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Determination of Carbamate Pesticide Residues in Vegetables and Fruits by Liquid Chromatography−Atmospheric Pressure Photoionization−Mass Spectrometry and Atmospheric Pressure Chemical Ionization−Mass Spectrometry

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A liquid chromatography−atmospheric pressure photoionization (APPI)−mass spectrometry method was developed for the determination of 22 carbamates including their metabolites in vegetables and fruits. For the optimization of APPI, several APPI ion source parameters were examined. As a result, many carbamates with APPI using the optimized parameter gave simple mass spectra, and a strong signal corresponding to $[M + H]^+$ was observed except for aldicarb. However, some carbamate metabolites gave ammonium adduct ions $[M + NH_4]^+$ as base peak ions. The mean recovery of each carbamate from grape and onion samples spiked at 5 ng/g was 81.7−105.7%, with relative standard deviations of 3.3−5.9%. Furthermore, matrix constituents did not significantly influence the ionization efficiency. The limit of detection ($S/N = 3$) in grape and onion was in the range of 0.33−3.33 ng/g. For the robustness of this method, this system has been used to analyze 50 samples, and the intensities for all carbamates were found to be unaffected by the contamination of the APPI source by sample matrix constituents. This result indicates that the method is reliable.

KEYWORDS: APPI; LC/MS; carbamates; fruits; vegetables; pesticides

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. However, because they are inhibitors of acetylcholinesterase, they are considered to be toxic for human beings (*1*). This impelled the introduction of regulations in the United States, European countries, and Japan. The maximum residue levels (MRLs) established by Japan are in the range of 0.02−7 μg/g. This severe limitation coupled with the complexities of the matrices in which carbamates may occur has compelled researchers to develop sensitive and specific analytical procedures for the determination of these target compounds. Owing to thermal lability of carbamates, traditional gas chromatography−mass spectrometry (GC-MS) methods are not of use (*2*). Thus, on the basis of the pionnering work by Moye et al. (*3*), various sensitive and selective liquid chromatography (LC) methods including postcolumn reaction to form fluorescent drivatives for simultaneously determining *N*-methyl carbamates in water and vegetables have been proposed (*4*, *5*). Furthermore, hyphenated techniques such as LC coupled to MS detection have been extensively developed and applied in the residual analysis in food. The high selectivity and sensitivity of MS detection methods associated with the resolution of LC provide decisive advantages to perform qualitative as well as quantitative analysis of a wide range of molecules at trace levels (*6*−*9*). In particular, the recent development of soft ionization methods such as electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) has made mass spectrometry an important tool in this area (*6*, *10*, *11*). APCI and ESI are affinity methods, and the most common reactions in positive ion mode are protonation, adduct formation, and charge exchange, and those in negative ion mode are deprotonation, adduct formation, electron capture, and charge exchange. These processes are generally associated with polar compounds. Therefore, common problems for ionization methods such as APCI and ESI include the suppression of the desired analyte signal by compounds with higher ionization efficiencies than the target compounds and/or that are present in large excess, and this suppression effect is a more significant problem with ESI than with APCI. Adduct formation with background and sample constituents is prevalent in ESI and is also observed in APCI.

Atmospheric pressure photoionization (APPI) is a new alternative ionization technique for LC-MS (*12*, *13*). The APPI source is based on a high-frequency gas discharge lamp that generates vacuum-ultraviolet (VUV) photons of 10 and 10.6 eV energy. The photon energy of this discharge lamp is normally greater than the first ionization potential (IP) of many analytes because many organic compounds have IPs in the range of 7−10 eV. The principal mechanism for APPI of molecule M is photoabsorption and electron ejection to form the molecule

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radical cation $[M]^{\bullet+}$. In the presence of water vapor or protonic solvents, $[M]^{\bullet+}$ can extract an H^- atom to form a protonated molecule $[M + H]^+$. This tends to occur if M has a high proton affinity. On the other hand, the most commonly used LC mobile phase solvents have IPs higher than the photon energy (water, IP = 12.6 eV; methanol, IP = 10.8 eV; acetonitrile, IP = 12.2 eV). Hence, the total ion production from these solvents has been quite low. Bruins and co-worker have demonstrated (*12*) that the addition of a large quantity of a photoionizable dopant such as acetone or toluene can greatly increase the ionization yield of the target compounds. The principal mechanism for APPI of a molecule with dopant is charge exchange with dopant ions or proton transfer with protonated eluent molecules ionized by dopant ions. Thus, APPI may directly ionize only molecules of analyte molecules that have the relatively lower IPs and may overcome the suppression problem for the APCI and ESI mentioned above. However, to the best of our knowledge, it has never been applied for the analysis of residual pesticides in food.

This paper focuses on the optimization of the APPI parameters and the suitability of the APPI technique for the determination of carbamates in fruits and vegetables by comparison with APCI, which is the most common ionization technique.

MATERIALS AND METHODS

Chemicals. Carbamates (Aldicarbsulfone, aldicarbsulfoxide, aminocarb, bendiocarb, butocarboximsulfone, butocarboximsulfoxide, carbaryl, carbofuran, dithiocarb, ethiofencarb, fenobcarb, isoprocarb, methiocarb, methiocarbsulfone. methiocarbsulfoxide, pirimicarb, thiodicarb, thiofanoxsulfone. thiofanoxsulfoxide, and XMC) and carbamoyloximes (aldicarb and oxamyl) were obtained as individual products from Hayashi Pure Chemicals (Osaka, Japan). The purity of these compounds was >99%. Ammonium acetate, HPLC grade methanol, acetonitorile, and dichloromethane were obtained from Wako Chemicals (Osaka, Japan). These chemicals except HPLC grade solvents were of reagent grade. Deionized water was obtained from a Milli-Q water purification system (Millipore, Tokyo, Japan). All solvents were passed through a 0.45 μm cellulose filter (Millipore) before use. Bondesil C8, a silica base sorbent with an octyl functional group (particle size = 5 μm) was acquired from GL Sciences, Inc. (Tokyo, Japan).

Apparatus and Chromatographic Conditions. The liquid chromatography−mass spectrometry (LC-MS) system consisted of an Agilent 1100 series liquid chromatograph system (Agilent Technologies, Waldbronn, Germany) including a vacuum solvent degassing unit, a binary high-pressure gradient pump, an automatic sample injector, a column thermostat, a photodiode array detector, and an 1100 MSD benchtop mass spectrometer with ESI and APPI capabilities. LC separation was performed on a 150 × 3 mm i.d. column packed with 5 μm Zorbax Eclipse XDB C_{18} (Agilent Technologies, Palo Alto, CA) using linear gradient elution for 15 min with a mobile phase of methanol/water containing 10 mM ammonium acetate (from 30:70 to 100:0, v/v). The flow rate was set at 500 μL/min. The Agilent 1100 series MSD single-quadrupole instrument was equipped with the orthogonal spray APCI or APPI (Agilent Technologies, Palo Alto, CA). Nitrogen generated from pressurized air by a Whatman model 75-72 nitrogen generator (Whatman, Haverhill, MA) was used as nebulizer gas and drying gas (350 °C). Capillary voltage for the ion transmission, fragmentor voltage for in-source fragmentation, and vaporizer temperature were optimized by using the analytical column with carbamate standard at 10 ng/mL. The nebulizer gas, the drying gas, the capillary voltage, the fragmentor voltage, and the vaporizer temperature were set at 50 psi, 7 L/min, 4000 V, 100 V, and 300 °C, respectively. For APCI, a corona current was set at 20 μA. The skimmer and the entrance lens voltage in the ion source were automatically optimized by the calibrant delivery system using a calibration standard (Agilent Technologies, Palo Alto, CA) at 0.1 mL/min and set to 25 and 78 V, respectively. The LC-APPI-MS determination was performed by operating the MSD in the positive ion mode. Mass spectra of all carbamates were acquired over the scan range *m*/z 100−500 using a step size of 0.1 u and a scan speed of 0.5 scan/s. Quantitative analysis was carried out using the selected ion monitoring (SIM) mode of base peak ions as shown in **Table 1**.

Table 1. Time-Scheduled SIM Conditions for Monitoring 22 Carbamates

carbamate	target mass (*m*/*z*) APCI	target mass (*m*/*z*) APPI	dwell time (ms)	fragmentor voltage (V) APCI	fragmentor voltage (V) APPI	retention window (min)
butocarboximsulfoxide	207	207	100	80	80	0.0–12.0
aldicarbsulfoxide	224	207	100	80	80	0.0–12.0
butocarboximsulfone	240	240	100	80	80	0.0–12.0
aldicarbsulfone	240	240	100	80	80	0.0–12.0
oxamyl	237	237	100	120	120	0.0–12.0
thiofanoxsulfoxide	252	178	500	80	120	12.0–15.0
thiofanoxsulfone	268	268	165	80	80	15.0–17.5
methiocarbsulfoxide	242	242	165	120	120	15.0–17.5
dithiocarb	224	224	165	120	120	15.0–17.5
methiocarbsulfone	275	275	500	120	120	17.5–19.5
aldicarb	116	116	500	120	120	19.5–22.0
carbofuran	222	222	165	120	120	22.0–25.1
bendiocarb	224	224	165	120	120	22.0–25.1
aminocarb	209	209	165	120	120	22.0–25.1
carbaryl	219	219	85	80	80	25.1–30.0
ethiofencarb	226	226	85	120	120	25.1–30.0
XMC	180	180	85	120	120	25.1–30.0
thiodicarb	355	355	85	120	120	25.1–30.0
pirimicarb	239	239	85	120	120	25.1–30.0
isoprocarb	194	194	85	120	120	25.1–30.0
fenobucarb	208	208	250	120	120	30.0–33.0
methiocarb	226	226	250	120	120	30.0–33.0

Standard Solutions. Individual standard solutions were prepared by dissolving 100 mg of each carbamate in 100 mL of methanol and were stored at 4 °C in the dark until use. For the working standard of LC-MS analysis, at first, an aliquot of each stock solution in the range from 100 to 10 μL was equally pipetted and transferred into a 100 mL volumetric flask and then these were diluted to mark with the initial mobile phase to make the working solutions with concentrations of 1000, 500, 200, and 100 ng/mL. Furthermore, an aliquot of the working solution with 100 ng/mL in the range from 5000 to 100 μL was transferred to a 10 mL volumetric flask, and it was diluted to mark with the mobile phase to make the working solutions with concentrations of 50, 20, 10, 5, 2, and 1 ng/mL.

Sample Preparation. For the sample preparation, matrix solid-phase dispersion (MSPD) was used for this work according to the method of Fernandez et al. (*11*). Briefly, the samples analyzed (grape and onion) were obtained from the local market. A representative portion of sample (200 g of grape and onion) was first chopped and mixed well with a food chopper. Then, a 1 g portion was weighed and placed into a mortar. For the preparation of spiked samples, 100 μL of the standard working solution with a concentration of 50 ng/mL was added to 1 g of sample. They were then allowed to stand at room temperature for 2 h. The concentration level of the spiked sample was 5 ng/g. The 1 g portion in the glass mortar was blended with 0.5 g of Bondesil C_8 for 5 min using a pestle to obtain a homogeneous mixture. This mixture was introduced into a 100 × 10 mm i.d. glass column followed by adding 10 mL of dichloromethane/acetonitrile (60:40, v/v) to the column. The sample was eluted at ~5 mL/min by applying a slight vacuum. The eluent was evaporated under a stream of nitrogen, and the residue was redissolved with 0.5 mL of methanol/10 mM ammonium acetate aqueous solution (30:70, v/v). Ten microliters of the final extract was injected into the LC-APPI-MS system.

RESULTS

Optimization of APPI Parameters. Twenty-two 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 signal weakening occurred

Table 2. Mass Spectra of Each Carbamate Using APCI and APPI

	APCI			APPI		
carbamate	$(M + H)^+$	$(M + NH_4)^+$	$(M + H - CH_3NCO)^+$	$(M + H)^+$	$(M + NH_4)^+$	$(M + H - CH_3NCO)^+$
butocarboximsulfoxide	207 (100)	224 (165)	150 (34)	207 (100)	224 (9)	
aldicarbsulfoxide	207 (54)	224 (100)	207 (100)	224 (32)		
butocarboximsulfone	223 (15)	240 (100)		223 (21)	240 (100)	
aldicarbsulfone	223 (09)	240 (100)		223 (11)	240 (100)	
oxamyl	237 (100)			237 (100)		
thiofanoxsulfoxide	235 (34)	252 (100)	178 (21)	235 (42)	252 (54)	178 (100)
thiofanoxsulfone	251 (11)	268 (100)		251 (18)	268 (100)	
methiocarbsulfoxide	242 (100)		185 (69)	242 (100)		185 (71)
dithiocarb	224 (100)		167 (34)	224 (100)		167 (21)
methiocarbsulfone	275 (100)			275 (100)		
aldicarb			116 (100)[a]			116 (100)[a]
carbofuran	222 (100)			222 (100)		
bendiocarb	224 (100)			224 (100)		
aminocarb	209 (100)		152 (31)	209 (100)		152 (27)
carbaryl	202 (40)	219 (100)	145 (12)	202 (100)		145 (15)
ethiofencarb	226 (100)	243 (34)	169 (17)	226 (100)		169 (13)
XMC	180 (100)	197 (34)	123 (22)	180 (100)		123 (15)
thiodicarb	355 (100)			355 (100)		
pirimicarb	239 (100)			239 (100)		
isoprocarb	194 (100)	211 (79)		194 (100)		
fenobucarb	208 (100)	225 (47)		208 (100)	225 (3)	
methiocarb	226 (100)	243 (72)		226 (100)	243 (17)	

[a] $m/z = 116$ is the other fragment ion.

for both ionizations. A possible explanation for the APCI results is that acetonitrile is not an ionizable solvent compared with methanol (*6*, *11*). On the other hand, this seems to be related to the different mechanisms of APPI because both solvents cannot be ionized by photons.

To optimize the APPI conditions, all parameters that influence ionization efficiency and the appearance of the mass spectra were investigated because APCI has already been investigated by Fernandez et al. (*11*). These parameters are the drying gas flow, the nebulizer gas pressure, the vaporizer temperature, the capillary voltage, the fragmentor voltage, and the mobile phase. This study was conducted using the chromatographic conditions mentioned under Materials and Methods with MS monitoring in SIM mode using the target ion in **Table 1**. The target ions of aldicarbsulfoxide and thiofanosulfoxide by APPI were different from those by APCI because of the difference of the base peak ions. The concentration of the carbamate standard solution used in these optimization tests was 1 ng/mL. It was found that modification of the drying gas flow rate and the nebulizer gas pressure did not drastically improve the sensitivity of any carbamates. Furthermore, although acetone (IP = 9.70 eV) as photoionizable dopant was added into the APPI source at a flow rate of 50 μL/min to increase the ionization efficiency, addition of the dopant also did not influence the peak intensities of all carbamates. Therefore, only the effects of the fragmentor voltage, the capillary voltage, the vaporizer temperature, and the flow rate of the mobile phase are described.

Effect of Fragmentor Voltage or Mass Spectra. The fragmentor voltage is applied to the exit of the capillary and affects the transmission efficiency and fragmentation of sample ions by in-source collision induced dissociation (CID) for this reason (*14*, *15*). In general, the optimum fragmentor voltage is compound-dependent (*9*, *18*). First of all, mass spectra of APCI and APPI were obtained at the low fragmentor voltage (100 V) to evaluate the spectral information of these carbamates. As shown in **Table 2**, no signal corresponding to the $(M)^+$ radical cation was observed in APPI mass spectra of all carbamates. The predominant ions observed in all carbamates except some metabolites and aldicarb were protonated molecular ions $(M + H)^+$. Other main ions were ammonium adduct ions $(M + NH_4)^+$ and fragment ions $[M + H - (CH_3)_2NCO]^+$. Especially, some metabolites generated $(M + NH_4)^+$ as the base peak ions. These results seem to indicate that the $(M)^+$ radical cation of carbamate might extract hydrogen from water or methanol to form $(M + H)^+$ because almost all carbamates have low IPs (<10 eV). It is beyond the scope of this paperarticle to expound on the ion−molecule chemistry of the APPI source.

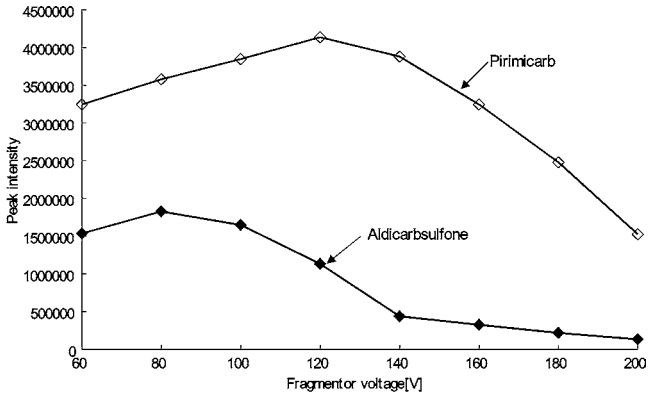

Figure 1. Effect of fragmentor voltage on peak intensity of aldicarbsulfone (◇) and primicarb (◆). Concentration = 1 ng/mL. For other conditions, see Materials and Methods.

On the other hand, **Table 2** shows that mass spectra by both ionizations were very similar except for the intensities of $(M + NH_4)^+$ in the mass spectra of some carbamates, in which their intensities with APCI were much higher than those with APPI. The effect of modifying the fragmentor voltage in the intensity of the base peak ion in APPI is shown in **Figure 1** for aldicarbsulfone and pirimicarb. These results showed that 80 and 120 V were found to be optimum for aldicarbsulfone and pirimicarb, respectively, because the base peak ions of these mass spectra were different [aldicarbsulfone, $(M + NH_4)^+$; pirimicarb, $(M + H)^+$].

Some carbamates that produce $(M + NH_4)^+$ as the base peak ion showed lower optimum fragmentor voltage because of the instability of $(M + NH_4)^+$. On the other hand, the other

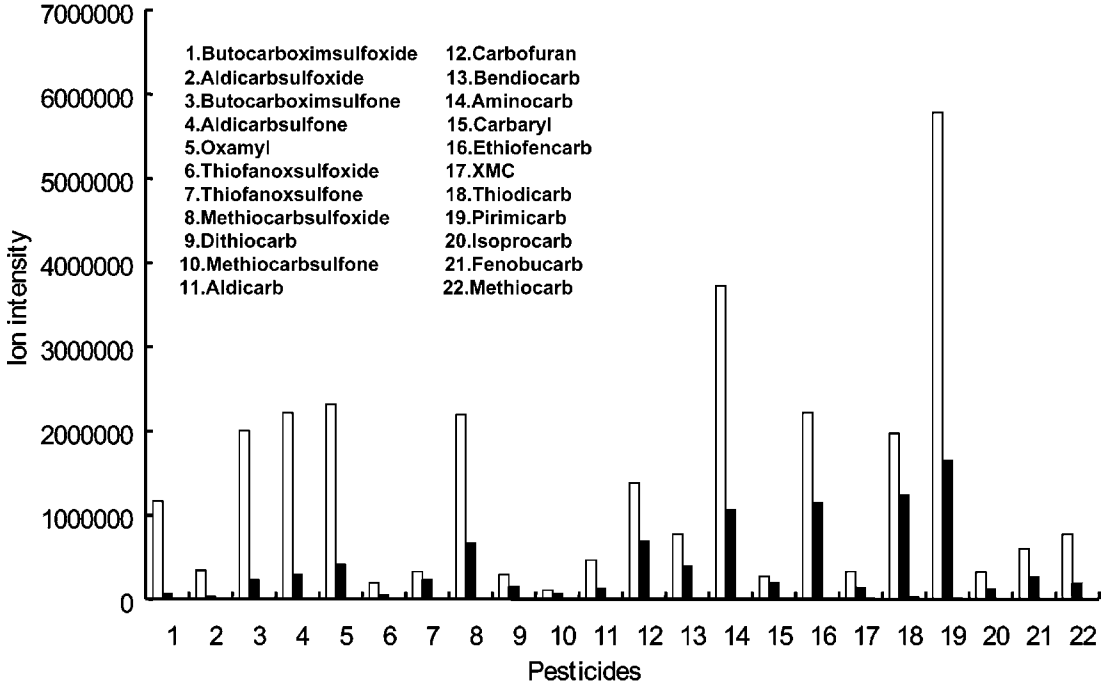

Figure 2. Effect of capillary voltage on peak intensity of carbamates. Concentration = 1 ng/mL: (white bars) 1500 V; (black bars) 2500 V; (slashed bars) 3500 V. For other conditions, see Materials and Methods.

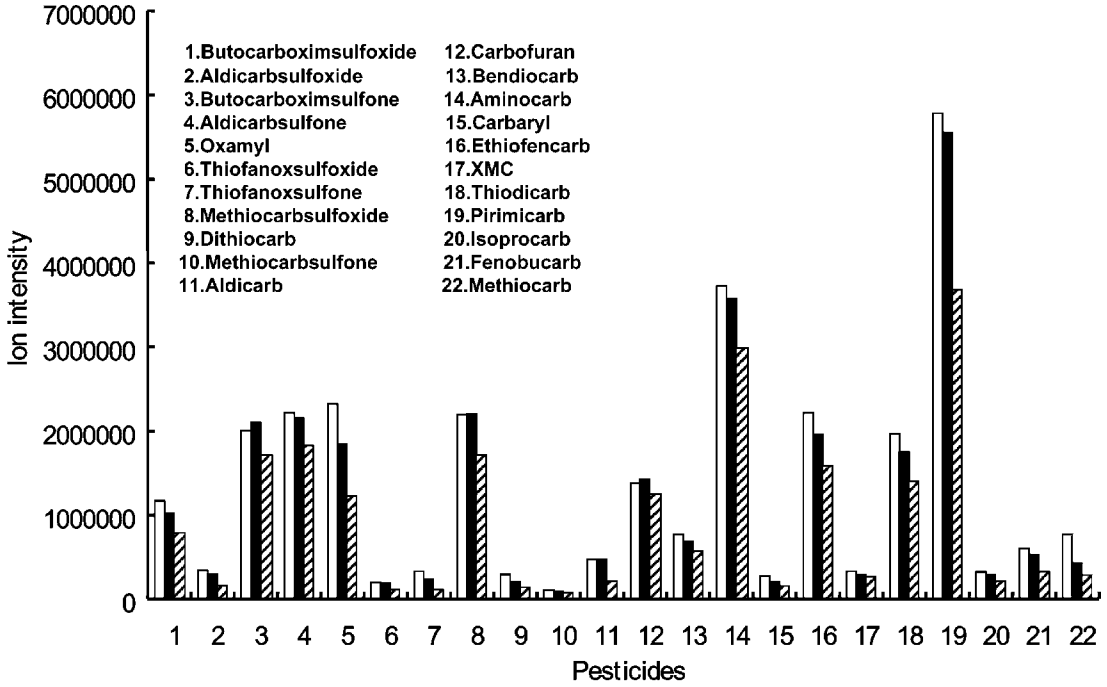

Figure 3. Effect of vaporizer temperature on peak intensity of carbamates: (white bars) 300 °C; (black bars) 350 °C; (slashed bars) 400 °C. Concentration = 1 ng/mL. For other conditions, see Materials and Methods.

carbamates that produce $(M + H)^+$ as the base peak ion showed higher optimum fragmentor voltage because of the higher transmission efficiency between the skimmer and the exit of the capillary at the higher fragmentor voltage. Optimum fragmentor voltages of all carbamates are shown in **Table 1**.

Effect of Capillary Voltage. The capillary voltage is applied to the inlet of the capillary and influences the transmission efficiency of the ions through a capillary sampling orifice. There is one additional feature of the APPI source: ion formation by APPI does not require any element within the ionization region to help ionization at a high potential, unlike ESI and APCI, where the spray needle and corona discharge needle are operated at several kilovolts. The APPI source is essentially field free. This characteristic allows for the independent optimization of the capillary voltage, which may have a tremendous effect upon the sensitivity of the method. To establish the optimum capillary voltage, this parameter was set to 1500, 2500, and 3500 V. As shown in **Figure 2**, 1500 V was found to be optimum, and a tremendous effect of this parameter on the intensity of all carbamates was observed as the intensities of all carbamates at 3500 V were <1% of those at 1500 V.

Furthermore, the ion current measured at the capillary inlet using APPI was much lower than when APCI was used. This result indicates that the ionization efficiency of APPI is very

Table 3. Relative Intensities and Signal to Noise (*S*/*N*) Ratios of Each Carbamate Using APCI and APPI

carbamate	relative intensity[b] (%)	*S*/*N* ratio[a] APPI	APCI
butocarboximsulfoxide	103	22.1	16.4
aldicarbsulfoxide	76	15.5	4.3
butocarboximsulfone	69	10.8	7.3
aldicarbsulfone	72	11.0	6.5
oxamyl	74	19.1	15.3
thiofanoxsulfoxide	25	9.1	7.2
thiofanoxsulfone	34	8.3	7.5
methiocarbsulfoxide	82	31.5	34.4
dithiocarb	42	6.7	7.3
methiocarbsulfone	32	15.6	14.2
aldicarb	45	11.7	6.5
carbofuran	42	22.4	18.2
bendiocarb	33	8.7	7.7
aminocarb	64	23.8	18.4
carbaryl	22	3.1	7.7
ethiofencarb	62	17.7	18.7
XMC	56	8.6	9.5
thiodicarb	72	22.4	21.3
pirimicarb	74	19.9	18.4
isoprocarb	45	8.9	10.5
fenobucarb	53	9.2	8.1
methiocarb	51	7.8	8.5

[a] *S*/*N* ratios are calculated from the results of standard at 5 ng/mL. [b] Relative intensities are based on an intensity of standard at 5 ng/mL with APCI.

low but that high sensitivity could be achieved by optimizing the transmission efficiency of the ions through a capillary sampling orifice by capillary voltage. On the basis of the above results, the capillary voltage was set at 1500 V in APPI.

Effect of Vaporizer Temperature. In general, under APCI conditions, the ionization is carried out at a temperature of ~350 °C. Using the APPI source, higher temperatures have to be applied for a better ionization. The increase of the vaporizing temperature leads to a reduction of the aerosol droplet sizes and yields an improvement in the evaporation of the solvent and, therefore, a better ionization efficiency. **Figure 3** shows that the optimal temperature for a maximum intensity is at 300 °C when the vaporizer temperature was varied between 300 and 400 °C. The intensities of some carbamates declined as the vaporizer temperature increased to >350 °C. Therefore, the deterioration of the intensity at >350 °C seems to be a result of the thermal degradation. On the basis of the above results, the vaporizer temperature was set at 300 °C.

Optimization of Chromatographic Conditions. As described in the above section, methanol was suitable for obtaining high intensity of all carbamates because methanol can easily supply hydrogen to the radical ion of carbamates. For the concentration of ammonium acetate, no effects were observed. Thus, methanol and 10 mM ammonium acetate were used as the mobile phase.

Finally, the flow rate of the mobile phase was investigated. The transmission efficiency of gas-phase ionizations such as APPI and APCI is not affected by the flow rate of the mobile phase. However, the ionization efficiency may be affected by the flow rate. Then, the flow rate was varied from 0.2 to 1 mL/min in order to optimize the intensity of the carbamates. As a result, the maximum intensity of carbamates was found at 0.5 mL/min and the higher flow rate of the mobile phase decreased the ion intensity and the associated signal-to-noise (*S*/*N*) ratio. This result indicates that the ionization efficiency in APPI positive mode was affected by the flow rate of the mobile phase. Thus, the flow rate was set at 0.5 mL/min.

Comparison of LC-APPI-MS and LC-APCI-MS Systems. At first, the sensitivities of the optimized LC-APPI-MS and LC-APCI-MS methods were investigated using the standard mixture of 22 carbamate pesticides in pure solvent at 5 ng/mL. To achieve optimum sensitivity, all experiments were carried out under SIM mode to monitor the base peak ions shown in **Table 1**. The relative intensities of APPI for APCI and *S*/*N* ratio of both ionization modes are shown in **Table 3**. The relative

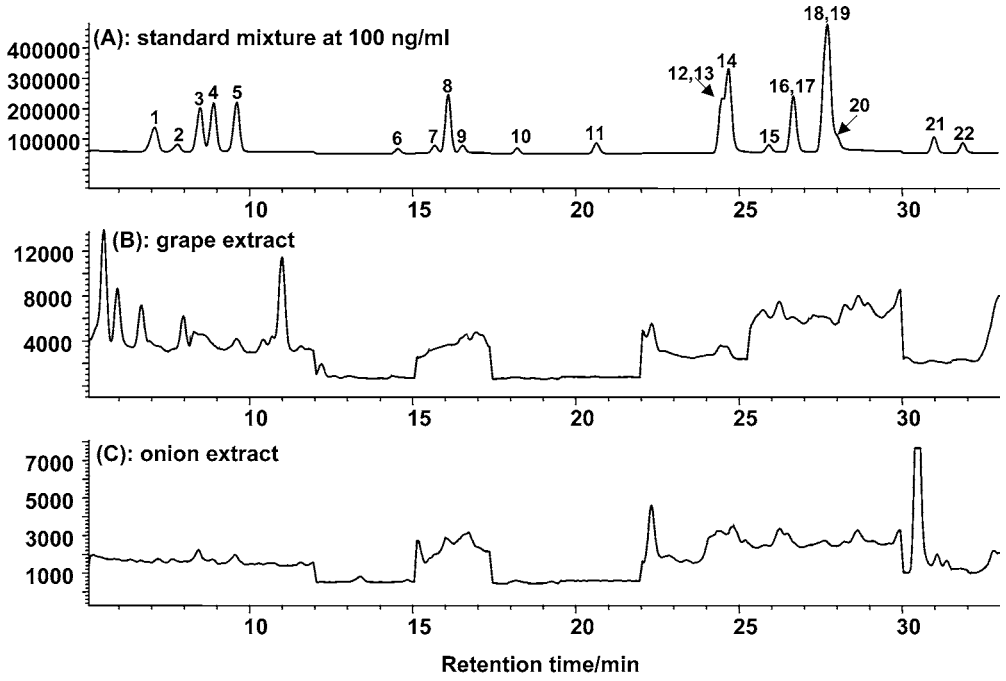

Figure 4. Total ion chromatograms of standard mixtures at 100 ng/mL (**A**), a grape extract (**B**), and an onion extract (**C**) by SIM mode. Peaks: 1, butocarboximsulfoxide; 2, aldicarbsulfoxide; 3, butocarboximsulfone; 4, aldicarbsulfone; 5, oxamyl; 6, thiofanoxsulfoxide; 7, thiofanoxsulfone; 8, methiocarbsulfoxide; 9, dithiocarb; 10, methiocarbsulfone; 11, aldicarb; 12, carbofuran; 13, bendiocarb; 14, aminocarb; 15, carbaryl; 16, ethiofencarb; 17, XMC; 18, thiodicarb; 19, pirimicarb; 20, isoprocarb; 21, fenobucarb; 22, methiocarb.

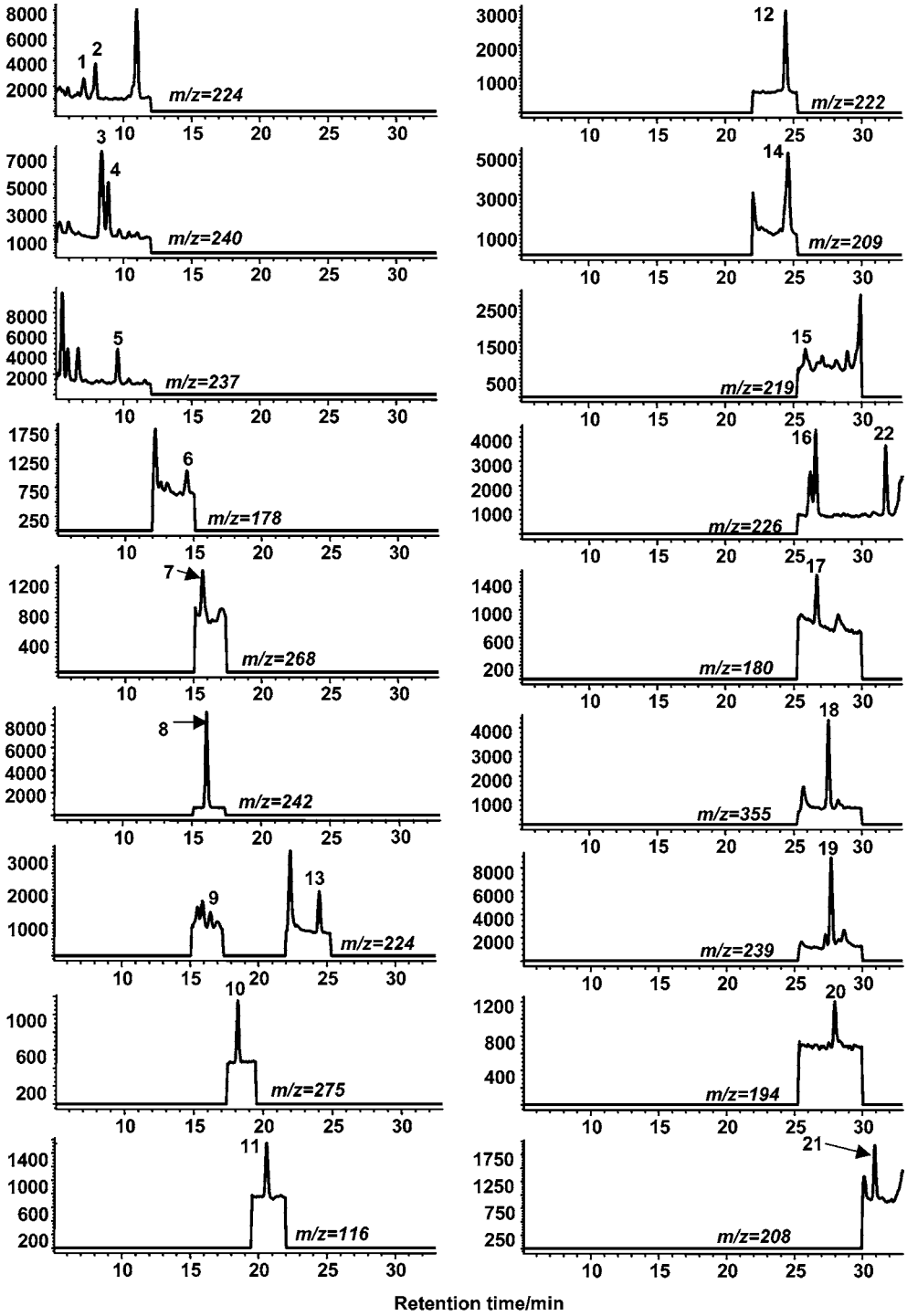

Figure 5. SIM chromatograms of a grape sample spiked at 5 ng/g with a 22 carbamate mixture. Peaks: 1, butocarboximsulfoxide; 2, aldicarbsulfoxide; 3, butocarboximsulfone; 4, aldicarbsulfone; 5, oxamyl; 6, thiofanoxsulfoxide; 7, thiofanoxsulfone; 8, methiocarbsulfoxide; 9, dithiocarb; 10, methiocarbsulfone; 11, aldicarb; 12, carbofuran; 13, bendiocarb; 14, aminocarb; 15, carbaryl; 16, ethiofencarb; 17, XMC; 18, thiodicarb; 19, pirimicarb; 20, isoprocarb; 21, fenobucarb; 22, methiocarb.

intensities of carbamates with APPI ranged from 22 to 103%, but the *S*/*N* of these compounds did not show significant differences between APPI and APCI. These results confirm that APPI is also a suitable technique for the residual analysis of carbamates.

Sensitivity, Linearity, and Precision of LC-APPI-MS System. The analytical performance characteristics of the LC-APPI-MS method were determined from the standard mixture of 22 carbamates in pure solvent using SIM mode. The calibration curves of all carbamates showed good linearity in the range from 1 to 1000 ng/mL with correlation coefficients (r^2) above 0.999. The repeatability of the LC-APPI-MS method was calculated on the basis of five replicates at 5 ng/mL analyzed on the same day. The relative standard deviations (RSDs) of all carbamates ranged from 1.3 to 3.2%. The limits of detection (LODs) calculated with *S*/*N* of 3 from each SIM chromatogram at 5 ng/mL ranged from 0.5 to 5.0 ng/mL.

Analysis of Vegetables and Fruits. Generally speaking, affinity-based ionization techniques, such as APCI and ESI, are susceptible to competition for ionization from some matrix components that coelute with analytes. Therefore, the matrix effect in the APPI determination was investigated by comparing

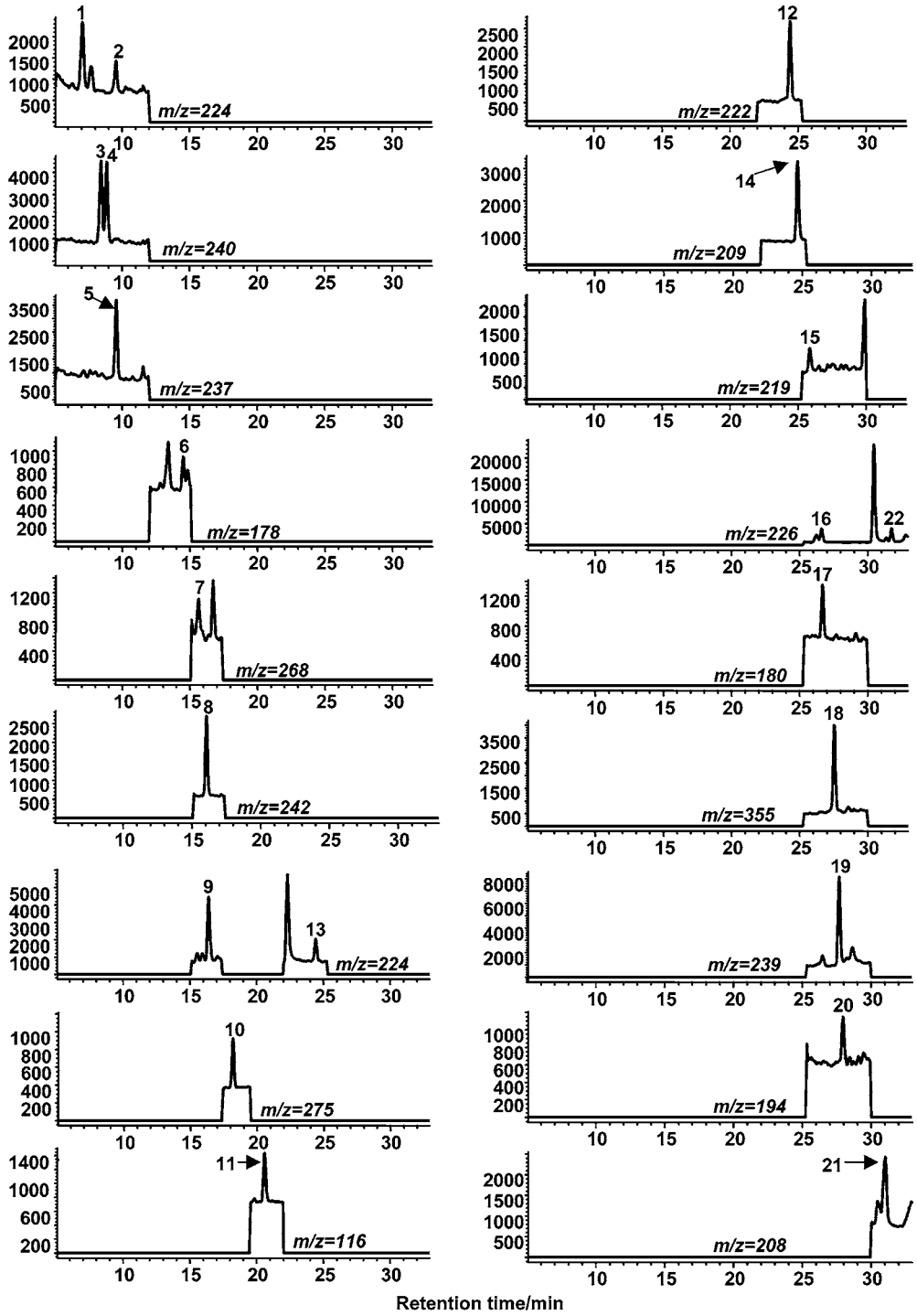

Figure 6. SIM chromatograms of an onion sample spiked at 5 ng/g with a 22 carbamate mixture. Peaks: 1, butocarboximsulfoxide; 2, aldicarbsulfoxide; 3, butocarboximsulfone; 4, aldicarbsulfone; 5, oxamyl; 6, thiofanoxsulfoxide; 7, thiofanoxsulfone; 8, methiocarbsulfoxide; 9, dithiocarb; 10, methiocarbsulfone; 11, aldicarb; 12, carbofuran; 13, bendiocarb; 14, aminocarb; 15, carbaryl; 16, ethiofencarb; 17, XMC; 18, thiodicarb; 19, pirimicarb; 20, isoprocarb; 21, fenobucarb; 22, methiocarb.

SIM chromatograms obtained for a standard solution in pure solvent with those obtained from the analyte-free sample extract. As shown in **Figure 4**, it was observed that both sample matrixes led to alterations in the chromatograms, including some additional peaks. However, these additional peaks caused no interference, because they were well separated from the peaks of all carbamates. Furthermore, average changes in retention time and peak intensity of all carbamates obtained from matrix-matched standard solutions prepared from analyte-free sample were less than 1.0 and 10.0%, respectively. These properties of APPI make it a very useful technique for high-throughput applications because it minimizes the need to prepare matrix-matched standards. The recoveries of all carbamates for the proposed method were calculated using spiked carbamate-free samples of grape and onion. Five samples of different grapes and onions were spiked with the 22 carbamate mixture at 5 ng/g. Typical SIM chromatograms from extracts of grape and onion spiked are shown in **Figures 5** and **6**, respectively. Average recovery and RSD of each carbamate from these samples were in the ranges of 81.7−105.7 and 3.3−5.9%, respectively (**Table 4**). The LODs of the 22 carbamates in grape and onion were determined by the signal corresponding to

Table 4. Recovery and LOD of Each Carbamate from Spiked Grape and Onion Samples Using APPI[a]

carbamate	recovery (RSD,[b] %)		LOD[c] (ng/g)	
	grape	onion	grape	onion
butocarboximsulfoxide	92.1 (4.2)	85.7 (3.7)	1.83	1.69
aldicarbsulfoxide	89.4 (3.8)	91.3 (4.5)	1.43	1.81
butocarboximsulfone	88.2 (3.9)	87.5 (3.8)	1.39	1.24
aldicarbsulfone	93.4 (4.1)	95.7 (4.9)	1.76	1.36
oxamyl	82.1 (5.4)	86.3 (4.6)	1.65	1.74
thiofanoxsulfoxide	87.2 (3.9)	81.7 (5.3)	1.90	1.88
thiofanoxsulfone	96.7 (4.1)	91.4 (3.7)	2.21	2.42
methiocarbsulfoxide	102.1 (4.5)	97.6 (3.4)	0.33	0.36
dithiocarb	86.2 (5.1)	88.9 (4.7)	2.88	1.97
methiocarbsulfone	98.7 (5.7)	92.4 (3.5)	0.71	0.83
aldicarb	91.4 (3.7)	88.1 (3.2)	0.99	1.53
carbofuran	86.6 (4.2)	89.8 (5.3)	1.22	1.44
bendiocarb	90.8 (3.3)	96.2 (4.5)	1.15	1.40
aminocarb	86.2 (4.5)	87.4 (3.8)	1.53	0.80
carbaryl	95.4 (3.6)	92.1 (5.7)	3.33	1.69
ethiofencarb	103.4 (4.1)	105.7 (4.9)	1.18	1.47
XMC	88.1 (4.2)	85.7 (4.8)	1.29	1.36
thiodicarb	90.4 (5.2)	86.6 (5.4)	1.12	1.61
pirimicarb	84.1 (5.3)	88.1 (5.6)	1.38	1.42
isoprocarb	93.7 (5.7)	89.6 (4.4)	1.69	1.72
fenobucarb	93.9 (5.4)	90.9 (5.9)	1.47	1.76
methiocarb	88.0 (5.0)	83.7 (4.5)	0.98	0.56

[a] Concentration of each carbamate = 5 ng/g. [b] Five different spiked samples at the same amount were analyzed. [c] Limit of detection is defined as $S/N = 3$ for the spiked grape and onion.

Table 5. Precision of Each Carbamate in Grape Using APPI

carbamate	quantitative result[a] [ng/g (RSD, %, $n = 5$)]	
	repeatability[b]	reproducibility[c]
butocarboximsulfoxide	5.4 (2.4)	5.2 (5.7)
aldicarbsulfoxide	4.7 (1.9)	4.9 (4.8)
butocarboximsulfone	5.1 (1.6)	4.8 (6.2)
aldicarbsulfone	4.9 (2.4)	4.7 (3.9)
oxamyl	4.7 (3.7)	5.3 (6.0)
thiofanoxsulfoxide	4.9 (2.7)	5.4 (4.1)
thiofanoxsulfone	5.3 (3.2)	5.1 (4.9)
methiocarbsulfoxide	5.1 (2.5)	5.3 (5.2)
dithiocarb	4.6 (2.1)	4.8 (4.3)
methiocarbsulfone	4.7 (1.6)	4.8 (3.6)
aldicarb	5.1 (3.7)	4.8 (3.8)
carbofuran	5.4 (2.6)	5.2 (4.1)
bendiocarb	4.7 (2.9)	4.9 (4.8)
aminocarb	5.4 (2.7)	5.3 (3.7)
carbaryl	4.7 (2.0)	4.9 (5.1)
ethiofencarb	5.1 (1.4)	5.3 (3.4)
XMC	4.7 (2.2)	4.6 (4.7)
thiodicarb	5.4 (2.8)	5.2 (4.1)
pirimicarb	4.7 (1.9)	5.1 (3.7)
isoprocarb	4.5 (2.1)	4.7 (4.1)
fenobucarb	4.7 (2.7)	4.9 (5.1)
methiocarb	5.3 (3.1)	5.1 (3.9)

[a] Calculated for grape spiked at 5 ng/g. [b] Repeatability was calculated on the basis of five replicates at 10 ng/g within 1 working day. [c] Reproducibility was calculated on the basis of a single analysis at 10 ng/g per day for 5 days.

3 times the background noise in the SIM chromatograms for the samples spiked at 5 ng/g. As shown in **Table 4**, the LODs of the carbamates ranged from 0.33 to 3.33 ng/g. The intraday precision (repeatability) was evaluated as the RSD of the quantitative results obtained by injecting carbamate-free grape spiked at 5 ng/g five times during a working day. The interday precision (reproducibility) was calculated by analyzing the same sample over 5 working days. As these results show in **Table 5**, the repeatability and reproducibility for each carbamate were in the ranges of 1.4−3.7 and 3.4−6.2%, respectively. The inaccuracy ranged from −10 to 8% for grape.

As described in the above section, the current sample preparation by MSPD is relatively rapid because it does not have any cleanup step after the MSPD step. Thus, on repeated analysis of crude extracts, the APPI source could be contaminated to such a point that intensities for the analytes become irreproducible over time. Then, the feasibility of using APPI as an ionization technique for routine determination of carbamates in fruits and vegetables was evaluated over 1 day of heavy use of the instrumentation by injecting a final extract of the sample spiked at 5 ng/g into the LC-APPI-MS system. The resulting data showed that intensities of all carbamates did not show any definite tendency to decrease over 1 day. The RSD from peak areas for each carbamate ranged from 2.2 to 4.5%. These figures were in fair agreement with those obtained by analyzing in five replicates the standard solution of all carbamates in pure solvent, with the APPI source still uncontaminated by sample matrix. Thus, it appears that a moderate contamination of the APPI source did not affect the accurate quantitation of the analytes because there is no significant ion suppression due to the dirty ion source. We could analyze at least 50 samples without any termination of the system. These results indicate that this LC-APPI-MS method is suitable for the analysis of residues of carbamates in fruits and vegetables.

In conclusion, we have demonstrated that the APPI technique is an ideal ionization technique for the determination of carbamates in fruits and vegetables because of its high sensitivity and high selectivity. Another advantage of using APPI for carbamate determination in fruits and vegetables is the low matrix effect. Consequently, the proposed method eliminates the need of matrix-matched standards, which is more tedious for the samples from different origins. Furthermore, validation data demonstrate that this method is convenient for the routine analysis of carbamate residues in fruits and vegetables at trace levels.

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