An Evaluation of Liquid Chromatography/Mass Spectrometry with Atmospheric Pressure Chemical Ionization for the Rapid and Simultaneous Measurement of Carbamate Pesticides and Organophosphorus Pesticides

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Liquid chromatography/mass spectrometry with an atmospheric pressure chemical ionization interface (LC/APCI/MS) is evaluated for the simultaneous determination of carbamate pesticides and organophosphorus pesticides in a single chromatographic analysis. APCI mass spectra of those compounds were obtained to study their ionization characteristics. APCI provided abundant ions such as protonated molecules and characteristic fragment ions for carbamate pesticides and organophosphorus pesticides. To evaluate the feasibility of the LC/APCI/MS for a routine quantitative analysis, the linearity and repeatability of LC/APCI/MS were examined by measuring standard solution mixtures of five carbamate pesticides and four organophosphorus pesticides over the range of 1 to 100 μg/mL. The peak areas in chromatograms of characteristic ions for those compounds showed less than 3% of variation from run to run. The standard calibration curves for the nine pesticides show good linearity in the concentration range. The detection limits of the LC/APCI/MS system for those compounds range from 0.006 to 0.2 ng.

Introduction

Since the use of most of organochlorine pesticides was prohibited due to their high toxicity and slow degradation rate, relatively "short-life" organophosphorus pesticides and carbamate pesticides have replaced those "long-life" pesticides in agriculture.[1] Meanwhile, the increasing use of carbamate pesticides in agriculture demands development of highly sensitive analytical methods for the determination of trace level residues of these compounds in food and drinking water. Gas chromatography (GC) with appropriate detection systems such as flame ionization detector (FID), nitrogen-phosphorus detector (NPD), and mass spectrometry has been widely accepted as a reliable method for the analysis of organophosphorus pesticides.[1-5] However, analytical methods for carbamate pesticides have been rather limited. The thermal lability of most of carbamate pesticides prevents their direct analysis using GC.[1,6,7] A few complicated derivatization methods have been developed to overcome the thermal lability.[7,8] High performance liquid chromatography (HPLC) can overcome the thermal lability, but the lack of a sensitive and selective detector limited this approach to only a few specific carbamates.[9,10] The HPLC-fluorescence detection system using post-column hydrolysis and derivatization is currently the most widely used technique for the determination of carbamate pesticides[11,12] in water[5,13] and foodstuffs.[2,14,15] Therefore, screening of widely used organophosphorus pesticides and carbamate pesticides in water and foodstuffs demands both GC and HPLC analysis.

Liquid chromatography combined with mass spectrometry (LC/MS) has been regarded as a potential candidate of pesticide analysis due to the coupling of the capability of the mass spectrometric detection and the applicability of LC to the wide range of molecules.[16] However, the lack of reliable interfaces between LC and MS hampered the routine use of LC/MS. Recently, several atmospheric pressure ionization (API) techniques such as thermospray ionization (TSI),[17,18] electrospray ionization (ESI),[19,20] and atmospheric pressure chemical ionization (APCI)[21,22] have been developed as robust and reliable interfaces for the application of LC/MS to routine analyses of pesticides and other biological and industrial chemicals.[21-25] Among those API techniques, APCI has been outstanding for the quantitative analysis of pesticides as it provides a good sensitivity and a wide linear dynamic range in LC/MS.[20,23-25] A few recent investigations by Pleasance *et al.*[25] and by Kawasaki *et al.*[23] demonstrated that LC/MS systems with APCI interfaces (LC/APCI/MS) can be applicable to the quantitative analysis of carbamate pesticides. We note that the APCI can also provide good ionization efficiencies to widely used organophosphorus pesticides. Most of organophosphorus pesticides are polar, and they are likely to form protonated molecules in APCI processes. Therefore, LC/APCI/MS is expected to be applicable to the determination of both organophosphorus pesticides and carbamates in a single chromatographic analysis, and to be used for the fast screening of those regulatory pesticides.

This report presents an evaluation of LC/APCI/MS method for the analysis of both organophosphorus pesticides and carbamate pesticides. The pesticides studied in this work are five carbamate pesticides (aldicarb, aldicarbsulfone, methiocarb, methiocarbsulfoxide, and carbaryl) and four representative organophosphorus pesticides (parathion, malathion, diazinon, and fenitrothion). The four organophosphorus pesticides and carbaryl in drinking water are regulated in Korea and the other pesticides are chosen to represent carbamate pesticides widely used in Korea and their metabolites. The

LC/APCI/MS conditions are described for the separation and detection of the nine pesticides.

Experimental Section

All of the pesticides used in this study were obtained from Dr. Ehrenstorfer Reference Materials repository, Augsburg, Germany. Their structures and common names are shown in Figure 1. Individual stock solutions were prepared by dissolving a weighed portion (around 2 mg) of each pesticide in 2 mL of methanol. A composite stock solution was prepared by mixing appropriate amounts of the individual stock solutions and diluting with methanol to the concentration of 100 μg/mL for each compound except carbaryl, which was prepared to be 80 μg/mL without any specific intention. Standard solution mixtures used to make calibration curves were prepared by diluting the composite stock solutions with methanol. HPLC grade methanol was obtained from Burdick and Jackson, Inc. 99.5% acetic acid were obtained from Hayashi Pure Chemical Industries Ltd. Pure water was prepared by using a membrane-filtering system and further purified by passing through a Millipore Corp Milli-Q RG purification system.

Analysis was performed on a Hewlett-Packard 1050 series liquid chromatography (LC) with a solvent delivery system and an autosampler, which was coupled to a quadrupole ion trap mass spectrometer (LCQ, Finnigan) equipped with an APCI interface. Nitrogen was used as a nebulizing gas. The corona discharge needle of the APCI interface was maintained at 4.5 kV. Ions were extracted by a heated capillary tube, focused by a tube lens, passed through a skimmer, and guided to the mass analyzer by an octapole lens.

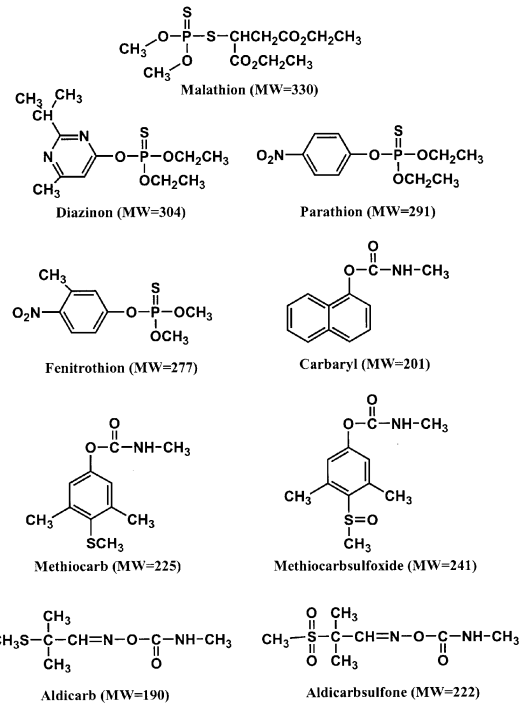

Figure 1. Structures and common names of pesticides considered in this study.

APCI mass spectra of the nine pesticides were obtained by injecting 10 μL of the individual stock solutions or their diluted solutions through an injection valve equipped between the HPLC system and the APCI interface on the mass spectrometer. In this case, HPLC system was used to deliver mobile phase, mixture of methanol and 10-20% (v/v) of aqueous solution, at a flow rate of 0.2 mL/min. The mobile phase contained 0.1% acetic acid. APCI interface and MS conditions were optimized with monitoring ion signals of the individual pesticide solutions.

To analyze the standard solution mixtures, a Waters 2.1 mm $\times$ 25 cm column packed with 5 μm C18 stationary phase was used for the separation of the nine pesticides. 1 μL of each standard solution mixture was loaded to the column by the autosampler. The initial composition of mobile phase was 80/20 (v/v %) methanol/water and was held for 7 min. Then, methanol content was linearly increased to 100 % within 14 min. The mobile phase contained 0.1% acetic acid. The flow rate of the mobile phase was 0.2 mL/min. We note that selected ion monitoring (SIM) mode and full-scan mode provide similar sensitivity due to the inherent mass selection method of the quadrupole ion trap techniques.[26] Thus, the mass spectrometer was operated on full-scan mode (m/z 100-400) during the acquisition of chromatograms.

Results and Discussion

Both carbamate pesticides and organophosphorus pesticides considered in this study provided good APCI ion signals under the conditions mentioned in the previous section. As it is well known, the voltage difference between the tube lens and the skimmer of the APCI interface played an important role in controlling the levels of collisionally induced dissociation (CID) of primary ions originally generated by APCI processes. Thus, the voltage difference was maintained at 20 V throughout the experiment to minimize the levels of CID and maximize the ion extraction efficiency for most of the pesticides. The full scan APCI mass spectra of the individual pesticides were obtained under the same conditions. APCI mass spectra of a few selected pesticides are shown in Figure 2. Listed in Table 1 are the major ions observed and their relative intensities of the nine pesticides considered in this work. Protonated molecules, $[M+H]^+$, and several fragment ions are observed in most of the spectra. In the spectra of malathion, diazinon, carbaryl, methiocarb and methiocarbsulfoxide, $[M+H]^+$ is dominant and a few weak fragment ion peaks are observed. Two peaks at m/z 223 and 255 are dominant in the spectrum of aldicarbsulfone. The peak at m/z 223 corresponds to $[M+H]^+$, but it is interesting to see a strong peak at m/z 255. The peak is assigned as methanol additives of the protonated molecules, $[M+H+CH_3OH]^+$. On the other hand, the spectra of fenitrothion and parathion are dominated by the fragment ions at m/z 124 and 110, respectively. The spectrum of aldicarb shows a strong fragment peak at m/z 116 and the $[M+H]^+$ ion is undetectable. CID studies of $[M+H]^+$ ions using the MS/MS algorithm of the ion trap mass spectrometer ensured that the

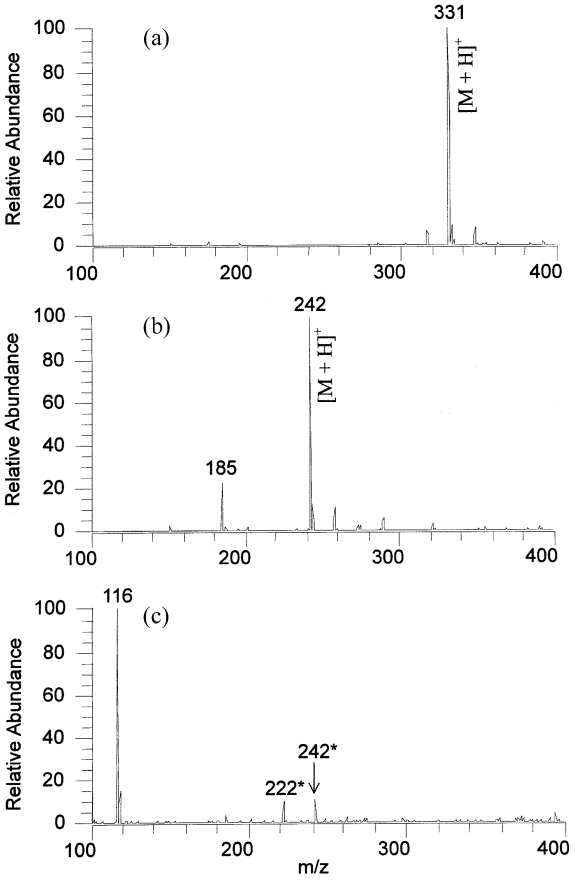

Figure 2. Mass spectra of (a) malathion, (b) methiocarbsulfoxide and (c) aldicarb obtained by APCI/MS. In spectrum (c), the peak a 242 is $[M+H]^+$ of residual methiocarbsulfoxide run right before obtaining this spectrum, and the peak at 222 is from other background residues.

peaks at lower m/z values than $[M+H]^+$ in the corresponding spectrum were from the fragmentation of $[M+H]^+$ ions.[27] The peak assignments listed in Table 1 are based on the CID studies and the characteristic structures of the corresponding molecules.

Figure 3(a) shows the total ion current (TIC) chromatogram obtained by injecting 1 μL of the composite stock solution containing the nine pesticides using the conditions mentioned above. The scanning range of the mass spectrometer was from m/z 100 to m/z 400. The nine compounds are adequately resolved except the slight overlaps between aldicarbsulfone and methiocarbsulfoxide and between malathion and methiocarb. For the quantitative analysis, the selected ion chromatograms representing the individual pesticides were extracted using the data reduction program implemented in the data system of the mass spectrometer. The results are shown in Figure 3(b) ~ (h). Here, the selected ions are the characteristic ions with the highest intensity in the mass spectra of the corresponding compounds. The selected ions for the nine compounds are listed in Table 1. The compounds overlapped in the TIC chromatogram are well resolved in the selected ion chromatograms. Thus, the LC conditions were not further optimized to separate the compounds that are partially overlapped in the TIC chromatogram. It demonstrates that the relatively low resolving power of LC can be overcome by the mass selecting capability of the MS detector.

For the positive identification of a target pesticide from an unknown sample, one needs to detect two or more characteristic ions of the compound and compare their relative intensity in addition to matching of its LC retention time with that of the compound. As shown in Table 1, most of the pesticides studied here, except aldicarb and malathion, have at least two characteristic ions under the APCI ionization conditions used. Figure 4 shows chromatograms of ions at m/z 292, m/z 262, and m/z 110, which are characteristic for parathion. The ion chromatograms were extracted from the LC/MS run of the mixture of nine pesticides at the individual levels of 100 μg/mL. The area ratios and relative peak heights of the three ions agree with the parathion mass spectrum, indicating that a target compound can be identified by the detection of its characteristic ions. We note that all the nine pesticides show higher levels of fragmentation to give more characteristic fragment ions when the voltage difference between the tube lens and the skimmer of the APCI

Table 1. Major ions observed by LC/APCI/MS and its detection limits for the nine pesticides considered in this study

Compounds	M.W.	APCI Ions[a]	Selected Ion[b] (m/z)	Detection Limit[c] (ng)
Aldicarb	190	$[M + H - (O_2CNHCH_3 + H)]^+_{116}$ (100)	116	0.2
Aldicarbsulfone	222	$[M + H + CH3OH]^+_{255}$ (100), $[M + H]^+_{223}$ (90)	255	0.06
Methiocarb	225	$[M + H]^+_{226}$(100), $[M + H - OCNCH_3]^+_{169}$ (10)	226	0.01
Methiocarbsulfoxide	241	$[M + H]^+_{242}$ (100), $[M + H - OCNCH_3]^+_{185}$ (30)	242	0.03
Carbaryl	201	$[M + H]^+_{202}$ (100), $[M + H - OCNCH_3]^+_{145}$ (10)	202	0.02
Diazinon	304	$[M + H]^+_{305}$(100), $[S=P(OC_2H_5)_2]^+_{153}$ (15)	305	0.01
Fenitrothion	277	$[M + H]^+_{278}$ (5), $[M + H - CH_4]^+_{262}$ (10), $[M + H - C_2H_6]^+_{248}$ (5), $[S=P(OCH_3)(OCH_2)]^+_{124}$ (100)	124	0.06
Malathion	330	$[M + H]^+_{331}$(100)	331	0.006
Parathion	291	$[M + H]^+_{292}$ (20), $[M + H - CH_4]^+_{276}$(15), $[M + H - C_2H_6]^+_{262}$ (30), $[H_2(P=S)OC_2H_5]^+_{110}$ (100)	110	0.05

[a] $[M]^+_x$ (y): Subscript x denotes the mass to charge ratio (m/z) of the M^+ ion; value y in the parenthesis denotes the intensity of the ion, normalized with respect to the base peak of each spectrum. The peak assignments are based on the CID studies[27] and the characteristic structures of the individual compounds. [b] Selected ions used for the quantitative analysis. [c] The detection limits for S/N > 3 in the selected ion chromatograms. The uncertainties of the given values are estimated to be 20% of the values.

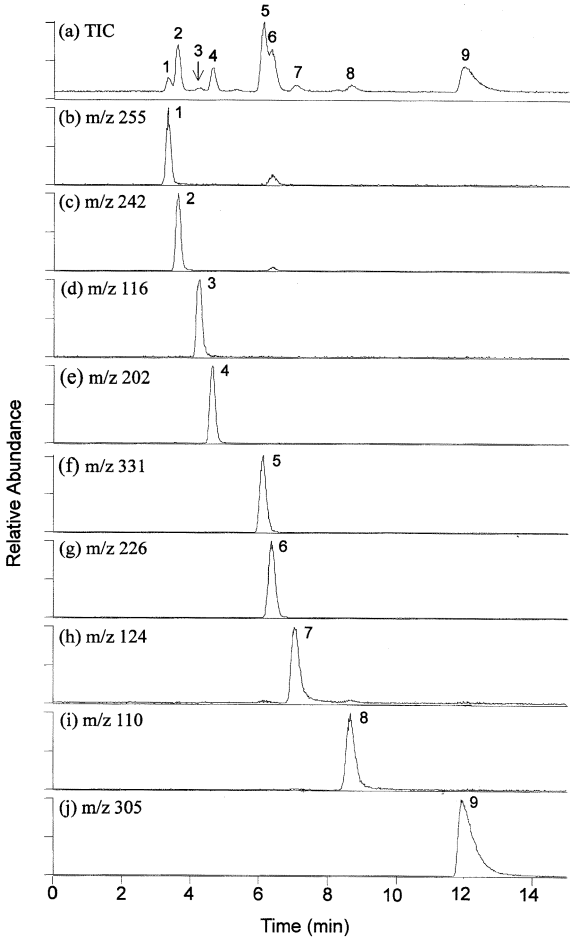

Figure 3. LC/APCI/MS run of a standard solution mixture containing five carbamate pesticides and four organophosphorus pesticides at the individual levels of 100 μg/mL (except carbaryl: 80 μg/mL): 1, aldicarbsulfone; 2, methiocarbsulfoxide; 3, aldicarb; 4 carbaryl; 5, malathion; 6, methiocarb; 7, fenitrothion; 8, parathion 9, diazinon. (a) Total ion current chromatogram. (b)-(h) Selected ion chromatograms of the nine compounds. Waters 2.1 mm i.d.× 25 cm column packed with 5 μm C18 stationary phase was used. The initial composition of mobile phase was 80/20 (v/v %) methanol/water, and was hold for 7 min. Then, methanol content was linearly increased to 100% within 14 min. The mobile phase contained 0.1% acetic acid. The flow rate of the mobile phase was 0.2 mL/min.

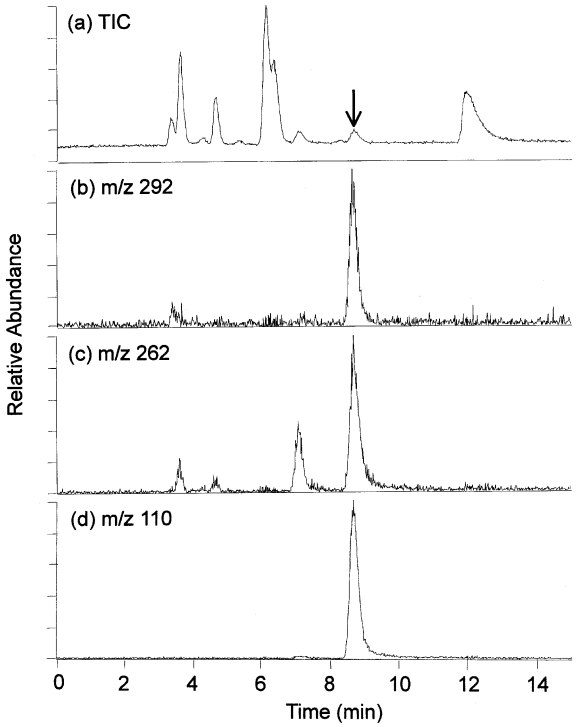

Figure 4. Selected ion chromatograms for parathion, extracted form the LC/MS run of the nine pesticide mixture at the individual levels of 100 μg/mL. (a) Total ion chromatogram. (b), (c) and (d) are ion chromatograms of three characteristic ions for parathion at m/z 292, m/z 262, and m/z 110, respectively.

interface increases. Thus, moderately higher voltage difference is preferable for the identification of target pesticides from an unknown sample.

To test feasibility of the LC/APCI/MS for the routine quantitative analysis of organophosphorus pesticides and carbamate pesticides, the linearity and repeatability of the LC/APCI/MS were examined. Calibration curves shown in Figure 5 were prepared by injecting 1 μL of standard solution mixtures containing 1 to 100 μg/mL of the nine pesticides under the same conditions mentioned above. This concentration range represents the working range of general pesticide analysis.[2,5] The peak areas used in the calibration curves were obtained from the selected ion chromatograms of the corresponding compounds. The peak areas are the mean values of three measurements. The relative standard deviations of the areas from repeated measurements are within 3% for all compounds in the concentration range. The calibration curves given in Figure 5 are obtained from the linear regression of the experimental data. The regression coefficients (r^2) for all nine compounds are better than 0.999. All nine compounds show good linearity in the concentration range. These results indicate that the LC/APCI/MS is a good analytical method for the quantitative analysis of both

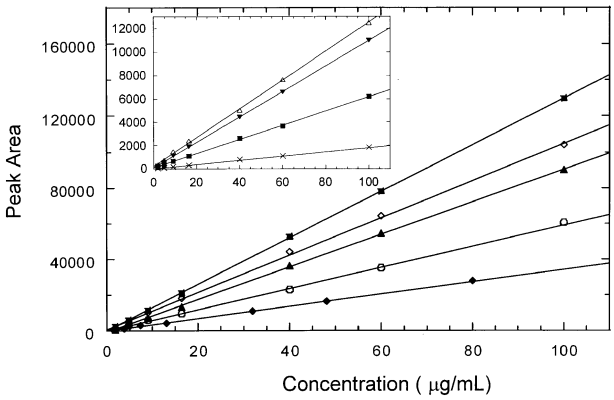

Figure 5. LC/APCI/MS calibration curves for diazinon (■), malathion (◇), methiocarbsulfoxide (▲), methiocarb (○), and carbaryl (◆). Inset shows calibration curves of pesticides with relatively low sensitivities: fenitrothion (△), parathion (▼), aldicarbsulfone (□), and aldicarb (×). The areas are obtained from chromatograms of selected ions for the individual pesticides as listed in Table 1, and are the means of three measurements.

carbamate pesticides and organophosphorus pesticides.

The slopes of the calibration curves for the individual pesticides in Figure 5 reflect the relative LC/APCI/MS sensitivities. LC/APCI/MS sensitivities are in the order of diazinon > malathion > methiocarbsulfoxide > methiocarb > carbaryl > fenitrothion > parathion > aldicarbsulfone > aldicarb. The detection limits for the individual pesticides in the experimental conditions were derived from the signal to noise levels of their selected ion chromatograms at appropriate concentrations. Listed in Table 1 are their estimated detection limits with the signal to noise ratios of 3. The range of detection limits is between 0.006 ng (malathion) and 0.2 ng (aldicarb). Here, 1 ng of a pesticide equals to the solution concentration of 1 μg/mL when 1 μL is injected for analysis. The detection limits of this method for the carbamate pesticides are comparable with those of the LC/fluorescence detection method with post-column derivatization, whose detection limits for these compounds were known to be near 0.1 ng.[2] The detection limits of the LC/APCI/MS system for the organophosphorus pesticides are lower than GC-FID (generally 1 ng), but higher than GC-NPD (~0.001 pg). However, the detection limits of the LC/APCI/MS system for the nine pesticides are much lower than required in general regulatory pesticide analysis in food and environmental material (1 ng or 1 μg/mL for 1 μL injection). Therefore, the LC/APCI/MS is sensitive enough to simultaneously analyze both carbamate pesticides and organophosphorus pesticides in routine single chromatographic analysis.

This study applied LC/APCI/MS to the analysis of solution mixtures containing both carbamate pesticides and organophosphorus pesticides. The application of the LC/APCI/MS system to the analysis of extracts of foodstuffs and environmental samples is currently under investigation. As the APCI processes generate a few characteristic ions, such as protonated molecules and their fragments, the mass spectrometric detection method is expected to provide strong selectivity for target compounds and thus to filter out background interferences originated from the sample matrix. Also, the adoption of MS/MS techniques is expected to provide secondary filtering effects when a target compound overlaps with background interferences both in chromatogram and in mass spectrum. The collisionally induced dissociation pathways of the characteristic ions of the pesticides considered in this study were also investigated to provide information for the application of MS/MS techniques to the identification of detected compounds and their quantification.[27] Details of the CID studies are too extensive and not included in this report.

Conclusion

LC/APCI/MS techniques were evaluated for multi-residue analysis of both carbamate pesticides and organophosphorus pesticides. All of the five carbamate pesticides and the four organophosphorus pesticides considered in this study provided good ion signals with the APCI ionization method. The APCI mass spectra of most of the pesticides show protonated molecules and their characteristic fragment ions. The levels of the fragmentations vary among the pesticides. The calibration curves of the pesticides present good linearity in the general working concentration range of 1-100 μg/mL. The peak areas in selected chromatograms show less than 3% variation from run to run. The detection limits for the nine pesticides are lower than 1 ng. In conclusion, LC/APCI/MS has proved to be a good analytical tool for the routine simultaneous analysis of both carbamate pesticides and organophosphorus pesticides.

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