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# Accurate mass analysis of ethanesulfonic acid degradates of acetochlor and alachlor using high-performance liquid chromatography and time-of-flight mass spectrometry

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**Abstract**

Degradates of acetochlor and alachlor (ethanesulfonic acids, ESAs) were analyzed in both standards and in a groundwater sample using high-performance liquid chromatography–time-of-flight mass spectrometry with electrospray ionization. The negative pseudomolecular ion of the secondary amide of acetochlor ESA and alachlor ESA gave average masses of 256.0750±0.0049 amu and 270.0786±0.0064 amu respectively. Acetochlor and alachlor ESA gave similar masses of 314.1098±0.0061 amu and 314.1153±0.0048 amu; however, they could not be distinguished by accurate mass because they have the same empirical formula. On the other hand, they may be distinguished using positive-ion electrospray because of different fragmentation spectra, which did not occur using negative-ion electrospray. Published by Elsevier Science B.V.

*Keywords:* Water analysis; Environmental analysis; Acetochlor; Alachlor; Pesticides; Ethanesulfonic acids; Organosulfur compounds

## 1. Introduction

The advent of liquid chromatography–mass spectrometry (HPLC–MS) quadrupole instruments has made analysis of polar pesticides in groundwater a common procedure [1,2]. During the past 5 years many papers have been published on the analysis of pesticides and their degradation products by quadrupole HPLC–MS [3–12]; however, there are several analytical shortcomings yet to be overcome. For example, analysis of polar pesticides often gives only a molecular-ion adduct or a weak fragment ion, especially when the interface is electrospray ionization. The fragmentor or cone voltage is used to enhance collision-induced dissociation (CID) in the source and transport region of the electrospray source. This fragmentation voltage may vary substantially among different analytes and sources, which makes fragmentation difficult to predict in an analysis of unknown compounds. Second, there are no universal libraries available for pesticide analysis by HPLC–MS as in electron-impact gas chromatography–mass spectrometry (GC–MS). This problem makes identification of unknown pesticides or their degradates nearly impossible by simple quadrupole HPLC–MS analysis.

These analytical shortcomings may be overcome partially by the application of liquid chromatog-

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raphy–time-of-flight mass spectrometry (HPLC–TOF-MS), which gives the accurate mass of the molecular ion, which in turn may be used to determine the empirical formula of the unknown. This may be quite helpful in the determination of unknowns when no standards exist, especially for pesticides where the mass of the molecular ion is typically less than 350 amu.

In this paper the accurate mass analysis of secondary and tertiary ethanesulfonic acid (ESA) degradates of acetochlor and alachlor are described by HPLC–TOF-MS. During methods development, accurate masses were determined for all standards, and these masses were compared to the known accurate mass. The unknown groundwater sample was analyzed and the retention time and spectra compared to the accurate mass from the standards for identification. This paper is new in that it presents the use of TOF-MS for pesticide identification and the use of both positive and negative ion electrospray for the identification of degradates of acetochlor and alachlor ESAs.

## 2. Experimental

### 2.1. Materials and methods

HPLC-grade acetonitrile, methanol, acetone, and water along with reagent-grade acetic acid, hydrochloric acid, and sodium sulfite were obtained from Fisher Scientific (Pittsburgh, PA, USA). The analytical standards for acetochlor and alachlor were obtained from Chem Service (West Chester, PA). The analytical standard for acetochlor ESA was obtained from Zeneca Agrochemicals (Fernhurst, Haslemere, UK), and the standard for alachlor ESA was obtained from the US Environmental Protection Agency Repository (Cincinnati, OH, USA). Standards of the secondary amide of acetochlor and alachlor ESA were synthesized according to the method of Thurman et al. [13], which is described in the next section. Standard solutions were prepared in methanol at 100 and 200 μg/ml.

The solid-phase extraction (SPE) procedure was performed using an automated Autotrace workstation (Tekmar, Cincinnati, OH, USA) as described by Ferrer et al. [12]. The SPE cartridges (Sep-Pak) were obtained from Waters-Millipore (Milford, MA, USA). They contained 500 mg of 40-μm $C_{18}$ bonded silica. Each $C_{18}$ cartridge was preconditioned as follows: 2 ml methanol, 2 ml ethyl acetate, 2 ml methanol, followed by 2 ml distilled water. A 123-ml sample was passed through the cartridge at a flow-rate of 10 ml/min, and the cartridge was purged with air to remove excess water. The cartridge was eluted with 3 ml ethyl acetate, followed by 3 ml methanol. The ethyl acetate removed the parent pesticide and secondary amide of the parent compound. The methanol eluted the secondary and tertiary amide of the ESA, which are the ionic degradates of the parent pesticide. The eluate was taken to dryness under nitrogen and re-dissolved in mobile phase for HPLC–TOF-MS analysis.

HPLC–TOF-MS in negative-ion mode of operation was used to determine the accurate mass of ESA analytes. The analytes were separated by using a series 1100 Agilent liquid chromatograph (Palo Alto, CA, USA) equipped with a reversed-phase $C_{18}$ analytical column (RESTEK Ultra Aqueous $C_{18}$, Bellefonte, PA, USA) of 150×2.1 mm and 5-μm particle diameter. Column temperature was maintained at 65°C. The mobile phase used for eluting the analytes from the HPLC column consisted of solvent A (0.3% acetic acid in water) and solvent B [0.3% acetic acid in methanol–acetonitrile (1:2) at 0.4 ml/min]. The gradient consisted of 40% B increasing linearly to 70% B over 4 min. The volume of injected sample was 10 μl. This HPLC system was connected to a time-of-flight mass spectrometer, Leco Jaguar TOF-MS (Leco, St. Joseph, MI, USA) system equipped with an electrospray ionization (ESI) source. Operating conditions of the MS system were optimized in the full-scan mode (scan range: $m/z$ 90–1500) in the negative-ion mode. Acquisition rate was 2 spectra/s. The drying gas flow was set at 6 L/min, the nebulizer pressure was 25 Pa, the drying gas temperature was 300°C, the capillary voltage was 3100 V, and the nozzle-to-skimmer voltage was set at a difference of 100 V. Analyses were carried out in the Leco Separation Science Applications Laboratory, St. Joseph, MI, USA.

### 2.2. Preparation of secondary-amide standards

The acetochlor and alachlor secondary amide ESAs were prepared by a method described by Thurman et al. [13]. In brief, the method consists of refluxing 100 mg of either acetochlor or alachlor in a mixture of acetone and 0.5 *M* HCl. This procedure synthesizes the secondary amide of acetochlor and alachlor (see Fig. 1). Next, the acetone solution is diluted 1:10 with deionized water, and 1.4 g sodium sulfite is added per milliliter of deionized water to form a 0.1 *M* solution. The solution is refluxed overnight to form the ESA of the secondary amide of either acetochlor or alachlor. The solution then is desalted by reversed-phase flash chromatography and is ready for analysis.

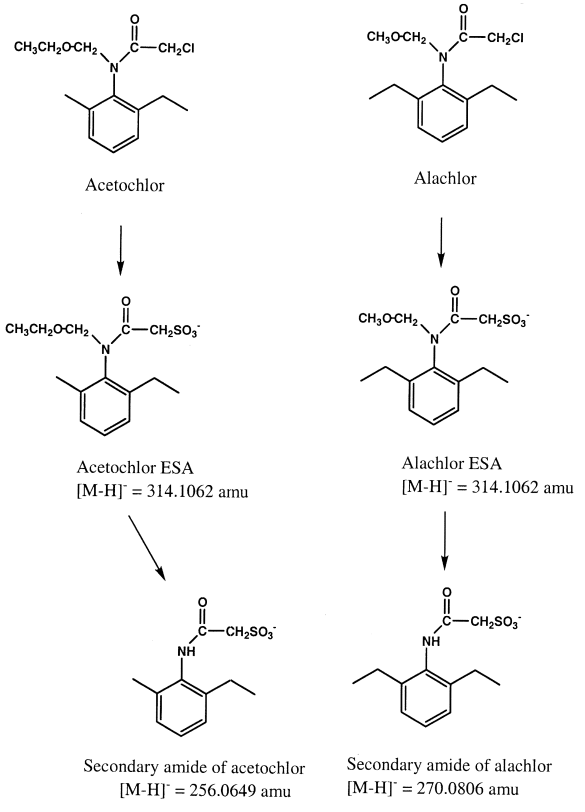


Fig. 1. Chemical structures and accurate masses of acetochlor and alachlor and their ESA degradates.

### 2.3. Sample collection

The groundwater sample was collected from a well in Minnesota as part of a groundwater survey of point-source contamination. The water was filtered through 0.7-μm glass-fiber filters (Whatman GF/F, Maidstone, UK) and stored on ice and shipped to the US Geological Survey in Lawrence, KS, USA. Samples were processed according to the method just described for SPE and for HPLC–TOF-MS analysis.

## 3. Results and discussion

Fig. 2a and b show the chromatographic results and mass spectrum obtained from the analysis of the secondary amide of acetochlor ESA. The shorter, narrower diameter column (Fig. 2) produced a reasonably fast analysis. Some peak tailing was observed. This tailing may be a result of the age of the column. The mass spectrum showed the appropriate single negatively-charged pseudomolecular ion with mass of 256.0750 amu and with mass accuracy of 0.0049 amu of the expected molecular ion at 256.0649 amu and a standard deviation of 0.0100 amu based on five injections (see Table 1). The expected pseudomolecular ion was determined in negative-ion electrospray by taking the exact mass of the analyte and subtracting the mass of a proton (257.0722 amu − 1.00728 amu = 256.0649 amu) in the case of the secondary amide of acetochlor ESA.

A similar chromatographic result and mass spectrum were obtained for the secondary amide of alachlor ESA (data shown in Table 1 only). Again, the shorter, narrower diameter column produced a reasonably fast analysis with some peak tailing, similar to the secondary amide of acetochlor ESA, shown in Fig. 2. This tailing also may be a result of the age of the column. The mass spectrum showed the appropriate single negatively charged pseudomolecular ion. The average observed pseudomolecular ion at 270.0786 amu was within 0.0020 amu of the expected molecular ion at 270.0806 amu based on five injections with a standard deviation of 0.0064 amu (Table 1). The expected molecular ion was determined in negative-ion electrospray by taking the exact mass of the analyte and subtracting

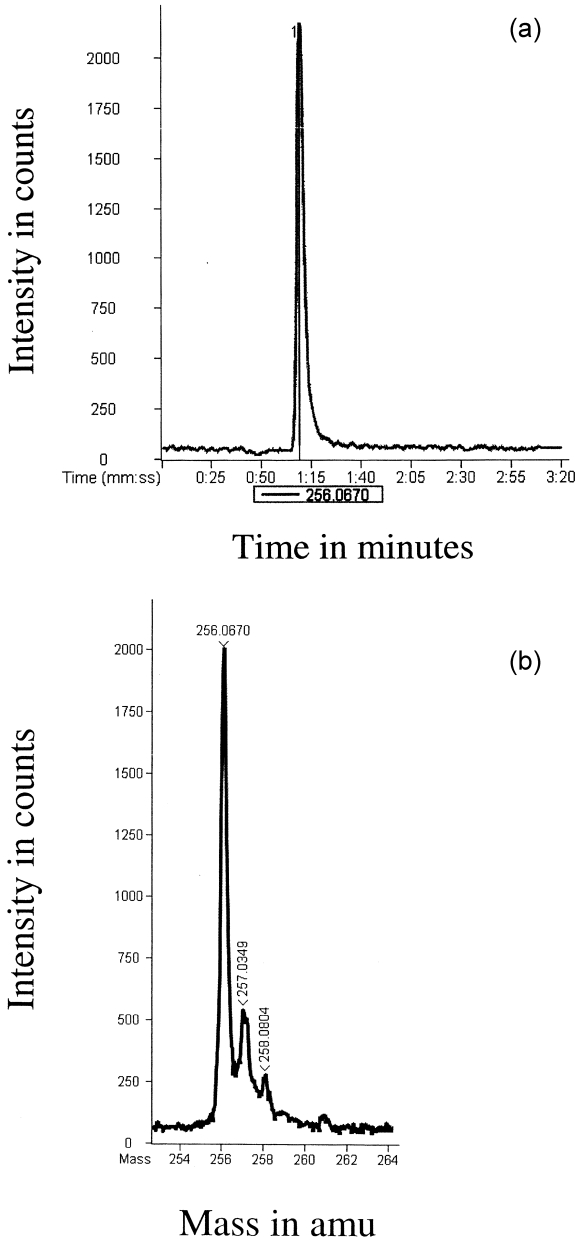


Fig. 2. (a) Extracted ion profile of $m/z$ 256.0670 indicating peak shape for the standard of the secondary amide of acetochlor ESA and (b) mass spectrum obtained from HPLC–TOF-MS analysis of secondary amide of acetochlor ESA.

the mass of a proton (271.0879 amu − 1.00728 amu = 270.0806 amu) in the case of the secondary amide of alachlor ESA.

We obtained a standard deviation of 0.0049 amu for the secondary amide of acetochlor ESA and 0.0064 amu for the secondary amide of alachlor ESA (Table 1). The commercially available mass calibration standard provided a negative-ion signal at $m/z$ 431.98 and 601.98. Ideally, an ion with a mass-to-charge ratio less than the exact mass of the secondary amide of acetochlor ESA (256.0649 amu) also should be chosen for mass axis calibration. Best calibration results are obtained when the range of expected mass-to-charge ratios is bracketed with internal standards during sample analysis. Perhaps a better choice of internal standards could improve the results obtained for the secondary amides of acetochlor ESA and alachlor ESA, and this is being considered for future work.

Despite the mass delta observed, the accuracy was considerably better than accuracy of a single HPLC–MS quadrupole analysis for the secondary amides of acetochlor ESA and alachlor ESA by a factor of 20–50 times. Furthermore, this mass accuracy is an improvement in detection reliability over the conventional method of monitoring only the 256 amu or 270 amu ions using quadrupole mass spectrometry [13]. Electrospray ionization is a very mild ionization technique typically resulting in the formation of molecular ions. With only a molecular ion to use for analyte confirmation, accurate masses become critical in obtaining confidence in analyte identification. It is much more difficult to confidently confirm an analyte with only a nominal mass of 270±0.1 than it is to confirm an analyte knowing the molecular ion is 270.0786±0.0060 amu (Table 1). The number of potential empirical formulas is reduced from hundreds to fewer than 20, also reducing the error of an incorrect mass assignment for single ion monitoring of pesticides in water.

However, the secondary amides of acetochlor ESA and alachlor ESA were not detected by HPLC–TOF-MS, although they were present in the sample at concentrations of 15 μg/l and 10 μg/l, respectively, based on a quadrupole analysis [13]. These data indicated that the HPLC–TOF-MS is less sensitive than the quadrupole analysis by a factor of 100, which is a disadvantage for this class of compounds.

Table 1 also shows the mass accuracy results obtained for five replicate analyses of acetochlor

Table 1
Mass accuracy results obtained from five replicate analyses of the secondary amide of acetochlor ESA and the secondary amide of alachlor ESA

| Compound | Exact mass (u) | Mean observed mass (u) | Standard deviation of observed (u) |
|---|---|---|---|
| Acetochlor 2nd amide ESA | 256.0649 | 256.0750 | 4.9 |
| Alachlor 2nd Amide ESA | 270.0806 | 270.0786 | 6.4 |
| Acetochlor ESA | 314.1062 | 314.1098 | 6.1 |
| Alachlor ESA | 314.1062 | 314.1153 | 4.8 |

ESA and alachlor ESA (see Fig. 1 for structures). The standard deviations were 0.0061 amu for acetochlor ESA and 0.0048 amu for alachlor ESA. Because both compounds (pseudomolecular ions in negative-ion mode) have the same accurate mass of 314.1062, they cannot be distinguished either by their nominal mass of 314 amu or by their accurate mass of 314.1062. The difficulty of distinguishing acetochlor ESA and alachlor ESA based on the 314 amu ion has been discussed in several papers dealing with the measurement of the ESA degradates of alachlor and acetochlor [13,14]. Thus, HPLC–MS–MS has been used to separate and to distinguish between these two compounds [14]. Upon fragmentation of the 314 amu ion, acetochlor has a different fragmentation than alachlor in MS–MS giving the 146 ion for acetochlor ESA and the 160 ion for alachlor ESA. However, these ions are not found using single quadrupole analysis [13,14]. Therefore, acetochlor ESA and alachlor ESA must be separated chromatographically for identification using the 314 amu ion only [15].

Fig. 3 shows the analysis of the groundwater sample using the negative-ion electrospray HPLC–TOF-MS for both acetochlor and alachlor ESA. There was only a strong signal at 314.1064, equivalent to an acetochlor ESA or an alachlor ESA response of 200 μg/l. Previous analysis of this sample by HPLC–MS quadrupole indicated a concentration of 75 μg/l for acetochlor ESA and 120 μg/l for alachlor ESA. Thus, the combined concentration of acetochlor ESA plus alachlor ESA was within 10% of the value obtained by the quadrupole method, where the two compounds were separated.

Because the TOF-MS system was equipped with peak location and mass spectral deconvolution capabilities of the software program, called ChromaTOF (Leco), different signals at the 314 amu mass can be separated. For example, analytes exhibiting a specified peak width and signal-to-noise ratio are automatically located by the peak find algorithm, even those analytes found beneath the baseline of the total ion current chromatogram or buried beneath high concentration matrix interferences. In the case where analytes are coeluting, the spectral deconvolution algorithm automatically associated the signals belonging to each analyte into a pure spectrum of the analyte free of interferences from neighboring compounds and sample background. This software was useful in the analysis of the groundwater sample but only the single 314.1064 ion was observed, which is further evidence for the accurate mass of the acetochlor ESA and alachlor ESA.

A second approach was accurate mass analysis in the positive ion mode for acetochlor ESA and alachlor ESA. Acetochlor ESA gave a $[M+H]^+$ ion of $316.1208 \pm 0.0100$ amu (Fig. 4) based on a mass-spectral scan. The fragment ion was 270.0873 for acetochlor ESA, which is the loss of ethanol from the amide nitrogen (Fig. 1 and 4). The fragment ion was 284.1027 for alachlor ESA, which is the loss of methanol from the amide nitrogen (data not shown). Another advantage of the HPLC–TOF-MS system over either quadrupole or triple quadrupole analysis is the accurate mass obtained from the loss of neutral fragments. For example, the loss of ethanol has an exact mass loss of 46.0935 for acetochlor ESA and the loss of methanol for alachlor ESA was 32.0283. These data give more confidence in understanding fragmentation, which is a valuable part of unknown identification. Thus, HPLC/TOF-MS is a valuable complimentary tool to both HPLC–MS quadrupole and HPLC–MS–MS analysis for environmental unknowns.

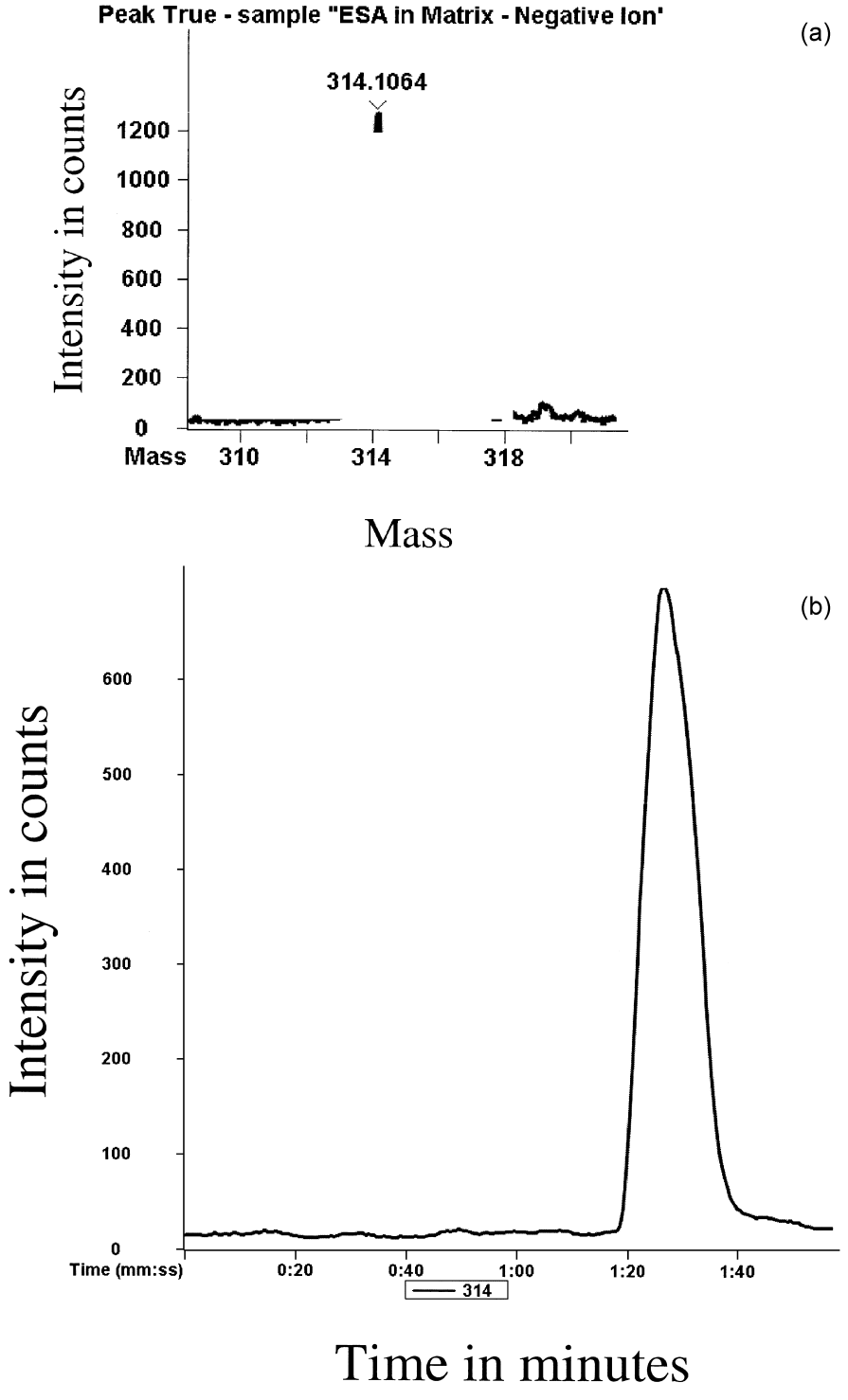


Fig. 3. (a) Extracted 314 amu ion in groundwater sample (b) accurate mass determination for groundwater sample.

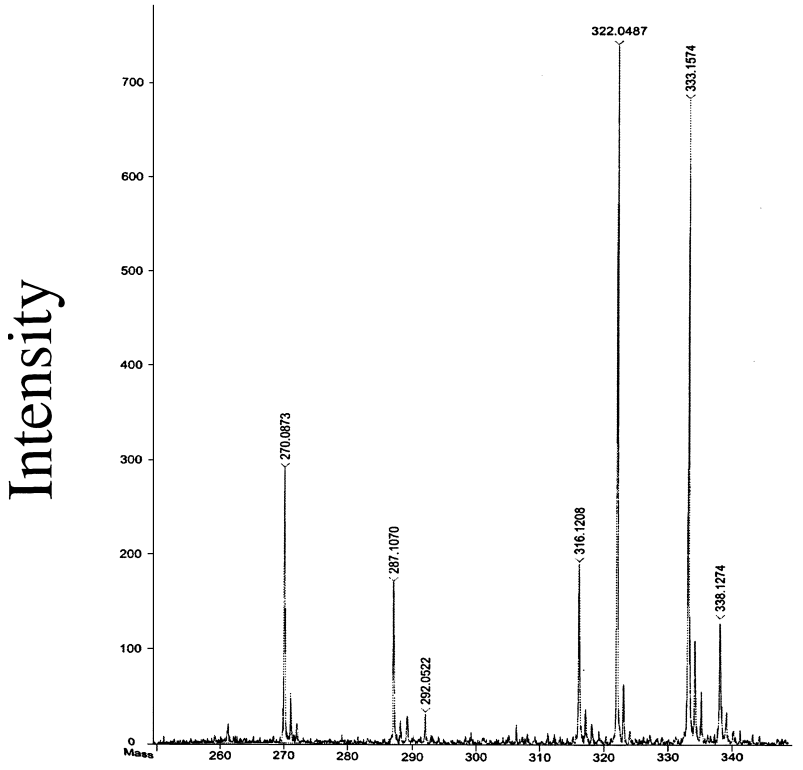


Fig. 4. Positive ion spectrum of acetochlor ESA.

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The use of brand, trade or firm names is for identification purposes only and does not constitute endorsement by the US Geological Survey. The authors thank Ray Matejczyk formerly of Leco Corporation for assistance in sample analysis.

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