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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY–NEGATIVE CHEMICAL IONIZATION MASS SPECTROMETRY OF ORGANOPHOSPHORUS PESTICIDES

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SUMMARY

Eighteen organophosphorus pesticides were studied by combined high-performance liquid chromatography–negative chemical ionization mass spectrometry (HPLC–NCI-MS). The LC separation was done on a reversed-phase C_8 column, using acetonitrile–water (60:40) as mobile phase. The MS analysis was done on-line, using a direct liquid-insertion probe LC–MS interface. The negative-ion mass spectra obtained under these LC–MS conditions are very simple and are very similar to those reported for methane-enhanced negative ionization. Molecular ions are generally not present in either mode, but intense fragment ions containing useful structural information are usually observed.

INTRODUCTION

Organophosphorus pesticides are widely used in agriculture, especially in recent years since the use of the more persistent chlorinated hydrocarbon pesticides has been curtailed. As a result, there is much interest in methods for the detection of these compounds in environmental matrices, and analytical methods have been developed for the determination of organophosphorus compounds in animal tissues[1–3], in plant tissues[4–8], water[2,5,9–11], soil[5], sewage sludge[10] and human urine[7].

At present, analysis of organophosphorus compounds is done mainly by gas chromatography (GC)[7,10,12]. Several researchers have studied these compounds by high-performance liquid chromatography (HPLC), using both normal-phase and re-

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versed-phase columns. Normal-phase techniques used include adsorption chromatography on silica gel[13] and diatomaceous earth[8], as well as on polar stationary phases such as β,β′-oxydipropionitrile coated on Zipax[2] or Corasil[14]. Reversed-phase separations have been done on μBondapak/Corasil[9] and Zorbax-ODS[11], both of which are octadecylsilane (C_{18}) stationary phases.

Several mass spectrometric (MS) studies of these compounds have been performed on individual pesticides by direct-probe analysis in the electron-impact ionization mode[6,15], and by direct-probe analysis in the methane positive chemical ionization (CI) mode[16]. Chloride-attachment negative CI (NCI) has also been used for organophosphorus compounds[17]. Combined GC–MS has been done on several organophosphorus pesticides, and the relative sensitivities in various positive and negative ionization modes, with different combinations of methane and oxygen, have been compared[18]. Various on-line LC–MS systems have been developed[19–23] and have been used for a variety of analytical applications[24,25].

Based on the increased sensitivity observed for organophosphorus compounds in NCI modes[18], it was decided to study the applicability of combined LC–NCI-MS to this class of compounds in order to investigate a method for its possible use in trace analysis of organophosphorus pesticides, and/or their possibly more polar or thermally labile metabolites.

EXPERIMENTAL

Equipment

The HPLC system consisted of two Waters 600A pumps (Waters Assoc., Milford, MA, U.S.A.), a Waters 660 Solvent Programmer, and a Perkin-Elmer LC-55 variable-wavelength UV detector (Perkin-Elmer, Norwalk, CT, U.S.A.). The HPLC column used was an RP-8 reversed-phase column, 10 μm particle size, 10 cm × 4.6 mm I.D. (Brownlee Labs., Santa Clara, CA, U.S.A.). The mobile phase used was acetonitrile–water (60:40), and the flow-rate was 1 ml/min. The UV detector wavelength was 254 nm.

The LC–MS interface was an unmodified Hewlett-Packard Direct Liquid Insertion Probe[26] (Hewlett-Packard, Palo Alto, CA, U.S.A.), which is a variable split-type interface. The usual split ratio is *ca.* 1:99 (mass spectrometer to fraction collector) (Fig. 1). This means that, with an LC flow-rate of 1 ml/min, *ca.* 10 μl/min of mobile phase enters the mass spectrometer source.

The mass spectrometer used in this research was a Finnigan 3300 chemical ionization mass spectrometer (Finnigan-MAT, Sunnyvale, CA), previously modified for NCI operation[27]. The mass spectrometer was interfaced to a Finnigan/Incos 2300 data system. The standard Finnigan 3300 1/4 in. I.D. direct-probe inlet system was replaced by a 1/2 in. I.D. inlet system, and a "desolvation chamber" was threaded into a modified direct-probe inlet-adapter plate on the Finnigan ion source (Fig. 2). The LC–MS interface probe slides into the desolvation chamber, maintaining source tightness for CI. The droplets of the jet are vaporized in the desolvation chamber, and the resulting sample–solvent gas mixture then enters the source, where it is ionized. Since the solvent is present in excess, it acts as a CI reagent gas[19] and as an electron-energy moderator, and the mode of ionization is probably electron capture and dissociative electron capture. No modification of the mass spectrometer pumping system

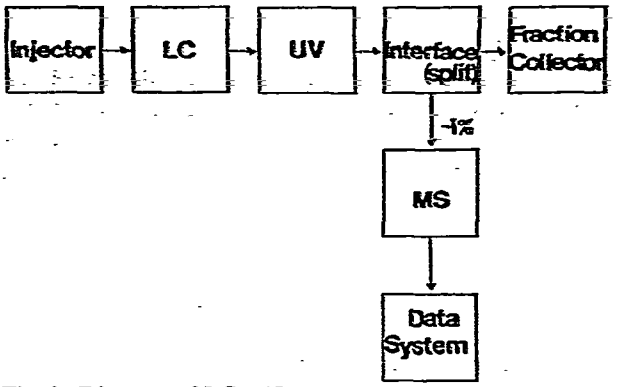


Fig. 1. Diagram of LC–MS system.

was necessary. Details of operation of the LC–MS system have been given earlier[28]. Since source temperature has been shown to have a profound effect on NCI mass spectra[29,30], source temperature was held at approximately 185°C.

Samples and solvents

Samples of the organophosphorus pesticides were obtained from the U.S. Environmental Protection Agency. Standard solutions were prepared using HPLC-grade acetonitrile. All sample solutions were stored in a freezer when not in use. The stated purities of these compounds ranged from 92 to 100%, with the exception of mevinphos, which was stated to be 64.5% pure. Since HPLC was used for sample introduction, no other purification was attempted prior to analysis.

Solvents used in this study were HPLC-grade acetonitrile (Fisher Scientific, Fair Lawn, NJ, U.S.A.), and HPLC-grade water (J.T. Baker, Phillipsburgh, NJ, U.S.A.). The acetonitrile was filtered through a 0.5-μm filter; the water was filtered through a 0.45-μm filter (Millipore, Bedford, MA, U.S.A.).

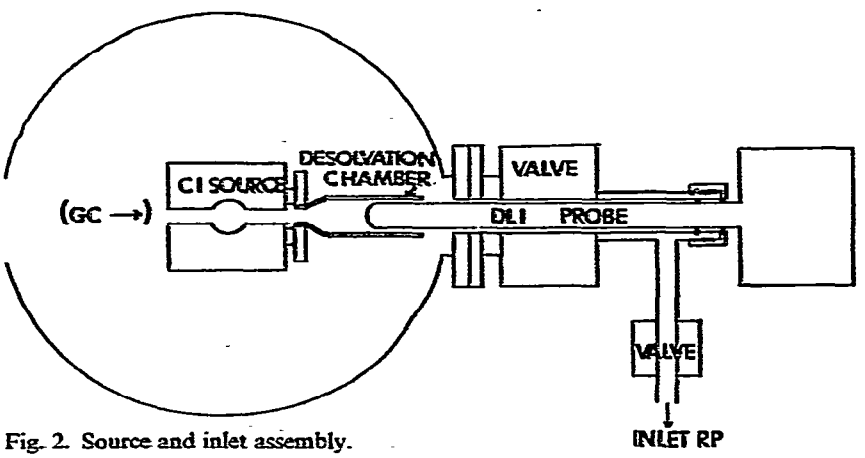


Fig. 2. Source and inlet assembly.

RESULTS AND DISCUSSION

Four classes of compounds were studied: phosphates (monocrotophos, dicrotophos and mevinphos), phosphorothioates (ronnel, bromophos, dichlofenthion, ethyl parathion, chlorpyrifos and diazinon), phosphorodithioates (dimethoate, malathion, azinphos-methyl, azinphos-ethyl, phorate, carbophenthion and menazon), and phenylphosphorothioates (leptophos and EPN).

The mass spectra and LC retention times are shown in Tables I–V. The methane-enhanced negative-ion (MENI) and methane oxygen-enhanced negative-ion (MOENI) mass spectra of most of the compounds studied have been reported previously[18]. The LC–NCI-MS mass spectra were simple and were similar to the reported MENI spectra, with more of the ion current being carried by the lower-mass fragments in the LC–NCI-MS spectra. All major peaks in the LC–NCI-MS spectra are also found in the MENI spectra. Molecular ions are not usually found, but diagnostic fragment ions giving structural information are usually present (Table V). The similarity between the GC–MS and the LC–MS spectra indicates that there is not much thermal decomposition occurring during GC analysis for the compounds studied.

Phosphates

For the three members of this compound class studied, almost all of the ion

TABLE I

COMPOUNDS STUDIED

Compound class	*Compound*	*MW*	*Retention time (min:sec)*	*Structure*
Phosphates	Monocrotophos	223	1:55	$(CH_3O)_2(PO)OC(CH_3)CHCONHCH_3$
	Dicrotophos	237	7:27	$(CH_3O)_2(PO)OC(CH_3)CHCON(CH_3)_2$
	Mevinphos	224	2:11	$(CH_3O)_2(PO)OC(CH_3)CHCO(OCH_3)$
Phosphorothioates	Ronnel	320	6:46	$(CH_3O)_2(PS)OC_6H_2Cl_3$
	Bromophos	364	6:58	$(CH_3O)_2(PS)OC_6H_2Cl_2Br$
	Dichlofenthion	314	8:04	$(C_2H_5O)_2(PS)OC_6H_3Cl_2$
	Ethyl parathion	291	4:43	$(C_2H_5O)_2(PS)OC_6H_4NO_2$
	Chlorpyrifos	349	8:12	$(C_2H_5O)_2(PS)OC_5NHCl_3$
	Diazinon	304	5:03	$(C_2H_5O)_2(PS)OC_4N_2H_2CH_3CH(CH_3)_2$
Phosphorodithioates	Dimethoate	229	2:11	$(CH_3O)_2(PS)SCH_2CONHCH_3$
	Malathion	330	3:45	$(CH_3O)_2(PS)SCH(COOC_2H_5)CH_2(COOC_2H_5)$
	Azinphos-methyl	317	3:09	$(CH_3O)_2(PS)SCH_2N_3COC_6H_4$
	Azinphos-ethyl	345	4:02	$(C_2H_5O)_2(PS)SCH_2N_3COC_6H_4$
	Phorate	260	5:20	$(C_2H_5O)_2(PS)SCH_2SC_2H_5$
	Carbopenthion	342	9:09	$(C_2H_5O)_2(PS)SCH_2SC_6H_4Cl$
	Menazon	281	2:07	$(CH_3O)_2(PS)SCH_2C_3N_3(NH_2)_2$
Phenylphosphonothioates	Leptophos	410	11:45	$(CH_3O)(C_6H_5)(PS)OC_6H_2Cl_2Br$
	EPN	323	6:05	$(C_2H_5O)(C_6H_5)(PS)OC_6H_4NO_2$

TABLE II

LC–NCI MASS SPECTRA OF PHOSPHATE PESTICIDES

MW = Molecular weight.

Compound	*Mass*	*Relative abundance (%)*	*Tentative identification*
Monocrotophos	114	<0.1	$[M-(CH_3O)_2PO]^-$
$(CH_3O)_2\overset{O}{\overset{\|}{P}}OC(CH_3)=CHC\overset{O}{\overset{\|}{N}}HCH_3$	124	0.3	
MW = 223	125	100.0	$(CH_3O)_2PO_2^-$
	126	1.4	
	127	0.3	
Dicrotophos	125	100.0	$(CH_3O)_2PO_2^-$
$(CH_3O)_2\overset{O}{\overset{\|}{P}}OC(CH_3)=CH\overset{O}{\overset{\|}{C}}-N(CH_3)_2$	127	0.3	
CH_3	128	0.8	
MW = 237	129	0.6	
	222	1.9	$[M-(CH_3)]^-$
Mevinphos	125	100.0	$(CH_3O)_2PO_2^-$
$(CH_3O)_2\overset{O}{\overset{\|}{P}}OC(CH_3)=CHCOCH_3$	127	0.1	
MW = 224			

current was carried by the peak at m/z 125, which corresponds to $(CH_3O)_2PO_2^-$ (Table II). This ion is apparently characteristic of this class of compounds. In addition, the spectrum of monocrotophos contains a small peak corresponding to $[M - (CH_3O)_2PO]^-$, and the spectrum of dicrotophos a peak for the loss of CH_3 from the molecular ion.

Phosphorothioates

Of these compounds, two (ronnel and bromophos) are dimethoxy compounds and four (dichlofenthion, ethyl parathion, chlorpyrifos and diazinon) are diethoxy compounds (Table III). This class of compounds shows common fragments corresponding to $(RO)_2PSO^-$ at m/z 141 when R = CH_3, and at m/z 169 when R = C_2H_5. Also present are peaks corresponding to the thiophenate ions produced by S migration. The phenoxide ion was observed in the LC–NCI-MS spectra of dichlofenthion, ethyl parathion and chlorpyrifos at m/z 161, 177 and 196, respectively. The substitution reaction involving addition of O_2 (from trace amounts of air in the source) and loss of $ClO^{\bullet}$ giving an overall decrease of 19 a.m.u. is seen for the thiophenate ions in ronnel, dichlofenthion and chlorpyrifos.

The m/z 79 ion found in the spectra of ronnel, chlorpyrifos and dichlofenthion corresponds to a combination of PO_3^- and PSO^-, PO_3^- probably being the predominant species since the PO_3^-:PSO^- ratio reported for chlorpyrifos was 4:1[31]. The m/z 79 ion in bromophos is the base peak in the spectrum, with the ^{81}Br isotope giving a relative intensity of 87%. The intensity difference between the m/z 79 and m/z 81 peaks is probably due to the PO_3^- and PSO^- contributions to the $^{79}Br^-$ peak. Bromophos also shows a trace of $(M - CH_3)^-$ and $(M - CH_3Br)^-$ at m/z 351 and 270, respectively.

TABLE III

LC–NCI MASS SPECTRA OF PHOSPHOROTHIOATE PESTICIDES

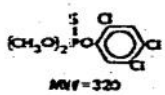
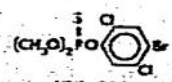
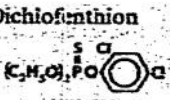
Compound	*Mass*	*Relative abundance (%)*	*Tentative identification*
Ronnel	79	2.3	PSO^-
	95	10.9	PSO_2^-
	110	0.3	$(CH_3O)PSO^-$
	126	0.2	$(CH_3O)PSO_2^-$
MW=320	139	<0.1	
	141	100.0	$(CH_3O)_2PSO^-$
	192	1.8	
	194	1.2	$[SC_6H_2Cl_3^- + O_2 - ClO^-]$
	196	0.3	
	210	0.4	
	211	82.5	
	213	86.6	$SC_6H_2Cl_3^-$
	215	30.6	
	217	1.9	
	234	0.2	$[M - CH_3 - HCl_2]^-$
	270	0.1	$[M - CH_3 - Cl]^-$
	305	0.1	$[M - CH_3]^-$
Bromophos	77	<0.1	
	79	100.0	$^{79}Br^-$ and PSO^-
	81	86.1	$^{81}Br^-$
	95	1.2	PSO_2^-
MW=364	140	0.1	
	141	23.6	$(CH_3O)_2PSO^-$
	143	0.3	
	220	0.2	$SC_6H_3ClBr^-$
	221	<0.1	
	254	<0.1	
	255	14.2	
	257	25.5	$SC_6H_2Cl_2Br^-$
	259	13.3	
	261	1.2	
	270	2.1	
	271	1.5	
	272	0.9	$[M - CH_3 - Br]^-$
	273	0.4	
	351	<0.1	$[M - CH_3]^-$
Dichlofenthion	63	2.4	$COCl^-$
	79	1.1	PSO^-
	93	1.3	
	95	100.0	PSO_2^-
MW=314	97	4.2	
	124	0.7	$(C_2H_5O)PSO^-$
	157	0.8	
	158	17.3	$[SC_6H_2Cl_2^- + O_2 - CO^-]$
	160	4.5	
	161	1.4	$OC_6H_2Cl_2^-$
	163	0.9	
	169	62.1	$(C_2H_5O)_2PSO^-$
	170	1.7	

TABLE III *(continued)*

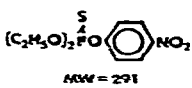
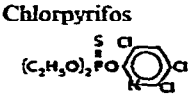
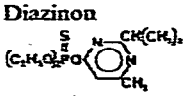
Compound	*Mass*	*Relative abundance (%)*	*Tentative identification*
	177	10.4	$SC_6H_3Cl_2^-$
	179	6.5	
	250	1.7	$[M - C_2H_5 - Cl]^-$
	251	13.6	$[M - COCl]^-$
	253	6.6	
	278	1.7	$[M - HCl]^-$
	280	0.5	
	285	4.2	$[M - C_2H_5]^-$
	287	3.4	
Ethyl parathion	138	5.0	$OC_6H_4NO_2^-$
	154	100.0	$SC_6H_4NO_2^-$
	155	7.7	
	156	4.0	
MW = 291	157	<0.1	
	169	16.1	$(C_2H_5O)_2PSO^-$
	170	0.5	
	171	0.4	
	262	<0.1	$[M - (C_2H_5)]^-$
	290	0.5	$[M - 1]^-$
Chlorpyrifos	79	1.9	PSO^-
	95	62.1	PSO_2^-
	96	2.2	
	97	1.4	
MW = 349	124	0.6	$(C_2H_5O)PSO^-$
	141	2.2	
	161	0.8	
	169	100.0	$(C_2H_5O)_2PSO^-$
	170	3.2	
	171	3.8	
	189	1.3	
	191	0.9	
	193	3.4	$[SC_5NHCl_3 + O_2 - ClO]^-$
	195	2.2	
	196	0.8	
	198	0.8	
	211	1.1	
	212	57.7	$SC_5NHCl_3^-$
	214	56.8	
	216	19.3	
	217	1.5	
	218	1.5	
	288	0.6	
	313	1.0	$[M - HCl]^-$
	315	1.4	
	316	1.4	
Diazinon	151	0.4	$[M - (C_2H_5O)_2PO]^-$
	168	0.5	
	169	100.0	$(C_2H_5O)_2PSO^-$
	170	3.5	
MW = 304	171	2.8	

TABLE IV

LC–NCI MASS SPECTRA OF PHOSPHORODITHIOATE PESTICIDES

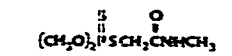
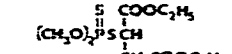
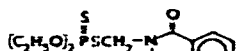
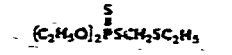
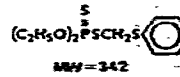
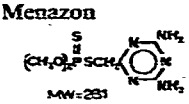
Compound	*Mass*	*Relative abundance (%)*	*Tentative identification*
Dimethoate	104	<0.1	$[M - ((CH_3O)_2PS)]^-$
	141	6.3	$(CH_3O)_2PSO^-$
	142	0.8	
MW = 229	143	0.1	
	155	0.1	
	157	100.0	$(CH_3O)_2PS_2^-$
	159	8.8	
	161	<0.1	
Malathion	110	<0.1	$(CH_3O)PSO^-$
	125	0.3	$(CH_3O)_2PS^-$
	129	0.1	
MW = 330	142	0.5	
	157	100.0	$(CH_3O)_2PS_2^-$
	159	9.1	
	161	<0.1	
	203	<0.1	
Azinphos-methyl	120	0.1	
	125	0.2	$(CH_3O)_2PS^-$
	132	0.6	$CH_2N_3C_6H_4^-$ or $CHNCOC_6H_4^-$
	134	<0.1	
MW = 317	142	0.8	
	146	0.1	$N_3COC_6H_4^-$
	157	100.0	$(CH_3O)_2PS_2^-$
	159	10.1	
	164	0.8	$[M - (CH_3O)_2PS - N_2]^-$ or $[M - (CH_3O)_2PS - CO]^-$
Azinphos-ethyl	95	0.2	PSO^- and PS_2^-
	111	0.1	
	120	0.1	
	132	0.6	$CH_2N_3C_6H_4^-$ or $CHNCOC_6H_4^-$
	134	0.5	
MW = 345	146	0.1	$N_3COC_6H_4^-$
	152	0.1	$(C_2H_5O)_2PS^-$
	154	0.1	
	164	1.1	$[M - (C_2H_5O)_2PS - N_2]^-$ or $[M - (C_2H_5O)_2PS - CO]^-$
	169	0.1	$(C_2H_5O)_2PO^-$
	185	100.0	$(C_2H_5O)_2PS_2^-$
	187	10.4	
	189	<0.1	
Phorate	61	0.8	$SC_2H_5^-$
	79	0.5	PSO^-
	95	3.0	PSO_2^- and PS_2^-
MW = 260	112	<0.1	
	124	0.1	
	153	3.0	$(C_2H_5O)_2PS^-$
	157	<0.1	
	184	0.1	
	185	100.0	$(C_2H_5O)_2PS_2^-$
	187	9.7	

TABLE IV *(continued)*

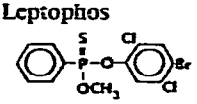
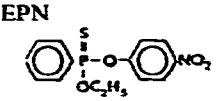
Compound	*Mass*	*Relative abundance (%)*	*Tentative identification*
Carbophenthion	95	0.1	PS_2^-
	112	0.1	
	143	100.0	$SC_6H_4Cl^-$
	145	37.4	
MW = 342	147	0.5	
	153	0.4	$(C_2H_5O)_2PS^-$
	183	0.2	
	185	82.3	$(C_2H_5O)_2PS_2^-$
	187	7.0	
Menazon	157	100.0	$(CH_3O)_2PS_2^-$
	158	4.6	
	159	3.9	
MW = 281	280	0.4	$[M - 1]^-$

Phosphorodithioates

Characteristic ions for this class of compounds are $(RO)_2PS_2^-$, giving intense peaks at *m/z* 157 (when R = CH_3) and *m/z* 185 (when R = C_2H_5) (Table IV). In the

TABLE V

LC–NCI MASS SPECTRA OF PHENYLPHOSPHONOTHIOATE PESTICIDES

Compound	*Mass*	*Relative abundance, %*	*Tentative identification*
Leptophos	78	0.2	
	79	100.0	$^{79}Br^-$ and PSO^-
	81	81.8	$^{81}Br^-$
MW = 410	187	6.1	$C_6H_5PS_2OOCH_3^-$
	238	0.3	
	239	21.3	$OC_6H_2Cl_2Br^-$
	241	40.2	
	243	14.6	
	245	1.2	
	255	4.1	$SC_6H_2Cl_2Br^-$
	257	7.5	
	258	0.1	
	259	2.8	
EPN	122	1.6	$C_6H_4NO_2^-$
	136	<0.1	
	138	100.0	$OC_6H_4NO_2^-$
MW = 323	140	3.3	
	141	0.2	
	153	0.1	
	154	19.1	$SC_6H_4NO_2^-$
	155	1.1	
	156	0.6	
	201	4.3	$C_6H_5PSOOC_2H_5^-$
	203	0.2	
	204	<0.1	
	322	0.1	$[M - 1]^-$

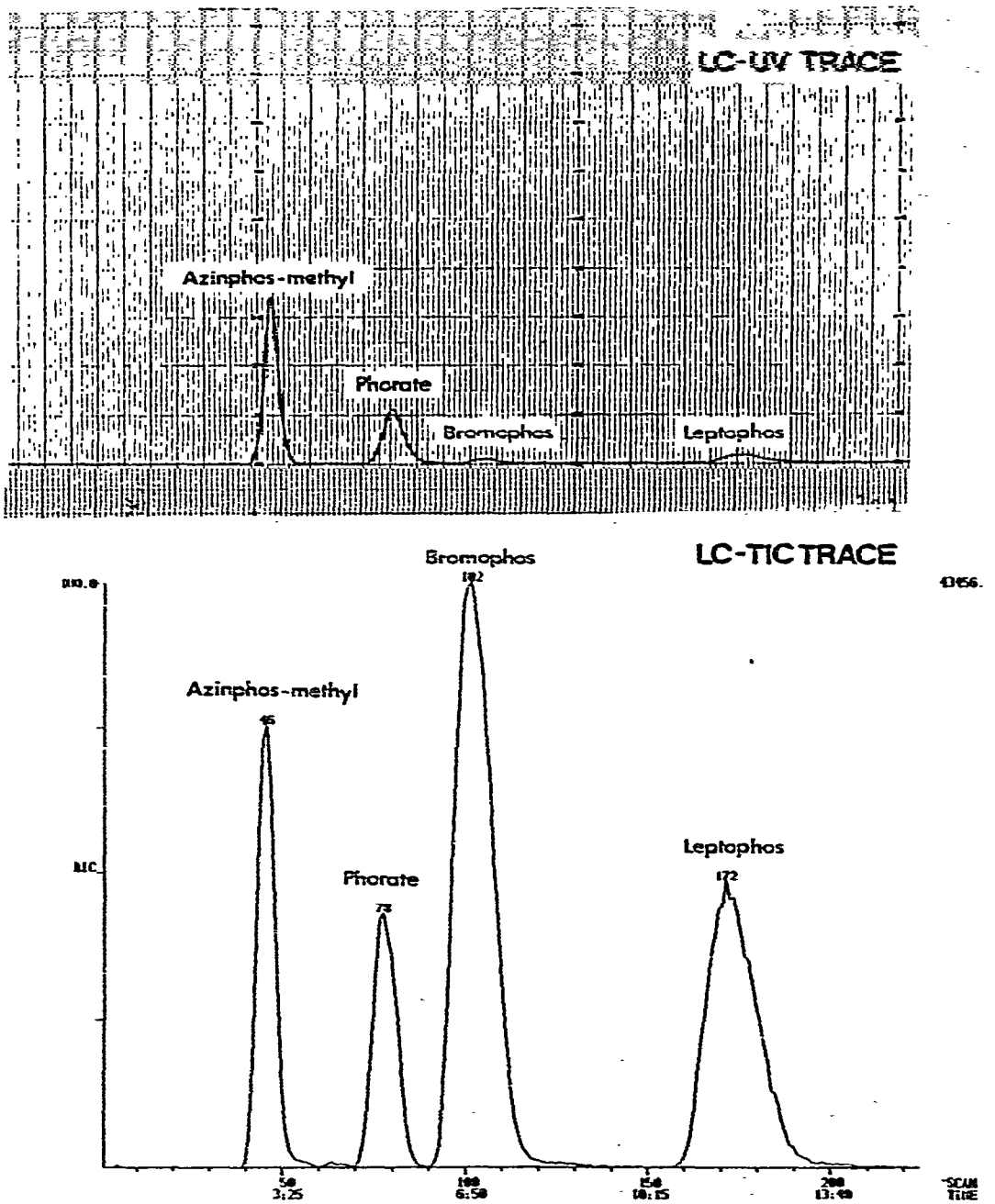


Fig. 3. LC–UV and LC–TIC traces for a mixture of bromophos, azinphos-methyl, phorate and leptophos.

aromatic phosphorothioates, some of the ion current is also carried by the aromatic portion of the molecule, for example, $SC_6H_4Cl^-$ is the base peak for carbophenthion, and there is a trace of $N_3COC_6H_4$ at *m/z* 146 in azinphos-ethyl and traces of

$CH_2N_3C_6H_4^-$ at *m/z* 132 in both azinphos-methyl and azinphos-ethyl. A peak at *m/z* 132 has been reported in the electron-impact mass spectrum of these compounds and has been ascribed to the loss of CO from the $CH_2N_3COC_6H_4^+$ ions[15]. Another rearrangement ion in azinphos-ethyl, at *m/z* 164, probably corresponds to $[M - (C_2H_5O)_2PS - N_2]^-$ or $[M - (C_2H_5O)_2PSCO]^-$ to give $SCH_2N_3C_6H_4^-$.

Other small but commonly occurring fragments include $[(RO)_2PS^-]$ at *m/z* 125 and *m/z* 152 for R = CH_3 and R = C_2H_5, respectively. This fragment is found in all of the dithioates studied with the exception of dimethoate and menazon. The $(CH_3O)_2PSO^-$ ion, apparently the result of oxygen/sulfur migration, gives a small peak at *m/z* 141 in dimethoate, and the corresponding $(CH_3CH_2O)_2PSO^-$ ion appears as a small peak at *m/z* 169 in azinphos-ethyl.

Other fragments include *m/z* 79 and *m/z* 95, corresponding to PSO^- (ref. 31) and a combination of PSO_2^- and PS_2^- (ref. 32), respectively.

Phenylphosphonothioates

As can be seen in Table V, diagnostic ions for this class of compounds are *m/z* 187 and *m/z* 201, corresponding to $C_6H_5P(OR)SO^-$, where R = CH_3 and R = C_2H_5, respectively. Peaks are also observed for the phenoxide ions from both compounds, $OC_6H_4NO_2^-$ at *m/z* 138 from EPN and $OC_6H_2Cl_2Br$ at *m/z* 239 from leptophos. The corresponding thiophenate-S-migration ions are also observed, at *m/z* 154 and *m/z* 255, respectively. Peaks at *m/z* 79 and 81 in leptophos are probably due to $^{79}Br^-$ and PSO^-, and $^{81}Br^-$, respectively. A small $(M - 1)^-$ ion is observed for EPN.

HPLC chromatograms resulting from UV and MS detection

LC–UV chromatograms and the corresponding LC–total ion current (TIC) traces for two mixtures of organophosphorus pesticides are shown in Figs. 3 and 5; Figs. 4 and 6 show the mass spectra obtained from these runs. Approximately 10–20 μg of each component were injected on to the LC column, which means that approximately 100–200 ng of each component entered the mass spectrometer ion source. Subsequent runs on these mixtures were made to determine approximate mass spectral detection limits. At levels of 100–200 ng injected onto the LC column (1–2 ng into the source), the major fragment ions could be detected in the reconstructed ion chromatograms, whereas no HPLC peak could be detected in the TIC trace. Thus, since at least a 10-fold increase in sensitivity would be expected by the use of single-ion monitoring, LC–MS sensitivities should be the same as those reported for MENI, which is 25–100 pg into the mass spectrometer source[18]. Since, obviously, the mass spectrometric detection limits are determined by the amount of material entering the source, the 99:1 split ratio would mean that about 100 times as much material must be injected on to the LC column. The use of a micro LC probe[25,33–35], where all of the HPLC effluent enters the mass spectrometer source and the need for a 99:1 split is eliminated, would be desirable for trace analysis.

Applicability to analysis of water samples

Oisuki and Takaku[9] reported a procedure for the analysis of Abate in water samples in which the water sample was pumped through the LC column, thus preconcentrating the pesticide at the front end of the column. The solvent was then programmed from 100% water to 100% acetonitrile, and a peak corresponding to the pesticide was observed in the UV trace.

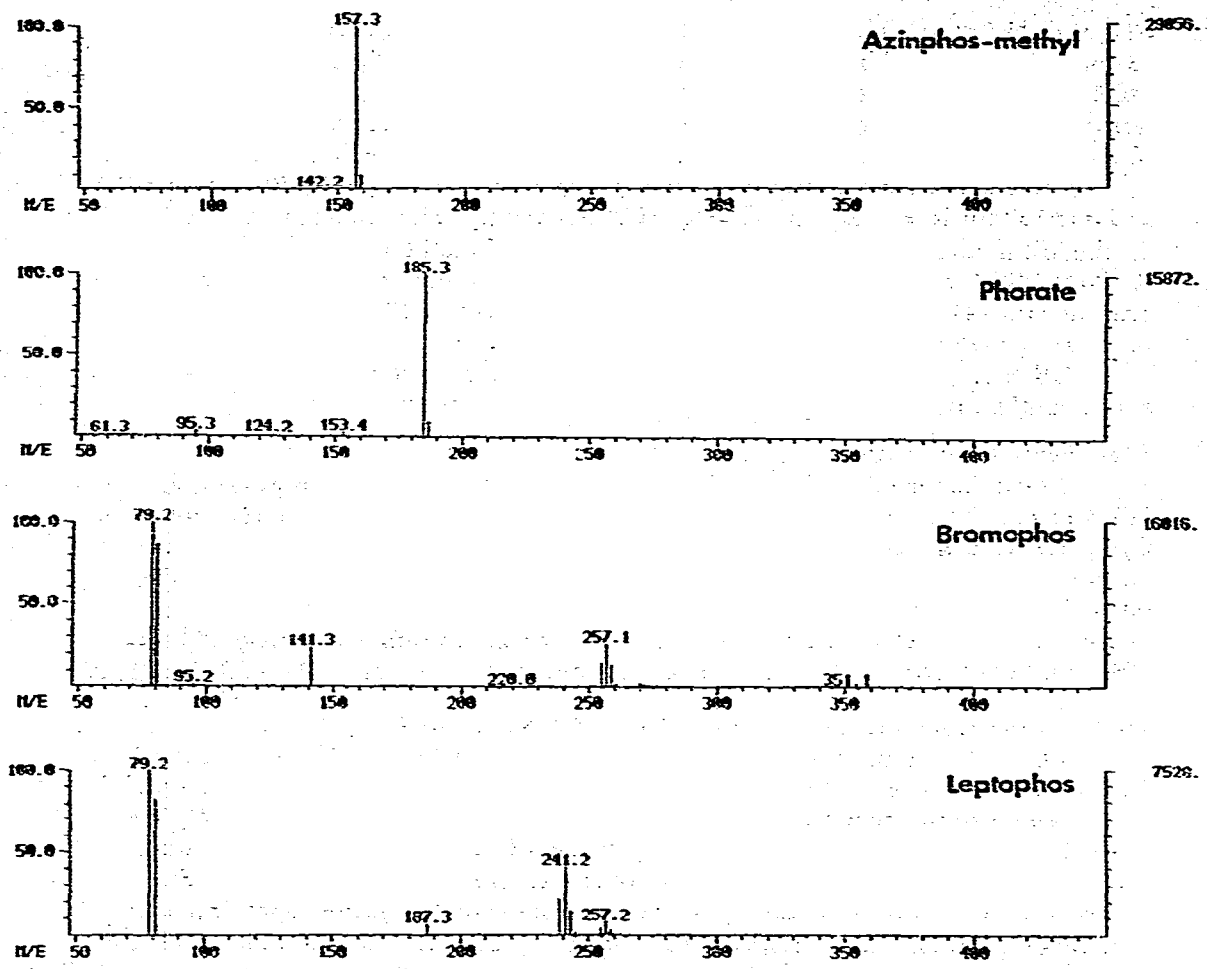


Fig. 4. LC–NCI mass spectra of bromophos, azinphos-methyl, phorate and leptophos.

In the analysis shown in Fig. 7, this idea was extended for use with mixtures of pesticides. The procedure used was to pump through the column 50 ml of water containing *ca.* 100 ng/ml each of monocrotophos, bromophos, leptophos, azinphos-methyl and phorate, followed by another 50 ml of water. The solvent programmer was then stepped to acetonitrile–water (60:40). A slight shift in relative retention times was observed, probably due to the column not having completely equilibrated to the change in solvent, but good LC peak shape and resolution were observed, indicating that little band spreading occurred during the pre-concentration step. There was no change in elution order, and no change in the mass spectra was observed since the solvent mixture, which becomes the mass spectrometer "reagent gas" remains in the same 60:40 ratio.

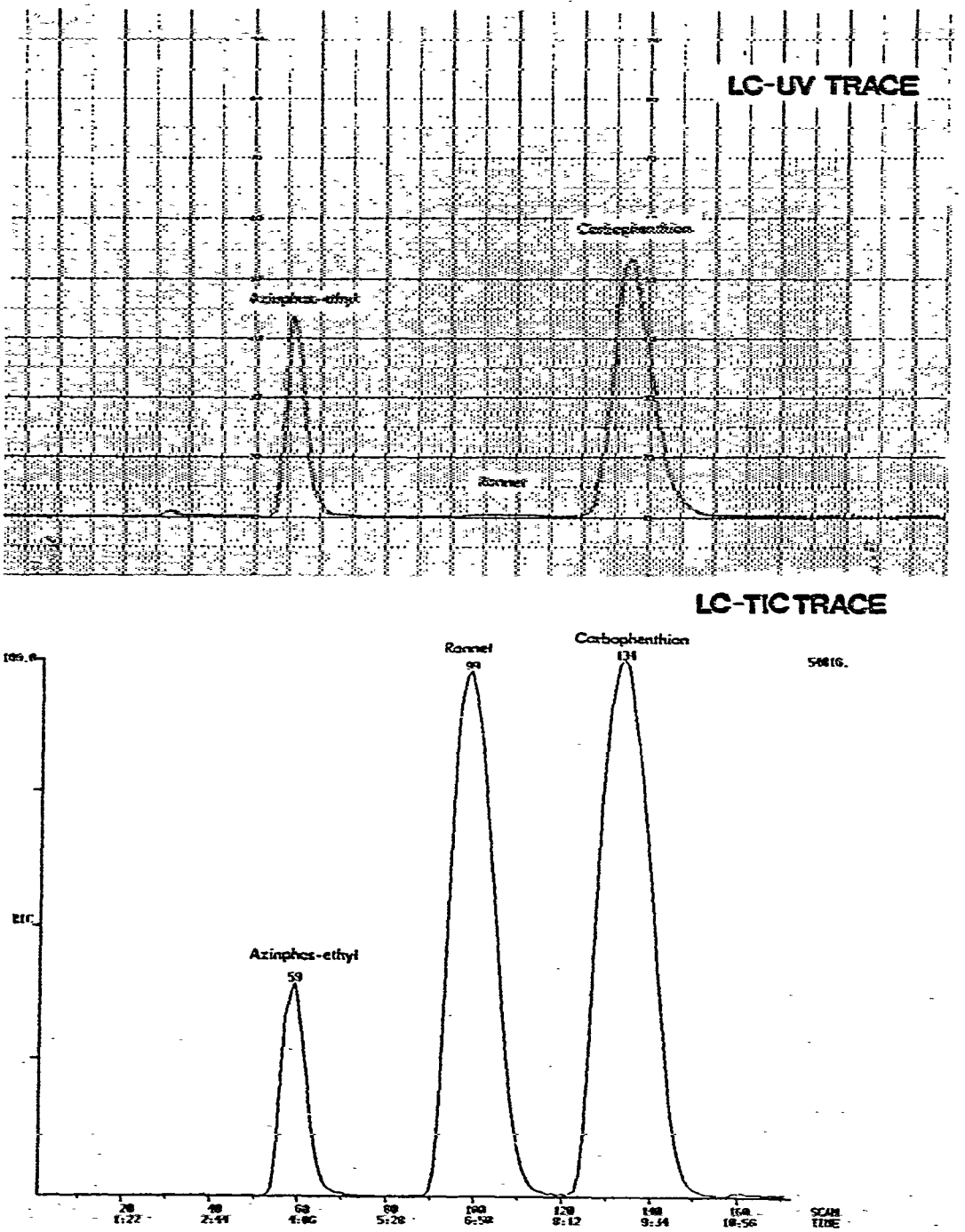


Fig. 5. LC–UV and LC–TIC traces for a mixture of ronnel, carbophenthion and azinphos-ethyl.

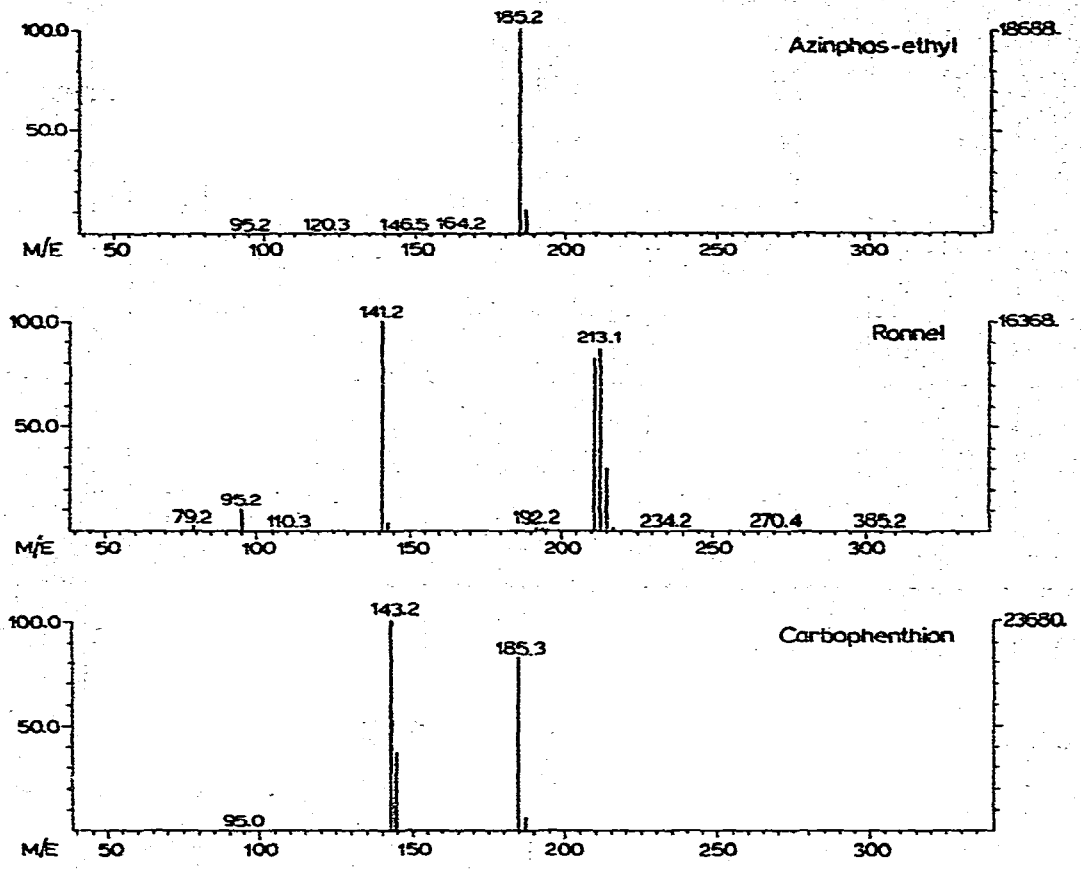


Fig. 6. LC–NCI mass spectra for ronnel, carbophenthion and azinphos-ethyl.

CONCLUSIONS

The technique of combined LC–NCI-MS shows promise for the analysis of organophosphorus pesticides. Combined with suitable extraction procedures, the MS sensitivities should make this technique applicable for residue analysis and the analysis of environmental samples. Since comparison of LC–MS results with those obtained by GC–MS shows that thermal degradation occurred during GC–MS separation, the main advantage of LC–MS over GC–MS would be for analysis of the possibly more polar or thermally labile metabolites of these pesticides. It should also be especially useful in the analysis of water samples, where the HPLC column can be used to pre-concentrate the pesticides in addition to providing the separation for the analysis. By combining LC retention-time data with MS fragmentation reaction data LC–NCI-MS provides a convenient and specific method for organophosphorus pesticides. In addition, it appears that negative ion spectra obtained with methane as reagent gas are sufficiently similar to those obtained with acetonitrile–water that existing information on MENI sensitivities and library spectra may be used.

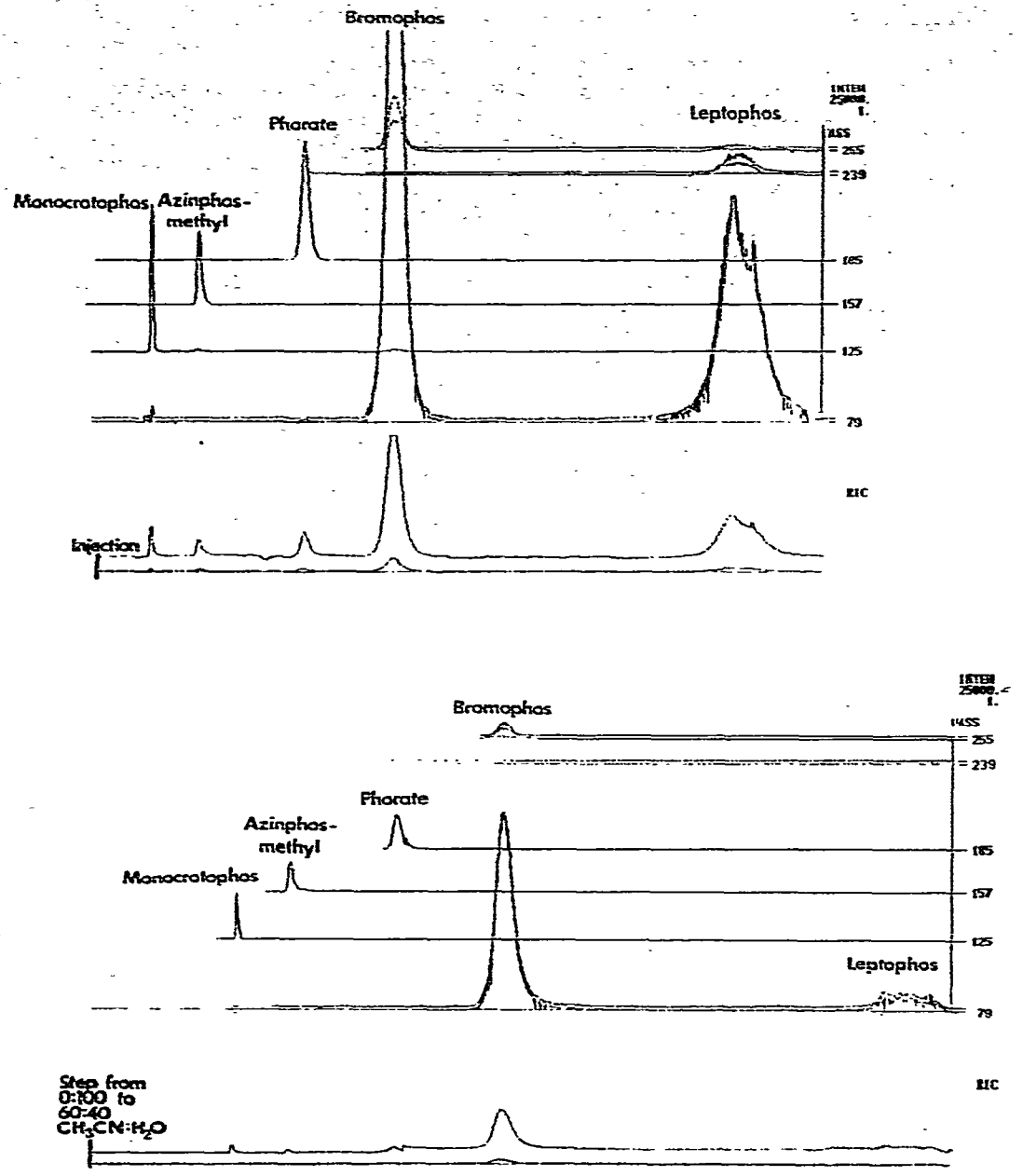


Fig. 7. Reconstructed Ion Chromatogram traces for a mixture of monocrotophos, bromophos, azinphos-methyl, phorate and leptophos under isocratic and "stepped" conditions.

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