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Short communication

Improved chromatographic method for the simultaneous determination of ten phenylurea herbicides and some of their degradation products in soil

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Abstract

A new analytical method is presented for the simultaneous determination of ten phenylureas and six degradation products. This is the first multiresidue method for these herbicides that includes the most prominent metabolites in soil, the monodesalkyl derivatives and, therefore, complements and improves existing methods. After extraction, the compounds are separated on two coupled columns with Nucleosil-100 C_{18}, 3 μm with a gradient of acetonitrile in water. The method has a detection limit of 0.02 mg/kg soil and can be applied for the determination of phenylureas and their degradates without clean-up.

Keywords: Pesticides; Phenylureas

1. Introduction

Substituted phenylurea herbicides are widely used in agriculture as herbicides and, consequently, can give rise to residues in crops, soil and surface waters [1]. Compounds belonging to this group are thermally unstable and hence their direct analysis by gas–liquid chromatography is not feasible. High-performance liquid chromatography, however, has emerged as an important method for the analysis of these pesticides. Although residue methods of single phenylureas are still of current interest [2,3], multiresidue methods for the determination of these compounds in soil [4,5], water [6–9] and plant materials [10] have gained importance and were published previously.

UV detection was most often applied for the determination of phenylureas in soil and plant samples. Electrochemical detection has proved to be more sensitive only for the analysis of water [5]. Fluorescence detection required derivatization without improving the detection limit in soil [11]. Photoconductivity is not suitable for all phenylureas under investigation [10].

Our aim was to develop an improved analytical method for the chromatographic separation of ten important phenylureas. According to our own degradation studies [12], monodesalkylated products of the phenylureas are the only products accumulating in significant amounts. Therefore, we wanted to include these compounds. The method was developed for degradation studies and the determination of adsorption distribution coefficients.

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2. Experimental

2.1. Chemicals

Herbicide standards and metabolites are listed in Table 1. They were all purchased from Riedel de Haen (Seelze, Germany) or kindly provided by Hoechst (Frankfurt, Germany), Ciba Geigy (Basle, Switzerland) and Bayer (Leverkusen, Germany). Acetonitrile (HPLC-grade) was purchased from Riedel de Haen (Seelze, Germany). Methanol, for the extraction of the soil, was from Merck (Darmstadt, Germany) and was distilled prior to use.

2.2. Apparatus

The instrumental system consisted of a pump 300 C and a gradient former 250 B from Gynkotek (Munich, Germany), an autosampler 2157 and an oven 2155 from LKB Pharmacia (Uppsala, Sweden), and a variable-wavelength detector SPD-6A from Shimadzu (Kyoto, Japan).

2.3. Columns

Compound separation was performed using two coupled columns (250.0×4.0 mm) with Nucleosil 100 RP-18, 3 μm (material manufactured by Machery and Nagel (Düren, Germany), packed by Knauer, Berlin, Germany) and a 5.0×4.0 mm guard column with the same material. The temperature of the column was kept at 37°C and UV absorbance was measured at 240 nm.

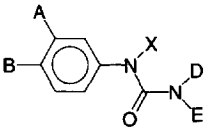
2.4. Mobile phase

The mobile phase was acetonitrile in water at a flow-rate of 0.55 ml/min (35% acetonitrile for 45 min, followed by a linear gradient from 35 to 50% acetonitrile over 30 min and a final elution with 50% acetonitrile for 10 min; the columns are then reconditioned with 35% acetonitrile in water for 20 min before starting the next chromatogram).

2.5. Sample preparation

Stock solutions were prepared with 50% acetoni-

Table 1
Formulae of the phenylureas investigated



Compound	A	B	X	D	E
Fenuron	-H	-H	-H	$-CH_3$	$-CH_3$
Monuron	-H	-Cl	-H	$-CH_3$	$-CH_3$
Diuron	-Cl	-Cl	-H	$-CH_3$	$-CH_3$
Isoproturon	-H	$-CH(CH_3)_2$	-H	$-CH_3$	$-CH_3$
Chlorotoluron	-Cl	$-CH_3$	-H	$-CH_3$	-CHd3
Monolinuron	-H	-Cl	-H	$-OCH_3$	$-CH_3$
Linuron	-Cl	-Cl	-H	$-OCH_3$	$-CH_3$
Metobromuron	-H	-Br	-H	$-OCH_3$	$-CH_3$
Chlorbromuron	-Cl	-Br	-H	$-OCH_3$	$-CH_3$
Methabenzthiazuron	-benzthiazolyl		$-CH_3$	$-CH_3$	-H
3-(3,4-Dichlorophenyl)-1-methoxyurea	-Cl	-Cl	-H	$-OCH_3$	-H
3-(3,4-Dichlorophenyl)-1-methylurea	-Cl	-Cl	-H	-H	$-CH_3$
3-(4-Chlorophenyl)-1-methoxyurea	-H	-Cl	-H	$-OCH_3$	-H
3-(4-Chlorophenyl)-1-methylurea	-H	-Cl	-H	-H	$-CH_3$
3-(4-(1-Methylethyl)-phenyl)-1-methylurea	-H	$-CH(CH_3)_2$	-H	$-CH_3$	-H
3-(3-Chloro-4-methylphenyl)-1-methylurea	-Cl	$-CH_3$	-H	$-CH_3$	-H

trile in water. The samples were centrifuged (12 000 *g*) prior to analyses. The injection volume was 100 μl. Concentrations of the individual compounds were determined using rectilinear standard curves (concentration range: 0.1–10.0 μg/ml). The minimum quantitation level at λ=240 nm was 0.1 μg/ml for all compounds investigated.

3. Results and discussion

We focused on reversed-phase chromatography with C_{18}-bonded silica columns, following the recommendations of most published investigations [2–9,13,14]. Attempts to separate phenylureas with nitrile- and amino-bonded stationary phases have been published previously [10,15], but not all of the compounds tested could be separated. Reversed-phase against normal-phase chromatography of phenylureas has also been tested previously and was recommended for the simultaneous determination of the corresponding aniline degradation products [16]. C_{18}-bonded silica columns, however, gave better separation of the parent compounds [4]. Furthermore, the use of normal-phase columns does not allow traces of water to be present in the injection solution [4], thereby complicating the preparation of environmental samples. Ion-interaction reversed-phase high-performance liquid chromatography was especially useful when compounds such as thiourea, phenylurea, and ethylenethiourea were included in the investigation [17]. However, these compounds do not occur as products of commercially available pesticides in soil.

As the reversed-phase material has a significant influence on the separation, investigations with material from different manufacturers (Nucleosil, Lichrospher, Eurospher and Hypersil) were undertaken. With 5 μm Lichrospher 100 RP 18 and 5 μm Eurospher 100 C_{18}, no good separation, especially of compounds 10–14 (see Table 2), could be obtained. No 3 μm material was available from the manufacturer. With 3 μm Hypersil 120 ODS, compounds 3 and 4 (see Table 2) could not be separated at all. The best separation was obtained with Nucleosil C-100, 3 μm, RP 18 columns. However, even on this column, complete baseline separation of compounds 6–14 (see Table 2) could not be achieved, either with isocratic or gradient elution, using mixtures of water and methanol or acetonitrile. Therefore, a second column was coupled to the first one via a short capillary. It was also found that column temperature

Table 2
Average extraction recoveries with relative standard deviations (±R.S.D.) of phenylurea herbicides and their metabolites in two soil samples at three spiking levels (2.00, 0.20 and 0.02 mg/kg, n=5)

No.	Compound	Recovery (%)±R.S.D. (%)
1	Fenuron	94±6
2	3-(4-Chlorophenyl)-1-methylurea	94±3
3	3-(4-Chlorophenyl)-1-methoxyurea[a]	76±3
4	Monuron	94±3
5	3-(3-Chloro-4-methyl)-phenyl)-1-methylurea	98±6
6	3-(4-(1-Methylethyl)-phenyl)-1-methylurea	99±3
7	3-(3,4-Dichlorophenyl)-1-methylurea	94±6
8	Methabenzthiazuron	85±7
9	Chlorotoluron	96±12
10	3-(3,4-Dichlorophenyl)-1-methoxyurea[a]	84±1
11	Monolinuron	101±15
12	Isoproturon	100±9
13	Diuron	94±5
14	Metobromuron	97±8
15	Linuron	106±7
16	Chlorbromuron	94±11
17	Chloroxuron	Standard

[a] These compounds are subject to fast chemical hydrolysis. Therefore, the extraction procedure was modified [evaporation of only 20 ml of the soil extracts and the use of spiking levels of 2.00 and 0.20 mg/kg, (n=3)].

had a notable influence on the separation and must be optimized between 35 and 41°C for each batch. If the temperature is too low, compounds 8 and 9, and 11 and 12, are not separated. Temperatures that were too high resulted in insufficient resolution of the peaks of the phenylureas 8 and 9. Fig. 1 shows the chromatogram of the extract of a fortified soil.

The performance of the method for environmental samples was assessed with two soil samples fortified at three concentration levels. The results are demonstrated in Table 2. The monodesalkylated products of fenuron, chlorbromuron and metobromuron could not be included in the method due to the lack of standards. Degradation studies with the single compounds [12], however, proved that no interference of products with any of the other phenylureas in the chromatograms occurred. The detection limits of 0.02 mg/kg in soil (recoveries >75%, variation coefficients <16%, $n=5$, based on a signal-to-noise ratio ≥2) meet, or surpass, those reached for single compounds [13,14,18]. Extraction and solvent evaporation were performed as described earlier [14], except that a 40-ml volume instead of a 20-ml volume of the extraction solution was used and the addition of phosphoric acid was omitted. The method is suitable for degradation studies, as residues of 1% of the initial concentration can be determined. Clean-up is not required for this concentration range, which is a main advantage of the method. However, guard columns must be used to protect the analytical column. They were changed after about 30 injections.

4. Conclusion

Our method enables the separation and determination of ten important phenylurea herbicides and their monodesalkyl derivatives, which are the principal degradation products in soil. So far, these products have only been taken into consideration in residue methods for single pesticides such as isoproturon and linuron [13,14,19]. For multiresidue methods, only anilines were included as degradates

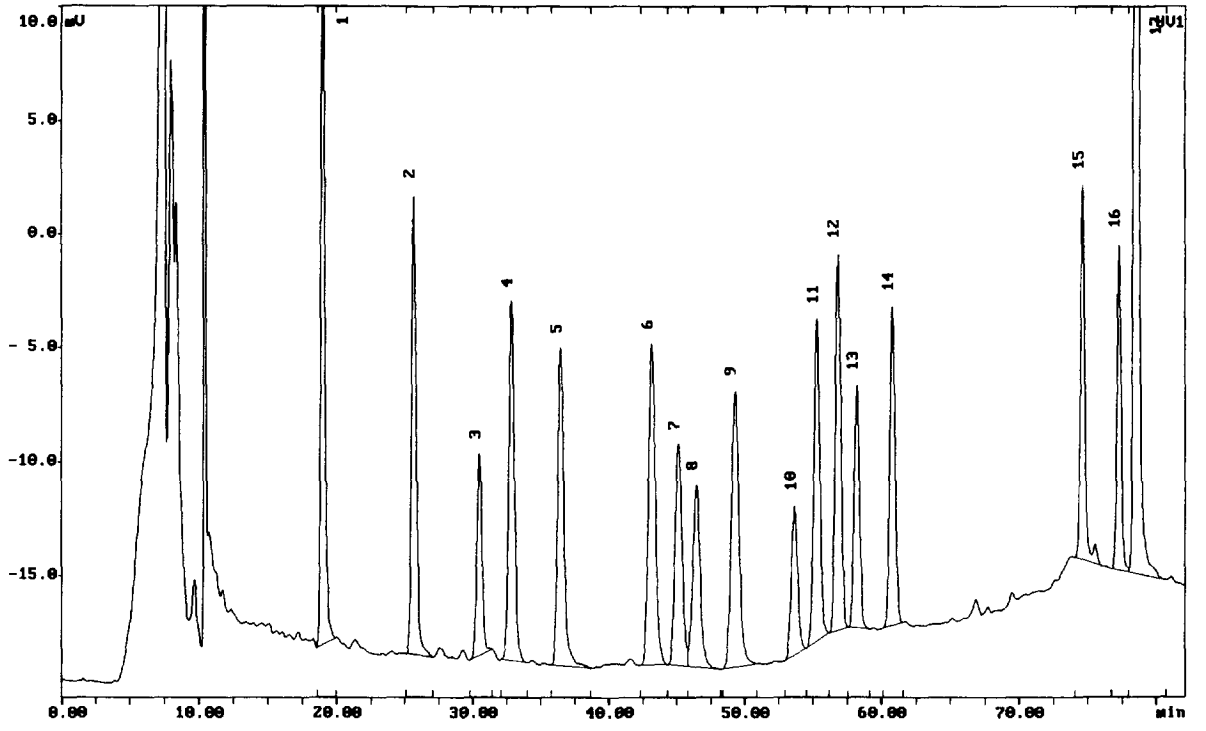


Fig. 1. RP-HPLC of the extract of a fortified soil sample (0.20 mg/kg). See Table 2 and text for the different compounds and the chromatographic conditions.

of phenylureas in water [6]. The method presented is currently used for the determination of the DT-90 values (disappearance time for 90% of the compound) of phenylurea herbicides in soil [12].

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