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Use of ion chromatography–electrospray mass spectrometry for the determination of ionic compounds in agricultural chemicals

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Abstract

This paper reports the use of ion chromatography–electrospray mass spectrometry for the separation and structure elucidation of anionic compounds in a complex organophosphate matrix. Conventional HPLC with ion pairing reagents, pH adjustments or use of buffer solutions have limited compatibility with mass spectrometry, as the ion pairing reagents or buffers have to be volatile to be introduced into the mass spectrometer. The choice of volatile additives is limited, resulting in poor chromatography. This paper demonstrates the use of ion-exchange chromatography (IC) for the separation of ionic compounds, followed by mass spectrometry (MS) for the structure elucidation of unknowns. The anionic impurities are separated using an anion-exchange column with aqueous sodium hydroxide as the eluent. Electrospray in the negative ion mode is used to obtain the mass spectra. The elemental composition of an unknown component in the sample is determined by high-resolution mass spectrometry. The coupling of IC to MS provides a new analytical tool to chemists faced with the challenge of separating and analyzing ionic compounds in complex matrices.

Keywords: Pesticides

1. Introduction

The separation of ionic compounds in complex matrices, followed by structure elucidation using mass spectrometry is a challenging task. The use of conventional HPLC with ion pairing, pH adjustment or use of buffers has limited application, as the mobile phase additives have to be volatile to be compatible with mass spectrometry. The choice of volatile additives is limited resulting in poor peak shape and limited retention.

Ion-exchange chromatography provides an alternative and efficient way to separate ionic compounds. A wide range of high-capacity and organic solvent-compatible columns are available. The drawback of using ion-exchange chromatography (IC) with mass spectrometry is the incompatibility of the mobile phase. To circumvent this problem, an online desalting device — a suppressor, can be incorporated after the IC column, to remove salts from the mobile solvent before it enters the mass spectrometer [1].

In spite of the demonstrated usefulness of this technique, there have not been many publications on the combination of IC and MS. Some of the first studies that showed the successful coupling of IC to MS used the thermospray, ion spray and the particle beam interfaces [2–5]. Recent investigations demonstrate the usefulness of coupling IC to MS using the

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electrospray interface for the determination of inorganic compounds [6] and for the analysis of polar organic compounds in water [7].

In a previous publication, we have demonstrated the use of IC–MS for the analysis of ionic compounds using the thermospray and the electrospray interfaces [8]. This paper further establishes the use of IC–MS for the identification and structure elucidation of ionic compounds in complex matrices such as agricultural chemicals.

2. Experimental

2.1. The mass spectrometer

The mass spectrometer is a VG (Micromass) Autospec-Q (M-series) with electrospray option. It is a high resolution double focusing instrument with a geometry of the type EBEQQ. The molecular mass range at full accelerating voltage (8 kV) is 4500. The data system is a Vax 4000 (90), operating on VMS 5.5 and using Opus 3.6.

2.2. The IC system

IC separations were performed on a Dionex ion chromatograph model 4000i, equipped with an anion-exchange IonPac AS11 analytical column, an IonPac AG11 guard column and a conductivity detector. The AS11 anion-exchange column is specially designed for using hydroxide eluent systems. It is stable between pH 0 and pH 14 and is compatible with eluents containing 0–100% organic solvents. Hydroxide gradient elution was used with the hydroxide concentration increasing from 4 m*M* sodium hydroxide to 36 m*M* sodium hydroxide in 15 min. The eluent flow-rate was 1 ml/min.

Two kinds of suppressors were used for these analyses, an Alltech electrochemically regenerated ion suppressor (ERIS) 1000 HP and the ASRS-I self-regenerating suppressor from Dionex.

The ERIS consist of two cells, packed with a strong cation exchange resin in the hydrogen form for anion suppression or with an anion-exchange resin in the hydroxide form for cation suppression. The ions from the mobile solvent are exchanged with hydrogen ions or hydroxide ions in the cell to form water which goes to the detector. The analytes are converted to their acid or base forms and can be detected using a conductivity detector. A 10-port valve is used to switch mobile solvent between the two cells. While one cell is used to suppress the mobile phase, the other cell is electrochemically regenerated to produce the hydrogen or the hydroxide form of the resin.

The ASRS-1 is a membrane-based device that uses the electrolysis of water to produce the hydronium and the hydroxide ions required to neutralize the eluent.

The Alltech suppressor is easy to use and rugged but has limited capacity for ion-exchange. For mobile solvents containing more than 10 m*M* NaOH, a Dionex ASRS-I self-regenerating suppressor is used. The suppressor is installed between the analytical column and the detector.

The output of the conductivity detector is connected to the mass spectrometer to simultaneously record responses from the conductivity detector and the mass spectrometer. A schematic diagram of the IC–MS system is shown in a previous paper [8].

2.3. Experimental setup for electrospray

The flow-rates generally used for electrospray are in the 10–50 μl/min range. Because of this requirement, effluent from the suppressor was split 99:1 and mixed with an equal amount of acetonitrile–water (90:10) containing 0.5% ammonium hydroxide, before it entered the electrospray probe unit. The electrospray was operated in the negative ion mode. A syringe pump was used to deliver acetonitrile–water containing ammonium hydroxide. Ammonium hydroxide increases the pH of the mobile solvent and assists in negative ion formation. Calibration compounds were also introduced into the mass spectrometer using the syringe pump. The region between the sampling cone and the skimmer is an intermediate pressure region in the electrospray source. Ions with sufficient energy in this region can undergo collision-induced dissociation (CID), resulting in fragment ions. The voltage difference between the sampling cone and the skimmer was increased to assist in fragmentation of the $[M–H]^-$ ions. A scan range of m/z 50–600, with a scan time of 1.5 s/decade was used.

3. Results and discussion

3.1. Mass spectrometric determination of ionic compounds

Fig. 1 shows the chromatogram resulting from injecting a sample of a 0.5% solution of an organophosphate insecticide, using conductivity detection. The chromatogram shows the complex nature of the organophosphate matrix. Without the use of IC, It would not have been possible to separate and analyze the different ionic compounds present in the sample.

CID was used to fragment the molecular ion, in order to obtain mass spectral information. The voltage difference between the sampling cone and the skimmer was varied to a maximum difference of 200 V, to control the degree of fragmentation of the $[M–H]^-$ ion. Fig. 2 shows the resulting mass spectra. The mass spectrum of methyl sulfate in the top box in Fig. 2, shows mainly the $[M–H]^-$ ion, whereas the bottom box shows the $[M–H]^-$ ion as well as the fragment ions resulting from the dissociation of the molecular ion. The mass spectra of the different components in the sample were similarly recorded by using collision-induced dissociation.

The ion chromatograms resulting from the molecular ion, $[M–H]^-$, of some of the compounds identified in the sample are shown in separate boxes in Fig. 3. Boxes are labeled with the $[M–H]^-$ ion as well as the identity of the compound.

The first box in Fig. 3. shows the ion chromatogram due to m/z 111. The $[M–H]^-$ ions resulting from methyl sulfate and methyl phosphate have the same m/z of 111, yet their mass spectra are different. Methyl sulfate is clearly identified by its fragmentation pattern, as compared to the mass spectrum of methyl phosphate shown in Fig. 4.

The mass spectrum of methyl sulfate clearly shows the typical isotopic cluster resulting from the presence of sulfur in this compound. The isotopic cluster around m/z 111 for methyl phosphate, is consistent with lack of sulfur in this compound.

Two other compounds identified in the sample are *S*-methyl phosphoramidothioate and *O*,*S*-dimethyl phosphorothioate. The structures of these compounds are shown in Figs. 5 and 6 along with their mass spectra.

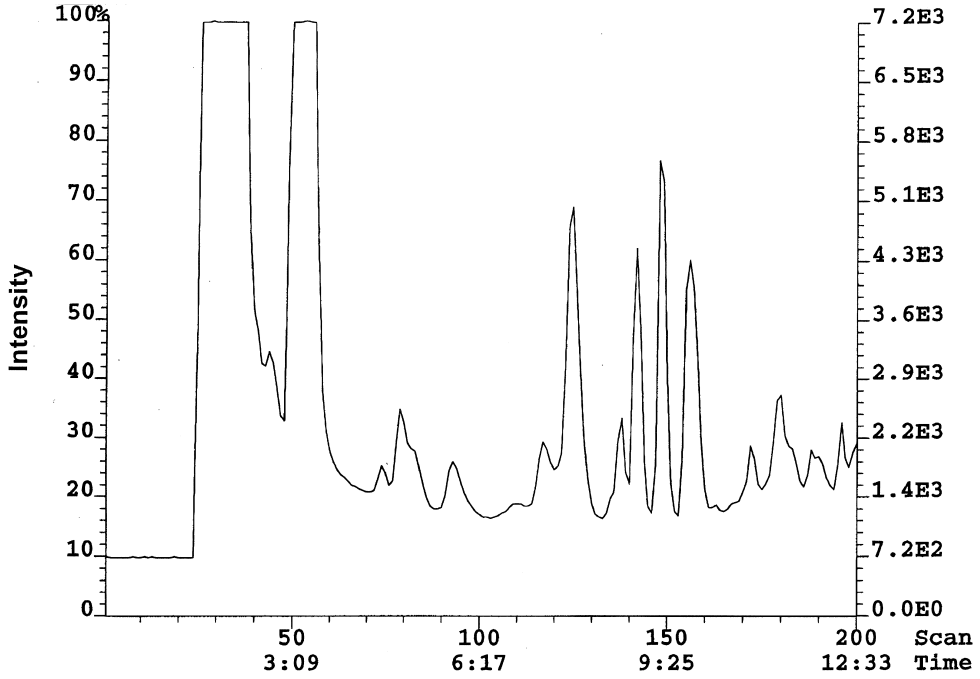

Fig. 1. IC trace of impurities in sample.

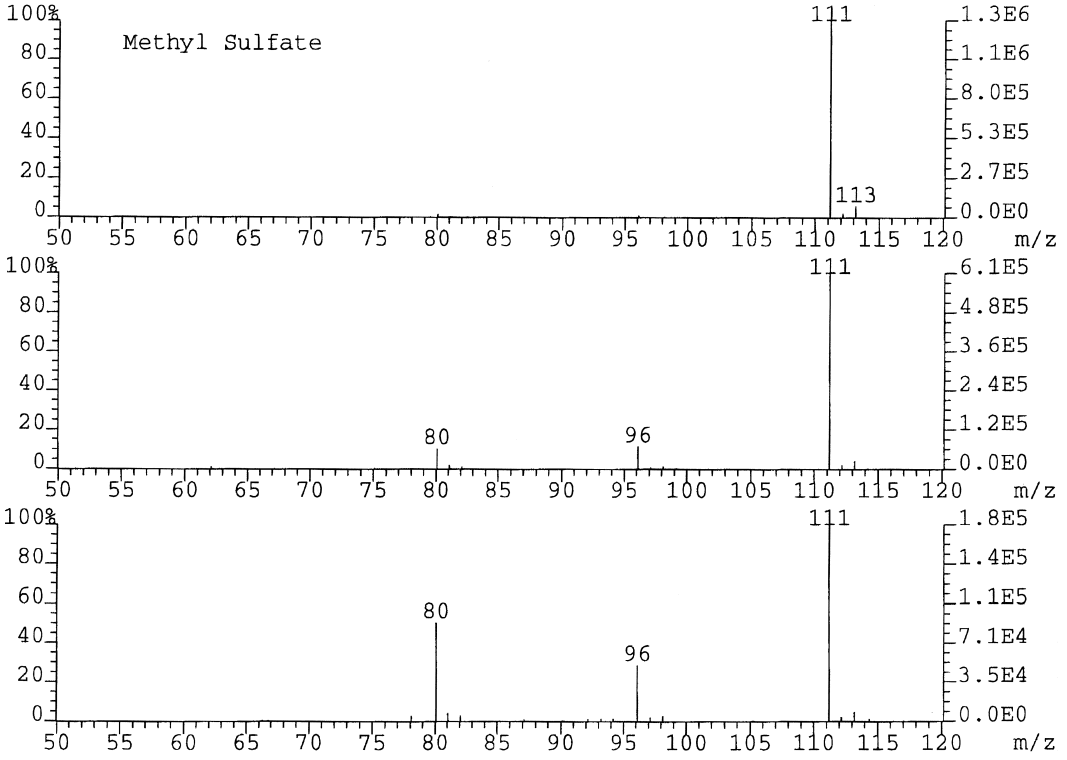

Fig. 2. Mass spectrum of methyl sulfate showing increase in fragmentation of m/z 111 due to CID.

The last box labeled m/z 343 in Fig. 3 shows a peak due to an unknown compound with a molecular weight of 344 ($[M-H]^-$ is 343). High-resolution MS was used to determine the elemental composition of this compound. This experimental procedure is described below.

3.2. High-resolution accurate mass determination

The elemental composition of m/z 343 was determined by high-resolution accurate mass measurement. Sucrose and oryzalin were used as internal reference compounds for the determination of the elemental composition of m/z 343. The structures of sucrose and oryzalin are shown in Fig. 7. Sucrose and oryzalin have molecular masses very close to and bracketing the molecular mass of m/z 343.

A reference file containing the accurate masses of the $[M-H]^-$ ion of sucrose and oryzalin was generated. The m/z of the $[M-H]^-$ ions of sucrose and oryzalin are 341.1084 and 345.0868, respectively. This reference file was used to identify the reference peaks in the scans. The reference peaks in each scan were identified and their expected position compared to their actual positions. This information was used to correct for errors in mass measurement. The mass of the unknown was calculated based on the mass of the reference peaks. The experiment was carried out at a resolution of 5000, in narrow range voltage scanning between m/z 340 and 350. The solution containing the reference compounds was continuously introduced into the mass spectrometer through the syringe pump

The elemental composition of m/z 343 was determined to be $C_4H_{13}N_2O_6P_3S_2$. The structure of the unknown is shown in Fig. 8 along with its mass spectrum.

A number of factors were used to predict the structure of the unknowns:

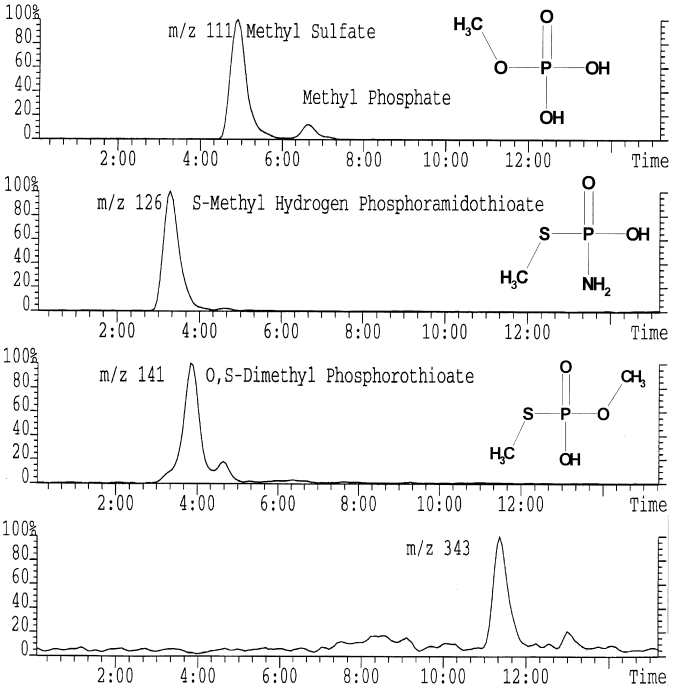

Fig. 3. Ion chromatogram of m/z 111, 126, 141 and 343.

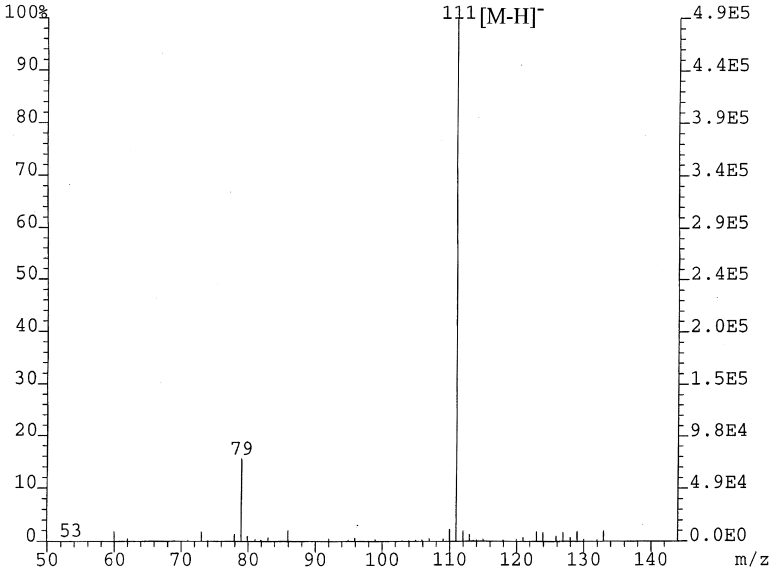

Fig. 4. Mass spectrum of methyl phosphate.

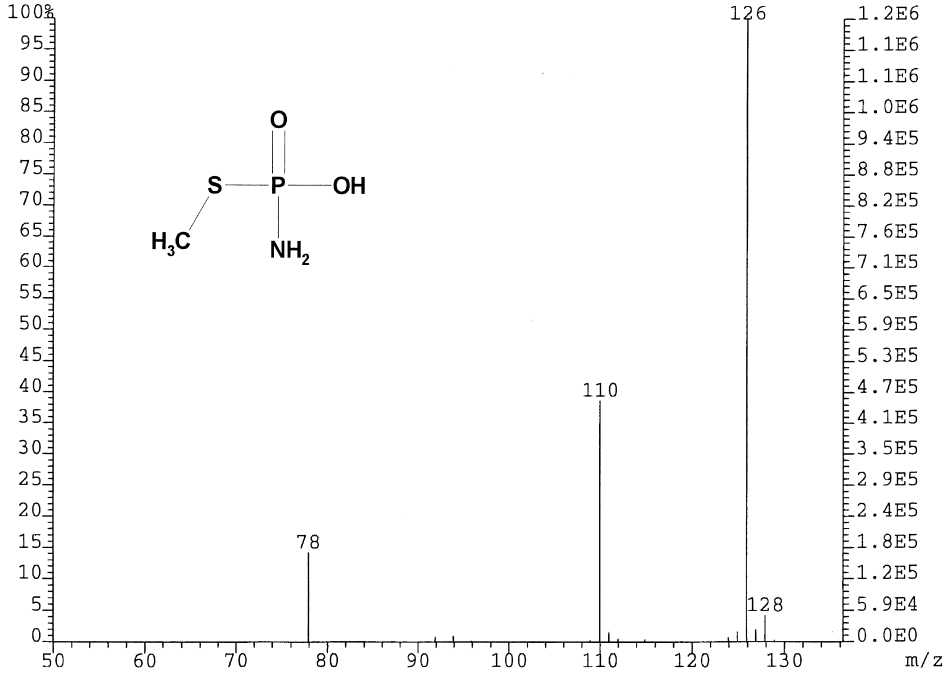

Fig. 5. Mass spectrum of *S*-methyl phosphoramidothioate.

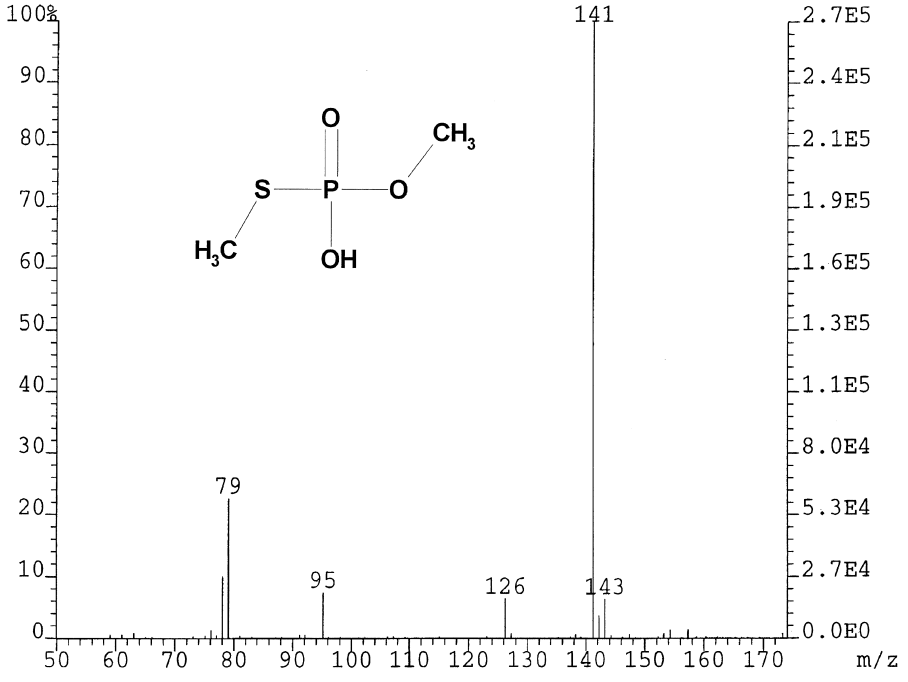

Fig. 6. Mass spectrum of *O*,*S*-dimethyl phosphorothioate.

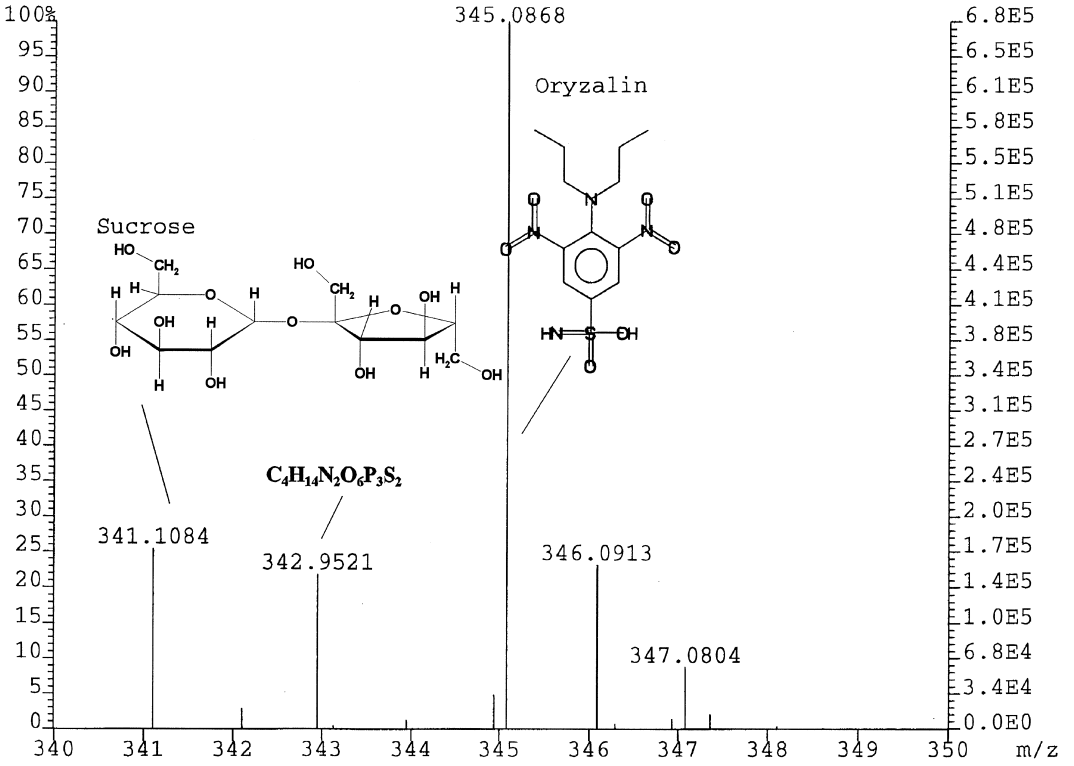

Fig. 7. High-resolution accurate mass determination of m/z 343.

(i) The retention time of the peak on the IC scan was used to estimate the number of ionizable protons present in the compound. In general, compounds with one ionizable proton elute early in the chromatogram, compared to compounds that have more than one ionizable proton. For example, dimethyl phosphate, with one ionizable proton, elutes at ~3 min, whereas phosphate elutes at ~13 min under the same conditions. Peaks eluting in between the retention times of dimethyl phosphate and phosphate were estimated to contain between one and three ionizable protons.

(ii) The formula molecular mass of the impurities gave an indication of whether the compound had an odd or an even number of nitrogen atoms.

(iii) Most peaks gave a fragmentation pattern that helped in proposing a structure.

(iv) The process chemistry of making the organophosphate eliminated a number of structural possibilities.

(v) The high-resolution accurate mass measurement provided elemental composition information.

4. Conclusions

Ion-exchange chromatography–electrospray mass spectrometry was successfully used for the separation and structure elucidation of ionic compounds in a complex matrix.

By changing the voltage between the sampling cone and the skimmer in the intermediate pressure region of the electrospray source, the molecular ions were fragmented to give their mass spectra, for structure elucidation.

High-resolution accurate mass measurement provided elemental composition information which helped in predicting the structures of unknowns.

Ion-exchange chromatography is ideally suited for

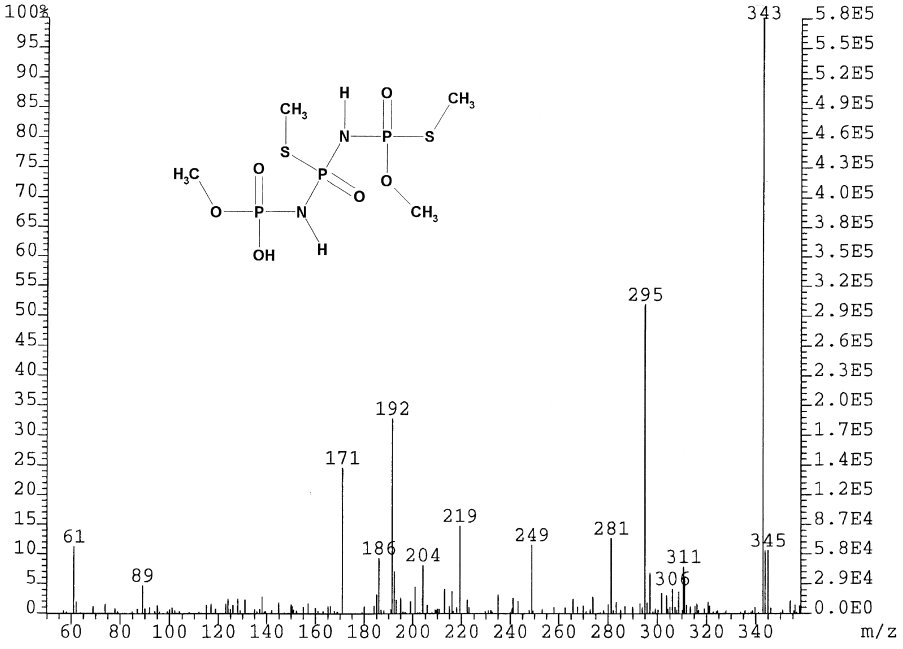

Fig. 8. Mass spectrum of m/z 343.

the separation of ionic compounds. Coupling of IC to the mass spectrometer provides a new tool for the separation and analysis of ionic compounds in complex matrices.

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