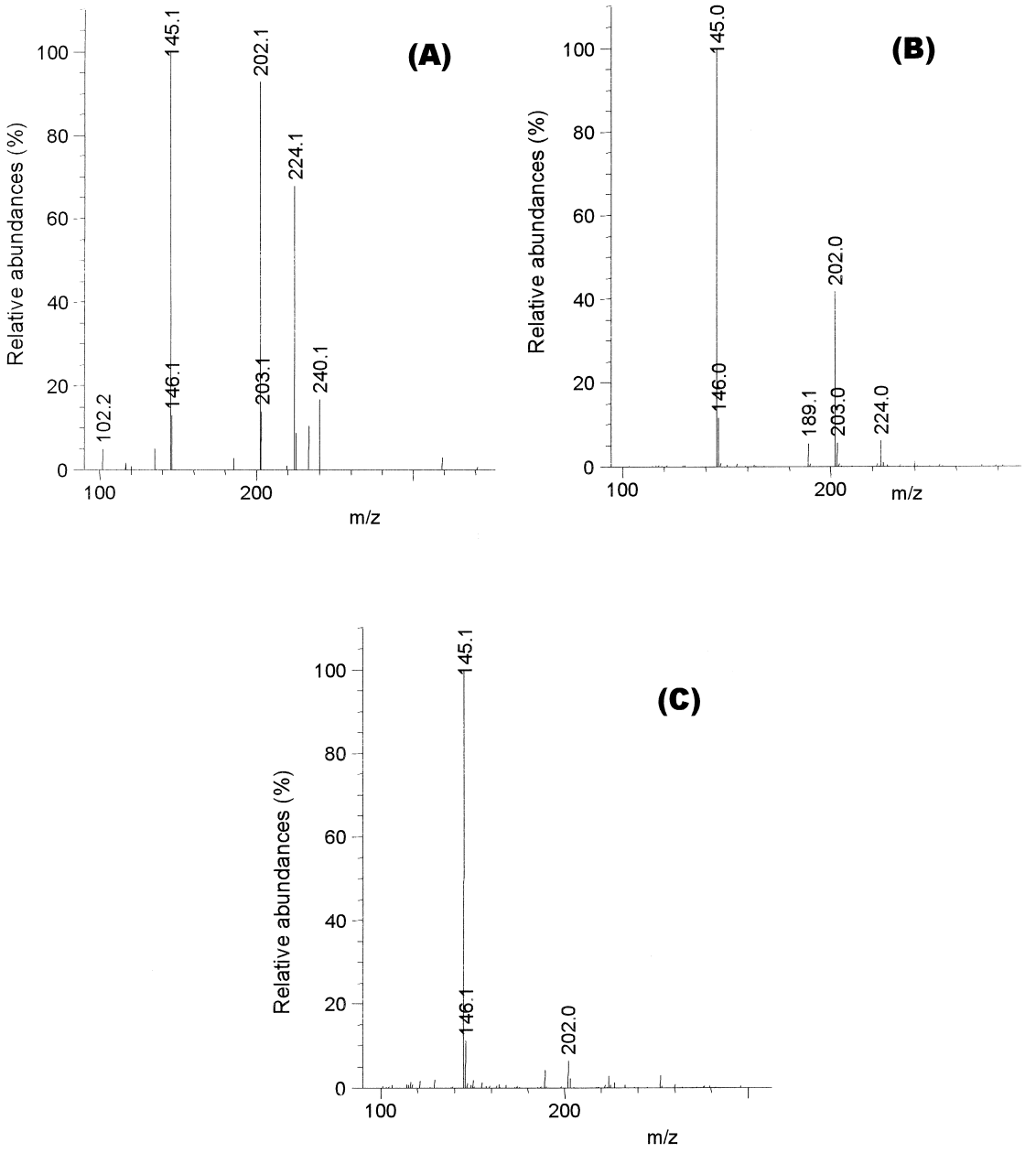

Fig. 2. Variation of the normalised absolute abundance (%) for some fragment ions of carbaryl vs. the extraction voltage: 20 V (A), 40 V (B), and 60 V (C) using LC–ES-MS conditions described in Section 2.3. Tentative assignation of ions m/z 224, $[M+Na]^+$; 202, $[M+H]^+$; 145, $[M+H-CH_3NCO]^+$.

The effect of the addition of H^+ or Na^+ to the LC mobile phase was checked to see if their presence was able to improve the carbamates signal intensity. According to other data reported [10], the results showed that addition of this compounds produced lower ion signal strength.

In LC–API-MS determination, the production of positive or negative ions depends considerably on the

acidity of the analyte in question [18,19]. Using APCI in negative mode, the formation of ions $[M–CONHCH_3]^-$ was observed for the most compounds; other authors previously reported this behaviour although they used thermospray-mass spectrometry (TPS–MS) [28]. The molecular ion $[M–H]^-$ of diethofencarb and fenoxycarb was clearly observed (see Table 1). Pirimicarb did not show any response. The fragmentor voltage requires higher values than in positive mode to obtain maximum sensitivity. Table 1 also lists the fragmentor voltage employed to determine each compound. The sensitivity is better in positive than in negative mode. Carbamates were not detected with the ES source in the negative acquisition mode at acceptable levels.

Separation of carbamates was carried out between 30 and 40 min depending of the mobile phase flow-rate. The low flow-rates required for the ES source did not constitute a problem for the peak resolution, except for fenobucarb and diethofencarb, which were poorly resolved; however, they could be easily identified and quantitated on selected ion chromatograms (SIM).

The system sensitivity was fully optimized using SIM. The time-schedule of SIM was performed following the procedure reported in Table 2. Although the quantification was carried out using the proposed SIM program in order to obtain lower detection limits, more fragment ions can be used for each compound to identify it.

ES ionisation is a softer ionisation technique than APCI and it induced lower fragmentation of some carbamates such as oxamyl. For the same reason, negative fragment ions of carbamates were usually obtained using the APCI in negative mode whereas they were not obtained with ES. APCI and ES sources appear to have high potential as identification methods and it is interesting to note that with both techniques, by increasing the fragmentor one can enhance fragmentation of analytes, and therefore, it is possible to identify compounds.

Quality parameters were calculated for all the interfaces and ionisation modes tested. LC with ES or APCI provided a linear response from amount injected in the range of 0.5 to 50 ng with a good correlation coefficient (r between 0.9864 and 0.9999). For the repeatability study of the LC–MS method, five replicate determinations on the same day of a standard solution (1 mg l^{-1} of each carbamate) were carried out under the optimal LC–MS conditions (precision run-to-run). Moreover, five injections of one solution performed on five different days allowed the establishment of day-to-day precision. Relative standard deviation (RSD) ranged from 5 to 12% for the run-to-run precision and from 8 to 16% for the day-to-day precision, showing a good reproducibility. Linear response and precision parameters did not show differences between the ES or APCI sources, or between positive or negative ionisation modes using the APCI source.

Table 2
Time scheduled SIM condition for monitoring carbamates pesticides in fruit and vegetable samples obtained by ES and APCI mode

Compound	ES positive		APCI positive		APCI negative	
	channel mass m/z	retention window, min	channel mass m/z	retention window, min	channel mass m/z	retention window, min
Oxamyl	242.0	1–11	163.0	1–5	161.0	1–5
Metolcarb	166.0	11–14	166.0	5–10	107.0	5–10
Propoxur	210.1	14–17	210.1	5–10	151.0	5–10
Carbofuran	222.1	14–17	222.1	5–10	163.0	5–10
Carbaryl	202.0	17–23	202.0	10–14	143.0	10–14
Ethiofencarb	226.0	17–23	226.0	10–14	167.0	10–14
Isoprocarb	194.1	23–25	194.1	14–17	135.0	14–17
Pirimicarb	239.1	23–25	239.1	17–22	–	–
Fenobucarb	208.1	25–30	208.1	17–22	149.0	17–22
Diethofencarb	268.1	25–30	268.1	17–22	266.0	17–22
Methiocarb	226.0	25–30	226.1	22–25	167.0	22–25
Fenoxycarb	302.1	30–40	302.1	25–30	185.2	25–30
Thiobencarb	258.0	30–40	258.0	25–30	132.2	25–30

Detection limits (LODs), obtained by direct injection of the standard mixture and calculated with a signal-to-noise ratio of three in SIM mode, were between 10 and 30 times lower using positive mode compared with negative mode. LODs ranged from 100 to 500 pg in APCI negative mode and from 5 to 50 pg in APCI or ES in positive mode. Comparing the LODs (based on the amount injected from standard solutions), it can be deduced that positive mode is required when carbamates should be determined at very low concentrations level; however, negative mode could be a useful tool for confirmation.

3.2. Optimization of the MSPD extraction

Different solid-phases C_{18}, C_8, cyano, phenyl and amine were tested following the procedure reported in Section 2.2. Sample preparation. The results are shown in Table 3. These data are the mean recoveries obtained analyzing, in triplicate, four samples of each fruit and vegetable studied spiked at a concentration of 1 mg kg^{-1}.

The results showed that the recoveries using polar (CN, and NH_2) and non-polar (C_{18}, C_8 and phenyl) bonded phases, resulted in acceptable recoveries (42–104%). CN provided the most effective recoveries of all polar phases and C_8 among reversed ones. C_8 was the preferred solid material for MSPD because it gave the best recovery averages of the compounds, the lowest variation in the values obtained and the cleanest extract. Although in some instances the recoveries for some compounds (e.g. oxamyl and isoprocarb) were unacceptably low, they were only obtained in sporadic cases and it was observed that they were mainly caused by temperature variations and large periods of concentration of the extract under the nitrogen stream. These results are in agreement with other work that made reference to the poor stability of these analytes at higher temperatures of 40–45°C [10]. The methylene chloride–acetonitrile (60:40) mixture was used as eluent because it required a short evaporation time and provided higher recoveries.

Characteristic examples of LC–MS chromatograms of an orange sample spiked at a level of 1 mg kg^{-1} are shown in Fig. 3. The background obtained from chromatograms was very low, but some interfering peaks appeared at the initial part of the chromatogram when LC–APCI-MS in positive mode was used. In the orange extract, oxamyl was eluted on the shoulder of a large peak from a unidentified substance that only appeared in the orange samples; grape, onion and tomato samples did not present that phenomenon. This finding indicates that monitoring ions of low m/z leads to problems of specificity.

Table 3
Recovery of 13 carbamates added at concentration level of 1 mg kg^{-1} to four different types of fruit and vegetables using different solid-phases for homogenisation

	Recovery% (max−min)				
	C_8	C_{18}	Phenyl	Amine	Cyano
Oxamyl	62 (76–49)	55 (66–48)	46 (49–42)	47 (49–44)	50 (54–45)
Metholcarb	90 (94–77)	64 (78–54)	72 (74–71)	76 (81–73)	82 (95–73)
Propoxur	89 (92–77)	73 (77–60)	71 (73–68)	80 (82–78)	80 (90–73)
Carbofuran	83 (85–74)	73 (87–61)	65 (67–61)	61 (69–56)	76 (83–67)
Carbaryl	65 (77–64)	66 (75–47)	42 (52–31)	43 (45–42)	59 (63–56)
Ethiofencarb	77 (84–71)	51 (64–43)	46 (50–40)	49 (59–47)	58 (63–54)
Isoprocarb	60 (66–53)	59 (65–52)	58 (63–52)	55 (62–51)	64 (69–58)
Dietofencarb	67 (69–62)	71 (84–68)	51 (56–48)	58 (63–55)	63 (67–58)
Fenobucarb	74 (76–67)	61 (67–59)	57 (64–53)	64 (70–58)	68 (74–62)
Methiocarb	72 (87–67)	71 (76–67)	61 (68–51)	66 (71–65)	76 (81–66)
Fenoxycarb	67 (70 –63)	64 (74–53)	48 (54–44)	55 (58–52)	65 (69–62)
Thiobencarb	103 (111–90)	98 (105–87)	102 (105–98)	104 (106–88)	92 (104–65)
Pirimicarb	89 (92–77)	72 (74–62)	59 (51–66)	69 (74–61)	73 (83–67)
Mean	77	67	59	64	70

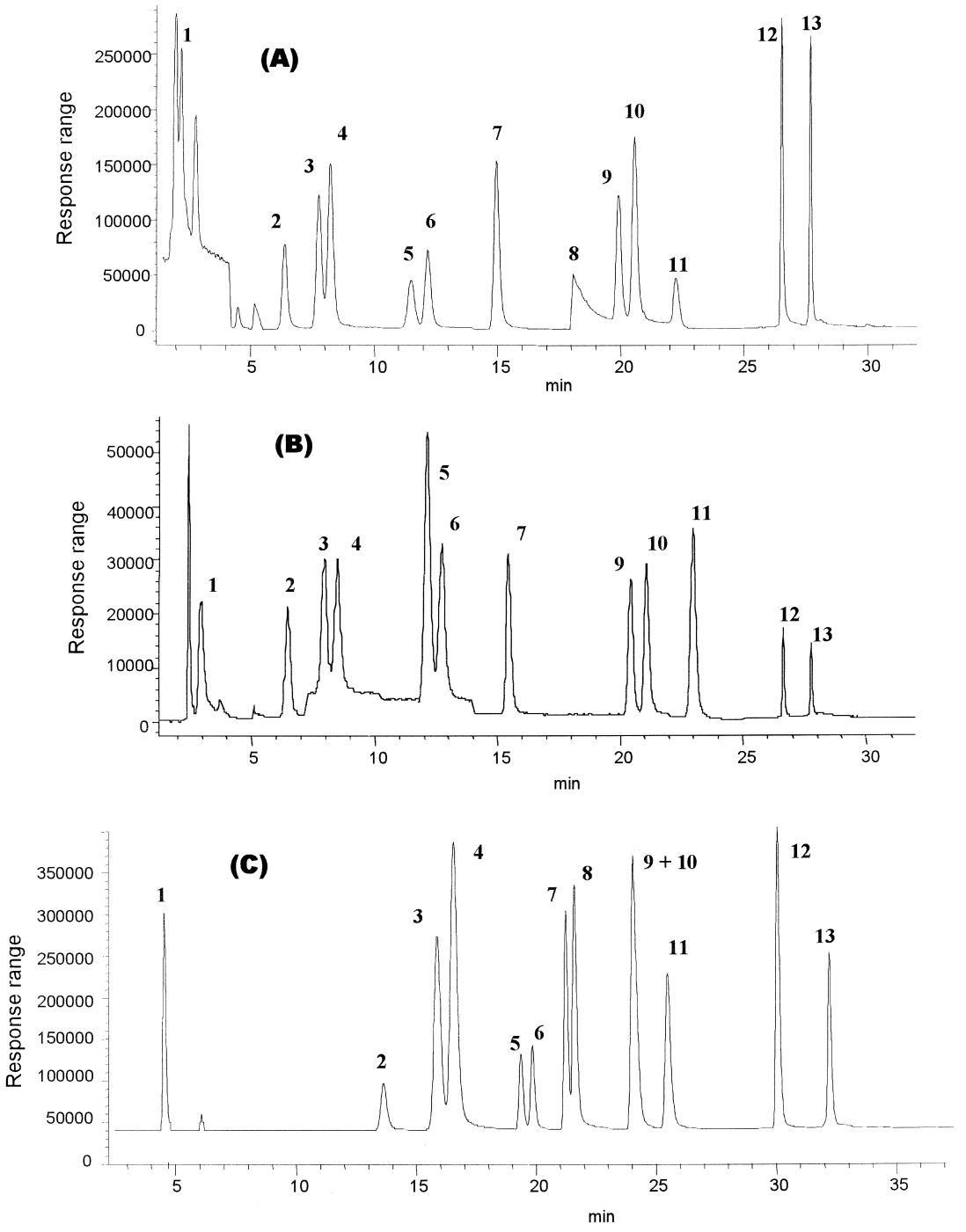

Fig. 3. SIM chromatograms of an orange sample spiked at 1 mg kg^{-1} with 13 carbamates standard mixture obtained by LC–APCI-MS in positive mode (A), LC–APCI-MS in negative mode (B) and LC–ES-MS in positive mode (C). Peaks 1. Oxamyl, 2. Metholcarb, 3. Propoxur, 4. Carbofuran, 5. Carbaryl, 6. Ethiofencarb, 7. Isoprocarb, 8. Pirimicarb, 9. Fenobucarb, 10. Diethofencarb, 11. Methiocarb, 12. Fenoxycarb and 13. Thiobencarb.

A clean-up procedure for fruit and vegetable extracts could be recommended. To this end, the addition of a silica layer in the bottom part of the glass column was used. Different authors previously reported silica as the most useful solid support for clean-up of vegetable extracts [9,23,24,26]. Because of this, it was selected for introducing the cleanup step to the method. The proposed cleanup maintained the recoveries. However, only a little improvement was observed in the elimination of interferences that were not totally removed at low carbamate concentrations.

Orange, grape, onion and tomato samples were fortified with a mixture of 13 carbamates at 10, 1, 0.1 and 0.01 mg kg^{-1}. Five replicate samples of each fruit or vegetable were extracted using C_8 as solid support for the dispersion and silica for the cleanup step. Recoveries and relative standard deviations obtained for grapes are summarised in Table 4. Mean recoveries ranged from 64 to 105% with relative standard deviation of 5 to 15%. Results were similar for the other matrices. Although, the data were calculated from the LC–ES-MS results, there were not significant differences with those estimated using APCI.

Table 5 shows the detection limits calculated for each pesticide. Comparing the LODs (based on ng injected) for standard solutions and fruit and vegetable samples, it can be deduced that matrix effects, even in complex samples with no clean-up, were not relevant when the described techniques are used, except for oxamyl in orange samples.

They were all satisfactory, being 10–100 times lower than maximum residue limits (MRLs) admitted by the European Union (EU) or by the Codex Alimetarius Commision in or on fruit and vegetables [29–31], and comparable or even lower that those obtained by other authors with LC–ES-MS [10,16].

Fig. 4 shows a chromatogram obtained by extracting an onion sample fortified with the analytes at the individual level of 0.01 mg kg^{-1} and analysing the final extract by SIM. As can be observed a clean extract is obtained in APCI positive mode.

The legal criteria for confirming the presence of contaminants usually insist on selecting molecular ion species plus characteristic ion fragments for each compound monitored. However, the correspondence of retention time and molecular weight could provide sufficient specificity for identification of a target compound in fruits and vegetables.

3.3. Application

Ten samples of each: onion, tomato, orange and grapes were tested for the presence of carbamate residues using MSPD followed by LC–ES-MS or LC–APCI-MS. Carbaryl, carbofuran, ethiofencarb, oxamyl and pirimicarb were detected at concentrations ranging from 0.02 to 1 mg kg^{-1} in fruit and vegetables taken from Valencian markets. Carbamate

Table 4
Recovery of 13 carbamates added at different concentration to the grape samples after MSPD with C_8 and LC–ES-MS

Concentration (mg kg^{-1})	Recovery% (x±RSD)			
	10	1	0.1	0.01
Oxamyl	67±9	64±9	73±12	68±15
Metholcarb	91±5	92±7	86±7	78±10
Propoxur	91±4	83±8	86±9	88±16
Carbofuran	85±3	84±5	79±6	86±12
Carbaryl	70±6	67±9	73±11	73±13
Ethiofencarb	86±4	78±12	74±13	85±15
Isoprocarb	67±6	63±8	69±9	69±11
Diethofencarb	76±2	67±6	78±7	69±8
Fenobucarb	78±8	74±7	79±9	81±10
Methiocarb	93±7	74±9	84±10	92±13
Fenoxycarb	87±4	69±6	79±8	83±9
Thiobencarb	106±5	104±8	99±12	104±13
Pirimicarb	91±3	87±9	83±11	94±11

Table 5
Maximum residue limits (MRLs) authorized and limits of detection (LODs)

Compound	MRLs (mg kg^{-1})		LODs (mg kg^{-1})		
	UE-Spain	FAO/WHO	APCI–MS positive	APCI–MS negative	ES–MS positive
Carbaryl	1–5	5–7	0.01	0.01	0.005
Carbofuran	0.1–2	0.1[a]	0.005	0.05	0.001
Diethofencarb	0.05–1	–	0.005	0.05	0.001
Ethiofencarb	1–2	–	0.01	0.1	0.01
Fenobucarb	–	–	0.01	0.01	0.005
Fenoxycarb	0.02–2	–	0.01	0.1	0.005
Isoprocarb	–	–	0.005	0.01	0.005
Methiocarb	0.01–0.2	0.05[a]	0.01	0.05	0.01
Metholcarb	–	–	0.01	0.05	0.005
Oxamyl	0.05–2	0.05[a]–5	0.01–1[b]	0.1	0.005
Pirimicarb	0.5	0.05[a]–1	0.001	–	0.001
Propoxur	3	0.05	0.01	0.05	0.01
Thiobencarb	0.1	–	0.01	0.1	0.01

[a] At or about the limit of determination.
[b] LOD in orange samples.

residues present in the real samples are shown in Table 6.

Although the concentrations found in the samples were always lower than the limits established (see Table 5), three samples (two of tomatoes and one of grape) showed carbamate amounts near to EU and Codex Alimentarius MRLs.

Representative chromatograms of a real tomato sample containing carbaryl at a concentration of 1 mg kg^{-1} are shown in Fig. 5. Non interfering peaks appear on the chromatograms. The fragmentor voltage used was 40 V and the other conditions were those reported in Section 2.3. Fig. 5A and B illustrate the APCI chromatograms in positive and negative mode, respectively and Fig. 5C represents the ES in positive mode, obtained for confirmatory purposes. In the upper and lower chromatograms, the ions used for identification in the tomato extract were 202 and 145, equivalent to the protonated molecular peak and to the acid hydrolysis reaction of the carbaryl with consequent formation of the protonated 1-naphtol. These ions are the base peak and the second most abundant depending on the interface used. Both API sources could be used for confirmation purposes. The high selectivity that was afforded for direct mixture analysis by these techniques greatly enhanced the utility of LC–MS for unambiguous compound identification in complex matrices.

4. Conclusions

Results in this work clearly prove that the LC–MS with APCI or ES sources in positive mode is able to analyse carbamates in fruit and vegetables at levels of regulatory relevance. The analysis of higher carbamate levels with LC–APCI-MS in negative mode is also possible and could be an interesting tool for confirmation.

The present procedure, involving a rapid and non-selective MSPD extraction and a LC–MS analytical technique, allows the simultaneous determination of thirteen carbamates in fruit and vegetables. It combines the advantages of LC–MS for the separation and unequivocal identification of carbamates in complex matrices, with those associated to MSPD in terms of time and solvent economies (removing concentration steps and reducing amounts of toxic solvent discarded), establishing an important alternative for residue analysis. From the results obtained from the real samples, it could be pointed out that the method is appropriate for the analysis of carbamate residues at levels down to MRLs in fruit and vegetables using only 0.5 g of samples.

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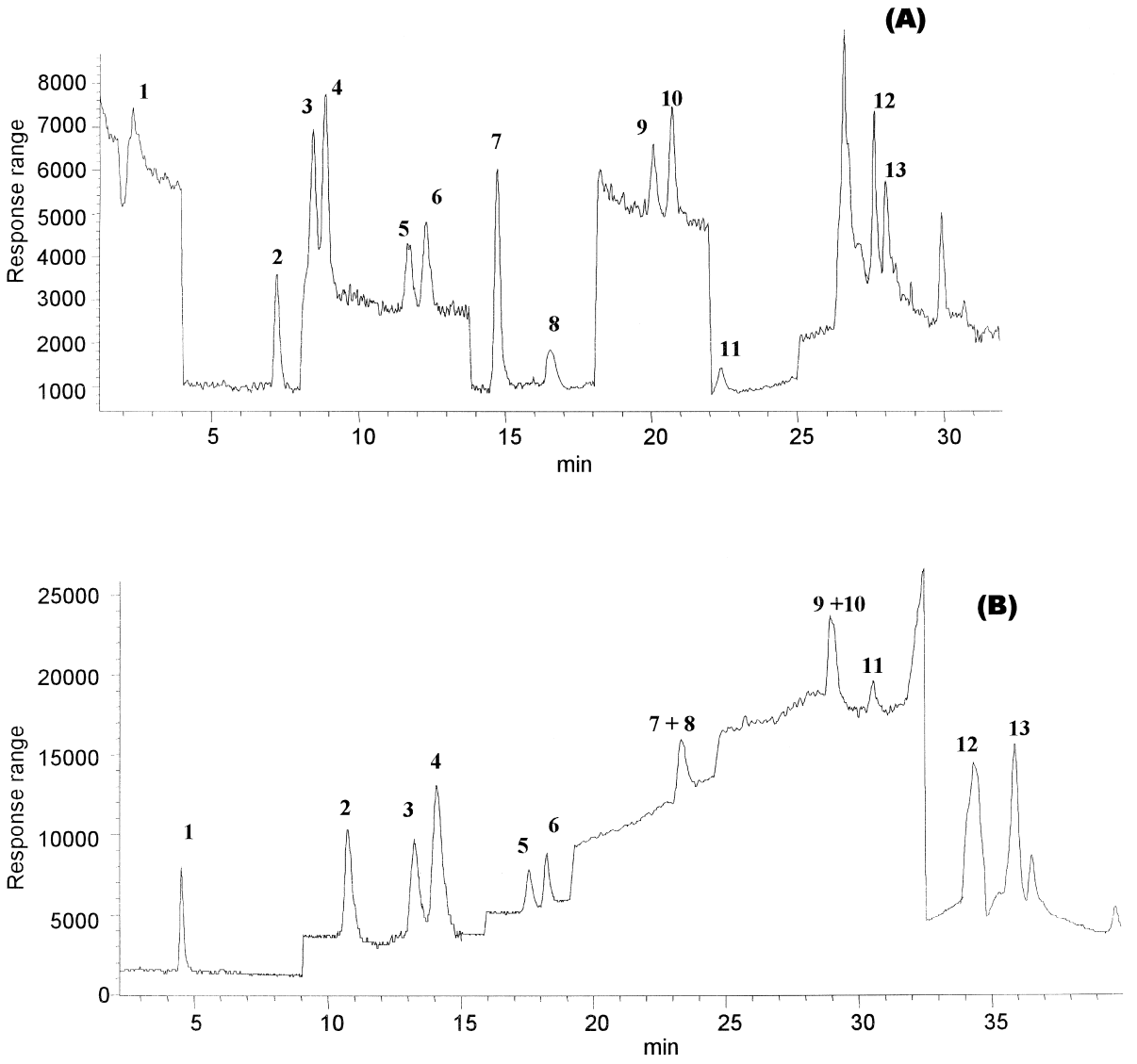

Fig. 4. SIM chromatogram of a grape sample untreated spiked at 0.01 mg kg^{-1} with 13 carbamates standard mixture obtained by LC–APCI-MS (A) and LC–ES-MS (B) both in positive mode. For compound numbers, see Fig. 3.

Table 6
Carbamate concentration in fruit and vegetables taken at the Valencian markets

Commodity	No. of positive samples	Pesticide	Concentration level (mg/kg)		
			APCI +	APCI −	ES +
Grape	1	Carbofuran	0.5	0.3	0.4
Orange	1	Ethiofencarb	0.02	–	0.03
	1	Pirmicarb	0.04	–	0.03
Tomato	1	Carbaryl	1.0	0.9	1.1
	1	Oxamyl	0.9	1.1	1.0
Onion	1	Oxamyl	0.02	–	0.02

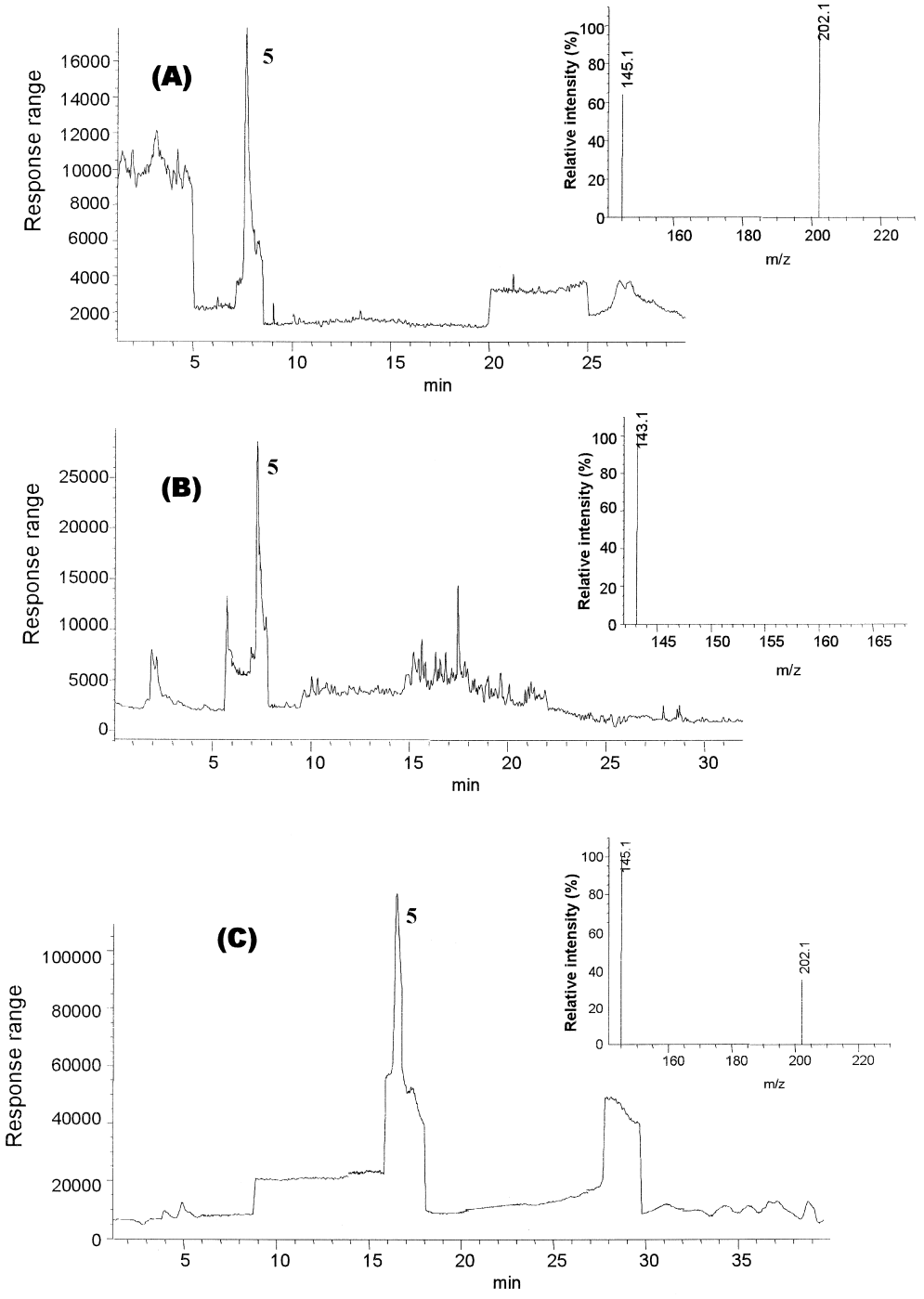

Fig. 5. Example of SIM chromatogram of a positive real tomato sample at 0.09 mg kg^{-1} of carbaryl under LC–APCI-MS in positive mode (A), LC–APCI-MS in negative mode (B), and LC–ES-MS in positive mode (C). For compound number, see Fig. 3.

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