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Application of liquid chromatography–atmospheric-pressure chemical-ionization mass spectrometry to pesticide analysis

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Abstract

An application of high-performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry for pesticides analysis is discussed. 21 types of organophosphorus and 8 types of N-methylcarbamate pesticides were selected for in this study.

We monitored both positive and negative ions to identify 21 organophosphorus pesticides. However, 8 N-methylcarbamate pesticides were detectable only with the positive ion measurement mode. Although the mass spectra obtained from this method showed a simpler pattern than those obtained with an electron impact method, those mass spectra were distinct enough to identify unknown peaks on the chromatogram. Because of the high specificity of this method, quick analysis could be performed with an extremely simple pre-treatment process. We suggest that this measurement method can be an extremely powerful means of pesticide analysis.

Keywords: Environmental analysis; Pesticides; Organophosphorus compounds; Methylcarbamates

1. Introduction

Environmental pollution is a worldwide problem in modern society. Pesticides, a major type of pollutant, are some of the most causative substances. However, pesticides are used increasingly in households, agriculture and for other areas (e.g. golf courses). Pesticides are indispensable chemicals in modern civilization, but we must not forget that the pesticides may be poisonous chemicals for mankind. Therefore, from the viewpoint of preventive medicine, it is extremely important to analyze the residual pesticides in food, drink or environmental water. Furthermore, it is also important in clinical medicine to analyze the pesticides that have inadvertently entered the human body.

Several hundred kinds of chemical substances are contained in the pesticides. Two groups of pesticides which are frequently used include organophosphorus pesticides (OPs) and carbamate pesticides (CPs). Because these two groups of pesticides inhibit acetyl-cholinesterase (Ac-ChE; E.C. 3.1.1.7) activity in the neurotransmission system, the surplus stimulation symptom is caused by Ac-ChE, and this may shorten life. In clinical laboratory tests, pesticide intoxication has caused a noticeable decrease in serum pseudo-cholinesterase (Ps-ChE; E.C. 3.1.1.8) activity. It has not been determined whether OPs or CPs cause intoxication. Furthermore, atropine sulfate and pralidoxime-iodide (PAM) are administered as a treatment of intoxication by these pesticides. Generally, although serum Ps-ChE activity in the cases of OP intoxication is recovered by administering PAM, Ps-ChE activity in the case of CP intoxication does

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not react to the same treatment. Rather, PAM administration tends to delay and restrict the natural recovery of Ps-ChE activity. For this reason, the establishment of a reference method of multiple pesticides analysis is crucial in clinical medicine as well as in preventive medicine.

Presently, many pesticides are generally analyzed by spectrophotometry, TLC, HPLC, GC or GC–MS. Previous reports have noted that the spectrophotometric method [1–3] and TLC [4] are inferior to the other methods in terms of detection sensitivity and specificity.

HPLC [4–8] is well known as a measurement method that has good operability, because it can be performed at room temperature. However, the separation efficiency of HPLC is inadequate when a complicated matrix like a bio-sample is analyzed. Therefore, the complex clean-up method [9–17] and the derivation reaction of a specimen on pre- or post-column [12,18–21] are often used in HPLC analysis.

Similarly, GC [15,22–31] is an easily operable analytical method. In recent years, the separation ability of this method has been improved remarkably by the spread of the capillary column, and the simultaneous measurement of many types of pesticides has become possible. On the other hand, GC is performed under high column temperatures and is unsuitable for thermally unstable substances [32–34]. Furthermore, the derivation reaction to convert the substance for measurement into a less polar compound is required in the analysis of a highly polar compound [35,36], this procedure requires time consuming and complicated pre-treatment of specimens. Moreover, the result is often controlled by the technical skill of the researcher.

There are also many previous reports regarding applications for GC–MS analysis of pesticides which is recognized as an analytical method that has an excellent specificity and sensitivity [24,35,37–43]. However, this method uses GC for sample separation procedure and hence involves the same problems encountered with GC analysis.

LC–MS is a comparatively new analytical method that was developed to improve the above problems of HPLC, GC and GC–MS. It is recognized as an ideal analytical method, which combines HPLC, which has great utility in the measurement of thermally unstable, highly polar or non-volatile substances, with MS that has excellent sensitivity and specificity. In this method, various types of ionization interface such as moving belt [44,45], silicone membrane separator [46], direct liquid introduction (DLI) [47,48], the heated wire [49], TSP [50–52] and API [53–58] were utilized to introduce the effluent from LC into MS. The applications of these interfaces for pesticides analysis have been previously reported [59–70]. Recently, even the applicability of super critical fluid chromatography (SCF)–MS [71] and tandem MS (MS–MS) [72] that were developed from LC–MS has been reported.

From this background, in this paper we describe an application of LC–MS using API interface adopted in our laboratory for OPs and CPs that affect human health.

2. Experimental

2.1. Pesticides

We determined the causative chemicals in pesticide intoxication patients in our laboratory. 21 OPs and eight N-methyl-CPs (N-MCPs), each having had an annual usage, in Saga Prefecture, Kyushyu, Japan, of over 100 tons or $100 \cdot 10^3$ l in 1994 were selected as the measurement objects (Tables 1 and 2). All these pesticides were purchased from GL Science (Tokyo, Japan). These pesticides were dissolved in CH_3CN, and were used in standard solutions.

2.2. Reagents

Distilled water was purified with a Milli-Q II system (Millipore, Bedford, MA, USA). HPLC-grade CH_3OH, CH_3CN and dichloromethane, and analytical-grade NH_4OAc was purchased from E. Merck (Darmstadt, Germany) and Wako (Osaka, Japan), respectively.

2.3. Instrumentation and chromatographic conditions

The HPLC apparatus consisted of an intelligent pump (Model L-6200; Hitachi, Tokyo, Japan), a sample injector (Model 7125, equipped with 200 μl

Table 1
List of organophosphorus pesticides examined

Compound	Formula	R	R′	M_r
Phosphates	$(RO)_2PO_2R'$			
Dichlorvos		CH_3	$CH{=}CCl_2$	221.0
Dimethylvinphos		CH_3	$C(C_6H_3Cl_2){=}CHCl$	331.5
Propaphos		C_3H_7	$C_6H_4SCH_3$	304.4
Phosphorothionates	$(RO)_2PSOR'$			
Chlorpyrifos		C_2H_5	C_5HNCl_3	350.6
Chlorpyrifos-methyl		CH_3	C_5HNCl_3	322.5
Diazinon		C_2H_5	$C_4HN_2(CH_3)CH(CH_3)_2$	304.4
EPN		C_2H_5,C_6H_5	$C_6H_4NO_2$	323.3
Fenitrothion		CH_3	$C_6H_3(CH_3)NO_2$	277.2
Fenthion		CH_3	$C_6H_3(CH_3)SCH_3$	279.3
Isoxathion		C_2H_5	$C_3HNOC_6H_5$	313.3
Parathion		C_2H_5	$C_6H_4NO_2$	291.3
Parathion-methyl		CH_3	$C_6H_4NO_2$	263.2
Pyridaphenthion		C_2H_5	$C_4H_2N_2(C_6H_5){=}O$	340.3
Phosphorothioates	$(RO)_2POSR'$			
Edifenphos		C_2H_5,C_6H_5S	C_6H_5	310.4
Iprofenfos		$(CH_3)_2CH$	$CH_2C_6H_5$	288.4
Phosphorodithioates	$(RO)_2PS_2R'$			
Dimethoate		CH_3	$CH_2CONHCH_3$	229.3
Disulfoton		C_2H_5	$(CH_2)_2SC_2H_5$	274.4
Ethion		C_2H_5	$CH_2S_2P(OC_2H_5)_2$	384.5
Malathion		CH_3	$CH(CH_2CO_2C_2H_5)CO_2C_2H_5$	330.4
Methidathion		CH_3	$CH_2C_2N_2S(OCH_3){=}O$	302.3
Phenthoate		CH_3	$CH(C_6H_5)CO_2C_2H_5$	320.4

sample loop; Rheodyne, Cotati, CA, USA), a column oven (Model 655A-52; Hitachi), and an ultraviolet spectrophotometer (Model L-4000; Hitachi). A Waters Nova-Pak C_{18} (15 cm×3.9 mm I.D., 4 μm average particle size; Millipore) was used as an analytical column. This HPLC setup was connected to the nebulizer unit in the APCI interface by a PTFE tube (1 m×1.6 mm O.D×0.25 mm I.D.), and it was also connected to a double-focusing mass spectrometer (Model M-2000; Hitachi) (Fig. 1).

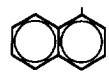
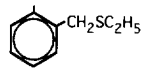
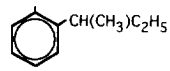
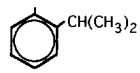
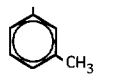
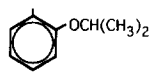
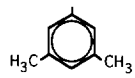
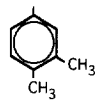
The measurement conditions of the HPLC, APCI interface and MS are shown in Table 3.

For GC–MS analysis as a control experiment, the GC apparatus (Model G-3000; Hitachi) equipped with a splitless sample injector was connected to the MS mentioned above. Ultra-2 capillary column (crosslinked 5% phenyl methyl silicon gum, 25 m× 0.2 mm I.D., 0.33 μm film thickness; Hewlett-Packard, Avondale, PA, USA) was used as an analytical column, and helium was used as the carrier gas at the back-pressure of 25 cm/s. The GC temperature program was set such that after being kept at 60°C for 2 min, the temperature of the column was increased to 250°C at a rate of 4°C/min. The temperatures of the sample injector and the separator (transfer line) were 210°C and 230°C, respectively. MS was operated under the conditions of 180°C of ion-source temperature, 200 μm of ion-source slit width, 150 μm of collector slit width, 70 eV of ionization voltage, 4 kV of acceleration voltage and 1.2 kV of secondary electronic step-up tube electrical potential supply. Isobutane was used as a reaction gas for CI measurement.

2.4. Sample pre-treatment

In the residual pesticide analysis, different pretreatment procedures for the samples must be used

Table 2
List of carbamate pesticides examined

Compound	Formula	R	M_r
N-*methylcarbamates*	R-OCONHCH$_3$		
Carbaryl			201.2
Ethiofencarb			225.3
Fenobucarb			207.3
Isoprocarb			193.3
Metolcarb			165.2
Propoxur			209.3
XMC			179.2
Xylilcarb			179.2

according to the type of specimen or the detection method. However, the sequential process which is used for the extraction and concentration of pesticide from the specimen is indispensable even if different types of matrixes, pesticides and measurement methods are adopted.

It is well known that OPs and N-MCPs disintegrate easily in water, acid or alkaline solutions, or ultraviolet rays [66,73,74]. Therefore, storage of the specimen must be avoided if possible and using a fresh specimen is desirable for accurate measurement. There are many reviews and previous papers regarding the pre-treatment method of a specimen, so we have summarised the description about the sample pre-treatment method.

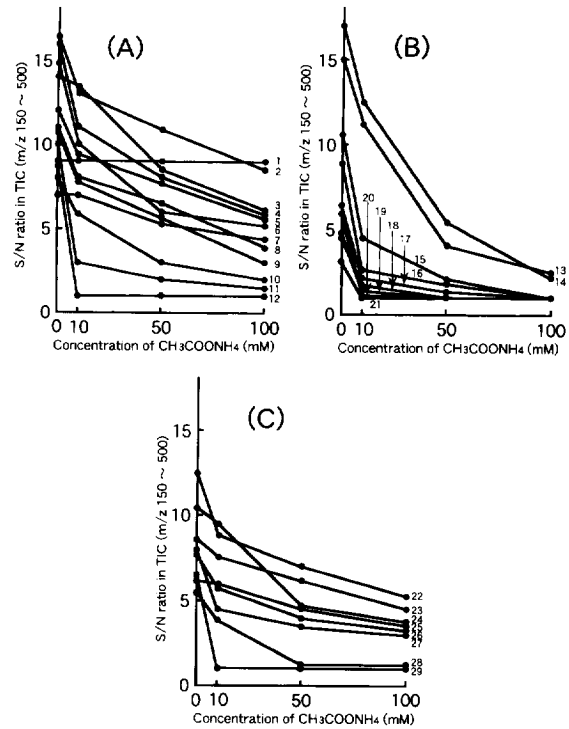


Fig. 1. Effects of ammonium acetate concentration in the mobile phase on determination sensitivity with total ion current monitoring. 1 μg of each pesticide was injected into the flow injection–APCI-MS system. 50% solution of methanol in 0, 10, 50 and 100 mM ammonium acetate buffer (pH=7.0) were used as the mobile phase. Other measurement conditions were the same as those shown in Table 3. (A) Positive-ion measurement mode in organophosphorus pesticides; (B) Negative-ion measurement mode in organophosphorus pesticides; (C) Positive-ion measurement mode in N-methyl carbamate pesticides. 1=diazinon; 2=dimethoate; 3=propaphos; 4=pyridaphenthion; 5=ediphenphos; 6=dimethylvinphos; 7=iprofenfos; 8=malathion; 9=isoxathion; 10=phenthoate; 11=dichlorvos; 12=fenthion; 13=parathion-methyl; 14=fenitrothion; 15=parathion; 16=methidathion; 17=EPN; 18=disulfoton; 19=ethion; 20=chlorpyrifos-methyl; 21=chlorpyrifos; 22=fenobucarb; 23=isoprocarb; 24=XMC; 25=metolcarb; 26=xylylcarb; 27=ethiofencarb; 28=carbaryl; 29=propoxur.

Biological fluids (e.g., serum or urine) from humans are used as measurement specimens, and the pre-treatment method was shown in Table 3 as an example. Like the figure, a simple clean-up method by the disposable solid-phase extraction cartridge was adopted. MS as a detection means has excellent specificity, and the recovery was more than 95% (data was abbreviated).

Recently, various kinds of solid-phase extraction

Table 3
Measurement conditions of organophosphorus and N-methylcarbamate pesticides examined

HPLC	
Column	Nova-Pak C_{18} (15 cm×3.9 mm I.D., 4 μm)
Elution	Linear gradient; CH_3OH in H_2O 35% (5 min) $\overset{25\ min}{\rightarrow}$ 55% (10 min)
Flow-rate	1.0 ml/min
UV	210 nm
APCI interface	
Vaporizer temperature	280°C
Nebulizer temperature	400°C
Ionization needle electrode flow	5 μA
Drift voltage	240 V
MS	
Vacuum pressure	1×10^{-4} Pa
Ion-source slit width	500 μm
Collector slit width	200 μm
Secondary electronic step-up tube electrical potential supply	1.2 kV
Scanned mass range	0~500
Scan intervals	8 s

cartridge columns such as Bond-Elut (Varian, Harbor City, CA, USA), Sep-Pak (Millipore), Excelpak (Hewlett-Packard) and LiChrolut (E. Merck) have been marketed.

3. Result and discussion

3.1. Selection of ionization mode (positive or negative ions) for measurement

After analyzing 1.0 μg of 21 OP standard solutions by LC–APCI-MS, the pesticides were classified into three groups that were detectable by only positive-ion measurement (group I), by only negative-ion measurement (group II) and by both positive- and negative-ion measurement (group III) (Table 4). In these three groups and four basic structures of OPs (phosphates, phosphorothionates, phosphorothioates or phosphorodithioates), no fixed relationship was recognized. However, the pesticides which contain a nitrogroup within the molecule (EPN, fenitrotion, parathion and parathion-methyl) were detected only with the negative-ion measurement mode. In group III, methidation was determined in the negative-ion mode whereas the other five pesticides were detected with high sensitivity in the positive-ion mode.

Table 4
Classification of organophosphorus and N-methyl carbamate pesticides by the detectable ionization mode in LC–APCI-MS

Group I	Group II	Group III
Phosphates		
Dichrorvos		
Propaphos		Dimethylvinphos (1)
Phosphorothionates		
Diazinon	Chlorpyrifos	Fenthion (1)
Isoxathion	Chlorpyrifos-methyl	Pyridaphenthion (1)
	EPN	
	Fenitrothion	
	Parathion	
	Parathion-methyl	
Phosphorothioates		
Iprofenfos		Edifenphos (1)
Phosphorodithioates		
Dimethoate	Disulfoton	Methidathion (2)
Malathion	Ethion	Phenthoate (1)
N-Methylcarbamates		
Carbaryl		
Ethiofencarb		
Fenobucarb		
Isoprocarb		
Metolcarb		
Propoxur		
XMC		
Xylylcarb		

1 μg of each pesticide was injected into the flow injection–APCI-MS system. Methanol–water (50:50, v/v) was used as a mobile phase solvent. Other measurement conditions were the same as those shown in Table 3. Group I = Pesticides which were detectable only with the positive-ion measurement mode; Group II = Pesticides which were detectable only with the negative-ion measurement mode; Group III = Pesticides which were detectable with both the positive- and negative-ion measurement mode. Numbers 1 and 2 indicate the pesticides which were detected with high sensitivity in positive- and negative-ion mode, respectively.

Furthermore, 8 N-MCPs were detected only by positive-ion measurement mode (Table 4).

In LC–API-MS determination, the production of positive or negative ions depends considerably on the acidity of the chemical in question. When the PA value of the LC mobile phase molecule is smaller than the PA value of the solute molecule, the LC mobile phase molecule acts as the Brönsted acid [75–79]. As a result, the positive-ion $[(M+H)^+]$ is

produced from the solute molecule. On the contrary, when the PA value of the LC mobile phase molecule is bigger than the PA value of the solute molecule, the LC mobile phase molecule acts as the Brönsted base, so the negative-ion $[(M-H)^-]$ is produced [80,81].

In this way, the selection of ionization mode in LC–APCI-MS measurement is decided by proton or hydride affinity of the solute and solvent. If the other solvent which includes acetonitrile is used as a LC mobile phase in this test, the classification in Table 3 might be changed. However, the chemicals which contain a nitro-group within the molecule act as a quasi-acid by electron attachment, so the change of ionization mode may have occurred by the little changes of mobile phase solvent.

3.2. Selection of LC mobile phase

In the reversed-phase LC, pH adjustment and the addition of a salt to the mobile phase solvents are often applied with the aim of enhancing the separation efficiency. However, as the LC effluent is introduced to the nebulizer via a capillary tube at high temperature, non-volatile salts cannot be used in LC–API-MS. Although some volatile buffers can be used as the mobile phase in LC–MS, acetic acid–ammonium acetate buffer is most often used because it is easily available, and its usefulness is well recognized [82]. Furthermore, Voyksner used five kinds of volatile salt [$(C_2H_5)_3N$, $(NH_4)_2CO_3$, $HCOONH_4$, NH_4HCO_3 and CH_3COONH_4] in OP measurement using LC–TSP-MS and examined the effect on the determination sensitivity of adding each salt to the mobile phase so the highest sensitivity was provided in addition to NH_4OAc [83].

We also examined the effect on the determination sensitivity of adding NH_4OAc to the mobile phase in OP and N-MCP measurement using the LC–APCI-MS system. The final concentrations of 0, 10, 50 and 100 m*M* of [acetic acid–NH_4OAc buffer (pH= 7.0)]–CH_3OH (1:1, v/v) were used as the mobile phase, and the signal-to-noise ratio (*S/N*) in TIC was measured when 1.0 μg of each pesticide standard solution was injected into this system (Fig. 1). It can be seen from the figure that NH_4OAc did not affect the determination sensitivity for diazinon. But for the other 20 OPs and eight N-MCPs marked decreases in detection sensitivity occurred as an increase of NH_4OAc concentration.

The protonated quasi-molecular ion $[(M+H)^+]$ was observed with high intensity when the mobile phase without NH_4OAc was used. However, the intensity of ammonia transferred quasi-molecular ion $[(M+NH_4)^+]$ increased in almost all pesticides as the NH_4OAc concentration increased. These findings are very useful to estimate the molecular mass of unknown peaks.

We did not examine the effect on the determination sensitivity of adding any salt to the mobile phase for the other volatile buffer. But the above findings suggest that the addition of NH_4OAc to the mobile phase is undesirable in OPs or N-MCPs analysis using APCI-MS.

3.3. Mass spectra

3.3.1. Positive-ion measurement mode

When methanol in water was used as a LC mobile phase solvent, the cluster ions derived from methanol [*m/z* 33 $(CH_3OH+H)^+$, *m/z* 65 $(2CH_3OH+H)^+$, *m/z* 97 $(3CH_3OH+H)^+$, *m/z* 129 $(4CH_3OH+H)^+$, *m/z* 161 $(5CH_3OH+H)^+$, *m/z* 193 $(6CH_3OH+H)^+$, etc.] were observed as a back-ground mass spectrum in positive-ion measurement APCI. Therefore, the mass spectral pattern in which the ions derived from the solute were piled up on the cluster ions derived from the LC mobile phase was obtained.

Acetonitrile is a solvent which is frequently used in reversed-phase LC. However, the mass number of the cluster ions derived from acetonitrile was larger than those derived from methanol because of its large molecular mass. This might cause the analysis of the mass spectral pattern to be difficult in the measurement of OPs or N-MCPs whose molecular mass is comparatively small.

The mass spectra by EI, gas-phase CI or APCI of propaphos, isoxathion, iprofenfos, malathion and ethiofencarb which represented for four groups in OPs (phosphates, phosphorothionates, phosphorothioates and phosphorodithioates) and N-MCPs, respectively, were compared. (Figs 2–11, respectively).

EI has also the identification ability for a known pesticide. However, sometimes the mass spectra obtained by EI show a pattern indicating that the

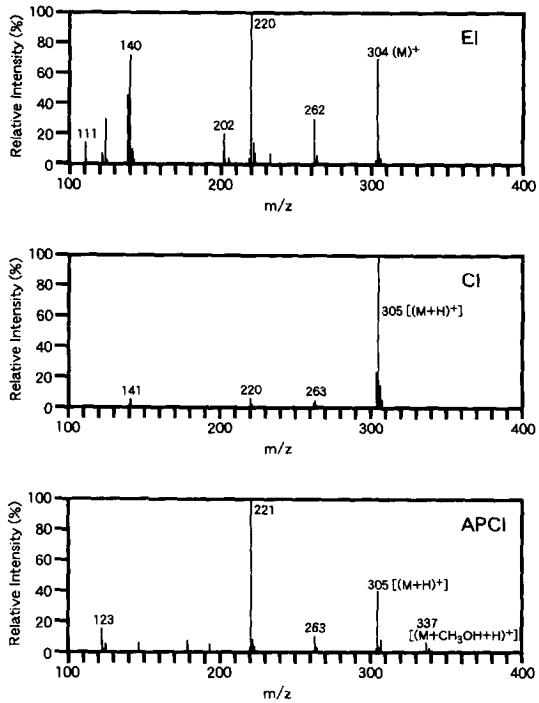


Fig. 2. Comparison of mass spectra for propaphos using different ionization methods.

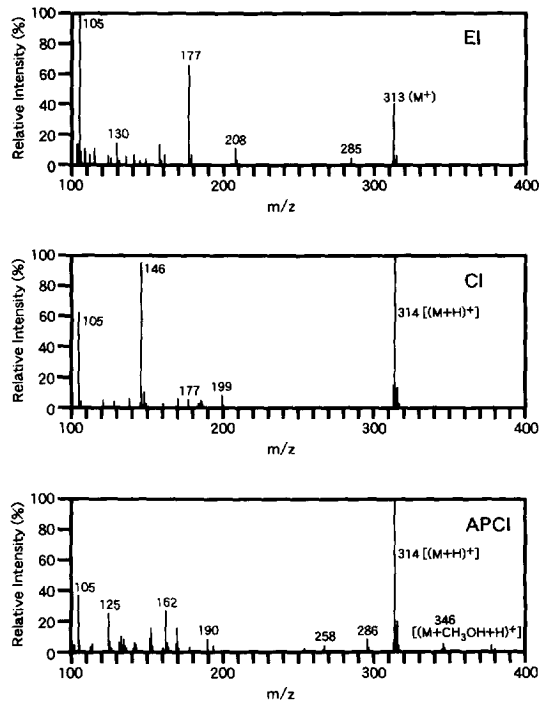


Fig. 4. Comparison of mass spectra for isoxathion using different ionization methods.

intensity of the molecule is extremely low (e.g. malathion and ethiofencarb; Figs. 8 and 10, respectively) or cannot be observed at all. As a result, confirmation of the molecular mass of the unknown sample may be difficult. On the other hand, many fragment ions are produced by EI, so it is well recognized that these fragment ions are extremely effective in the identification of unknown peaks. However, the mass spectrum shows a complex pattern from these many fragment ions. Moreover,

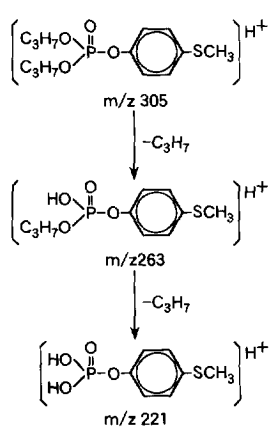


Fig. 3. Proposed fragmentation scheme of the protonated quasi-molecular ion at m/z 305 for propaphos using positive-APCI.

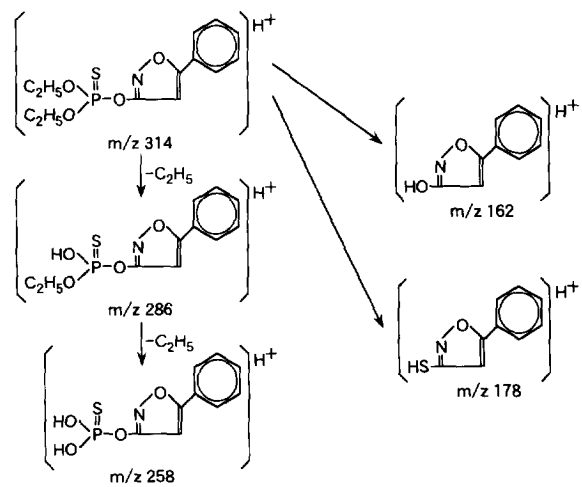


Fig. 5. Proposed fragmentation scheme of the protonated quasi-molecular ion at m/z 314 for isoxathion using positive-APCI.

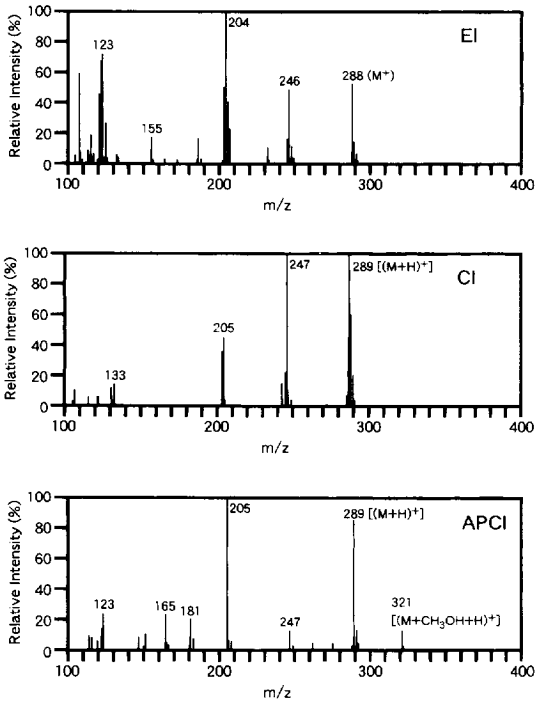


Fig. 6. Comparison of mass spectra for iprofenfos using different ionization methods.

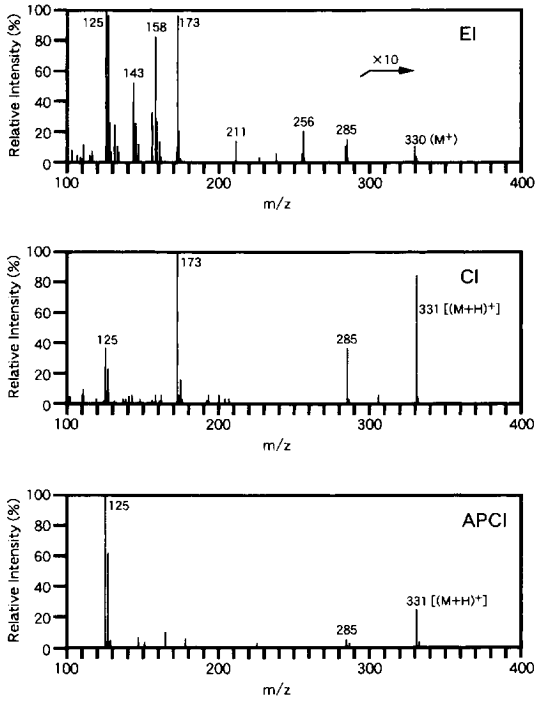


Fig. 8. Comparison of mass spectra for malathion using different ionization methods.

the metastasis during fragmentation may make it difficult to analyze the mass spectrum pattern.

In contrast, a protonated quasi-molecular ion was produced with high intensity in CI, and the fragment ions were few and of low intensity (e.g., propaphos;

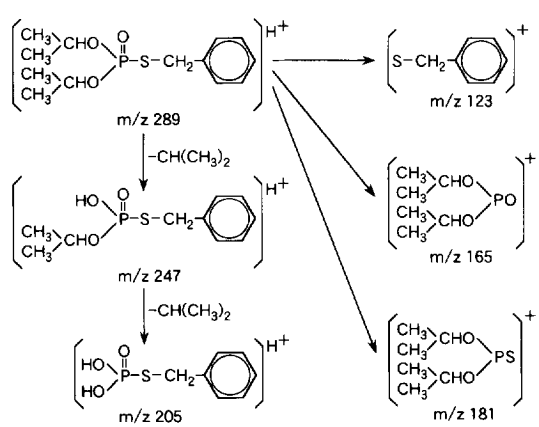


Fig. 7. Proposed fragmentation scheme of the protonated quasi-molecular ion at m/z 289 for iprofenfos using positive-APCI.

Fig. 2). This method is extremely effective for confirmation of the molecular mass of an unknown chemical, but it is ineffective for identification.

In APCI mass spectra, protonated quasi-molecular ions of high intensity were registered. These mass spectra patterns involved cluster ions derived from the LC mobile phase and one or two methanol-added quasi-molecular ions $[(M+nCH_3OH+H)^+]$. These ions made it possible to estimate the molecular mass of unknown peaks. The mass spectra obtained with this method involved a few fragment ions with

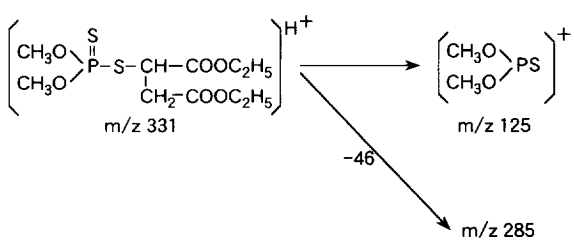


Fig. 9. Proposed fragmentation scheme of the protonated quasi-molecular ion at m/z 331 for malathion using positive-APCI.

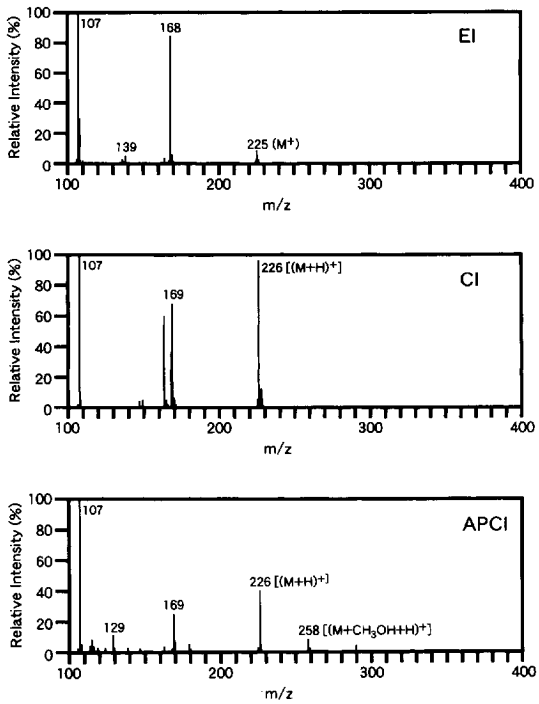


Fig. 10. Comparison of mass spectra for ethiofencarb using different ionization methods.

comparatively high intensity. These findings indicate that the APCI mass spectrum shows a simple pattern, but it has the ability to identify unknown chemicals.

3.3.2. Negative-APCI measurement mode

The cluster ions derived from the LC mobile phase solvent were scarcely observed in negative-ion measurement APCI even if methanol–water was used as a mobile phase. It is well known that an M^- or

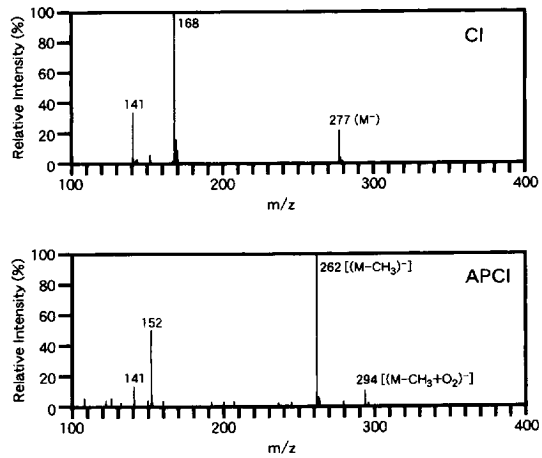


Fig. 12. Comparison of mass spectra for fenitrothion using different ionization methods.

$(M-H)^-$ ion of high intensity is generally produced in negative-ion measurement CI [84]. For nine OPs whose negative-ions were determined in this study, none produced any M^- or $(M-H)^-$ quasi-molecular ion. The seven OPs that are disulfton, ethion, EPN, fenitrothion, methidathion, parathion, parathion-methyl showed monodealkylation replaced by an ether bond (Figs. 12 and 13). Farran et al. reported to be similar in LC–TSP-MS determination [68]. For chlorpyrifos and chlorpyrifos-methyl which were interhalogen compounds, the ion with a chlorine atom came off from a molecule, and an oxygen atom was added, was registered (Figs. 14 and 15). Furthermore, the fragment ions which were the same as by gas-phase CI were registered in the mass spectra of these nine OPs.

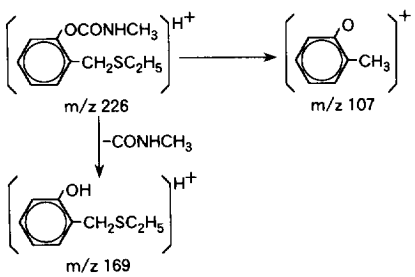


Fig. 11. Proposed fragmentation scheme of the protonated quasi-molecular ion at m/z 226 for ethiofencarb using positive-APCI.

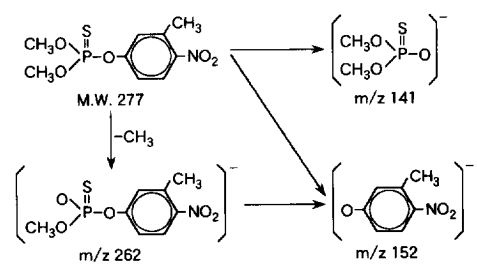


Fig. 13. Proposed fragmentation scheme for fenitrothion using negative APCI.

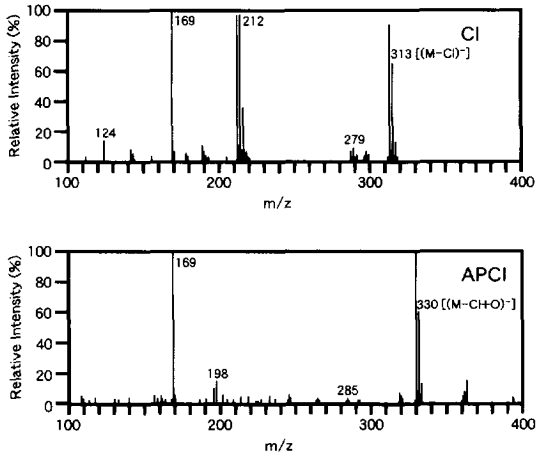


Fig. 14. Comparison of mass spectra for chlorpyrifos using different ionization methods.

3.4. Mass chromatogram

Under the same measurement conditions as shown in Table 3, the mass-fragmentgrams obtained by injecting 500 ng each of the pesticide standard solutions are presented in Fig. 16. Separation of the mixture was incomplete under these LC conditions. However, the separation ability that is equal in HPLC or GC analysis was not required, because the characteristic ions of a particular pesticide were monitored in this detection method.

For both diazinon and propaphos in positive-ion measurement (Fig. 16A), the protonated quasi-molecular ion (*m/z* 305) was monitored. But these two pesticides had different retention times and different

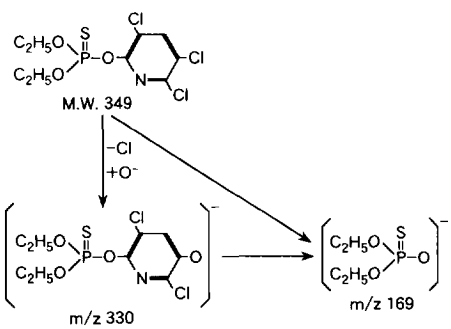


Fig. 15. Proposed fragmentation scheme for chlorpyrifos using negative APCI.

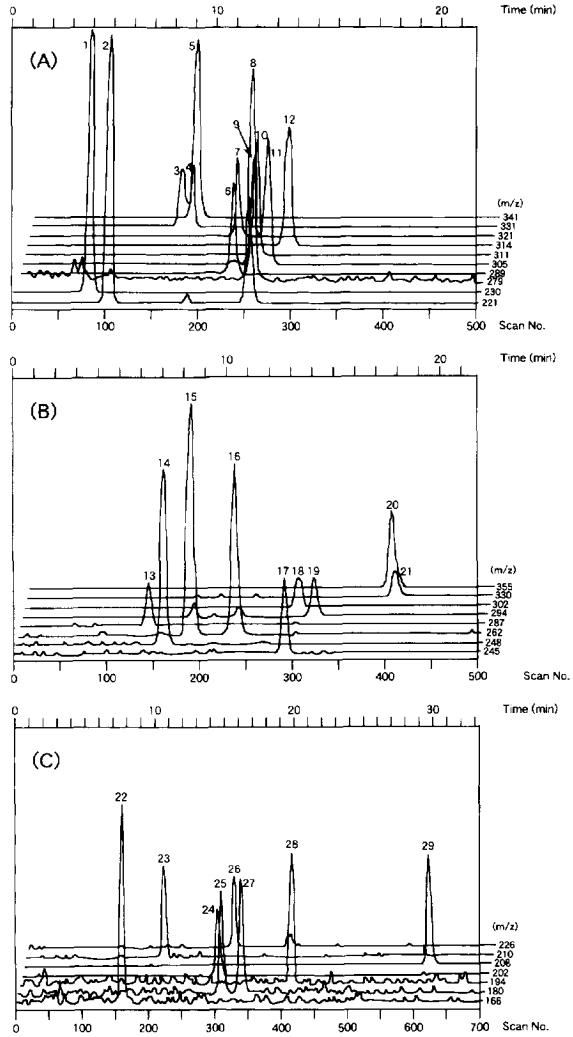


Fig. 16. Mass chromatograms. (A) Organophosphorus pesticides (positive-ion measurement mode); (B) Organophosphorus pesticides (negative-ion measurement mode); (C) N-methyl carbamate pesticides (positive-ion measurement mode). LC, APCI interface and MS conditions as in Table 3. Peaks: 1 = dimethoate (*m/z* 230); 2 = dichlorvos (*m/z* 221); 3 = malathion (*m/z* 331); 4 = dimethylvinphos (*m/z* 331); 5 = pyridaphenthion (*m/z* 341); 6 = iprofenfos (*m/z* 289); 7 = phenthoate (*m/z* 321); 8 = fenthion (*m/z* 279); 9 = propaphos (*m/z* 305); 10 = ediphenphos (*m/z* 311); 11 = diazinon (*m/z* 305); 12 = isoxathion (*m/z* 314); 13 = methidathion (*m/z* 287); 14 = parathionmethyl (*m/z* 248); 15 = fenitrothion (*m/z* 262); 16 = parathion (*m/z* 262); 17 = disulfoton (*m/z* 245); 18 = chlorpyrifos-methyl (*m/z* 302); 19 = EPN (*m/z* 294); 20 = ethion (*m/z* 355); 21 = chlorpyrifos (*m/z* 330); 22 = metolcarb (*m/z* 166); 23 = propoxur (*m/z* 210); 24 = carbaryl (*m/z* 202); 25 = xylylcarb (*m/z* 180); 26 = ethiofencarb (*m/z* 226); 27 = XMC (*m/z* 180); 28 = isoprocarb (*m/z* 194); 29 = fenobucarb (*m/z* 208).

mass spectrum patterns [69]. Therefore, these two pesticides could be distinguished easily.

Even negative-ion measurement of OPs (Fig. 16B), fenitrothion and parathion were monitored with the same ion mass number, monodealkylation ion at m/z 262. Similarly, these two pesticides have different retention times and different mass spectrum patterns, so they could be distinguished [69].

Moreover, in the mass-fragmentgram of N-MCP determination (Fig. 16C), numerous cluster ions derived from methanol and/or water in the LC mobile phase solvent caused an unstable base line with an ion mass of less than 200. This base line fluctuation might be avoided if the drift voltage was increased. However, in this case, the ions derived from the solute may be fragmented. As a result, the intensity of the protonated quasi-molecular ion may also decrease.

3.5. Specificity of LC–APCI-MS

Propoxur is a popular pesticide used as a cockroach insecticide in Japanese homes. To confirm the specificity of LC–APCI-MS, we performed the following test. After adding propoxur to drug-free serum (Bio-Rad Labs Richmond, CA, USA) to produce a final concentration of 0.2 μg/ml, propoxur was extracted with dichloromethane and concentrated ten-fold. Then we analyzed propoxur to compare the efficiency of the three methods for qualitative or quantitative analysis— HPLC–UV, GC–EI-MS, and LC–APCI-MS (Figs. 17–19, respectively).

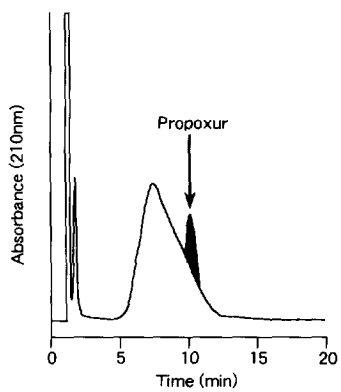


Fig. 17. Determination of propoxur in the spiked serum using HPLC–UV. Propoxur was added to the drug-free serum to produce a final concentration of 0.2 μg/ml, and the serum was used as a test sample. Propoxur was extracted with dichloromethane and concentrated by 10-fold. A 100 μl of this sample was injected. Propoxur peak rested upon the shoulder of a large peak thought to derive from a substance in the serum. LC conditions: column = Nova-Pak C_{18} (15 cm×3.9 mm I.D., 4 μm average particle size); mobile phase = methanol–water (4:6, v/v); flow-rate = 1.0 ml/min (45°C); monitoring wave range = 210 nm.

HPLC–UV produced the chromatogram with a propoxur peak on the shoulder of a large peak thought to derive from a substance in the serum (Fig. 17). This finding indicates that this method has problems with specificity and sensitivity.

On the other hand, GC–EI-MS is well recognized as an analytical method that has an excellent specificity and sensitivity. The low intensity of the molecular ion in the EI mass spectrum of propoxur made fragment-ion monitoring essential for quantitative analysis. For these reasons, simultaneous analysis of multiple chemicals with similar molecular structures (e.g., N-MCPs) using GC–EI-MS will cause a low specificity (Fig. 18). An approach such as gas-phase CI must be used in combination with GC–EI-MS in order to determine the molecular mass of the obtained peak.

In the mass spectrum of propoxur obtained by LC–APCI-MS, the high intensity of protonated quasi-molecular ion made it possible to achieve a high specificity (Fig. 19). This confirms that LC–APCI-MS is a very useful means to simultaneously analyze multiple chemicals with similar molecular structures.

3.6. Detection limits

Using LC–APCI-MS under the SIM mode, the detection limits of 21 OPs and eight N-MCPs that are utilized as the measurement object in our laboratory were shown in Tables 5 and 6, respectively. It is generally known that low pg analysis can be achieved by GC–MS under the SIM mode [37–43]. Comparison of the detection limits given by GC–MS showed the values were inferior. However, we believe that if the production of cluster ions derived from the LC mobile phase can be inhibited by any means, more sensitive analysis will be possible.

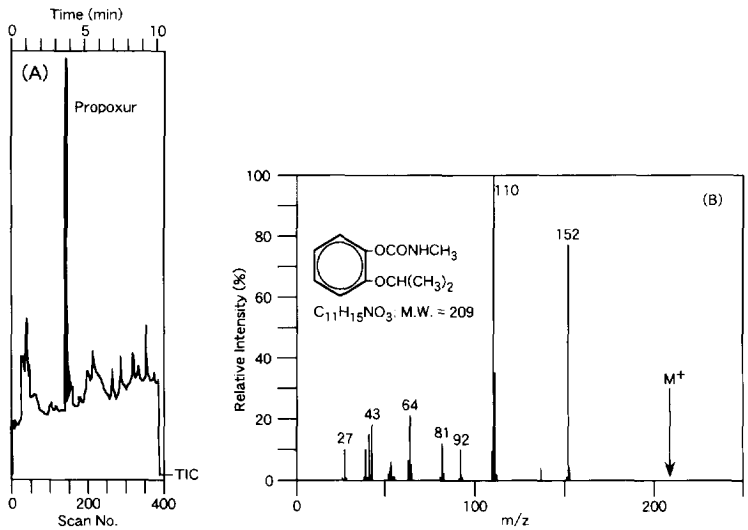


Fig. 18. Determination of propoxur in the spiked serum using GC–EI-MS. Propoxur was added to the drug-free serum to produce a final concentration of 0.2 μg/ml, and the serum was used as a test sample. Propoxur was extracted with dichloromethane and concentrated by 10-fold. A 1.0 μl volume of this sample was injected. In EI mass spectra, the molecular ion indicated very low intensity. Therefore, an approach such as chemical ionization must be used to determine the molecular mass of the obtained peak. (A) total ion current mass chromatogram, (B) EI mass spectrum. GC conditions: column = Ultra-2 capillary column (crosslinked 5% phenyl methyl silicon gum, 25 m × 0.2 mm I.D., 0.33 μm film thickness). After being kept at 60°C for 2 min, the temperature of the column was increased to 160°C at rate of 4°C/min. Injector temperature = 200°C; separator temperature = 220°C. MS conditions: ion-source temperature = 180°C; ion-source slit width = 200 μm; collector slit width = 150 μm; ionization potential = 70 eV; acceleration electrical potential = 4 kV; secondary electronic step-up tube electrical potential supply = 1.2 kV.

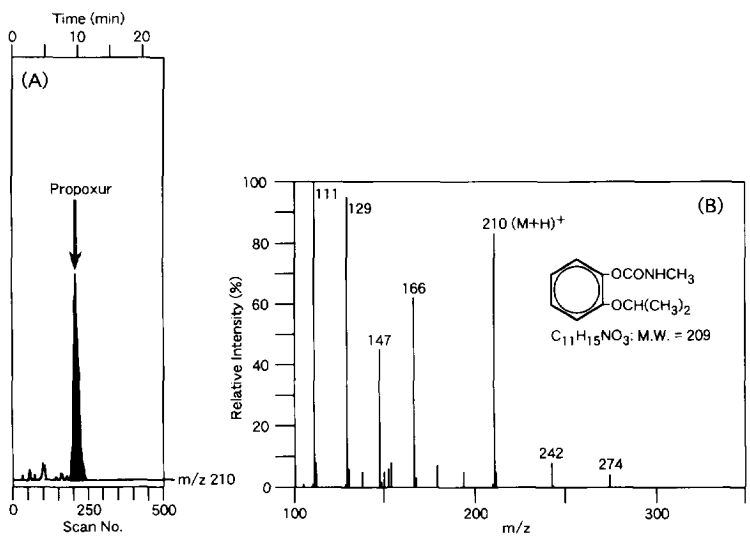


Fig. 19. Determination of propoxur in the spiked serum using LC–APCI-MS. Propoxur was added to the drug-free serum to produce a final concentration of 0.2 μg/ml, and the serum was used as a test sample. Propoxur was extracted with dichloromethane and concentrated by 10-fold. A 100 μl volume of this sample was injected. The protonated quasi-molecular ion $[(M+H)^+]$ of high intensity was registered. Therefore, highly specific analysis can be performed by monitoring this ion. (A) mass-fragmentgram during $(M+H)^+$ quasi-molecular ion (m/z 210) monitoring, (B) APCI mass spectrum. LC conditions were the same as those in Fig. 20, and APCI interface and MS conditions were the same as shown in Table 3.

Table 5
Detection limits of organophosphorus pesticides

Measurement mode	Pesticide	Monitoring ion m/z	Detection limit (ng)
Positive-ion	Dichlorvos	$221(M+H)^+$	2
	Dimethylvinphos	$331(M+H)^+$	5
	Propaphos	$305(M+H)^+$	5
	Diazinon	$305(M+H)^+$	5
	Fenthion	$279(M+H)^+$	20
	Isoxathion	$314(M+H)^+$	5
	Pyridaphenthion	$341(M+H)^+$	5
	Iprofenfos	$289(M+H)^+$	5
	Dimethoate	$230(M+H)^+$	2
	Malathion	$331(M+H)^+$	5
	Phenthoate	$321(M+H)^+$	5
Negative-ion	Chlorpyrifos	$330\ (M-Cl+O)^-$	50
	Chlorpyritos-methyl	$302\ (M-Cl+O)^-$	50
	EPN	$294\ (M-C_2H_5)^-$	50
	Fenitrothion	$262\ (M-CH_3)^-$	2
	Parathion	$262\ (M-C_2H_5)^-$	2
	Parathion-methyl	$248\ (M-CH_3)^-$	2
	Disulfoton	$245\ (M-C_2H_5)^-$	20
	Ethion	$355\ (M-C_2H_5)^-$	10
	Methidathion	$287\ (M-CH_3)^-$	10

Using 500 ng of each of the standard solutions, the detection limits during mass fragmentgraphy were derived from the S/N ratio as 3. LC, APCI interface and MS conditions were the same as shown in Table 3.

Furthermore, in negative-ion measurement LC–MS, it is reported that the addition of some interhalogen chemicals to the LC mobile phase solvent enhanced detection sensitivity [65,85]. Enhanced detection sensitivity can be further expected with various devices.

4. Conclusion

An application of LC–APCI-MS for pesticide analysis was described. We confirmed that this method is capable of rapid analysis, and produces a specificity equal to that of GC–MS. The monitoring

Table 6
Detection limits of N-methylcarbamate pesticides

Measurement mode	Pesticide	Monitoring ion m/z	Detection limit (ng)
Positive-ion	Carbaryl	$180(M+H)^+$	20
	Ethiofencarb	$226(M+H)^+$	25
	Fenobucarb	$208(M+H)^+$	10
	Isoprocarb	$194(M+H)^+$	60
	Metolcarb	$166(M+H)^+$	40
	Propoxur	$210(M+H)^+$	30
	XMC	$180(M+H)^+$	60
	Xylylcarb	$180(M+H)^+$	60

Using 500 ng of each of the standard solutions, the detection limits during mass fragmentgraphy were derived from the S/N ratio as 3. LC, APCI interface and MS conditions were the same as shown in Table 3.

of ions with a mass number of 200 or less gave an unstable base line, and the detection sensitivity was reduced. Furthermore, an appropriate internal standard does not exist in pesticide analysis using LC–APCI-MS as well as using GC–MS. Although some problems with this analytical method remain to be solved, there are many advantages in LC–MS.

The demand for pesticide analysis will increase in the future. But a reference method is not yet established in pesticide analysis. In this present situation, we are convinced that LC–APCI-MS will obtain an important position in the future.

5. List of abbreviations

OP(s)	Organophosphorus pesticide(s),
CP(s)	Carbamate pesticide(s),
NMCP(s)	N-methylcarbamate pesticide(s),
TLC	Thin-layer chromatography,
GC	Gas chromatography,
HPLC	High-performance liquid chromatography,
MS	Mass spectrometry,
EI	Electron impact ionization,
CI	Chemical ionization,
FAB	Fast atom bombardment,
TSP	Thermospray,
API	Atmospheric pressure ionization,
APCI	Atmospheric pressure chemical ionization,
TIC	Total ion current chromatogram,
SIM	Selected ion monitoring,
PA	Proton affinity.
H_2O	Water,
CH_3OH	Methanol,
CH_3CN	Acetonitrile,
NH_4OAc	Ammonium acetate.
Dichlorvos	2,2-dichlorovinyl dimethyl phosphate,
Dimethylvinphos	2-chloro-1-(2′,4′-dichlorophenyl)vinyl dimethyl phosphate,
Propaphos	4- methylthiophenyl dipropyl phosphate,
Chlorpyrifos	O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate,
Chlorpyrifos-methyl	O,O-dimethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate,
Diazinon	O,O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate,
EPN	O-ethyl O-4-nitrophenyl phenyl phosphorothioate,
Fenitrothion	O,O-dimethyl O-4-nitro-*m*-tolyl phosphorothioate,
Fenthion	O,O-dimethyl O-4-methylthio-*m*-tolyl phosphorothioate,
Isoxathion	O,O-diethyl O-5-phenylisoxazol-3-yl phosphorothioate,
Parathion	O,O-diethyl O-4-nitrophenyl phosphorothioate,
Parathion-methyl	O,O-dimethyl O-4-nitrophenyl phosphorothioate,
Pyridaphenthion	O,O-diethyl O-(2,3-dihydro-3-oxo-2-phenlpyridazine-6-yl)phosphorothioate,
Edifenphos	O-ethyl S,S-diphenyl phosphorodithioate,
Iprofenfos	O,O-diisopropyl S-benzyl phosphorothioate,
Dimethoate	O,O-methyl S-methylcarbomoylmethyl phosphorodithioate,
Disulfoton	O,O-diethyl S-2-ethylthioethyl phosphorodithioate,
Ethion	O,O,O′,O′-tetraethyl S,S′-methylene bis(phosphorodithioate),
Malathion	S-1,2-bis(ethoxycarbonyl)ethyl O,O-dimethyl phosphorodithioate,
Methidathion	S-2,3-dihydro-5-methoxy-2-oxo-1,3,4-thiadiazol-3-ylmethyl O,O-dimethylphosphorodithioate,
Phenthoate	S-α-ethoxycarbonylbenzyl O,O-dimethyl phosphorodithioate,
Carbaryl	1-naphthyl methylcarbamate,
Ethiofencarb	2-ethylthiomethylphenyl methylcarbamate,
Fenobucarb	o-sec.-buthylphenyl methylcarbamate,
Isoprocarb	o-cumenyl methylcarbamate,
Metolcarb	m-tolyl methylcarbamate,
Propoxur	2-isopropoxyphenyl methylcarbamate,

XMC 3,5-xylyl methylcarbamate,
Xylylcarb 3,4-xylyl methylcarbamate.

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