ELSEVIER

Journal of Chromatography A, 855 (1999) 575–582

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

# Determination of sulfonylurea degradation products in soil by liquid chromatography–ultraviolet detection followed by confirmatory liquid chromatography–tandem mass spectrometry

Rossana Bossi[a,*], Karl Vejrup[a], Carsten S. Jacobsen[b]

[a] *National Environmental Research Institute, Department of Environmental Chemistry, Frederiksborgvej 399, DK-4000 Roskilde, Denmark*

[b] *Geological Survey of Denmark and Greenland, Thoravej 8, 2400 Copenhagen, Denmark*

Received 13 November 1998; received in revised form 5 March 1999; accepted 9 June 1999

**Abstract**

A method based on liquid extraction followed by sample enrichment on reversed-phase solid-phase extraction was developed for the extraction of five degradation products of four sulfonylurea herbicides (chlorsulfuron, metsulfuron-methyl, thifensulfuron-methyl and tribenuron-methyl) from soil. The compounds have been quantified by LC–UV and identified by tandem LC–MS with electrospray ionization or atmospheric pressure chemical ionization. The limits of detection for the five compounds were between 10 and 50 μg $kg^{-1}$. The method has been applied to the extraction of soil samples after microbial degradation of sulfonylurea herbicides. © 1999 Elsevier Science B.V. All rights reserved.

*Keywords:* Soil; Sulfonylurea; Chlorsulfuron; Metsulfuron-methyl; Thifensulfuron-methyl; Tribenuron-methyl

## 1. Introduction

Sulfonylureas are low dose herbicides (10–15 g a.i. $ha^{-1}$) used to control broad leaved weeds in cereals.

Their persistence in soil is mostly influenced by their rate of chemical and microbial degradation [1]. The main chemical degradation reaction is the cleavage of the sulfonylurea bridge and the reaction products are a sulfonamide, a heterocyclic amine and carbon dioxide. The chemical hydrolysis is faster at low pH. Using often the same degradation pathways as chemical hydrolysis, microorganisms play also an important role for degradation of sulfonylurea herbicides in soil.

Several studies have investigated the degradation paths in aqueous solutions [2–6] and soil [7–11]. The degradation products have been analyzed by liquid chromatography–ultraviolet detection (LC–UV) [2,4,6,7,9,10], capillary electrophoresis (CE)–UV [3,5] and LC–mass spectrometry (MS) [2,7,11]. The structural identification of the degradation compounds have been performed by nuclear magnetic resonance [2,4] or MS [2,7,11]. Different possible metabolites have been synthesized and the products

*Corresponding author. Fax: +45-4630-1114.
*E-mail address:* rbo@dmu.dk (R. Bossi)

0021-9673/99/$ – see front matter © 1999 Elsevier Science B.V. All rights reserved.
PII: S0021-9673(99)00721-9

of aqueous or microbial degradation were identified on the basis of the chromatographic retention time.

Aqueous buffers or water, combined in different proportions with an organic solvent (methanol or acetonitrile) have been employed for extraction of sulfonylurea herbicides degradation products from soil. The metabolites of thifensulfuron-methyl were extracted with ammonium carbonate at pH 9–methanol (1:3, v/v) by Brown et al. [7]. Extraction at acidic or basic pH with a mixture of methanol–water (80:20, v/v) was employed by Cambon et al. [8] for the extraction of thifensulfuron-methyl metabolites. The degradation products of metsulfuron-methyl were extracted at acidic pH by Vega et al. [9] and Cambon and Bastide [10]. Shalaby et al. [11] extracted nicosulfuron, rimiduron and their degradation products from soil with acetonitrile–water (80:20, v/v).

Quantitative and qualitative determination of sulfonylureas degradation products in field samples has never been reported, due to their very low residual concentrations. However, laboratory investigations on the microbial degradation of the hydrolysis products should provide basic information on the fate of these compounds in soil.

For this purpose a specific extraction method for degradation products in soil is desirable.

The aim of this work was to develop an extraction method for quantitative determination of the main degradation products of four sulfonylurea herbicides (chlorsulfuron, metsulfuron-methyl, thifensulfuron-methyl and tribenuron-methyl) in soil. Degradation of sulfonylurea herbicides in soil in the reported literature [7–11] has been carried out with $^{14}C$-labeled compounds. The extraction efficiencies were reported as the total recovered radioactivity. In this way, it is not possible to evaluate the recovery of a single degradation product.

We have chosen to work with nonradiolabeled compounds and develop an extraction method which is specific for the main degradation products of sulfonylurea herbicides.

The developed method has been applied to the determination of degradation products of sulfonylurea herbicides after microbial degradation of the parent compounds. The identity of the compounds has been confirmed by LC–MS–MS, as a test of the new method's performance.

## 2. Experimental

### 2.1. Chemicals

Five degradation products of four sulfonylurea herbicides (chlorsulfuron, metsulfuron-methyl, thifensulfuron-methyl and tribenuron-methyl) were kindly donated by DuPont (Wilmington, DE, USA). The molecular structures of these compounds are given in Fig. 1.

2-Amino-4-methoxy-6-methyl-1,3,5 triazine (product 1) is the heterocyclic amine resulting from the hydrolysis of chlorsulfuron, metsulfuron-methyl and thifensulfuron-methyl, while 2-methylamino-4-methoxy-6-methyl-1,3,5 triazine (product 2) results from the hydrolysis of tribenuron-methyl.

2-(Aminosulfonyl)-methyl ester benzoic acid (product 3) is the sulfonamide common for metsulfuron-methyl and tribenuron-methyl.

3-(Sulfonyl)2-thiofene methyl carboxylate (product 4) and 2-chlorobenzenesulfonamide (product 5) are, respectively, the degradation products of thifensulfuron-methyl and chlorsulfuron.

All solvents used were LC grade from Merck (Darmstadt, Germany) or glass-distilled grade from Rathburn (Walkerburn, UK). The water used for standard dilution and LC mobile phase was purified with a Millipore Super Q system (Millipore, Bedford, MA, USA). The buffer solutions for soil extractions were prepared with PBS (phosphate-buffered saline) (0.01 *M* phosphate buffer, 2.7 m*M* potassium chloride and 138 m*M* sodium chloride, pH 7.4) from Sigma (St. Louis, MO, USA) or sodium hydrogencarbonate, pro analysis grade from Merck. Solid-phase extraction (SPE) was performed with t-$C_{18}$ (1 g) cartridges from Waters (Milford, MA, USA). Stock standard solutions (1 mg $ml^{-1}$) of each analyte were prepared in methanol (product 1) or acetonitrile (products 2–5). Standard dilutions were made in methanol–water (10:90, v/v).

### 2.2. Equipment

LC analyses were performed with a Waters system consisting of a Model 510 reciprocating pump, a WISP 712 autosampler and a UV detector Model 440 equipped with a cadmium lamp for detection at 229

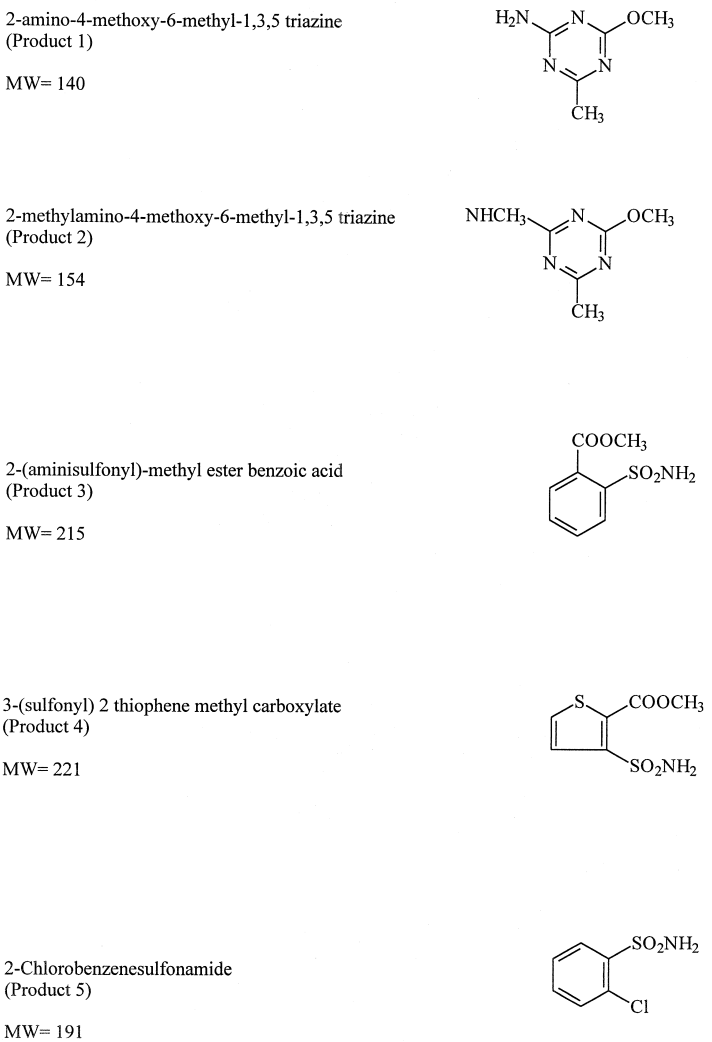


Fig. 1. Molecular structures of the five investigated compounds.

nm. The analytical column was a $C_{18}$ Hypersil (Phenomenex, Torrance, CA, USA) 250×4 mm, 5 μm particle size. The operating conditions were: mobile phase acetonitrile–0.05 *M* sodium hydrogenphosphate buffer, pH 7 (20:80, v/v), isocratic; flow-rate 1 ml $min^{-1}$; injection volume 100 μl.

For confirmatory analyses a LC–MS–MS system was employed. The LC system consisted of a Waters 600 MS solvent delivery system, a Waters 717 autosampler and a Hypersil BDS column (Shandon, Cheshire, UK) 250×2 mm, 5 μm particle size. The mobile phases were methanol–water (10:90, v/v) (A) and 100% methanol (B). The compounds were separated with the following gradient program: from 100% A at $t=0$ to 50% A at $t=3$ min; linear gradient from 50% A at $t=3$ min to 0% A at $t=30$; maintaining 0% A for 3 min and returning linearly to 100% A in 3 min. The flow-rate was 0.2 ml $min^{-1}$ and the injection volume was 50 μl.

The MS system was a Finnigan TSQ 700 (Finnigan MAT, San José, CA, USA) triple quadrupole equipped with an electrospray ionization (ESI) source or an atmospheric pressure chemical ionization (APCI) source. The instrument was operated in

the positive or negative ionization mode. The operating conditions for ESI were: sheat gas (nitrogen) pressure 65 p.s.i.; auxiliary gas (nitrogen) flow 2.1 L $min^{-1}$; spray voltage 5 kV and capillary temperature 250°C (1 p.s.i.=6894.76 Pa). For APCI the operating conditions were: sheat gas (nitrogen) pressure 40 p.s.i.; corona current 5 μA; vaporizer temperature 500°C; capillary temperature 170°C. The voltage in the pre-analyzer zone was kept low (30 V) in order to avoid fragmentation and obtain the quasi-molecular ion. For MS–MS analyses argon was used as collision gas at a pressure of 1 mTorr (1 Torr= 133.322 Pa).

### 2.3. Soil fortification

A sandy soil (coarse sand 78.8%; fine sand 10.0%; silt 3.8%; clay 4.1%; organic matter 3.4%; pH 7.0) was used for development of the extraction method. The soil (50 g) was fortified by adding 10 ml of an acetonitrile solution containing the five compounds. The slurry was mixed for 1 h and the solvent was allowed to evaporate at room temperature. After fortification the soil was kept at 4°C.

### 2.4. Soil Extraction

Different extraction solvents were tested: PBS solution (pH 7.4), 0.1 *M* sodium hydrogencarbonate buffer (pH 8.2), PBS–acetone (80:20, v/v) and PBS–acetonitrile (80:20, v/v).

A portion (10 g) of the fortified soil was extracted twice with 20 ml of extraction solvent for 30 min. The slurry was centrifuged at 3200 rpm on a Sorvall H-400 centrifuge (DuPont) and the supernatant was decanted into a glass bottle. When the extraction solvent contained acetonitrile or acetone, the organic solvent was removed by rotary evaporation in a water bath at 35°C and a pressure of 40 mbar.

The aqueous extract was concentrated with SPE. A t-$C_{18}$ cartridge was conditioned with 10 ml dichloromethane followed by 10 ml methanol and washed with 20 ml water. After passing the sample through the cartridge, residual water was removed by drying under vacuum for 30 min. The analytes were eluted with 10 ml dichloromethane. The solvent was evaporated to dryness under a stream of nitrogen and the sample reconstituted in 1 ml methanol–water (10:90, v/v).

## 3. Results and discussion

### 3.1. LC–UV analysis

The LC–UV system was used for quantitative analysis of soil extracts. A UV trace chromatogram of a standard containing the five investigated compounds at a concentration of 1 μg $ml^{-1}$ is shown in Fig. 2. The linearity of the chromatographic determination was examined for the concentration range 50 ng $ml^{-1}$ to 10 μg $ml^{-1}$, corresponding to a concentration of the compounds in soil, prior to extraction, of 0.05 to 1 mg $kg^{-1}$. The amount of the degradation products in soil was expected to lie in this concentration range after microbial degradation of sulfonylurea herbicides in laboratory experiments. Linear regression data are shown in Table 1. The calibration curves were linear in the investigated concentrations range. The soil extracts contained a compound that gave a peak very close to that of product 1, making it impossible to quantify it. For this compound quantification was performed with LC–MS.

### 3.2. LC–MS–MS analysis

In order to select the most abundant mass-to-charge ratio ($m/z$) for each compound, single standard solutions (20 μg $ml^{-1}$) were injected and a mass spectrum was recorded with both inlets in the positive or negative ionization mode. The scan range was 100–250 atomic mass units (amu) at 2 s dwell time. The ions used for selected ion monitoring (SIM) and the ionization mode for each compound are summarized in Table 2. For both heterocyclic amines (products 1 and 2) the highest response was obtained in the positive ionization mode and no significant difference was observed between ESI and APCI. Product 3 had the same fragmentation pattern for ESI and APCI in the positive ionization mode, with the most intense ion at $m/z$ 233. The intensity of $[M+H]^+$ ($m/z$ 216) was about five-times lower than that of $m/z$ 233. The intensity of these two ions was about ten-times higher with ESI than APCI.

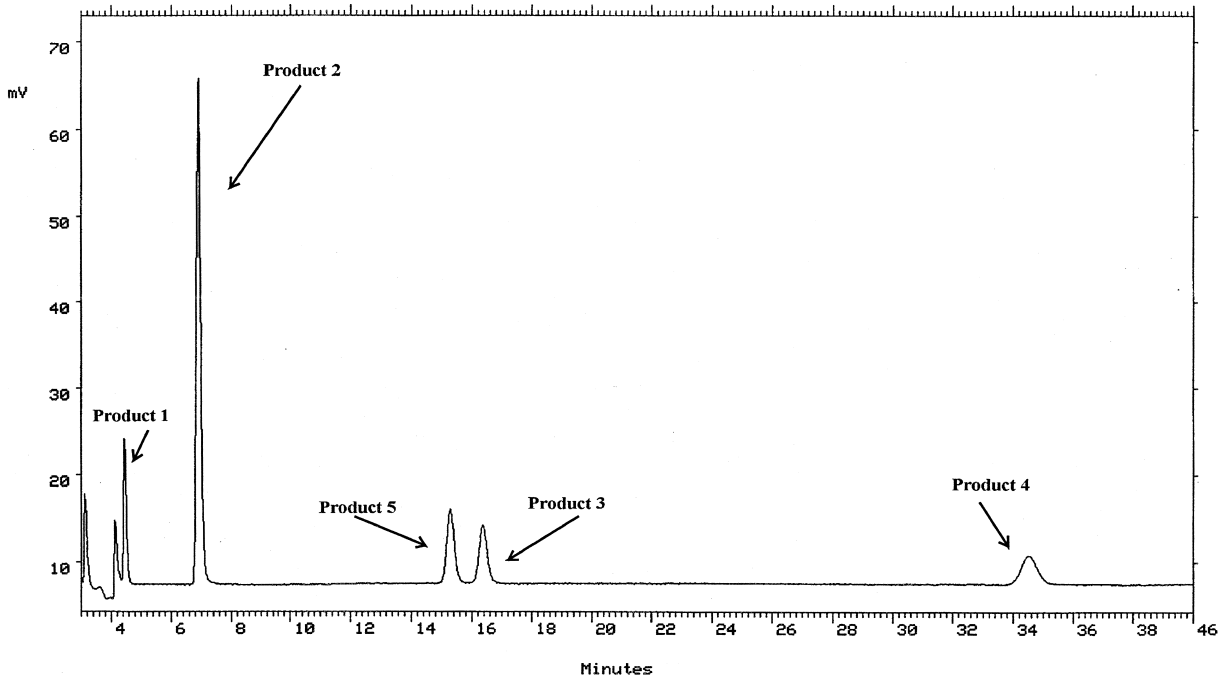


Fig. 2. LC–UV chromatogram of a standard containing the five investigated compounds (concentration: 1 μg $ml^{-1}$).

Table 1
Linear regression data for the five investigated compounds (LC–UV) [calibration range 0.05–1 μg $ml^{-1}$ (seven data points)]

| Compound | $y=bx+c$[a] | $r^2$ |
|---|---|---|
| Product 1[b] | $y=8.6470\cdot10^4x+1.1791\cdot10^2$ | 0.9436 |
| Product 2 | $y=1.5971\cdot10^4x+5.3895\cdot10^2$ | 0.9999 |
| Product 3 | $y=5.0462\cdot10^4x-1.3697\cdot10^2$ | 0.9862 |
| Product 4 | $y=1.5081\cdot10^4x-1.2121\cdot10^2$ | 0.9967 |
| Product 5 | $y=1.2736\cdot10^4x+1.3648\cdot10^2$ | 0.9862 |

[a] $x$=Injected concentration μg $ml^{-1}$; $y$=peak area.
[b] Linear regression data obtained with LC–MS.

Product 4 did not show any molecular ion. The most abundant ion was $m/z$ 158 in positive ionization mode with a signal four-times higher with ESI respect to APCI. This compound could not be ionized in negative ionization mode with any inlet. This result is in contrast with the results obtained by Brown et al. [7] for LC–MS of degradation products of thifensulfuron-methyl. These authors obtained a strong ion signal with negative ESI at $m/z$ 206, 188 and 162, corresponding to different fragments of product 4.

Product 5 gave an intense ion signal only with APCI in the negative ionization mode. For negative ESI the signal intensity was about 25-times lower.

In order to identify a compound, at least two typical ions are necessary. Both ESI and APCI are soft ionization techniques that give nearly no fragmentation of the produced protonated molecule. With

Table 2
MS parameters for the five investigated compounds

| Compound | Ionization mode | Parent ion ($m/z$) | Collision energy (eV) | Product ions (% relative abundance) |
|---|---|---|---|---|
| Product 1 | $APCI^+$ | 141 | 50 | 141 (100), 58 (85) |
| Product 2 | $APCI^+$ | 155 | 50 | 57 (100), 71 (65), 155 (85) |
| Product 3 | $ESI^+$ | 233 | 20 | 199 (100) |
| Product 4 | $ESI^+$ | 158 | 20 | 126 (100) |
| Product 5 | $APCI^-$ | 190 | 50 | 78 (100), 190 (70) |

tandem MS the protonated molecules are fragmented in the collision cell and the product ions are monitored in the third quadrupole. One or two of these ions are selected and the product ions are monitored in selected reaction monitoring (SRM). The high specificity of the LC–MS–MS analysis is given by the fact that only one selected ion is allowed to pass through the first quadrupole.

Daughter ion spectra in full scan were collected for each analyte by injecting a standard solution of 20 μg $ml^{-1}$. The pressure of the collision gas was kept constant so that the degree of fragmentation was only dependent on the applied voltage difference between the first quadrupole and the collision cell. The applied voltages were between 20 and 50 V.

For products 3 and 4 a voltage of 20 V was enough to obtain a complete fragmentation of the parent ion and one typical ion as most abundant product ion. For products 1, 2 and 5 a collision voltage of 30 V still gave the parent ion as the most abundant ion with a relative abundance of the fragment ions lower than 20%. For products 2 and 5 an applied voltage of 50 V resulted in a relative abundance of 100% for one of the fragments. The parent ions were still present at intensities from 50 to 90%. For product 1 the base peak in the daughter ion spectrum was still the parent ion with a voltage of 50 V, but the relative abundance of one of the fragment was increased to 85%. Application of higher voltages did not increased the relative abundance of the fragment ions. The $m/z$ values and the intensities of fragment ions (>50%) respect to the base peak and the collision voltage used to obtain the highest fragmentation of the parent ion are shown for each compound in Table 2.

SRM LC–MS–MS was used to confirm the identity of the degradation products in soil extracts after microbial degradation of sulfonylurea herbicides.

### 3.3. Extraction from soil

Four of the five investigated compounds contained a free amino group. An acidification of the extraction solvent, as proposed by Vega et al. [9] and Cambon et al. [8], would increase the protonation of the amino group, thereby increasing the adsorption of the compounds to the soil colloids by cation-exchange. Hence, only neutral or basic extraction solvents were investigated in this study.

In order to evaluate, in the first instance, the recoveries after solvent extraction, the soil was spiked with the five analytes to a high concentration (4 mg $kg^{-1}$) and the aqueous extract was directly analyzed by LC–UV. The average recoveries for three replicate extractions obtained with four different extraction solvents are summarized in Table 3. The recoveries obtained with each extraction solvent were statistically compared ($t$-test) to determine the probability of a significant difference ($P<0.01$) between each extraction treatment.

The low recoveries of products 2 and 4 with PBS might have been due to a stronger adsorption of these compounds into the soil organic matter. An attempt to extract higher amounts of the organic fraction was made by increasing the molarity and the pH of the extraction solvent or by addition of an aliquot (20%) of an organic solvent (acetone or acetonitrile) to the PBS solution.

Good recoveries for all compounds were obtained with PBS–acetone and PBS–acetonitrile. A significant difference was observed for product 2 between PBS–acetone and PBS–acetonitrile, giving the later extraction solvent a much higher recovery. This was probably due to a higher solubility of this compound in acetonitrile. Based on these results, PBS–acetonitrile (80:20, v/v) was chosen as extraction solvent for further experiments.

The analytes were concentrated from the aqueous

Table 3
Average recoveries from soil (RSD, %) obtained with four different extraction solvents (fortification level 4 mg $kg^{-1}$, $n=3$)

| Compound | PBS | Sodium hydrogencarbonate | PBS–acetone (80:20, v/v) | PBS–acetonitrile (80:20, v/v) |
|---|---|---|---|---|
| Product 1 | 88 (7.8) | 72 (20) | 90 (17) | 80 (8.7) |
| Product 2 | 59 (0.0) | 71 (2.4) | 78 (2.7) | 92 (1.7) |
| Product 3 | 90 (1.1) | n.d. | 87 (4.0) | 88 (1.1) |
| Product 4 | 64 (5.9) | 66 (3.8) | 71 (17) | 78 (2.2) |
| Product 5 | 95 (0.6) | 83 (1.8) | 91 (2.3) | 92 (2.1) |

extract with SPE. Recovery data for four fortification levels and limits of detection (LODs) are shown in Table 4. The LODs were determined as three-times the noise-background ratio for the five analyte peaks for a UV determination of a soil fortified to 0.05 mg $kg^{-1}$.

Product 4 gave low recoveries and high relative standard deviation (RSD) and for three fortification levels the compound was not recovered at all. However, product 4 could be extracted from soil with good average recoveries (Table 3). Poor recoveries might have been caused by a partial degradation of this compound during the two steps were the sample was heated (rotary evaporation of acetonitrile after soil extraction and evaporation of the solvent to dryness after elution of the SPE cartridge). For the other four compounds good average recoveries (60–112%) and good precision (0.8%< RSD<24.6%) were obtained. The recoveries of product 2 after SPE were significantly lower than the recovery obtained with PBS–acetonitrile extraction. For this compound breakthrough may have been occurred during the preconcentration step on SPE cartridge.

### *3.4. Application of the extraction method to real samples*

The extraction method was tested on soil samples spiked with four sulfonylurea herbicides (chlorsulfuron, metsulfuron-methyl, thifensulfuron-methyl and tribenuron-methyl) at a concentration of 10 mg $kg^{-1}$, and subjected to microbial degradation. The soil type was the sandy soil described in Section 2.3. The soil samples were incubated at 10°C and at a water content of the soil adjusted to 80% of field capacity. After 126 days of incubation, portions of 5–9 g soil were taken from each sample and air dried at room temperature. The soil samples were extracted with the method previously described and analyzed by LC–UV for degradation products.

For each sulfonylurea herbicide the corresponding degradation products were found in the extracts. Only for thifensulfuron-methyl the 3-(sulfonyl)2-thiophene methyl carboxylate (product 4) was not found, confirming the instability of this compound. The identity of the analytes was confirmed by SRM MS–MS. Fig. 3 shows the SRM ion trace chromatograms of the degradation products deriving from the hydrolysis of chlorsulfuron. Product 1 ($m/z$ 141→$m/z$ 58) was found as metabolite for chlorsulfuron, metsulfuron-methyl and thifensulfuron-methyl. Product 2 ($m/z$ 155→$m/z$ 57) was present in the extract of soil spiked with tribenuron-methyl, together with product 1 (0.5% of the total concentration of product 2), indicating that a demethylation of product 2 had occurred. Product 3 ($m/z$ 233→$m/z$ 199) was found as degradation product of metsulfuron-methyl and tribenuron-methyl, while product 5 ($m/z$ 190→$m/z$ 78) was found as degradation product of chlorsulfuron.

## 4. Conclusions

This method is suitable for the extraction of the degradation products of chlorsulfuron, metsulfuron-methyl, thifensulfuron-methyl and tribenuron-methyl from soil. 3-(Sulfonyl)2-thiophene methyl carboxylate, a degradation product of thifensulfuron-methyl, was quantitatively extracted from soil but poor recoveries were obtained after SPE, probably because of thermal instability. The LODs were too high to allow for the method to be used for monitoring sulfonylureas degradation products in field samples. However, the method can be useful for determination

Table 4
Average recoveries from soil (RSD, %) at indicated fortification levels after extraction with PBS–acetonitrile (80:20, v/v) followed by SPE on t-$C_{18}$ ($n$=3)

| Compound | 0.05 mg $kg^{-1}$ | 0.1 mg $kg^{-1}$ | 0.5 mg $kg^{-1}$ | 1 mg $kg^{-1}$ | LOD |
|---|---|---|---|---|---|
| Product 1[a] | 91 (5.2) | 75 (5.7) | 86 (3.1) | 92 (8.8) | 10 |
| Product 2 | 68 (13) | 68 (11) | 64 (18) | 63 (11) | 10 |
| Product 3 | 93 (6.5) | 97 (4.2) | 74 (8.4) | 71 (13) | 25 |
| Product 4 | n.d. | n.d. | 26 (38) | 26 (18) | 50 |
| Product 5 | 84 (9.3) | 83 (5.3) | 77 (7.8) | 76 (8.4) | 25 |

[a] LOD determined with LC–MS.

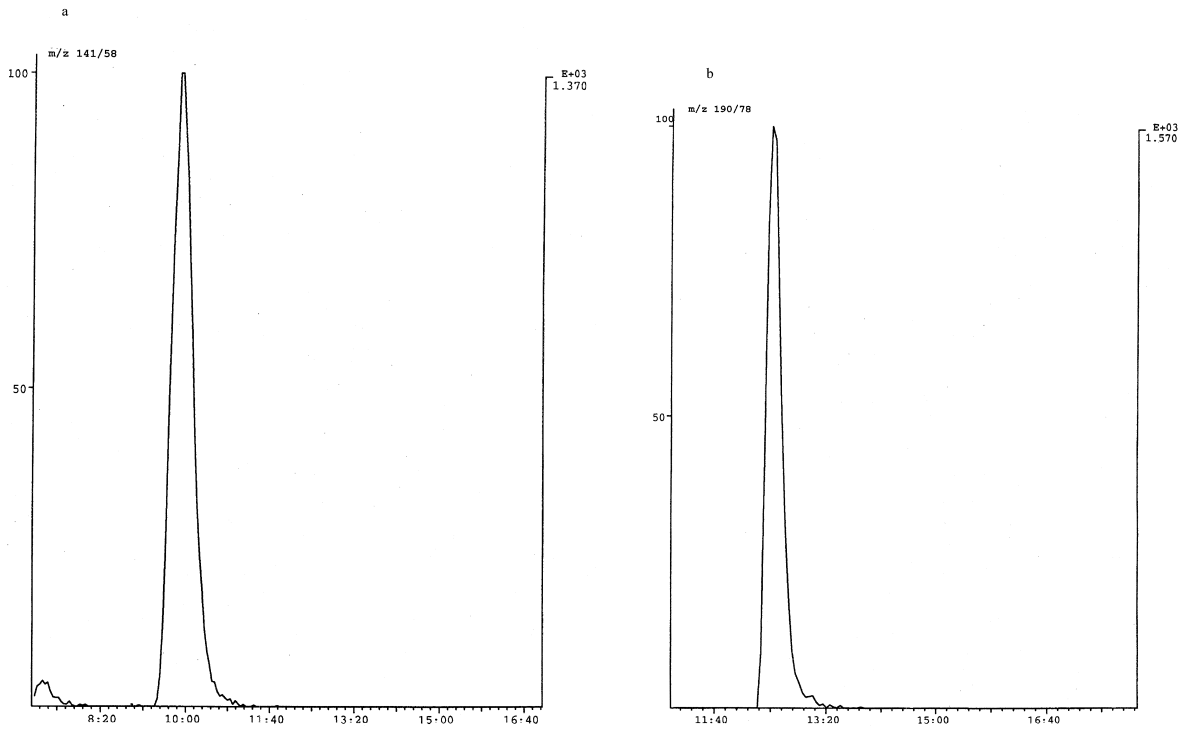


Fig. 3. SRM ion chromatogram for product 1 (a) and product 5 (b) analyzed in a soil extract from chlorsulfuron degradation.

of the microbial degradation rate of the degradation products during laboratory experiments, which are usually carried out at concentrations higher than field application rates. The information deriving from such experiments may provide additional knowledge about the fate of sulfonylurea herbicides in soil. The sensitivity of the method can be significantly improved by analysis of the soil extracts in SRM LC–MS–MS by monitoring the precursor-product ion transition for each analyte.

## Acknowledgements

This work was financially supported by a grant from The Danish Interministry Research Program on Pesticides. The authors would like to thank Du Pont de Nemours (Wilmington, DE, USA) for kindly donating the degradation products, as well as the mother compounds. Moreover, Mary A. Chrillesen and Annegrete Ljungqvist are thanked for helpful technical support.

## References

[1] E.M. Beyer, M.J. Duffy, J.V. Hay, D.D. Schlueter, in: P.C. Kearney, D.D. Kaufman (Eds.), Herbicides – Chemistry, Degradation and Mode of Action, Vol. 3, Marcel Dekker, New York, 1988, pp. 117–189.
[2] I. Braschi, L. Calamai, M.A. Cremonini, P. Fusi, C. Gessa, O. Pantani, A. Pausino, J. Agric. Food Chem. 45 (1997) 4495.
[3] G. Dinelli, A. Vicari, A. Bonetti, P. Catizone, J. Agric. Food Chem. 45 (1997) 1940.
[4] J.P. Cambon, J. Bastide, J. Agric. Food Chem. 44 (1996) 333.
[5] G. Dinelli, A. Vicari, A. Bonetti, J. Chromatogr. A 700 (1995) 195.
[6] S. Hemmamda, M. Calmon, J.P. Calmon, Pestic. Sci. 40 (1994) 71.
[7] H.M. Brown, M.M. Joshi, A.T. Van, T.H. Carski, J.J. Dulka, M.C. Patrick, R.W. Reiser, R.S. Livingston, J. Doughty, J. Agric. Food Chem. 45 (1997) 955.
[8] J.P. Cambon, J. Bastide, D. Vega, J. Agric. Food Chem. 46 (1998) 1210.
[9] D. Vega, J. Bastide, C. Poulain, Weed Res. 32 (1992) 149.
[10] J.P. Cambon, J. Bastide, Weed Res. 32 (1992) 357.
[11] L.M. Shalaby, F.Q. Bramble, P.W. Lee, J. Agric. Food Chem. 40 (1992) 513.