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Simultaneous Determination of Acetochlor and Propisochlor Residues in Corn and Soil by Solid Phase Extraction and Gas Chromatography with Electron Capture Detection

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Abstract A sensitive and simple method for simultaneous analysis of acetochlor and propisochlor in corn and soil has been developed. Two herbicides were extracted from soil and corn matrices with methanol/water and acetone, respectively, followed by solid phase extraction (SPE) to remove co extractives, prior to analysis by gas chromatography with electron capture detection (GC-ECD). Primary secondary amine (PSA) SPE cartridges (500 mg, 3 mL) were used for sample preparation. The analytes from corn and soil matrices were eluted with 5 mL petroleum ether-acetic ether (95/5, v/v) and 3 mL petroleum ether-acetic ether (95/5, v/v), respectively. The recoveries of two pesticides ranged from 73.8% to 115.5% with relative standard deviations (RSD) less than 11.1% and sensitivity of 0.01 mg/kg, in agreement with directives for method validation in residue analysis. The method was successfully applied to determine the fate of acetochlor and propisochlor in real corn and soil samples. For acetochlor and propisochlor, the half-life times (t1/2) in soil was 5.541 and 6.074 days, respectively. No acetochlor and propisochlor residues (<0.01 mg/kg) were detected in corn at harvest time withholding period of 2.5 months after treatments of the pesticides. Direct confirmation of the analytes in samples was achieved by gas chromatography–mass spectrometry (GC–MS).

Keywords Pesticide residue · Acetochlor · Propisochlor · Solid-phase extraction · GC-ECD

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Acetochlor and propisochlor are two important pre-emergence herbicides for control of annual grasses and broadleaf weeds in row crops such as soybean, peanut, cotton, especially in corn. When absorbed through the roots and shoots just above the seed of the target weeds, they act as a growth inhibitor by suppressing synthesis of protein (Cai et al. 2007; Gadagbui et al. 2010; Wang et al. 2007). Figure 1 illustrates the chemical structures of them. In China, 22% (acetochlor and propisochlor) commercial formulation suspoemulsion, which is 9% acetochlor and 13% propisochlor mixture, was frequently applied to corn fields to control stubborn weeds.

Several analytical methods for determining acetochlor or propisochlor in foodstuffs and environmental samples have been reported. There is, however, a little information available on the methods for the simultaneous analysis of the two herbicides. Two literatures (Nguyen et al. 2008, 2009) described a similar method for multiresidue analysis of hundreds of pesticides in rice paddy and herbs, in which analysis for acetochlor and propisochlor were achieved by expensive, time-consuming GC–MS. Konda and Pa'sztor (2001) have also studied environmental distribution of the two herbicides in sediment and runoff water samples. However, to the best of our knowledge no publication has documented the method of simultaneous analysis of acetochlor and propisochlor residues in corn and soil by gas chromatography with electron capture detection.

This paper presents a simple and rapid method based on GC-ECD coupled to SPE for simultaneous determination of acetochlor and propisochlor in corn and soil. The method is simple and proved to be effective in ensuring analysis at 0.01 mg/kg and other concentrations with sufficient accuracy and precision. The proposed method was applied to determine the pesticides levels in the corn and soil samples collected from the trial field.

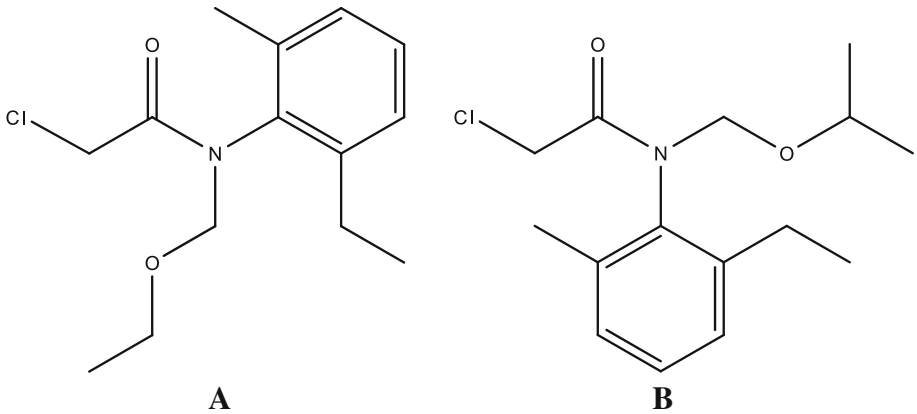

Fig. 1 The chemical structures of acetochlor (**a**) and propisochlor (**b**)

Materials and Methods

Acetochlor and propisochlor standards (purity >99%) were supplied by Shandong Qiaochang Chemical Co., Ltd. Other chemicals and solvents were from Dikma Limited (China) and were of analytical-grade. SPE columns (PSA, 500 mg, 3 mL) were purchased from Agela Technologies (China).

GC analyses were carried out on a Shimadzu 2014 gas chromatograph equipped with a split/splitless injector and an electron capture detector (ECD). A HP-5 capillary column (30 m × 0.25 mm × 0.25 μm film thickness) was used through out the entire experiment. Nitrogen (>99.999%) was used as carrier gas at a flow rate of 20 cm/s. Chromatographic separations were performed at the initial temperature held at 120°C for 1 min, then the temperature was ramped at 10°C/min to 240°C, then programmed at 3°C/min to 270°C, and finally programmed at 5°C/min to 290°C which was held for 2 min. Injector and detector temperatures were set at 280 and 310°C, respectively. Aliquots of 1 μl of the samples were injected under the splitless mode.

GC–MS analysis was performed with a Thermo POLARIS Q (EI) system. The GC column and oven temperature program described above were used. Samples (1 μL) were injected in splitless mode. Eluent from the GC column was fed into a 70-eV electron-impact ionization source maintained at 250°C. Spectra were acquired in full-scan mode (*m*/*z* 40–500). Helium (>99.999%) was used as carrier gas at a flow of 40 cm/s.

A mixture stock solution of 400 μg/mL of each pesticide was prepared in n-hexane. Working standard solutions (0.01, 0.05, 0.2, 0.5, 1 μg/mL), used for sample spiking and for preparation of standard curve, were obtained from stock solution by volumetric serial dilution.

Soil Sample

Soil samples were dried at room temperature and screened through 40-mesh sieves. A portion (20 g) of homogenized soil sample was weighed into an Erlenmeyer flask. A 80 mL mixture of methanol and water (1/1, v/v,) was added, and shaken for half an hour vigorously. The over layer was filtered through a 12-cm Buchner funnel. The solid residues were treated with an additional mixture (20 mL) of methanol and water; the filtrate was combined and transferred into a 250 mL separating funnel. The extracts were partitioned with 60 mL dichloromethane three times sequentially in the presence of 2% anhydrous sodium aqueous solutions (30 mL). The combined dichloromethane extracts were dehydrated on anhydrous sodium sulfate and concentrated to dryness by a rotary vacuum evaporation at 40°C. The resulting residue was dissolved with petroleum ether (10 mL) and for purification by PSA cartridges. A PSA cartridge was conditioned with petroleum ether (2 mL). Two mL extract solution was loaded on to the cartridge and the eluate was discarded. Analytes were eluted with 3 mL petroleum ether-acetic ether (95/5, v/v,) and the eluate was concentrated to dryness at 40°C under a stream of nitrogen. The resulting residue was re-dissolved in 2 mL n-hexane for GC-ECD analysis.

Corn Sample

Portion of chopped and homogenized corn sample was weighed (20 g) into a 250 mL Erlenmeyer flask and 60 mL acetone was added. The mixture was shaken for half an hour vigorously, followed by ultrasonic extraction for 3 min. The over layer was filtered through a Buchner funnel. The solid residues were subjected to ultrasonic extraction again with 20 mL acetone. The combined filtrate was partitioned with dichloromethane three times sequentially (20 + 20 + 20 mL) in the presence of 10 g sodium chloride. The combined dichloromethane extracts were then dried before concentration to dryness. The concentrated extract was dissolved with 10 mL petroleum ether and subjected to SPE. A PSA cartridge was preconditioned with petroleum ether (2 mL). 2 mL extract solution was loaded on to the cartridge and the eluate was discarded.

Analytes were eluted with 5 mL petroleum ether-acetic ether (95/5 v/v,) and the eluate was concentrated to dryness. The residue was re-dissolved in n-hexane (2 mL) for GC-ECD analysis.

Recovery Assay

Untreated corn and soil samples were fortified with known amounts of working standard solution (0.01, 0.1, 0.5 mg/kg) and processed according to the above procedure. Every recovery was done on three replicates.

Field Study

The trial was carried out in an experimental plot, located in a suburb of Beijing (China). A random block scheme was used, with three replications for each test, and each plot with a dimension of 30 m^2. The control plots were separated by guard rows to avoid contaminating by drift. Corn plants, receiving routine horticultural treatment, were sprayed with the commercial formulation of acetochlor and propislchlor (22% SE) at the dosage of 500 mL of active ingredient per acre (double the recommended by manufacturer), which were diluted 3,000–4,000 times with water. Corn samples were collected at harvest time. After picked, the samples were immediately put into polyethylene bags and transported to the laboratory where they were chopped, thoroughly mixed, and divided into three subsamples each. The sub-samples were kept deep-frozen (−30°C) until analysis.

Soil samples were collected at random from each plot at different time intervals at 0 day (2 h after application), 1, 3, 5, 7, 10, 14, 21, 28, 40 days after the herbicide application. Soil samples were collected from different depths ranging from 0 to 15 cm with a spade. Little stones and other unwanted materials were removed. All of the subsamples were kept in a deep-frozen (−30°C) environment until analyzed.

Results and Discussion

The calibration graphs obtained by plotting concentration versus average peak area (each sample injected in duplicate) were linear over the range of 0.01–1 μg/mL. The regression equations and correlation coefficients (R^2) for acetochlor and propisochlor as follows: $y = 90189x + 889.98$, $R^2 = 0.9994$; $y = 83182x + 460.1$, $R^2 = 0.9998$. Nominal retention times for acetochlor and propisochlor were about 13.8 and 14.1 min, respectively (Fig. 2).

Satisfactory results were found with recoveries of propisochlor in soil between 73.8% and 88.3%, and RSD between 0.77% and 11.1%, in corn between 82.8% and

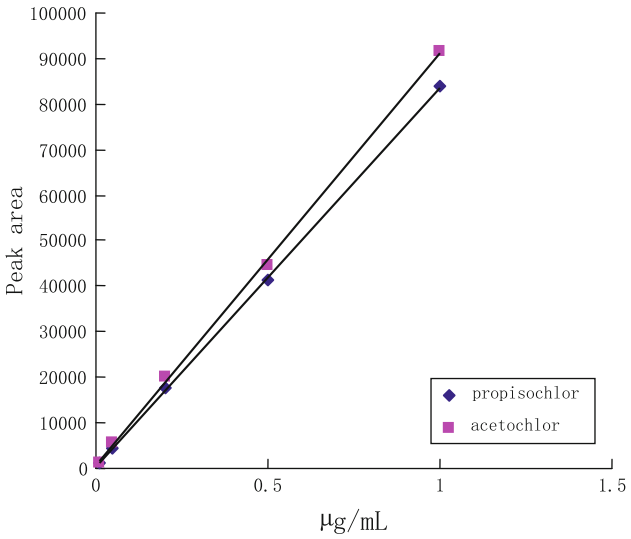

Fig. 2 External standard curve of acetochlor and propisochlor

115.5% and RSD between 1.69% and 5.30%; recoveries of acetochlor in soil between 82.7% and 95.4%, and RSD between 0.55% and 2.89%, in corn between 86.6% and 104.5% and RSD between 1.83% and 5.61% as showed in Table 1. Meanwhile blank corn and soil samples were analyzed and the results indicated the blank extracts did not contribute any interference with the target compounds. Figure 3 shows the chromatograms of the blank and different levels of the spiked samples. The limit of quantification (LOQ) for the method was defined as the lowest concentration of compounds in a sample that could be quantitatively determined with suitable precision and accuracy. LOQ of acetochlor and propisochlor was both 0.01 mg/kg in this method.

Acetochlor and propisochlor residues in soils and corn were extracted, purified, and analyzed according to the method. All residue data were subjected to statistical

Table 1 Recoveries of acetochlor and propisochlor residues in corn

Sample	Fortification level (mg/kg)	Pesticide	Average recoveries (%)	CV (%)
Soil	0.01	Propisochlor	87.9	11.1
		Acetochlor	82.7	1.71
	0.10	Propisochlor	88.3	8.08
		Acetochlor	95.4	2.89
	0.50	Propisochlor	73.8	0.77
		Acetochlor	90.2	0.55
Corn	0.01	Propisochlor	115.5	3.99
		Acetochlor	5.30	1.83
	0.10	Propisochlor	5.30	1.69
		Acetochlor	86.9	5.61
	0.50	Propisochlor	82.8	5.30
		Acetochlor	86.6	5.24

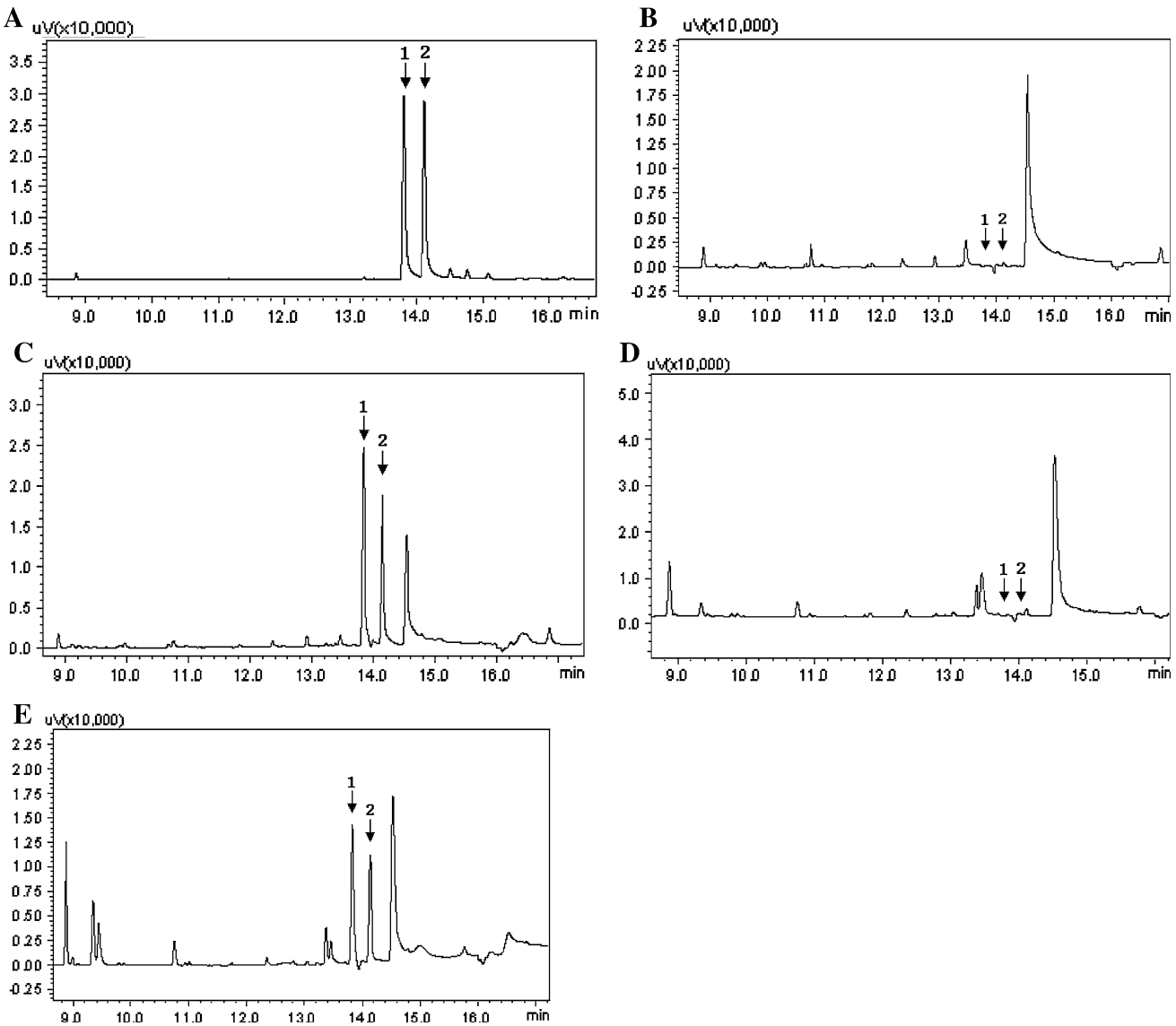

Fig. 3 The GC chromatogram of acetochlor and propisochlor in the studied samples (**a**—standard solutions **b**—soil blank; **c**—spiked soil at 0.5 mg/kg; **d**—Corn blank; **e**—spiked corn at 0.5 mg/kg)

Table 2 Acetochlor and propisochlor residues in soil at different time intervals

Time elapsed after treatment in days	Pesticide	Mean residues content (mg/kg)	RSD (%)	Dissipation rate (%)
0	Acetochlor	0.431	4.59	–
	Propisochlor	0.565	0.87	–
2	Acetochlor	0.389	4.54	9.67
	Propisochlor	0.529	8.42	6.73
7	Acetochlor	0.503	1.04	–
	Propisochlor	0.741	0.99	–
15	Acetochlor	0.119	2.29	72.3
	Propisochlor	0.150	2.16	73.4
22	Acetochlor	0.055	2.29	87.2
	Propisochlor	0.089	2.83	84.2
29	Acetochlor	0.011	1.83	97.4
	Propisochlor	0.021	5.12	96.2

BDL below determination limit

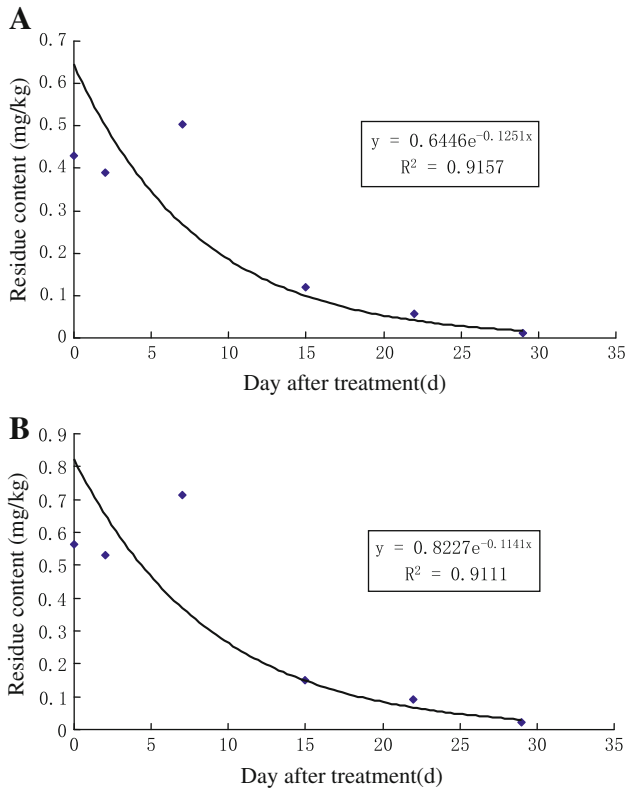

Fig. 4 Dissipation rate of acetochlor in soil (**a**); propisochlor in soil (**b**)

analysis to evaluate the dissipation of acetochlor and propisochlor according to time. No acetochlor and propisochlor residues (<0.01 mg/kg) were detected in corn at harvest time withholding period of 2.5 months after treatments of the pesticides. No MRLs of Acetochlor and propisochlor in corns were established by Chinese government, according to MRLs of acetochlor (0.05 mg/kg established by America and South Korea) and propisochlor (0.05 mg/kg South Korea) for corns, applying 22% suspoemulsion of acetochlor and propisochlor in corn field is safety. The soil under investigation was weak alkaline (7.82 pH), clay in texture. The organic matter content was 4.3%. The dissipation of acetochlor and propisochlor residues in soil with time can be described mathematically by a pseudo-first rate equation. The regression line equation for the concentration (C) related to time (t) was $Ct = 0.64465e^{-0.1251t}$ ($R^2 = 0.9157$) and $Ct = 0.8227e^{-0.1141t}$ ($R^2 = 0.9111$), for acetochlor and propisochlor, respectively, with the half-life times (t1/2) of the pesticides in soil was 5.541 and 6.074 days. These data clearly show a relatively fast decline of residues in field soil.

Rates of dissipation are influenced by physicochemical properties of the soil [such as pH and organic carbon (OC) content], biological properties (activity and distribution of microorganisms), and environmental conditions that control soil temperature and moisture content. Owing to the high temperature and frequent rain in the summer season of our experiment, the dissipation rates of the two pesticides are quickly.

The decrease in residue levels during the days after treatments in soil is presented in Table 2. Figure 4 shows the dissipation curves of acetochlor and propisochlor. Confirmation tests by GC–MS were used to determine whether or not peaks detected at the retention times of the analytes was just obtained from authentic samples (Fig. 5).

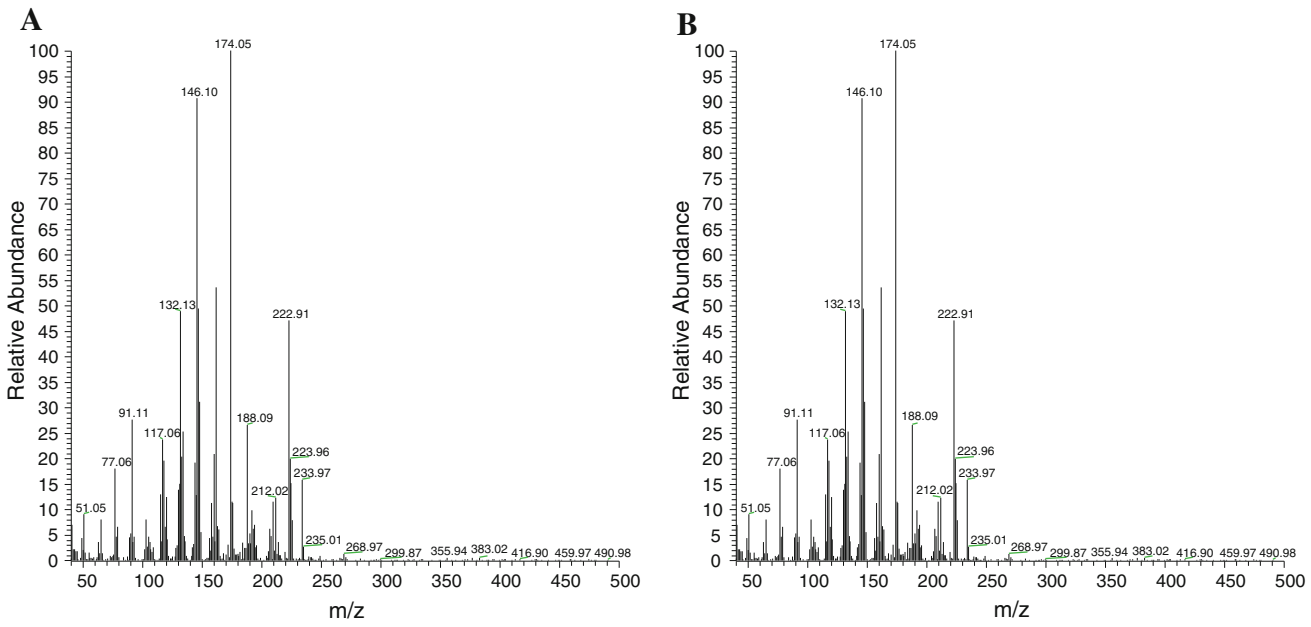

Fig. 5 Mass spectra of acetochlor (**a**) and propisochlor (**b**) obtained from real soil sample

In this paper, a simple and rapid GC-ECD method was developed and validated for the simultaneous determination of acetochlor and propisochlor residues in corn and soil. The developed method shows satisfactory validation parameters in terms of linearity, lower limits, accuracy and precision. The average recoveries of the two herbicides range from 73.8% to 115.5%. The uncertainty associated to the analytical method, expressed as RSD, was lower than 11.1%. LOQ of acetochlor and propisochlor is found to be 0.01 mg/kg. The developed method was successfully applied to determine the fate of acetochlor and propisochlor in corn and soil.

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