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Determination of oxanilic and sulfonic acid metabolites of acetochlor in soils by liquid chromatography–electrospray ionisation mass spectrometry

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Abstract

An analytical method is presented that describes the extraction and quantification of oxanilic and sulfonic acid metabolites of the herbicide acetochlor in soil samples. Experiments were performed on 50 g of soil using a solvent extraction technique with an acetonitrile–water (60:40) mixture in an acidic medium. Analysis was carried out by reversed-phase liquid chromatography and detection by electrospray ionisation mass spectrometry in single ion monitoring and negative modes. Four different soil matrices were spiked in triplicate with standards of each degradation compound at three concentration levels between 2 and 80 μg/kg. The average recoveries range from 90 to 120% for both the metabolites, with relative standard deviations lower than 15%. The limits of quantification are about 1 and 2 μg/kg for the ethanesulfonic acid and the oxanilic acid metabolites, respectively. The method has been applied to soils and solids recovered from the deeper unsaturated zone of a small French catchment closely monitored as part of the European project "Pesticides in European Groundwaters: detailed study of Aquifers and Simulation of possible Evolution scenarios (PEGASE)". © 2002 Published by Elsevier Science B.V.

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1. Introduction

Chloroacetanilide herbicides (e.g. alachlor, metolachlor, acetochlor), together with triazine compounds, are an important class of herbicides used to control grass weeds in various crops in the USA. Acetochlor was first applied in 1994 in the USA [1] and in 2000 in Europe, whereas alachlor and metolachlor have been widely used in the USA for more than 20 years.

The parent agrochemicals may degrade in soils and water to form oxanilic and sulfonic acid metabolites [2]. The typical half-life of the chloroaetanilide herbicides ranges from 15 to 30 days. These compounds can be detected in groundwater more often and at higher concentrations than their parent compounds [3,4]. Information on how and why these molecules reach groundwater is still very scarce, partly because of a lack of data concerning their presence and fate in the soil and unsaturated zone overlying aquifers [5,6]. Such studies are also very useful from a toxicological standpoint, enabling analytical verification of the metabolites.

Although a great number of papers have been published on the determination of these metabolites

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in waters [7–11], very few studies have been carried out on their determination in soils and solids.

An analytical method is presented that describes the extraction and quantification of the *N*-ethoxymethyl-2′-ethyl-6′-methyloxanilic acid (oxanilic acid, OXA) and *N*-ethoxymethyl-2′-ethyl-6′-methyl-2-sulfoacetanilide acid (ethanesulfonic acid, ESA) metabolites of acetochlor in soil samples. Samples of soils and solids were recovered from the deeper unsaturated zone at a depth of between 5 cm and 1 m and were subjected to a liquid–solid extraction process at temperatures below 40 °C in order to preserve the integrity of the metabolites.

The parent chloroacetanilide herbicides can be analysed either by gas chromatography (GC) or liquid chromatography (LC), whereas acid metabolites can only be analysed by LC due to their low volatility and ionic properties. In an attempt to reach the lowest detection limits and the best selectivity, diode array detection proved insufficient, and the detection method of choice was electrospray ionisation mass spectrometry (ESI–MS), extremely suited to determining thermolabile compounds as well as polar and acidic compounds.

This papers addresses the development of a method that has been applied to soils and solids recovered from the deeper unsaturated zone of a small French catchment closely monitored as part of the European project ''Pesticides in European Groundwaters: detailed study of Aquifers and Simulation of possible Evolution scenarios (PEGASE)''.

2. Experimental

2.1. Chemicals

Monsanto (St. Louis, MO, USA) provided the OXA and ESA metabolites of acetochlor. Acetonitrile (Carlo Erba, France) for high performance liquid chromatography (HPLC) was used for the extractions, as well as 85% Normapur phosphoric acid (Prolabo, France).

2.2. Equipment

A rotary evaporator (Buchi, Switzerland) was used during the evaporation stage. The chromatographic system was composed of a Varian 9100 autosampler and a Varian 9012 HPLC pump (Les Ulis, France). LC–MS analyses were performed using an SSQ 7000 Thermo-Finnigan instrument (Les Ulis) equipped with an ESI source. The analytes were detected in the negative ion mode as their deprotonated molecular ions $(M-H)^-$.

Methanol (Carlo Erba) for HPLC, water (BDH, UK) for HPLC, and 100% Normapur acetic acid (Prolabo) formed constituents of the mobile phases. The reversed-phase column chosen for all the experiments was an Omnispher C_{18} 100×3 mm I.D. with a 3-μm particle size (Varian-Chrompack, Les Ulis, France). Extracts were filtered through Spartan membranes (Schleicher–Schuell, Dassel, Germany) of 0.2-μm pore size and diameter 30 mm.

2.3. Sampling description

The soils were sampled within two plots at six dates during the experiment, which range from day 1 to day 355 from the day of acetochlor application. Each plot of ~2000 m^2 was divided into 16 sub-plots each with a 1-m-deep core, which was itself divided into ten sub-cores of 10 cm. It was not realistic to extract 2000 soil samples (160 soil samples per plot and per date), so a large number of sub-cores were gathered as composite cores depending on the depth. In all, ten composite cores plus four individual cores gave 50 soil samples per plot and per date.

2.4. Extraction procedure

A 200-ml solution of acetonitrile–water (60:40) was added to 50 g of dried soil (O.D.<2 mm). The mixture was shaken for 30 min and centrifuged at 8000 rpm for 20 min. After evaporation at 40 °C to 5 ml, 30 ml of acetonitrile (in 0.1% phosphoric acid) was added and the extract was filtered through a 0.2-μm membrane. A second evaporation stage at a temperature below 40 °C was then performed yielding an extract of ~3 ml. Finally, 3 ml (2×1.5 ml) of methanol was used to rinse the evaporation flask and added to the extract, which was stored at −18 °C until analysis. No extract clean-up was necessary prior to the HPLC–ESI–MS analysis.

Table 1
Characterisation of the soils studied

	Depth (cm)	Clay (%)	Silt (%)	Sand (%)	Total calcareous (%)	Organic matter (%)	CEC[a] (meq/100 g raw)	Organic carbon (%)
Pit A-1	**0–20**	**15.3**	**64.7**	**18.4**	**0**	**1.51**	**10.4**	**0.88**
Pit A-2	20–40	17.3	64.3	17.3	0	0.99	8	0.58
Pit A-3	40–65	20.5	64.7	14.1	0	0.74	10.93	0.43
Pit A-4	**65–90**	**25.8**	**61.7**	**12**	**0**	**0.55**	**13.1**	**0.32**
Pit A-5	90–110	30.0	54.6	14.9	0	0.52	15.0	0.30
Pit A-6	110–130	6.13	5.50	18.9	69	0.47	2.83	0.27
Pit B-1	**0–25**	**22.7**	**19.6**	**10.8**	**3.69**	**2.81**	**17.4**	**1.63**
Pit B-2	**25–50**	**22.8**	**18.2**	**10.4**	**7.03**	**2.27**	**15.4**	**1.32**

Soil matrices shown in bold were chosen as ''blank'' soils.
[a] CEC, cationic exchange capacity.

3. Results and discussion

3.1. Characterisation of the soils studied

The characterisation of the soils studied (Table 1) shows that they are contrasted under the same climatic conditions, with a deep silty soil and a more clayey and calcareous soil containing a higher proportion of organic matter and organic carbon. Four soil matrices (in bold in Table 1) were chosen as ''blank'' soils, taking into account the variability of the characterisation parameters. They were then sampled before acetochlor spreading.

3.2. Qualitative analysis by LC–ESI–MS

A gradient of two mobile phases at 0.4 ml/min was adopted in order to separate the two metabolites. Phase A consisted of MeOH (10%), water (89.9%), and acetic acid (0.1%), whereas phase B consisted of MeOH (99.9%) and acetic acid (0.1%). Phase A proportion was decreased from 100 to 50% in 6 min and from 50 to 30% in 14 min. These proportions (30% A and 70% B) were maintained for 5 min, and 10 min of equilibration time were included between each chromatographic run.

The MS operating conditions are given in Table 2.

The reversed-phase LC separation of the two metabolites exhibits two peaks for each compound, corresponding to the diastereomers only partially separated at room temperature (Fig. 1). By working at 60 °C, these diastereomers can be eluted in one single peak for each metabolite. The total ion current (TIC) chromatogram shows four peaks not sufficiently distinct to be quantified; their separation was resolved using the selective mass-to-charge ratio.

Bearing in mind that one of the main challenges was to reach very low detection limits in solid matrices, we carried out the analyses in the single ion monitoring (SIM) mode, at m/z 314 for the ESA metabolite and at m/z 146+264 for the OXA metabolite. The SIM analysis requires a certain degree of caution to ensure that the signals obtained can be attributed without doubt to the metabolites of interest. Thus, the occurrence of diastereomers at the relevant m/z and retention times provides additional information regarding the identity of the chromatographic peak in the SIM mode. The ion at m/z 314 corresponds to the base peak in the ESA mass spectrum, whereas that at m/z 146 is the base peak in the OXA mass spectrum, indicating the lower stability of the OXA metabolite compared to the ESA. The breakdown of the carbon–nitrogen bond forms the more stable fragment at m/z 146 (Fig. 2a and b).

Table 2
ESI–MS operating conditions

Parameter	Value
Sheath gas pressure	70 p.s.i.[a]
Auxiliary gas flow	20 ml/min
Source voltage	5 kV
CID offset	15 V
Ionisation mode	Negative
Capillary temperature	250 °C

[a] 1 p.s.i. = 6894.76 Pa.

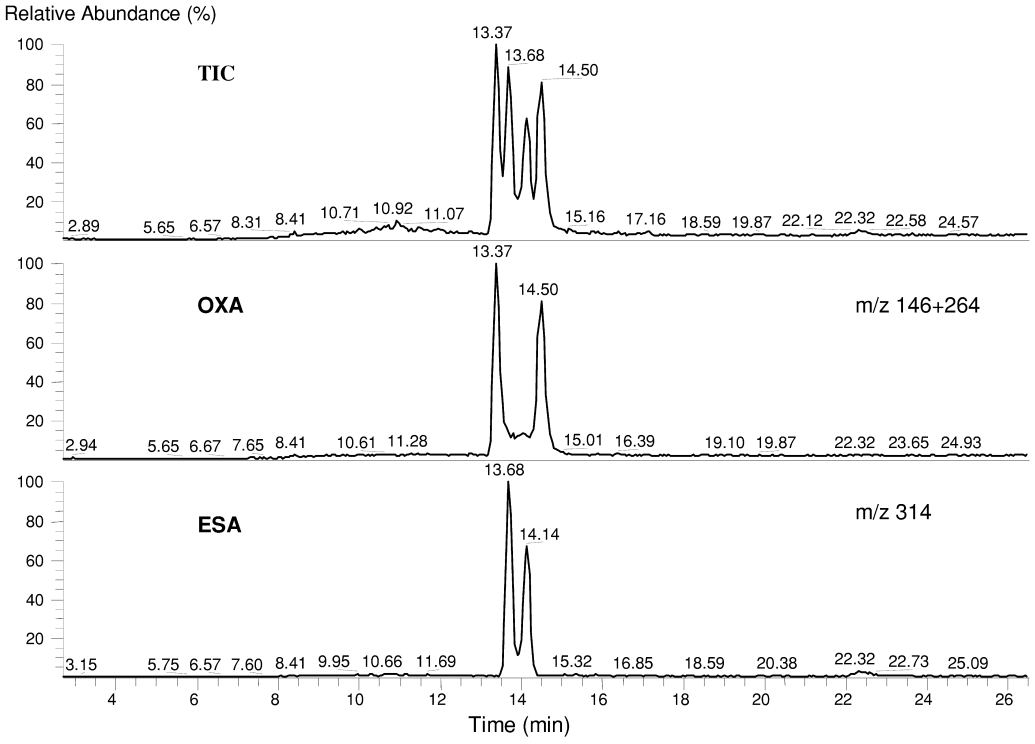

Fig. 1. (a) Chromatogram from an LC–ESI-full scan MS analysis of a P-B soil sample collected on date 2. (b) Reconstructed ion chromatogram for the same LC–ESI-full scan MS analysis (at m/z 146+264) of a P-B soil sample collected on date 2. (c) Reconstructed ion chromatogram for the same LC–ESI-full scan MS analysis (at m/z 314) of a P-B soil sample collected on date 2.

3.3. Quantitative results

3.3.1. Metabolite recoveries

The four ''blank'' soil matrices were spiked in triplicate at three concentration levels (low, medium, high) ranging from 2 (ESA) or 4 (OXA) to 80 μg/kg, with standard solutions of the ESA and OXA metabolites (Figs. 3–5).

All quantitations were performed using the standard addition method on ''blank'' soil samples. This method allowed us to overcome the matrix effects occurring during the ESI process prior to MS detection. These matrix effects are underlined by the response coefficients of each metabolite expressed as a function of the soil matrix and depth. The values in Table 3 were obtained after linear calibrations between 0 and 1 mg/l. These values show that the standard addition method provides accurate quantitative results in solid and soil matrices. It should be noted that the relative response coefficients are similar for the two soils sampled from the surface layer, irrespective of the plot.

Figs. 3–5 show that average recoveries range from 70 to 120% at the low spiking level and from 100 to 120% at the medium and high spiking levels. The relative standard deviations are lower than 15% in almost all cases.

3.3.2. Method performance

Correlation coefficients of 0.995–0.999 were observed for both the metabolites in all the soil matrices studied, between 0.05 and 1 mg/l, which are the common levels encountered in the soil extracts. The limits of quantification (LOQs) and limits of detection (LODs) were assessed for the four soil matrices after performing the extraction process on 50 g of soil (Table 4). The LOQ is between 1 and 2 μg/kg and the LOD between 0.3 and 0.7 μg/kg. The best performance is obtained for the ESA

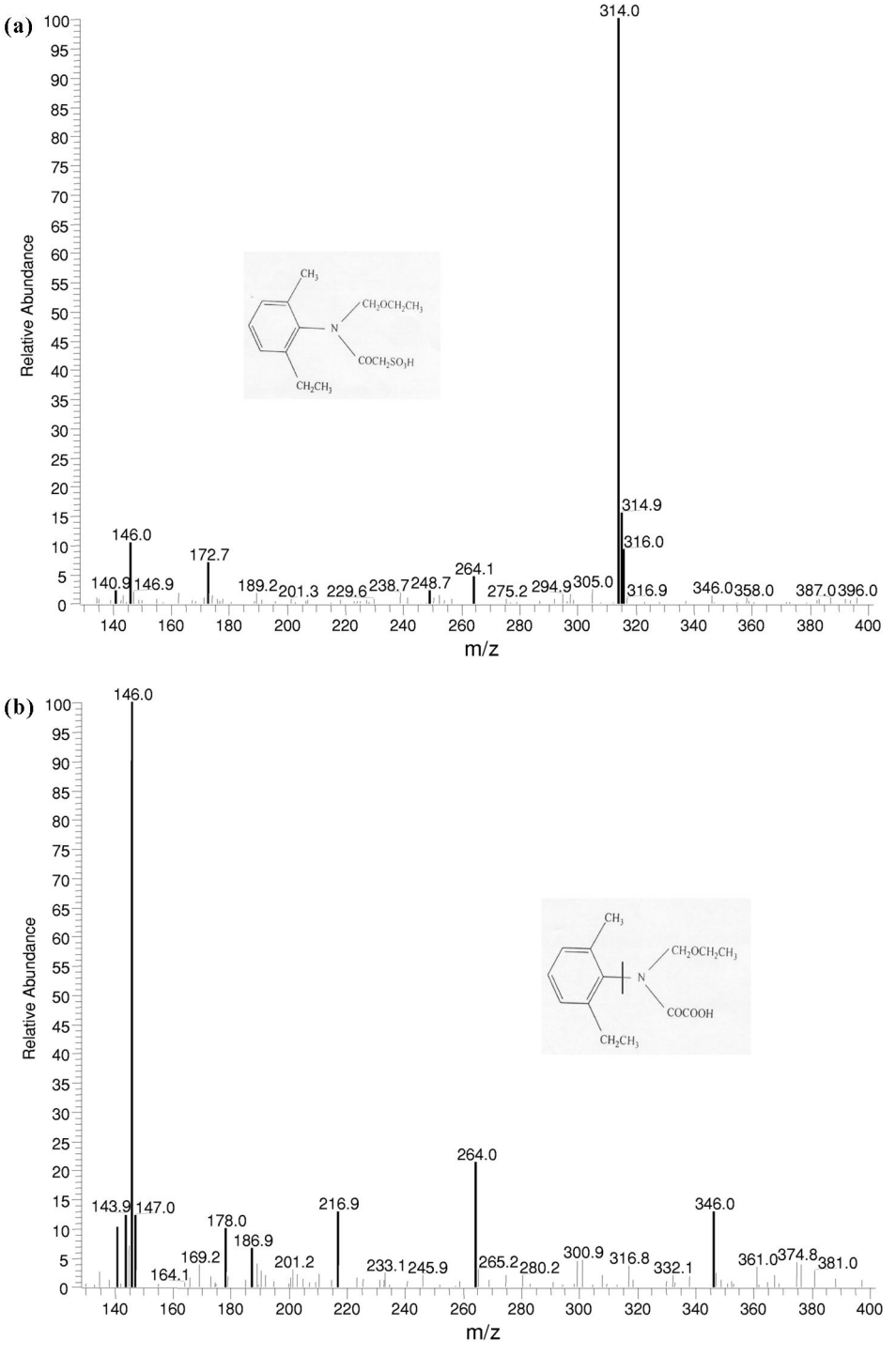

Fig. 2. Mass spectra of (a) the ESA metabolite of acetochlor and (b) the OXA metabolite of acetochlor.

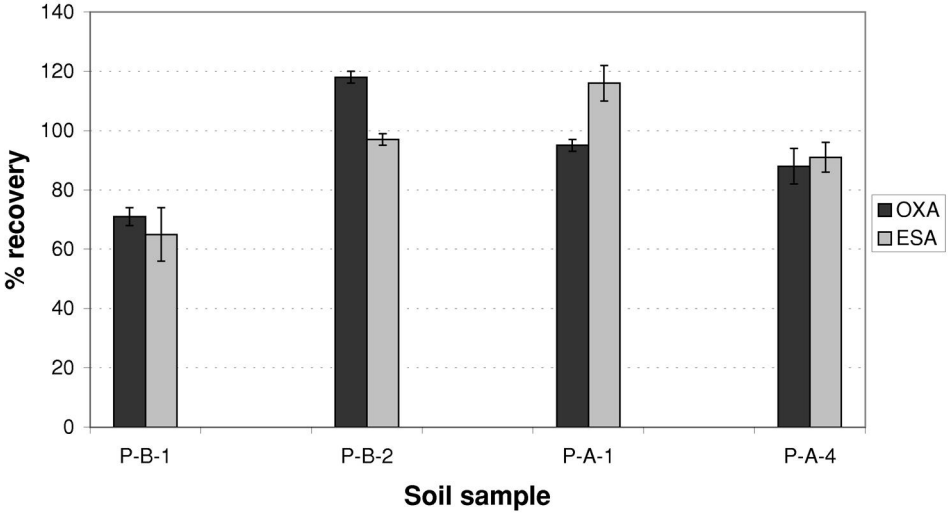

Fig. 3. Average recoveries of acetochlor metabolites in soils spiked at 2 μg/kg (ESA) and 4 μg/kg (OXA).

metabolite at the maximum sampling horizon for the luvisol, between 65 and 90 cm (P-A-4).

3.3.3. Application to soils spread with acetochlor

After optimising the overall methodology on spiked soils, real soil samples were subjected to the extraction process and the acetochlor metabolites were quantified using the standard addition method, which proved to be mandatory. Each soil extract was quantified by the ''blank'' soil showing a better match with the real soil in terms of physicochemical characteristics and depth. Figs. 6 and 7 present the data related to concentrations of acetochlor and its metabolites for two sampling dates. Acetochlor was

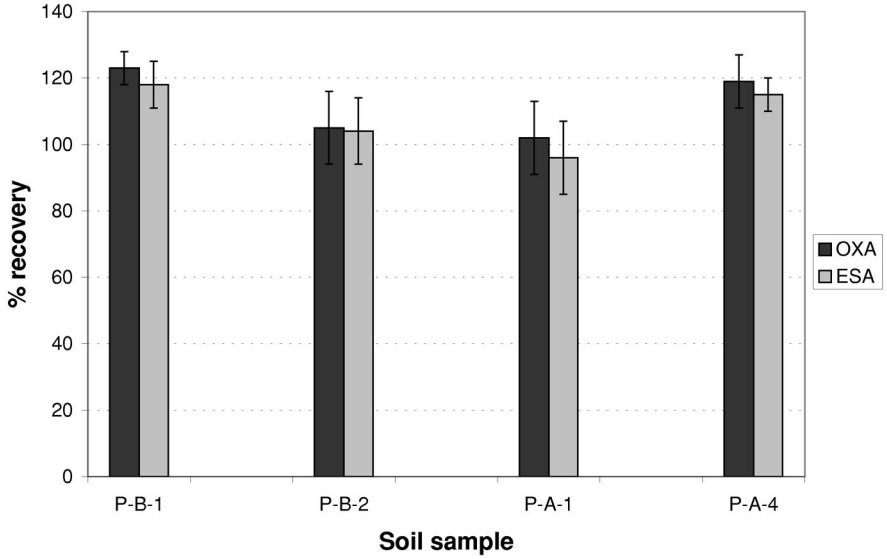

Fig. 4. Average recoveries of acetochlor metabolites in soils spiked at 20 μg/kg.

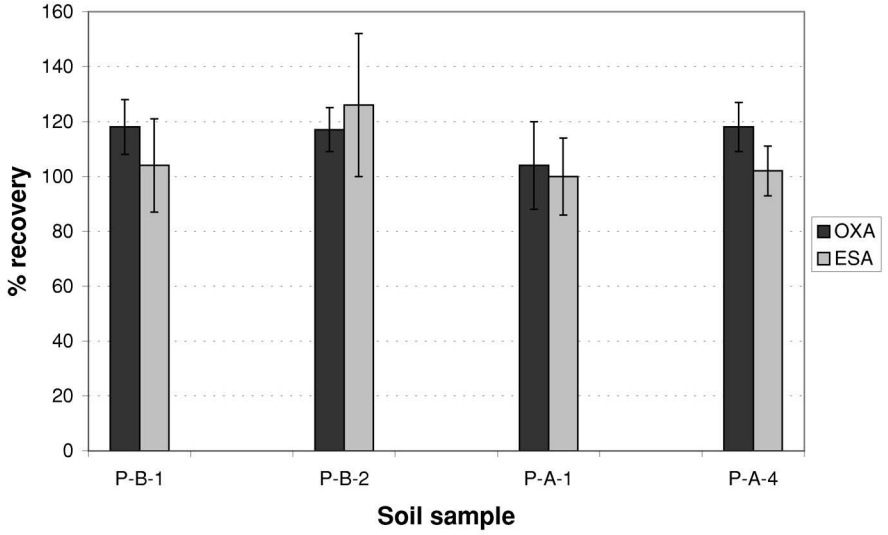

Fig. 5. Average recoveries of acetochlor metabolites in soils spiked at 80 μg/kg.

Table 3
LC–ESI–MS relative response coefficients of the ESA and OXA metabolites of acetochlor

Soil	ESA	OXA
P-A-1	1.19	1.12
P-A-4	1.43	1.39
P-B-1	1.18	1.13
P-B-2	1	1

extracted by pressurised fluid extraction and was analysed by GC–MS. All the experiments will be detailed in a forthcoming paper [12].

Acetochlor was only detected in the upper 5 cm of the soil. The detection of both the metabolites only 21 days after acetochlor application indicates a rapid onset of acetochlor degradation. In one luvisol core, traces of both the metabolites were still detected in the maximum sampling horizons 55 days after acetochlor application.

OXA metabolite concentrations in the luvisol surface layer are higher than in the calcosol, but higher ESA metabolite concentrations are observed in the calcosol, regardless of the time lapse or depth. Moreover, 55 days after acetochlor application, the OXA metabolite was only detected at the surface of the calcosol. Even if the OXA metabolite is less stable than ESA (see mass spectra in Fig. 2), its disappearance with time could indicate that it is more mobile, or that a greater proportion was metabolised in a un-extractable chemical form.

Table 4
Limits of quantification and detection of the ESA and OXA metabolites of acetochlor for the LC–ESI–MS coupling in SIM mode

Soil (50 g)	LOQ (μg/kg)		LOD (μg/kg)	
	ESA	OXA	ESA	OXA
P-A-1	1.1	1.7	0.4	0.5
P-A-4	0.9	1.4	0.3	0.5
P-B-1	1.1	1.7	0.4	0.6
P-B-2	1.3	2	0.4	0.7

4. Conclusions

The performance of the method, the metabolite recoveries for concentrations between 2 and 80 μg/kg, and the results for contrasted soils are conclusive and should allow the monitoring of chloroacetanilide metabolites in the unsaturated zone overlying aquifers.

However, in an attempt to improve analytical quality, the use of a deuterated surrogate, as well as an internal standard, will be developed.

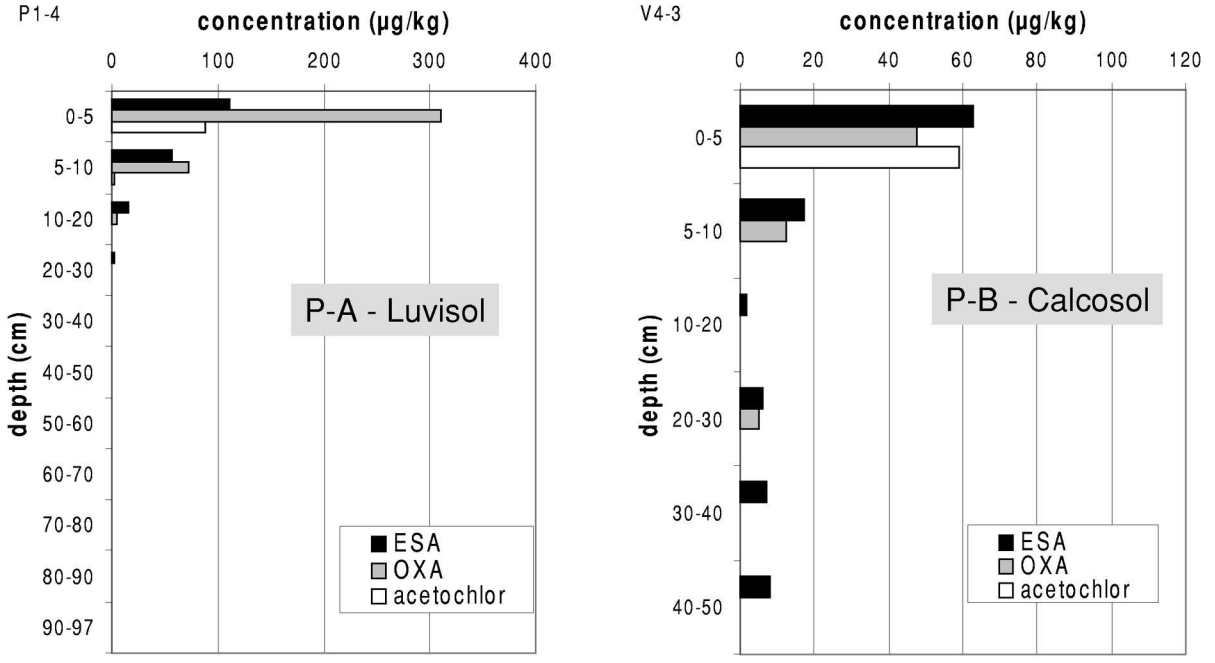

Fig. 6. Depth profiles of acetochlor and ESA and OXA acetochlor metabolites in contrasted soils 21 days after acetochlor application.

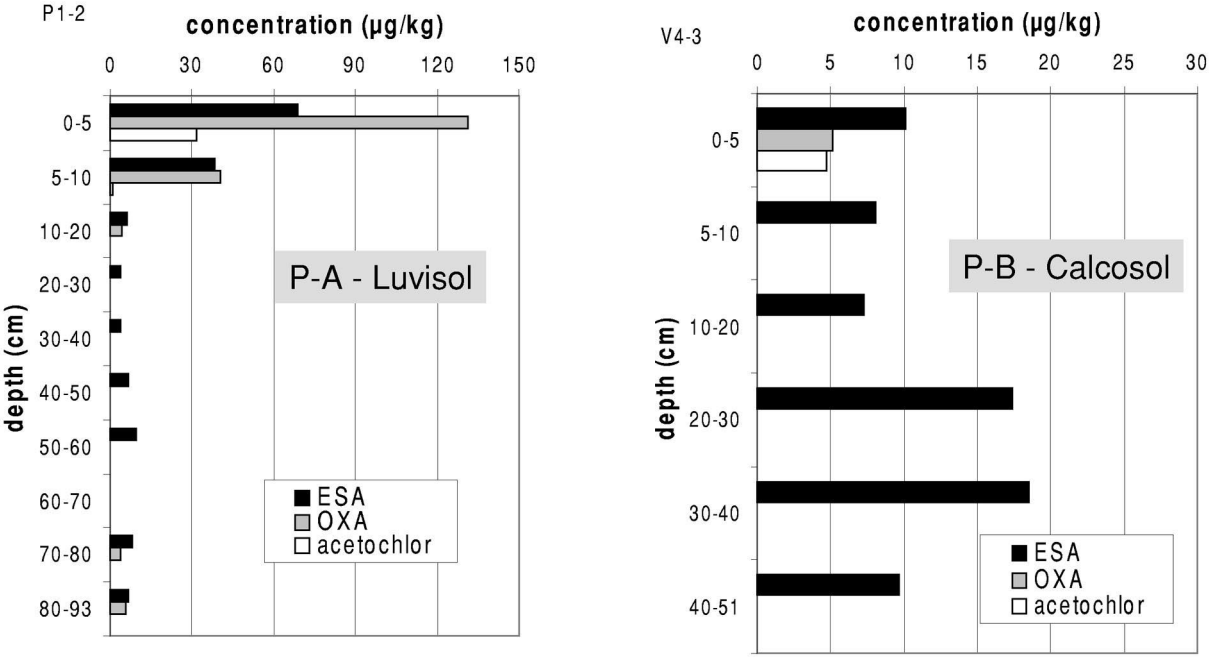

Fig. 7. Depth profiles of acetochlor and ESA and OXA acetochlor metabolites in contrasted soils 55 days after acetochlor application.

By using the MS–MS mode, a limit of detection below 0.1 μg/kg and limit of quantification below 0.5 μg/kg could be reached for the main chloroacetanilide metabolites.

Automated techniques might be necessary to improve the method at the extraction stage, but such techniques (pressurised fluid extraction or microwave assisted extraction for example) often induce temperature raising and cannot be used because of the weakness of the OXA metabolites.

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