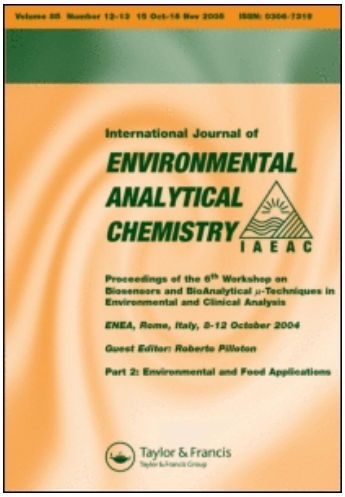

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ANALYSIS OF ORGANOPHOSPHORUS PESTICIDE RESIDUES BY LOW ENERGY TANDEM MASS SPECTROMETRY USING ELECTROSPRAY IONIZATION

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Electrospray mass spectrometry has aided the structural characterization of a series of phosphorothioates and phosphorodithioates as a means of developing a new method for detection of these organophosphorus pesticides. Possible fragmentation routes were first obtained by cone voltage dissociation. Low energy collision-activated dissociation (CAD) MS/MS analyses of the protonated molecular ion $[M+H]^+$ confirmed the characteristic fingerprint patterns obtained by cone voltage fragmentation for all investigated pesticides and also permitted differentiation of isomeric phosphorodithioates. MS/MS product and precursor ion spectra of selected intermediate fragments provided additional structural data and allowed rationalization of the fragmentation routes. Electrospray MS has proved to be a specific and very sensitive method for characterization of organophosphorus pesticides and allowed detection at levels as low as 10 μg/g.

KEY WORDS: Herbicide, organophosphorus, mass spectrometry, electrospray, detection, differentiation

INTRODUCTION

Three quarters of the earth's surface is covered with water. Any chemical that contaminates soil or the atmosphere has the potential for transfer to the hydrosphere and to pollute this medium. Pesticides are used in vast and ever-increasing amounts for the control of a wide variety of insect pests and many are relatively persistent and leave considerable residues in the soil or the atmosphere[1]. From these two media they are readily transferred to aquatic systems, through precipitation or in runoff and drainage from land to water. Once they reach large bodies of water they are transported by diffusion in water currents or in the bodies of aquatic organisms throughout any continuous aquatic system. In the aquatic systems, pesticides may either undergo degradation to simpler compounds, remain unchanged or move back into the atmosphere by volatilization[2].

Organophosphate pesticides have been shown to have bioaccumulation factors, in fish species, of up to 1682[3]. In fresh water, organophosphate exposure of fish has been shown to increase norepinephrine and dopamine levels in the cerebellum, and consequently alter

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their behavior which increases their susceptibility to predation[4]. In freshwater crab, organophosphorus pesticides caused a reduction of oxidative enzyme activity including that of acetylcholinesterase[5]. Algae have been reported to take up organophosphate pesticides from water[6] and consequently show reduction in both growth rate and standing crop[7]. In fish, the organophosphate pesticides are normally metabolized by hydrolysis or by demethylation followed by conjugation[8].

Statistical analysis of degradation rates of organophosphorus pesticide samples from two Gulf Coast estuaries, over a three-year period, indicated that biodegradation occurred only in the presence of sediment and was insignificant in water[9]. In water, the half life of organophosphate can be as short as 7.1 h for phosmet, to as long as 130 days for parathion[10]. The kinetics of disappearance of organophosphorus pesticides were studied in anaerobic sediment samples[11] and it was shown that in strongly reducing sediments the half-lives could be as short as a few minutes[11]. In water isolated from the sediments no reaction could be detected over a period of a week. In heat-sterilized sediments the disappearance rate constants were retarded about two orders of magnitude relative to non sterile sediments while in chemically treated sediments first-order disappearance rate constants were comparable to those in the nonsterile system.

Organophosphate pesticides are commonly analyzed by HPLC[12–15] but conventional spectrometric detectors lack the specificity and sensitivity necessary for the analysis of environmental samples. The mass spectrometry of organophosphorus pesticides, using a variety of ionization techniques, has been the subject of several investigations. Damico reported the electron impact mass spectrometry of these compounds and used high-resolution and metastable analysis to rationalize their fragmentation[16]. Chemical ionization (CI)[17–20] and thermospray (TSP)[21,22] mass spectrometry have also been used for the identification of organophosphorus pesticides. Recently, analyses for pesticide residues were reported[23,24] using MS/MS tandem mass spectrometry studies on the primary fragmentations observed for molecular ions or adducts of the molecular ion obtained from both the EI and CI modes.

Electrospray ionization (ESI) mass spectrometry is well established as a robust LC-MS technique which allows rapid, accurate and sensitive analysis of a wide range of analytes from low molecular weight polar compounds (less than 200 Da), to biopolymers larger than 100 kDa[25]. Under appropriate experimental conditions, gas-phase fragmentations are minimized and the subsequent ions which possess low internal energy are sufficiently stable to pass from the ion source to the detector without dissociation. This is common for ions produced under "very soft" ionization processes. If additional structural information is needed, dissociation of intact protonated molecular ions can be induced or activated by collision with a neutral gas phase species, usually in the pressurized collision cell of a tandem MS/MS instrument[26,27]. Ions that have undergone this collisional excitation process may subsequently fragment. This process is known as low energy collision-activated dissociation or CAD MS/MS. Another way to generate structural information by dissociation of intact protonated molecular ions can be induced by controlled adjustment of the voltage applied to the sampling cone of the electrospray source[28]. This procedure is also known as either cone voltage fragmentation or CAD in the atmospheric pressure/vacuum interface region under mild conditions. There are two advantages to using this approach : ion transmission remains high compared to MS/MS and an expensive MS/MS instrument is not required. The only disadvantage is that precursor ions are not selected prior to CAD.

The present study has been conducted within the Department of Fisheries and Oceans objectives to adopt new technologies and approaches to assist in predicting scientific issues affecting fisheries resources. The Canadian Green Plan for Toxic Chemicals

Program was undertaken to assess the state of our environment, As part of a Green Plan project aimed at the the determination of trace levels of toxic chemical residues in the Newfoundland environment and as a continuation of our interest in the tandem mass spectrometry of complex bioactive molecules[29–33] and low molecular weight pesticides[34], we now report on the structural characterization of phosphorodithiate and phosphorothiate pesticides using electrospray mass spectrometry without any prior chromatographic purification or preconcentration. Evidence of the possible fragmentation routes was first obtained by cone voltage fragmentation. Structural information was also derived from low energy tandem mass spectral analysis of the $[M+H]^+$ protonated molecules. Rationalization of the fragmentation routes was made by obtaining the product and precursor ion spectra of the various intermediate ions.

EXPERIMENTAL

Sample preparation

Samples of organophosphorus herbicides were obtained from Ultra Scientific, North Kingston, Rhode Island, U.S.A. The standard solutions used for LC-MS and LC-MS/MS were prepared with HPLC solvent grade methanol at a concentration of 50 pmol/μL. A 20 μL aliquot of sample was then introduced into the electrospray ion source by a continuous flow of acetonitrile: water (CH_3CN:H_2O, 1:1) at a flow rate of 20 μL/min using a Shimadzu LC-10AD pump connected to the Rheodyne injector with a 20 μL loop. Sensitivity studies were done with concentrations varying from 1 ppm (approximately 3 μM) to 1 ppb (approximately 3 nM) in water.

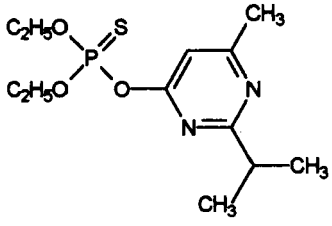
Mass spectrometry conditions

The electrospray MS spectra (positive ion mode) were recorded using a Fisons VG-Quattro quadrupole-hexapole-quadrupole mass spectrometer, equipped with an electrospray ionization source and capable of analyzing ions up to m/z 4000. A personal computer (486, 66 MHz processor) equipped with Fisons MASSLYNX Mass Spectrometry Data System Software, was used for data acquisition and processing. The temperature of the ES ionization source was maintained at 70°C. The operating voltage of the ES capillary was 3.50 kV and the high voltage lens was maintained at 0.50 kV throughout. ES mass spectra were recorded with a cone voltage setting varying from 30 to 75 volts. Conventional ES mass spectra were obtained by scanning in the Multi Channel Analysis (MCA) mode with a scan time of 1 second/250 a.m.u., Spectra are an average of 3–4 scans. Conventional ES and MS/MS spectra presented in this rationale have been background subtracted, smoothed and centered. The mass scale was calibrated in the positive ion mode using a polyethylene glycol mixture. MS/MS experiments were conducted using the same instrument. Product ion spectra of mass-selected ions were induced by collision with argon in the (RF only) hexapole. Argon collision gas was added to the enclosed chamber of the hexapole to give an indicated pressure of 2×10^{-5} mbar for collisional activation of the sample ions. The resulting fragments were analyzed by the second quadrupole. Collision energies of approximately 50 eV and a cone voltage of 50 to 75 V were used in all MS/MS experiments. Precursor ion scans were obtained by scanning the first quadrupole while selecting a given m/z value with the second quadrupole.

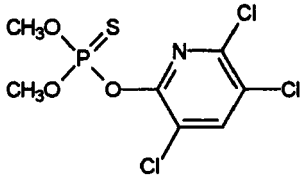
RESULTS AND DISCUSSION

Two classes of organophosphorus pesticides were studied in this rationale, namely, phosphorothioates and phosphorodithioates, whose structures are depicted in Figure 1.

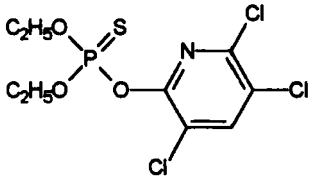
PHOSPHOROTHIOATES



1
Diazinon
$C_{12}H_{21}N_2O_3PS$, MWT 304

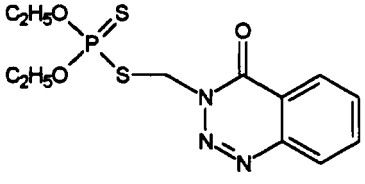


2
Chlorpyrifos-methyl
$C_7H_7Cl_3NO_3PS$, MWT 321

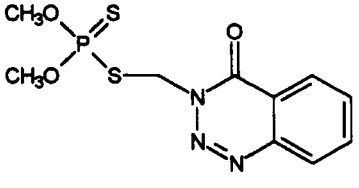


3
Chlorpyrifos
$C_9H_{11}Cl_3NO_3PS$, MWT 350

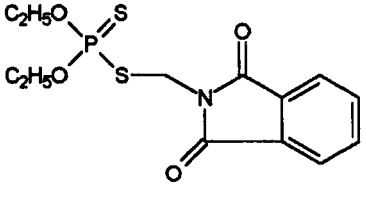
PHOSPHORODITHIOATES



4
Azinphos-ethyl
$C_{12}H_{16}N_3O_3PS_2$, MWT 345



5
Azinphos-methyl
$C_{10}H_{12}N_3O_3PS_2$, MWT 317



6
Phosmet
$C_{13}H_{16}NO_4PS_2$, MWT 345

Figure 1 Chemical structures of phosphorothioates and phosphorodithioates pesticides 1–6.

The electrospray mass spectrum (positive ion mode) of phosphorothioic acid *O,O*-diethyl-*O*-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl] ester 1, commonly known as diazinon (Figure 1), was recorded with a lower cone voltage (50 V) and gave the major protonated molecular ion $[M+H]^+$ at m/z 305. ESMS were also recorded with higher cone voltages to help promote the fragmentation of the $[M+H]^+$ protonated molecules in order to provide additional structural information. The ESMS of diazinon 1 were recorded with cone voltages of 50, 60, 75 and 100 volts and are shown in Figure 2.

The fragmentation route of the protonated molecular ion obtained by diverse cone voltage fragmentations has been tentatively rationalized and presented in Figure 3. In this rationale, the fragmentation of the protonated molecular ion $[M+H]^+$ at m/z 305 produces the fragment ion $[M+H-C_2H_4]^+$ at m/z 277 by the straightforward loss of a molecule of ethylene. The fragment ion $[M+H-2(C_2H_4)]^+$ at m/z 249 may originate from two different routes: either by loss of a molecule of ethylene from the fragment ion $[M+H-C_2H_4]^+$ at m/z 277, or directly from the protonated molecular ion by concerted losses of two molecules of ethylene. The fragment ion $[M+H-C_8H_{12}N_2]^+$ at m/z 169 occurs by the simple loss of a molecule of 6-methyl-2-(1-methylethyl)-4-pyrimidine. Finally, the fragment ion at m/z 153 has been assigned to $[M+H-C_4H_9O_2PS]^+$. The formation of the fragment ion $[M+H-C_4H_9O_2PS-C_2H_4]^+$ at m/z 125 can occur by three different routes: the simple loss of a molecule of carbon monoxide from the $[M+H-C_4H_9O_2PS]^+$ at m/z 153, the concerted losses of a molecule of $C_4H_9O_2PS$ and a molecule of carbon monoxide (or *vice versa*) from the protonated molecular ion, or by the concerted loss of a molecule of HO_2PS and a molecule of carbon monoxide from the fragment ion $[M+H-2(C_2H_4)]^+$ at m/z 249. The order of elimination of the molecules has not been studied further and is beyond the scope of the present work. In this context, it should be noted that "concerted losses" of two (or three) molecules in the MS experiment simply means that they are both lost within the time window of the same reaction region within the ES ionization source of the tandem mass spectrometer.

Low energy tandem mass spectrometric analyses were conducted to rationalize the pathways leading to the various fragmentations obtained in the conventional ES mass spectra by adjustment of the voltages (50 to 100 V) applied to the sampling cone of the electrospray source. Product ion spectra arising from fragmentation in the RF only hexapole collision cell of the quadrupole-hexapole-quadrupole instrument were obtained. The $[M+H]^+$ ion at m/z 305 was selected for the recording of the collision-activated dissociation (CAD) MS/MS. A significant advantage of the MS/MS technique is the elimination of all uncertainty as to the origin of the fragment ions. The CAD MS/MS of the ion at m/z 305 suggested the formation of the product ions at m/z 169, 153 and 125 as shown in Figure 4. In this CAD MS/MS experiment the product ions at m/z 277 and 249 were absent even when softer collision energies and lower gas collision pressures were used. This may be due to the fact that they fragment very rapidly into the fragment ion at m/z 125. Second generation product ions of the intermediate fragment ion $[M+H-C_2H_4]^+$ at m/z 277 were generated in a MS/MS experiment and afforded the product ions at m/z 169 and 153. Schematic representation of the different scan modes is conveniently represented on all MS/MS figures with symbols as already described in the literature[35]: filled circle indicates a fixed or preselected mass, open circle represents a scanned or variable mass.

In a different set of experiments, the precursors of the ions $[M+H-C_2H_4]^+$ at m/z 277 and $[M+H-2(C_2H_4)]^+$ at m/z 249 were sought using the precursor ion scan technique. Thus, the precursor ion scan of the fragment ion $[M+H-C_2H_4]^+$ at m/z 277 indicates that it originated from the protonated molecular ion (Figure 5a). The precursor ion scan of the fragment ion $[M+H-2(C_2H_4)]^+$ at m/z 249 indicates that it originated from the [M+H-

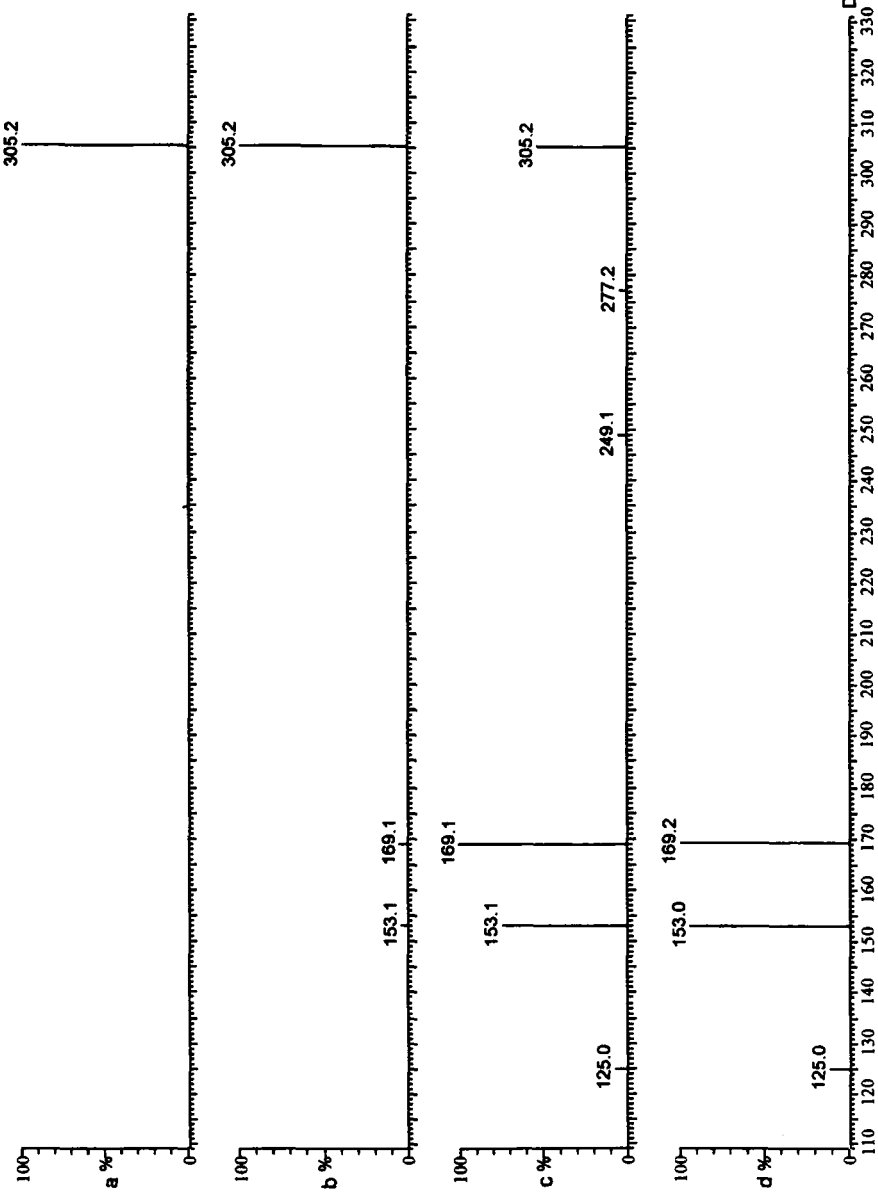


Figure 2 Electrospray mass spectra of phosphorothioic acid *O,O*-diethyl-*O*-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl] ester or diazinon 1 recorded with cone voltages of (a) 50 Volts, (b) 60 Volts, (c) 75 Volts and (d) 100 Volts.

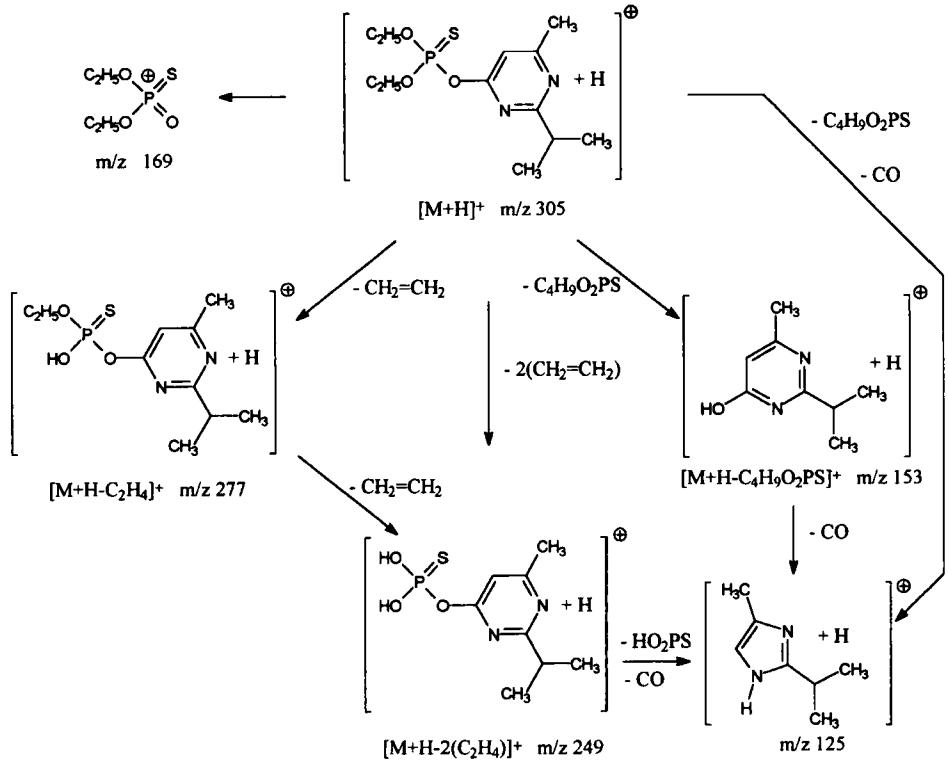


Figure 3 Major fragmentation routes of the $[M+H]^+$ ion of diazinon 1.

$C_2H_4]^+$ ion at m/z 277 (Figure 5b). Similarly, it was shown that the precursor ion of the fragment ion $[M+H-C_8H_{12}N_2]^+$ at m/z 169 (Figure 5c) was formed from the molecular ion. Also, the precursor ion scan of the fragment ion $[M+H-C_4H_9O_2PS]^+$ at m/z 153 (Figure 5d) indicates that it originated solely from the protonated molecular ion.

The electrospray mass spectra of phosphorothioic acid, *O,O*-dimethyl-*O*-(3,5,6-trichloro-2-pyridinyl) ester or chlorpyrifos-methyl 2 (Figure 1) were recorded with cone voltages of 50, 60, 75 and 100 Volts and are shown in Figure 6. In the ESMS of 2, recorded with a cone voltage of 50 V, we observed a cluster of isotopic molecular ions $[M+H]^+$ at m/z 322, 324, 326 and 328, with the correct ratio in accordance with the isotopic abundance of chlorine ^{35}Cl and ^{37}Cl atoms. Similarly, we noticed the presence of the sodiated adduct $[M+Na]^+$ at m/z 344, 346, 348 and 350.

The fragmentation route of the protonated molecular ion obtained for the different cone voltages is tentatively presented in Figure 7. Thus, the fragment ion $[M+H-CH_4]^+$ at m/z 306 is obtained from the protonated molecular ion $[M+H]^+$ at m/z 322 by the simple loss of a molecule of methane. The loss of a molecule of hydrogen chloride from the protonated molecular ion affords the fragment ion $[M+H-HCl]^+$ at m/z 286. The loss of a molecule of 3,5,6-trichloro-2-pyridine from the ion at m/z 322 affords the fragment ion $[M+H-C_5H_2Cl_3NO]^+$ at m/z 125. Similar fragment ions were obtained from the isotopic $[M+H]^+$ protonated molecular ions containing the different permutations of ^{35}Cl and ^{37}Cl isotopes. CAD MS/MS of the protonated molecular ion $[M+H]^+$ at m/z 322 afforded the product ions $[M+H-CH_4]^+$, $[M+H-HCl]^+$ and $[M+H-C_5H_2Cl_3NO]^+$ at m/z 306, 286 and

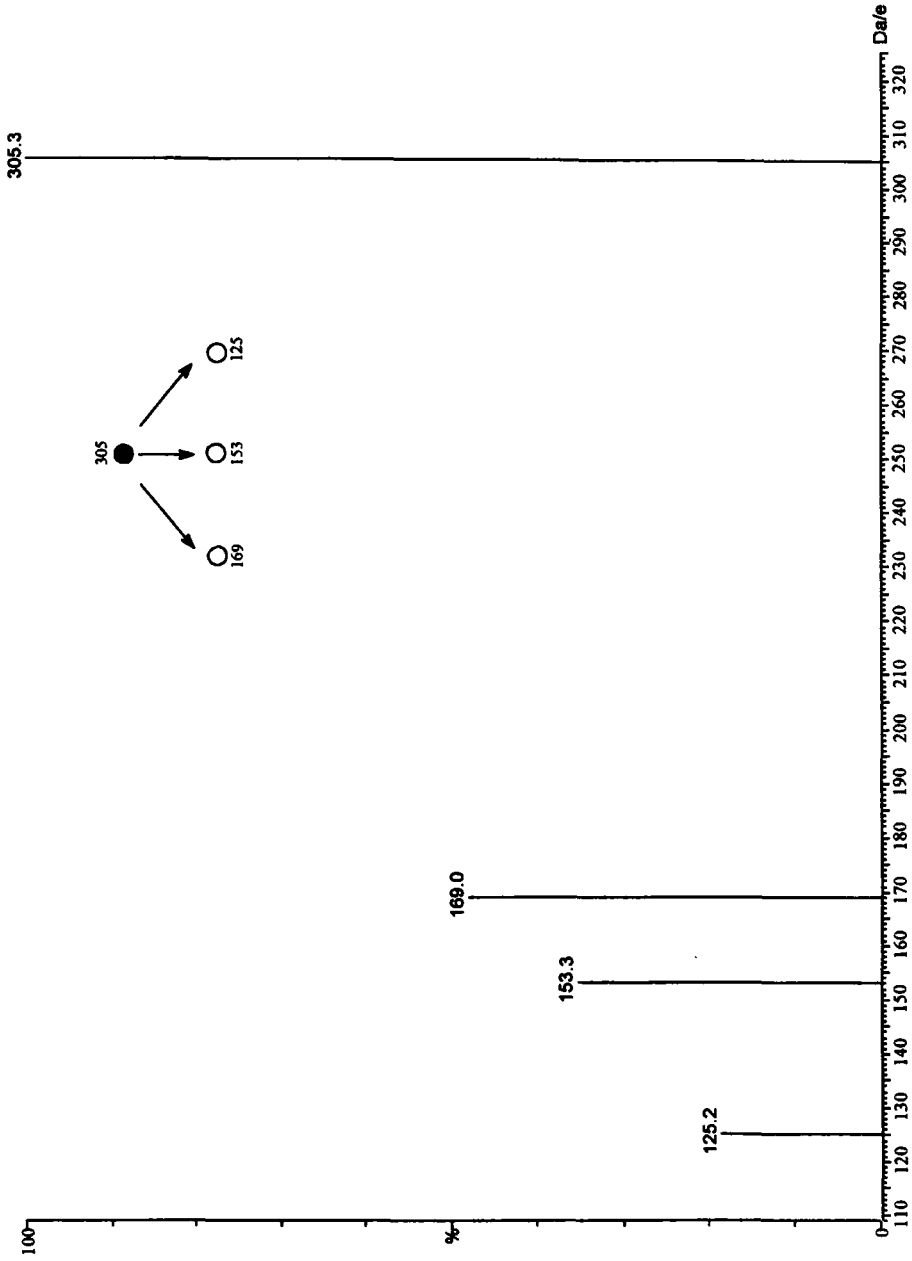


Figure 4 Low energy CAD MS/MS of the $[M+H]^+$ ion of diazinon 1.

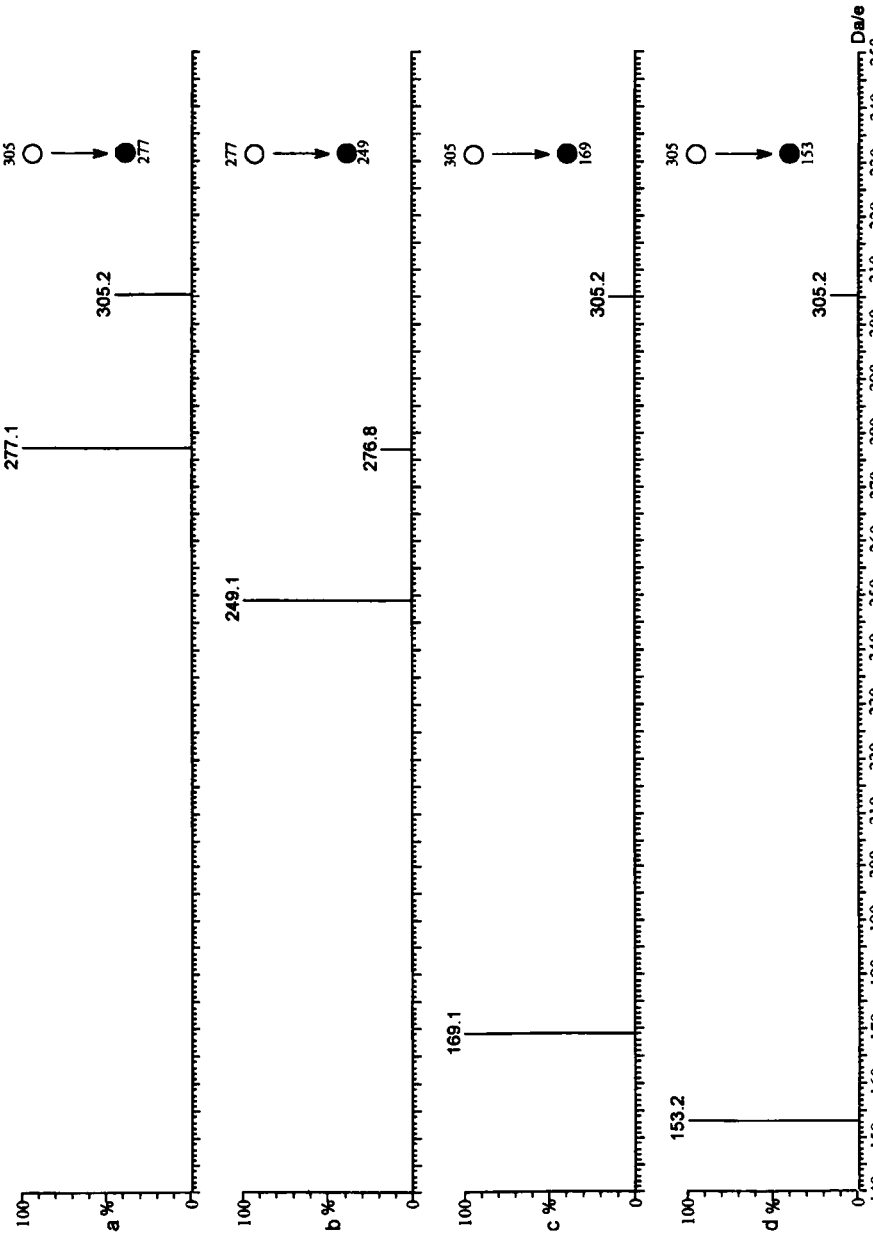


Figure 5 Precursor ion scan of (a) the fragment ion $[M+H-C_2H_4]^+$ at m/z 277, (b) the fragment ion $[M+H-2(C_2H_4)]^+$ at m/z 249, (c) the fragment ion $[M+H-C_2H_4]^+$ at m/z 277, (b) the fragment ion at m/z 169 and (d) the fragment ion $[M+H-C_4H_9O_2PS]^+$ at m/z 153 from diazinon 1.

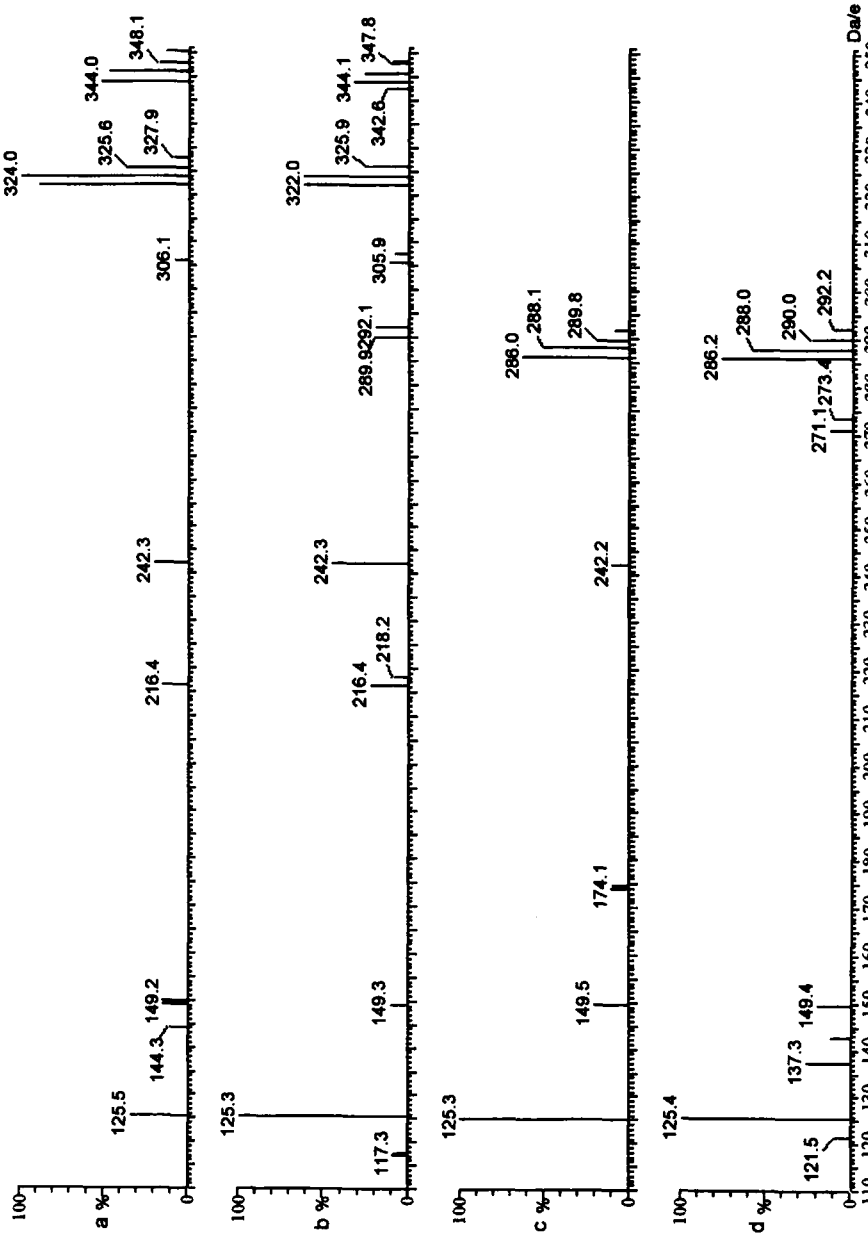


Figure 6 Electrospray mass spectra of phosphorothioic acid *O,O*-dimethyl-*O*-(3,5,6-trichloro-2-pyridinyl) ester or chlorpyrifos-methyl 2 recorded with cone voltages of (a) 50 Volts, (b) 60 Volts, (c) 75 Volts and (d) 100 Volts.

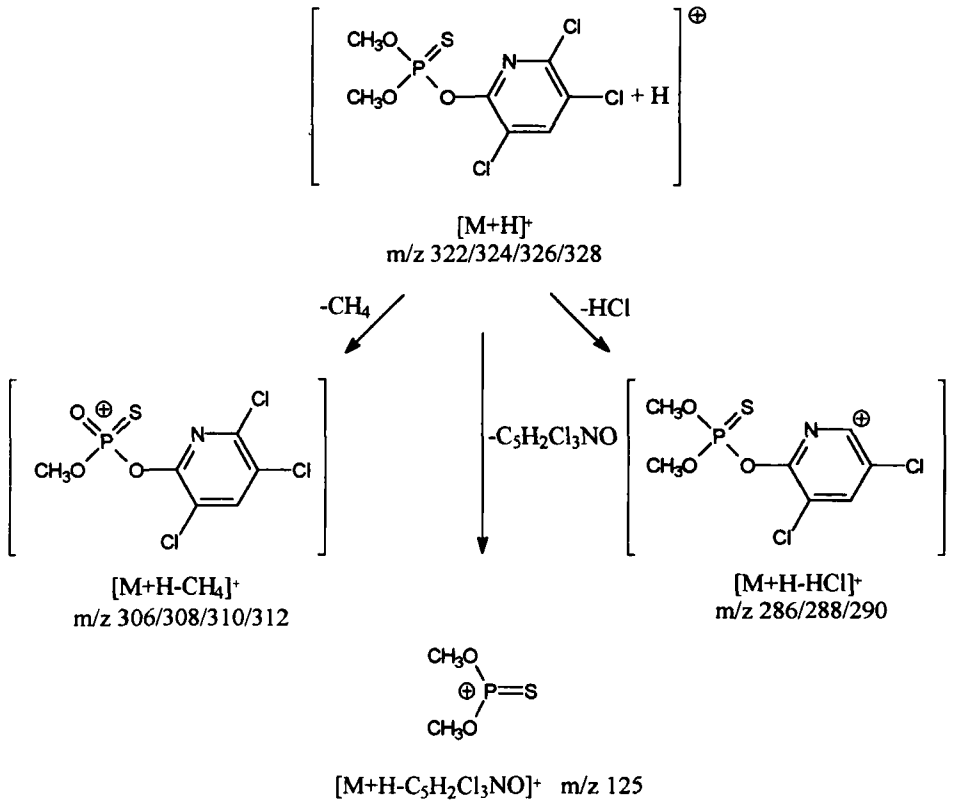


Figure 7 Major fragmentation routes of the $[M+H]^+$ ion of chlorpyrifos-methyl 2.

125, respectively. Precursor ion scan of the fragment ion $[M+H-HCl]^+$ at m/z 286 showed that it originated from the protonated molecular ion. Similarly, the precursor ion scan of the fragment ion $[M+H-C_5H_2Cl_3NO]^+$ at m/z 125 showed that it also originated solely from the protonated molecular ion.

The electrospray mass spectra of phosphorothioic acid *O,O*-diethyl-*O*-(3,5,6-trichloro-2-pyridinyl) ester or chlorpyrifos 3 (Figure 1) were recorded with cone voltages of 50, 60, 75 and 700 Volts, and are shown in Figure 8. As in the case of chlorpyrifos-methyl 2, we notice the presence of the cluster of isotopic protonated molecular ions $[M+H]^+$ at m/z 350, 352, 354 and 356 resulting from the isotopic permutation of the three chlorine atoms. Also, we noticed the presence of the isotopic cluster of sodiated adducts $[M+Na]^+$ at m/z 372, 374, 376 and 378, respectively. The fragmentation route of the protonated molecular ion $[M+H]^+$ obtained by diverse cone voltage fragmentations has been tentatively rationalized and is presented in Figure 9.

The protonated molecular ion $[M+H]^+$ may lose one or two molecules of ethylene to afford the ions $[M+H-C_2H_4]^+$ and $[M+H-2(C_2H_4)]^+$ at m/z 322 and 294 respectively. The fragment ion $[M+H-C_4H_9O_2PS]^+$ at m/z 198 is produced either by loss of a molecule of $C_4H_9O_2PS$ from the protonated molecular ion or by the loss of a molecule of $C_2H_5O_2PS$ from the $[M+H-C_2H_4]^+$ ion at m/z 322. The ion at m/z 198 may lose either a molecule of water or a molecule of hydrogen chloride to afford the ions $[M+H-C_4H_9O_2PS-H_2O]^+$ and

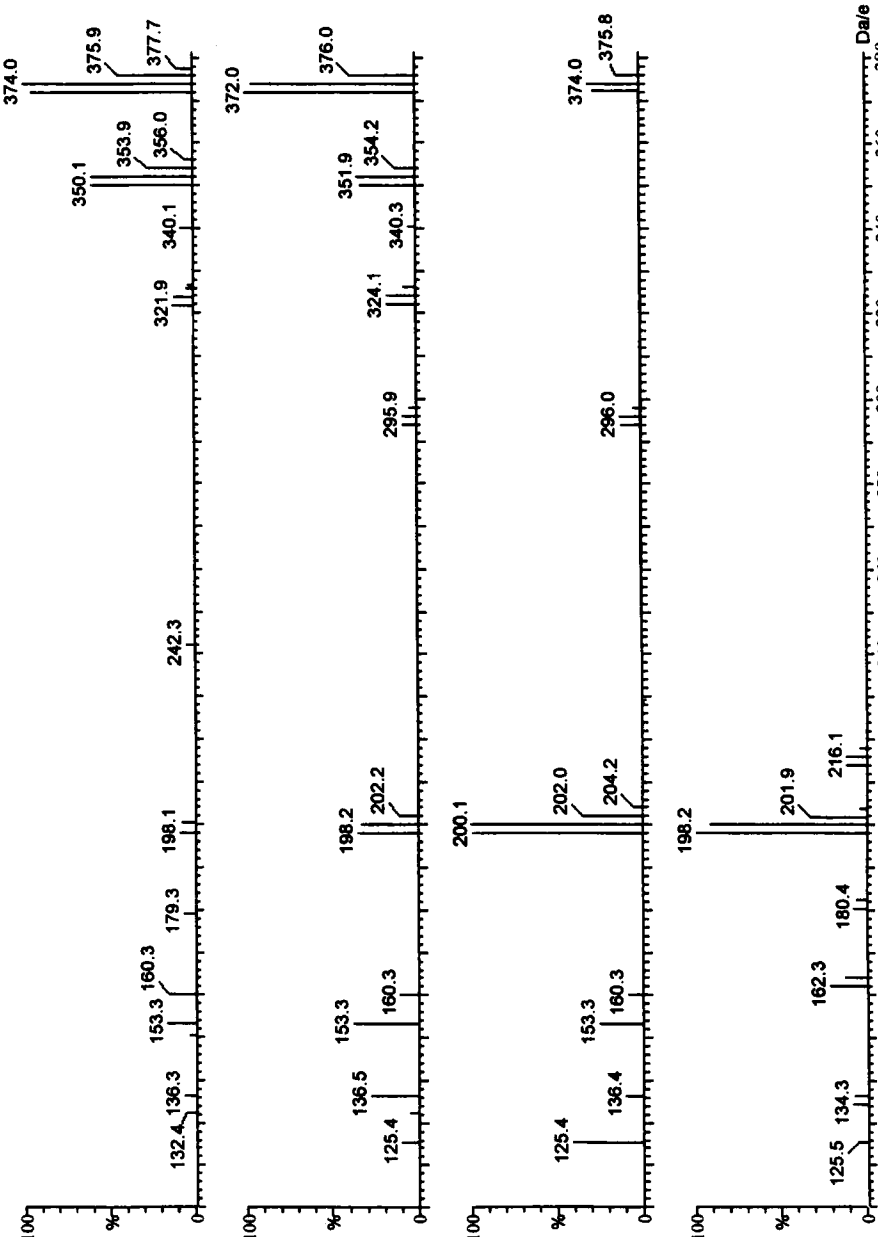


Figure 8 Electrospray mass spectra of phosphorothioic acid O,O-diethyl-O-(3,5,6-trichloro-2-pyridinyl) ester or chlorpyrifos 3 recorded with cone voltages of (a) 50 Volts, (b) 60 Volts, (c) 75 Volts and (d) 100 Volts.

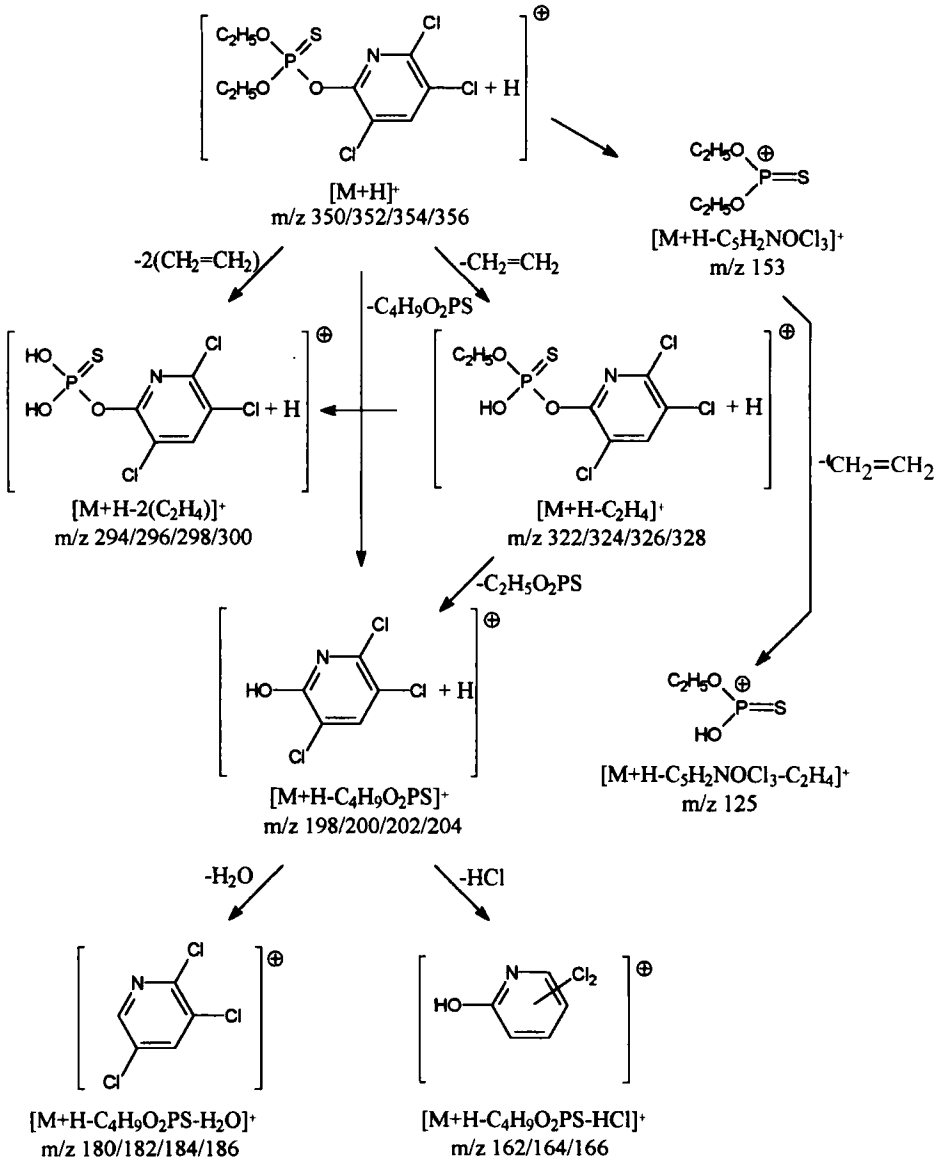


Figure 9 Major fragmentation routes of the $[M+H]^+$ ion of chlorpyrifos 3.

$[M+H-C_4H_9O_2PS-HCl]^+$ at m/z 180 and 162 respectively. The protonated molecular ion can lose a molecule of 3,5,6-trichloro-2-pyridine to afford the ion $[M+H-C_5H_2NOCl_3]^+$ at m/z 153. This latter ion loses a molecule of ethylene to afford the ion $[M+H-C_5H_2NOCl_3-C_2H_4]^+$ at m/z 125. Similar fragment ions were observed from the isotopic $[M+H]^+$ protonated molecular ions containing the different permutations of ^{35}Cl and ^{37}Cl isotopes. CAD MS/MS of the protonated molecular ion $[M+H]^+$ at m/z 350 afforded the product ions at m/z 322, 294, 198, 153, and 125. (Figure 10). Second generation product ions of the fragment ion at m/z 198, which was assigned as $[M+H-C_4H_9O_2PS]^+$ were generated in an MS/MS experiment and afforded the product ions at m/z 180 and 162.

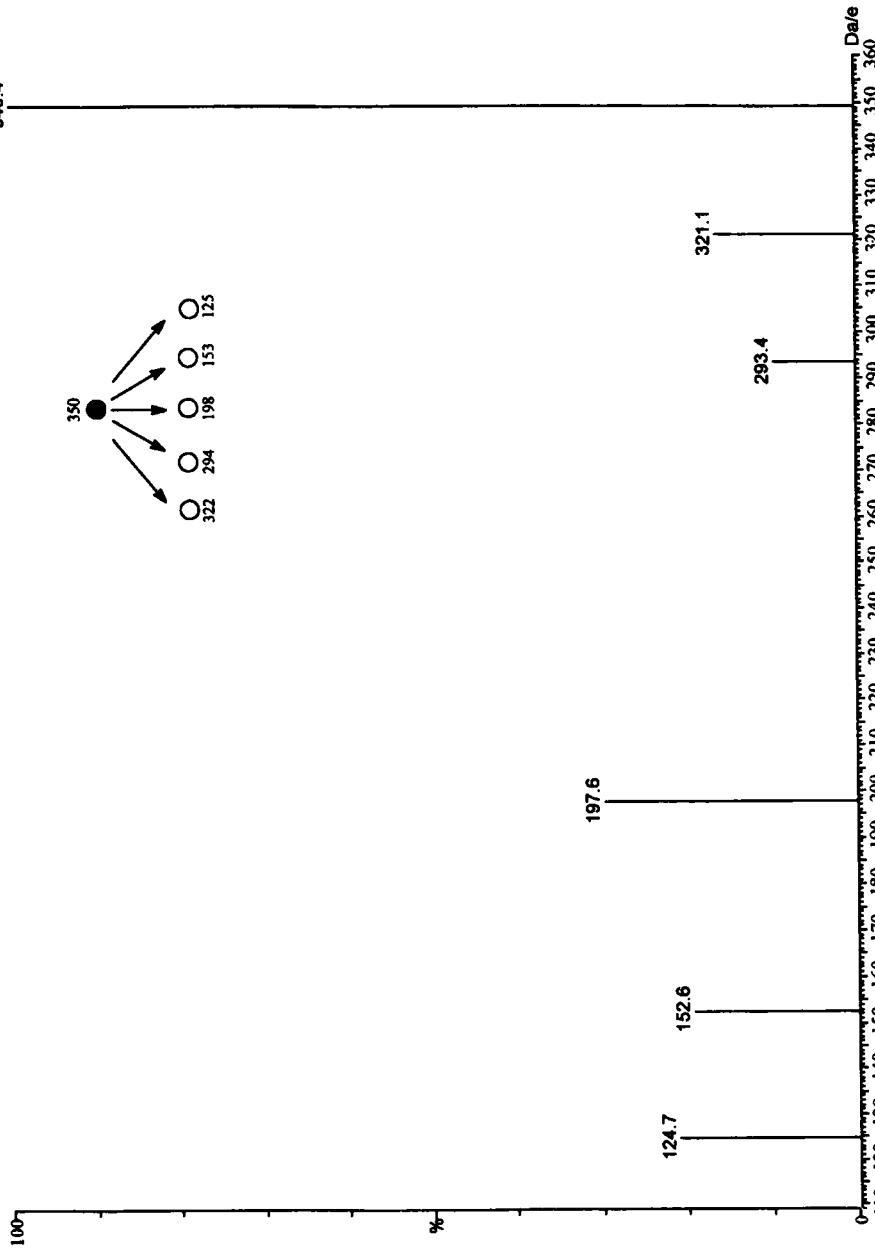


Figure 10 Low energy CAD MS/MS of the $[M+H]^+$ ion of chlorpyrifos 3.

The electrospray mass spectra of phosphorodithioic acid *O,O*-diethyl-S-[(4-oxo-1,2,3-benzotriazin-3(4H)-yl)methyl] ester or azinphos-ethyl 4 (Figure 1) were recorded with cone voltages of 50, 60, 75 and 100 volts respectively and are shown in Figure 11. The ES mass spectra recorded with voltages of 50 and 60 volts showed the protonated molecules $[M+H]^+$ at m/z 346, we also noticed the sodiated adduct $[M+Na]^+$ at m/z 368, an abundant major ion at m/z 160 (the base peak) and an ion at m/z 132. The ESMS recorded at 75 V showed that the ion at m/z 132 became the "base peak", whereas there was a considerable decrease in the relative intensity of the ion at m/z 160. Also, for this voltage there was an apparent increase in the relative intensities of the ions at m/z 105 and 78. For the ES mass spectra recorded at 100 V we noticed another change in the relative intensities of the ions. In this case the "base peak" was the ion at m/z 77.

The fragmentation route of the protonated molecular ion obtained for the various cone voltages has been tentatively rationalized and is presented in Figure 12. Thus, the protonated molecular ion $[M+H]^+$ at m/z 346 loses a molecule of methyl isocyanate by consecutive opening of the triazine ring to afford the fragment ion $[M+H\text{-}CH_3NCO]^+$ at m/z 289. The loss of a molecule of $C_4H_{11}O_2PS_2$ from the protonated molecule affords the fragment ion $[M+H\text{-}C_4H_{11}O_2PS_2]^+$ at m/z 160. This latter ion loses a molecule of carbon monoxide by contraction of the triazine ring to afford the $[M+H\text{-}C_4H_{11}O_2PS_2\text{-}CO]^+$ ion at m/z 132. The ion at m/z 160 can lose a molecule of HCN once more by the opening of the five membered ring to afford the $[M+H\text{-}C_4H_{11}O_2PS_2\text{-}CO\text{-}HCN]^+$ at m/z 105, which in turn loses a nitrogen molecule to afford the fragment ion $[M+H\text{-}C_4H_{11}O_2PS_2\text{-}CO\text{-}HCN\text{-}N_2]^+$ at m/z 77. In a different set of experiments, the precursors of the fragment ions $[M+H\text{-}C_4H_{11}O_2PS_2]^+$ at m/z 160 and $[M+H\text{-}C_4H_{11}O_2PS_2\text{-}CO]^+$ at m/z 132 were sought using the precursor ion scan technique. It was established that the ion at m/z 160 originated from the protonated molecule, whereas the ion at m/z 132 could be formed either from the protonated molecule or from the ion at m/z 160 by concerted losses of a molecule of $C_4H_{11}O_2PS_2$ and a molecule of carbon monoxide, or *vice versa* (Figure 13).

The electrospray mass spectra of phosphorodithioic acid *O,O*-dimethyl-S-[(4-oxo-1,2,3-benzotriazin-3(4H)-yl)methyl) ester or azinphos-methyl 5 (Figure 1) were recorded with cone voltages of 50 and 75 Volts and are shown in Figure 14. The fragmentation route of the protonated molecule $[M+H]^+$ is tentatively depicted in Figure 15. The loss of a molecule of $(CH_3O)_2PSH$ from the protonated molecule affords the $[M+H\text{-}C_2H_7O_2PS_2]^+$ ion at m/z 160. This latter ion loses a molecule of carbon monoxide with ring contraction to afford the $[M+H\text{-}C_2H_7O_2PS_2\text{-}CO]^+$ ion at m/z 132. The triazine ring opening of the protonated molecule followed by the loss of a molecule of methyl isocyanate affords the $[M+H\text{-}CH_3NCO]^+$ ion at m/z 261. CAD MS/MS of the $[M+H]^+$ ion afforded the product ions at m/z 261, 160 and 132. Precursor ion scan of the ion at m/z 160 indicated that it originated from the protonated molecule at m/z 318, whereas the precursor ion scan of the ion at m/z 132 indicated that it was formed exclusively from the ion at m/z 160.

The electrospray mass spectra of phosphorodithioic acid *O,O*-dimethyl-S-[(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)methyl] ester or phosmet 6 (Figure 1), isomeric with azinphos-methyl 5, were recorded with cone voltages of 50 and 75 Volts and are shown in Figure 16. In the ESMS of phosphorodithioate 6, recorded with a cone voltage of 50 V, we noticed the protonated molecular ion $[M+H]^+$ at m/z 318 and the sodiated adduct $[M+Na]^+$ at m/z 340, a fragment ion at m/z 230, which was assigned as $[M+H\text{-}CH_3CNO\text{-}CH_2O]^+$ and the "base peak" ion at m/z 160 assigned as $[M+H\text{-}C_2H_7O_2PS_2]^+$. The ES mass spectra, recorded with a cone voltage of 75 V, showed only the $[M+Na]^+$ ion at m/z 340 and the "base peak" ion at m/z 160. The fragmentation route of the protonated molecule $[M+H]^+$ of phosmet is tentatively depicted in Figure 17. Thus, the loss of a $(CH_3O)_2PSH$ molecule from the molecular ion produces the phthalimidomethylene ion [M+H-

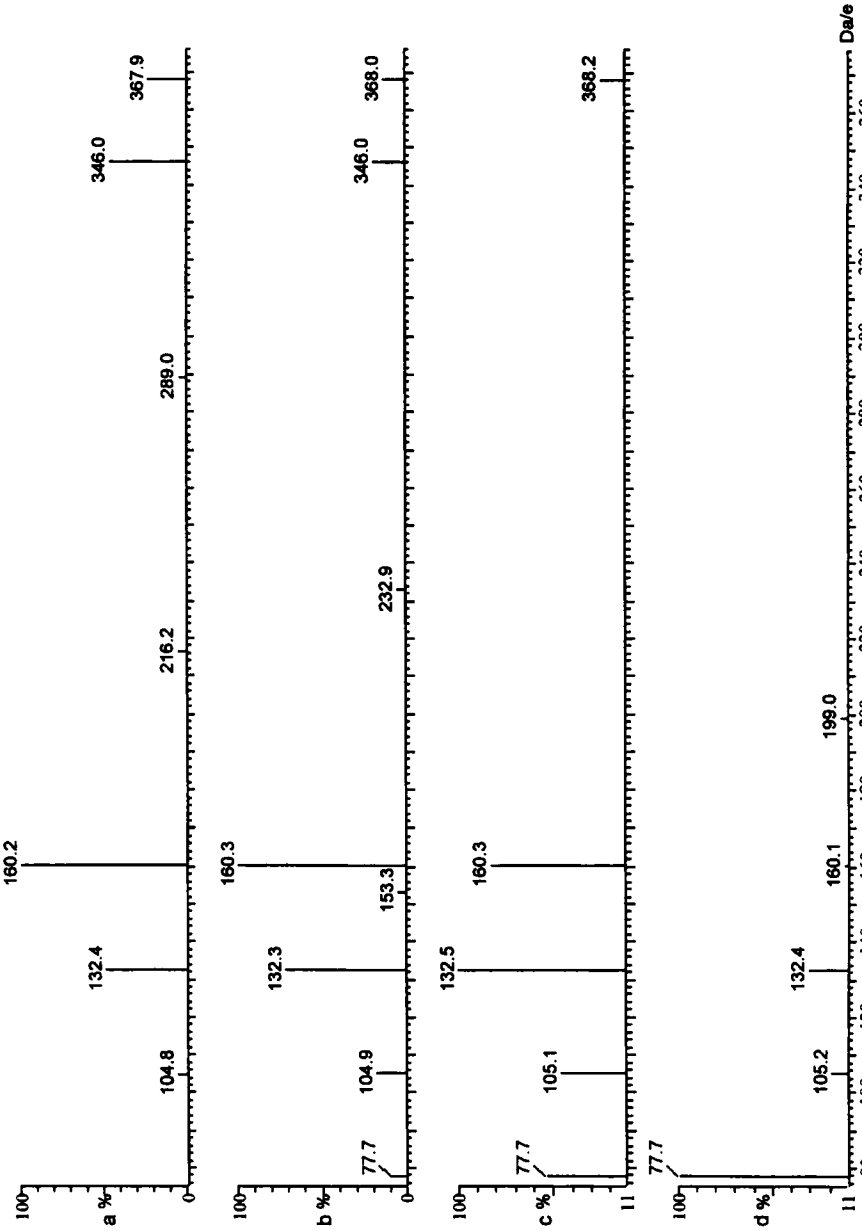


Figure 11 Electrospray mass spectra of phosphorodithioic acid O,O-diethyl-S-[(4-oxo-1,2,3-benzotriazin-3(4H)-yl)methyl] ester or azinphos-ethyl 4 recorded with cone voltages of (a) 50 Volts, (b) 60 Volts, (c) 75 Volts and (d) 100 Volts.

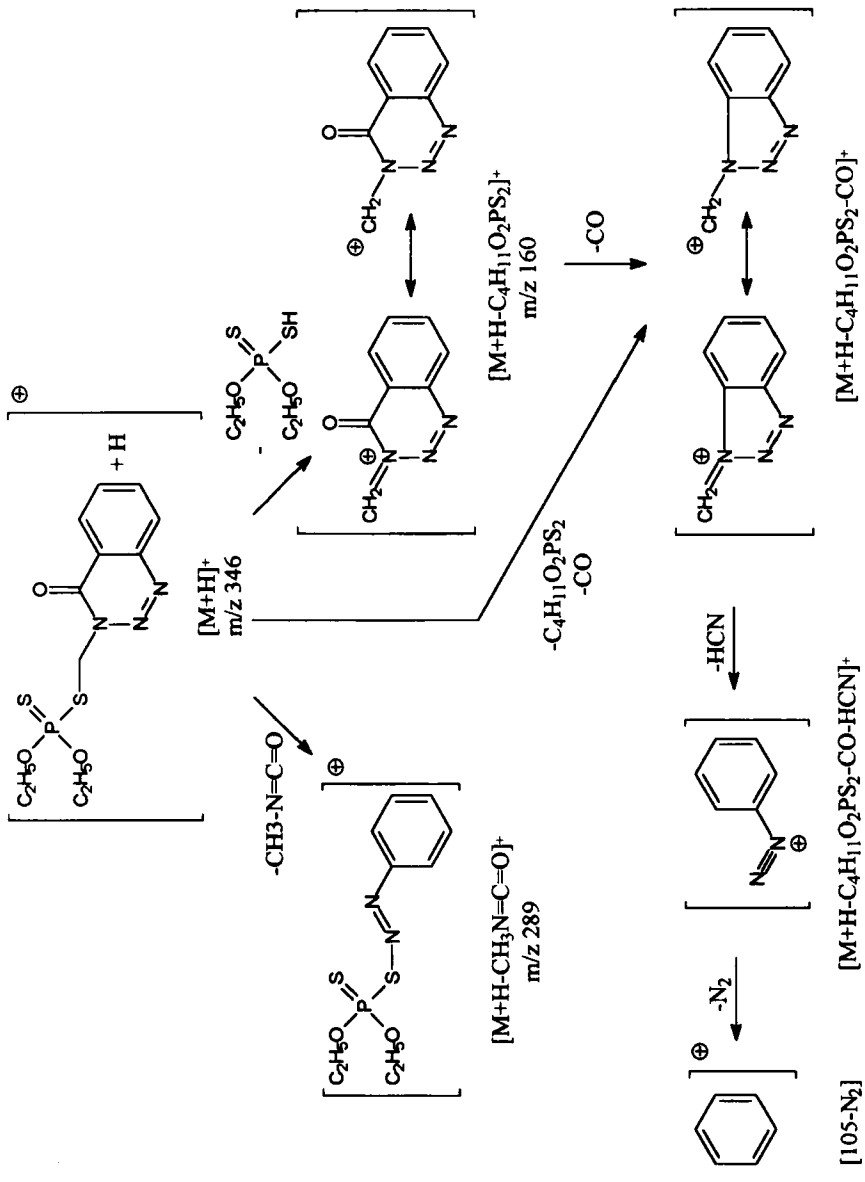


Figure 12 Major fragmentation routes of the $[M+H]^+$ ion of azinphos-ethyl 4.

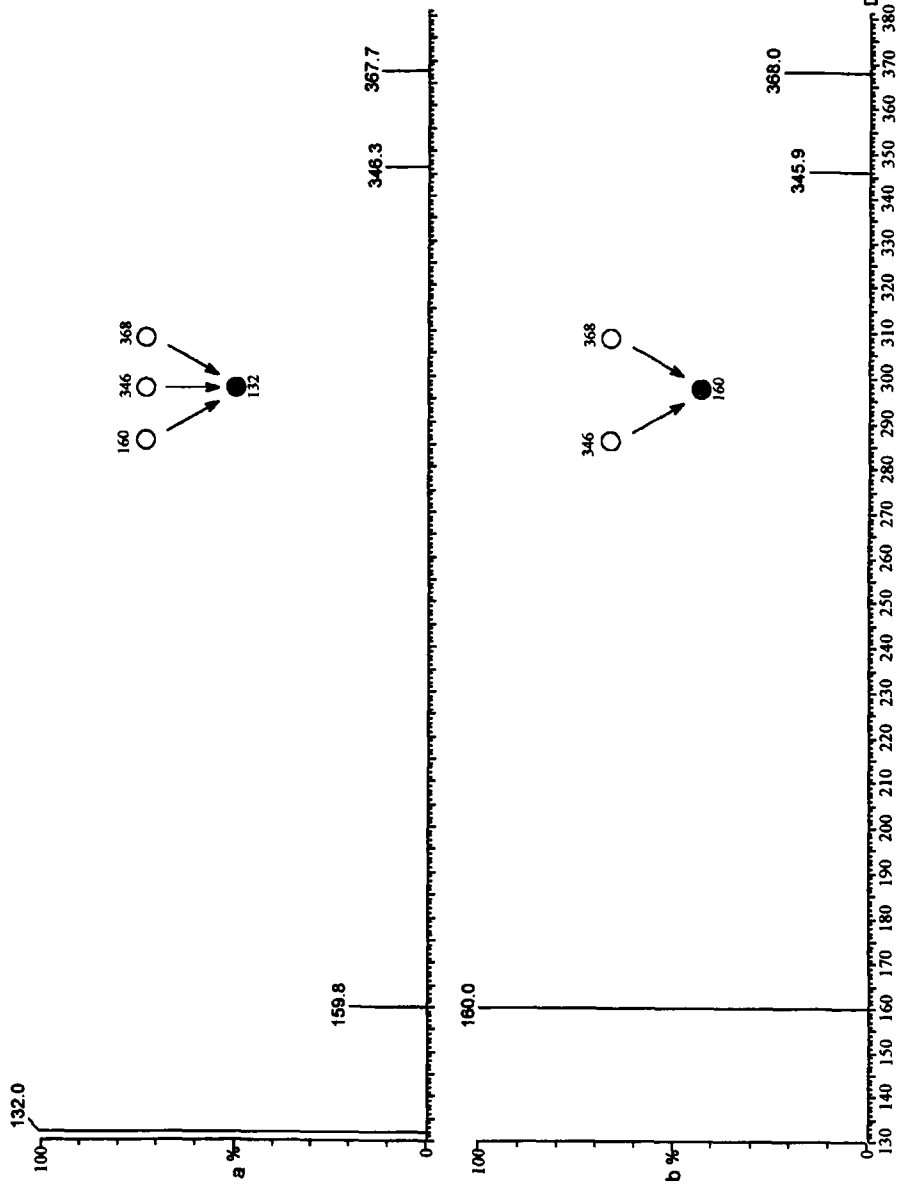


Figure 13 Precursor ion scan of (a) the fragment ion $[M+H\text{-}C_4H_{11}O_2PS_2\text{-}CO]^+$ at *m/z* 132 and (b) the fragment ion $[M+H\text{-}C_4H_{11}O_2PS_2]^+$ at m/z 160 from azinphos-ethyl 4.

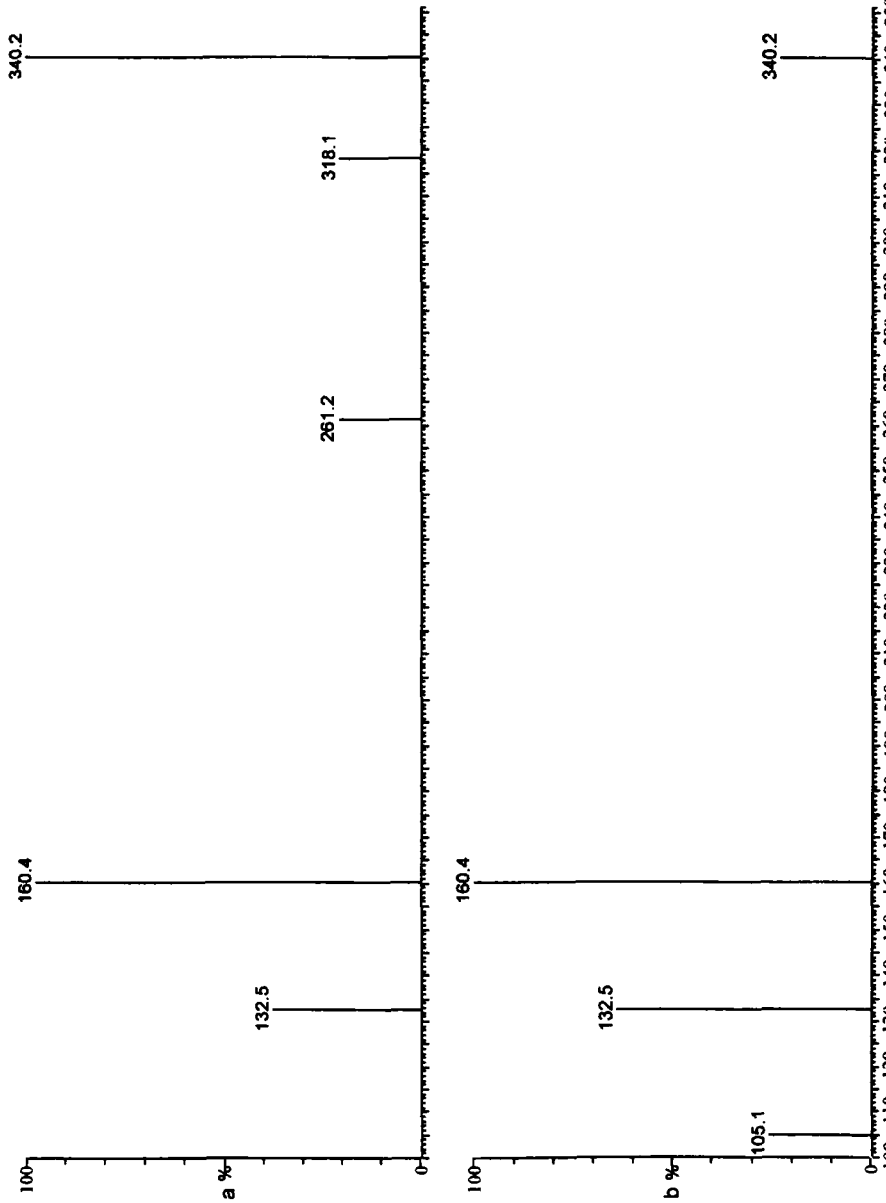


Figure 14 Electrospray mass spectra of pbosphorodithioic acid *O,O*-dimethyl-S-[(4-oxo-1,2,3-benzotriazin-3(4H)-yl)methyl] ester or azinphos-methyl 5 recorded with cone voltages of (a) 50 Volts and (b) 75 Volts.

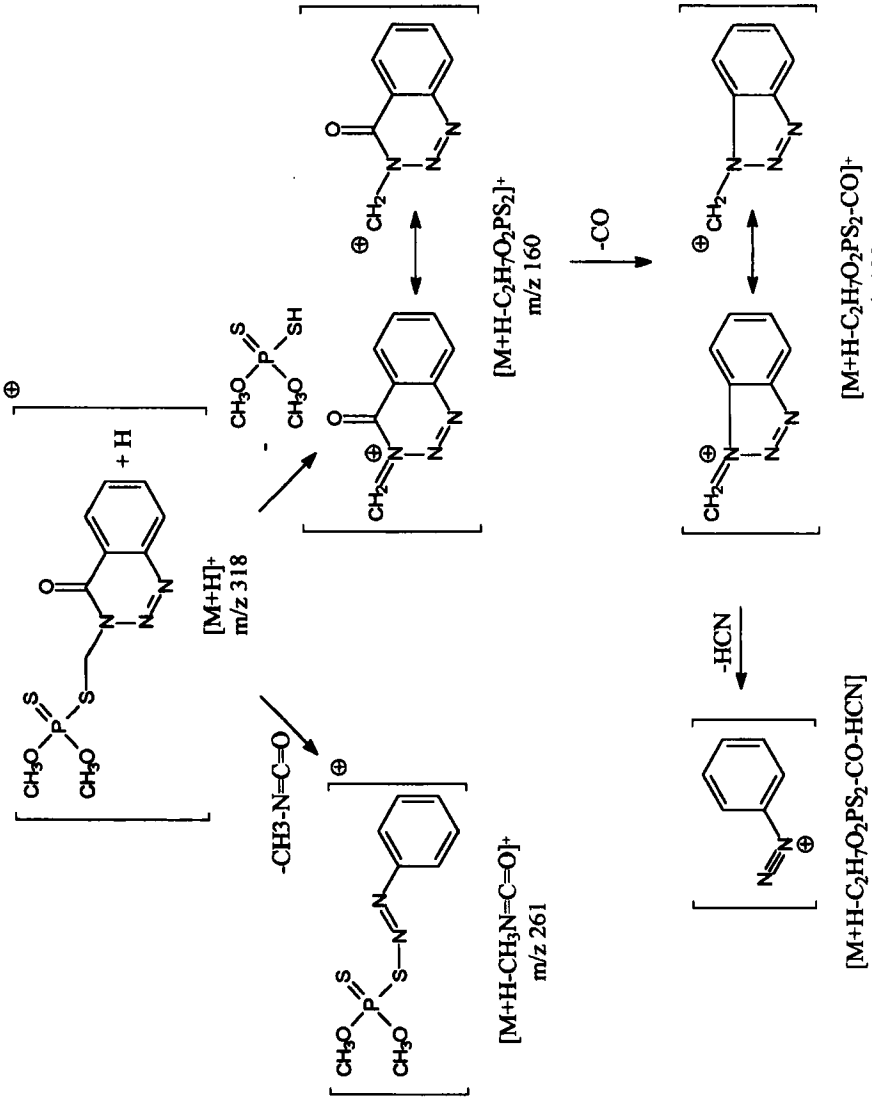


Figure 15 Major fragmentation routes of the $[M+H]^+$ ion of azinphos-methyl 5.

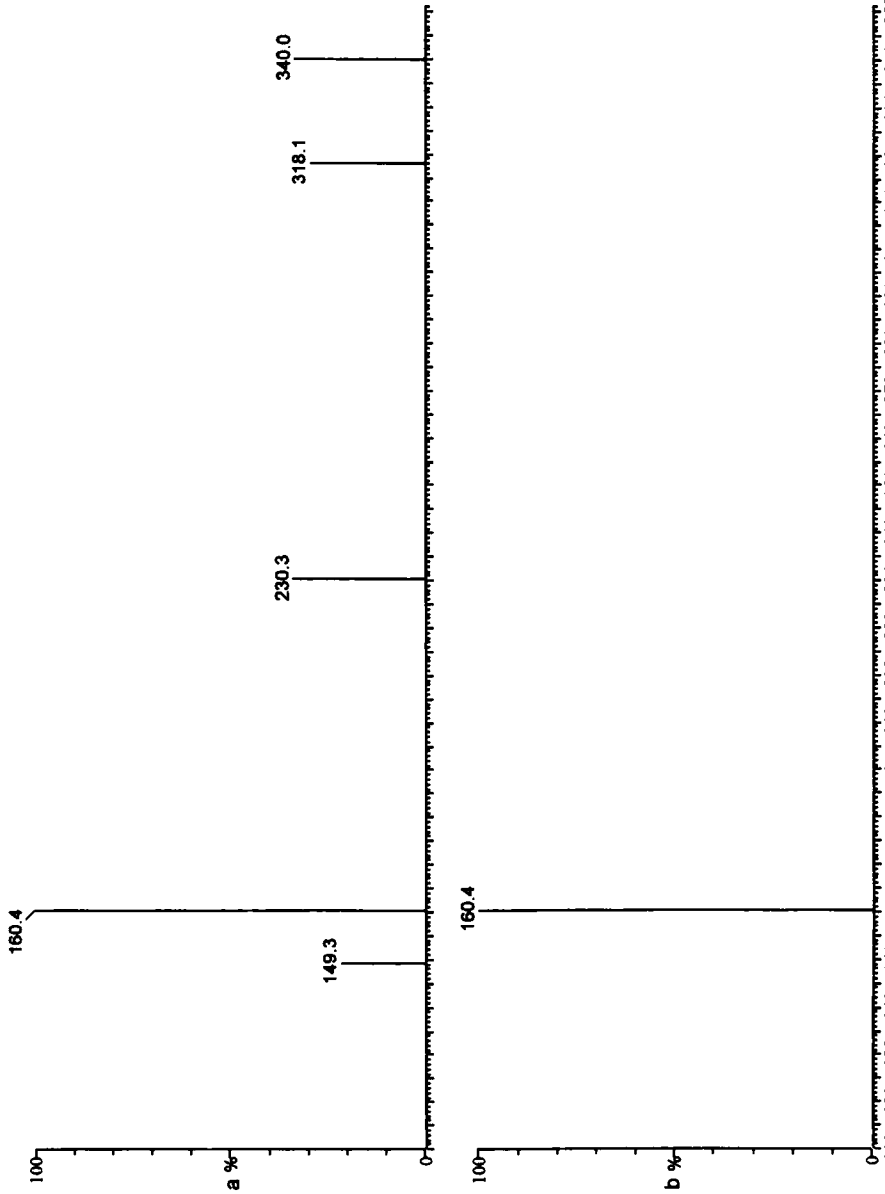


Figure 16 Electrospray mass spectra of phosphorodithioic acid O,O-dimethyl-S-[(1,3-dihydro-1.3-dioxo-2H-isoindol-2-yl)methyl] ester or phosmet 6 recorded with cone voltages of (a) 50 Volts and (b) 75 Volts.

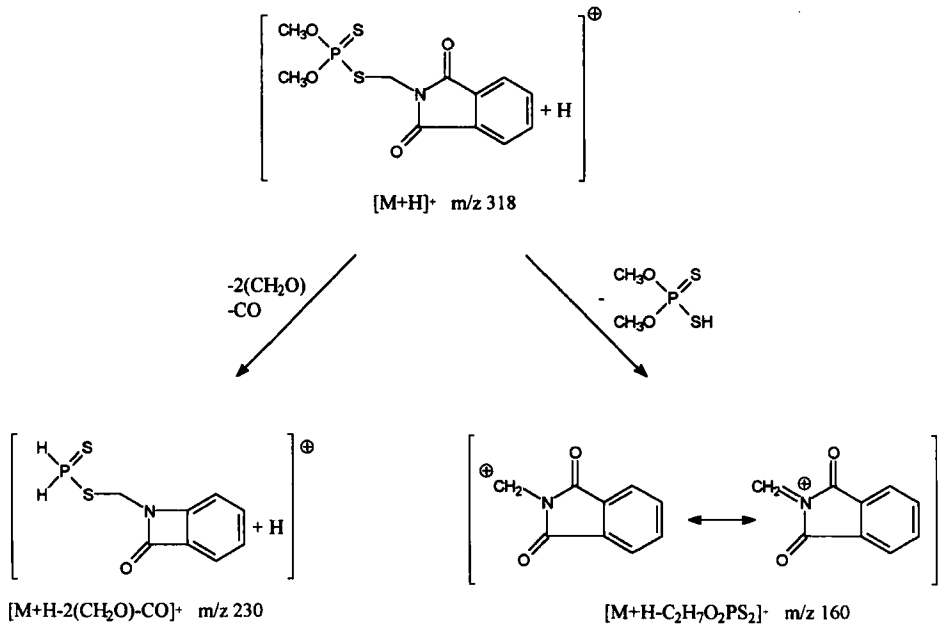


Figure 17 Major fragmentation routes of the $[M+H]^+$ ion of phosmet 6.

$C_2H_7O_2PS_2]^+$ at m/z 160 (base peak). The concerted loss of molecules of methyl isocyanate and formaldehyde resulting from the opening of the phthalimido ring of the protonated molecule produces the $[M+H-CH_3NCO-CH_2O]^+$ ion at m/z 230. CAD MS/MS of the $[M+H]^+$ ion afforded the product ions at m/z 230 and 160.

A quick perusal of the fragmentation patterns of the isomeric phosphorodithioates 5 and 6 (Figures 15 and 17) and of their respective ES mass spectra (Figures 14 and 16), will show that it is possible to distinguish between substantially different diagnostic ions. Indeed, we noticed the diagnostic ions for azinphos-methyl 5 at m/z 261 and 132 representing $[M+H-CH_3NCO]^+$ and $[M+H-C_2H_7O_2PS_2-CO]^+$, respectively and the diagnostic ion $[M+H-CH_3NCO-CH_2O]^+$ at m/z 230 for phosmet 6.

The Department of Fisheries and Oceans, under the umbrella of the "Green Plan", maintains a surveillance program for pesticide residues in local lakes and sediments. We have initiated this electrospray MS/MS study of organophosphorus pesticides to determine its potential applicability to the analysis of these pesticide residues. ES mass spectrometry has the potential to reduce the time required for these analyses and to extend the capability of existing methodologies to include this state-of-the-art technology. The following applications typify the uses of ES mass spectrometry in residue analysis to considerably reduce the clean-up required. The phosphorothiates 1–3 and phosphorodithioates 4–6 were dissolved in water in various concentrations and 20 µl of the solution were injected directly without any further purification, extraction or concentration, into the electrospray ionization source of the mass spectrometer. The electrospray mass spectrum was recorded with various cone voltages using a full scan procedure. Each compound of the mixture has been individually identified with certainty by its fragmentation pattern obtained either by cone voltage fragmentation or collision-activated dissociation (CAD) using product ions and precursor scans. The

detection/identification threshold was 0.1 ppm (approximately 0.3 μM, 6 pmoles injected) for compounds 2–6 and 10 ppb (33 nM, 0.66 pmoles injected) for compound 1. Please note that these detection/identification limits are specific to the ES tandem mass spectrometer and that, for water samples from polluted lakes, detection limits, can always be increased by various preconcentration methods such as liquid-liquid extraction (LLE), dynamic and static head-space analysis, solid-phase extraction or membrane processes[36–39]. Confirmation of the identity of this mixture of pesticides was achieved for pesticides 1–6 in the following manner: the ions at m/z 200 and 198 are diagnostic for chlorpyrifos 3; the ions at m/z 169 and 153 are diagnostic for diazinon 1; the ion at m/z 125 is diagnostic for chlorpyrifos-methyl 2 and the ion at m/z 289 is diagnostic for azinphos-ethyl 4. For the two isomeric dithioates azinphos-methyl 5 and phosmet 6 a common diagnostic ion is shared at m/z 160. However, the ions at m/z 261, 132 and 105 permit the discrimination and positive identification of azinphos-methyl 5 whereas the ion at m/z 230 is specific for phosmet 6.

Similar results were obtained from water samples from a local lake (Quidi Vidi,), spiked with the previous organophosphate pesticides at the same concentrations and filtered through a micropore membrane before initial injection in the electrospray ionization source. The same procedure could be paralleled for analysis of these pesticides in other matrices. Indeed, it is conceivable that an aliquot of the solution resulting from a simple extraction of solid matrix could be injected into the electrospray source and that the pesticides could be measured directly.

CONCLUSION

Although most official methods for pesticide analysis in water still use liquid-liquid extraction[40], these methods show some disadvantages : they are laborious, time consuming, expensive and are subject to problems arising from the formation of emulsions, the evaporation of large solvent volumes, and the disposal of toxic or inflammable solvents. As a consequence, the absolute compatibility of ES mass spectrometry with the detection of the organophosphorus pesticides, directly from solution and without either derivatization or further purification, provides a valuable method for the qualitative and quantitative analysis of these toxic chemicals.

Mass spectral analysis of the organophosphorus pesticides has been facilitated using electrospray ionization. Abundant signals corresponding to the protonated organophosphorus pesticide molecules were observed in all cases using this ionization technique. Collision-activated dissociations in the atmospheric pressure/vacuum interface were promoted by increasing the cone voltages and generated a wealth of structural information on the dissociation of precursor ions. It should be pointed out that ES mass spectra obtained by changing the cone fragmentation voltages did not allow the establishment of the different origins and fates of the fragment ions being studied. However, it did allow differentiation between the isomeric phosphorodithioates 5 and 6 by giving different fragment ions.

MS/MS spectra obtained using low energy collisional activation permitted the rationalization of the fragmentation pathways. Furthermore, product ion and precursor ion MS/MS spectra of selected intermediate ions formed during cone voltage fragmentation of the ionized species, allowed rationalization of the fragmentation behaviour.

ES spectra and ES MS/MS spectra of organophosphorus pesticides were shown to be valuable and very sensitive methods for the detection of the phosphorothioates and

phosphorodithioates which were characterized with certainty individually as well as in a mixture. This last aspect was of particular importance for the detection of these pesticide residues directly from lakes[41].

Acknowledgments

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