SOLID-PHASE EXTRACTION FOLLOWED BY HPLC/DAD FOR DETERMINATION OF SULFONYLUREA HERBICIDE IN SOIL

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Abstract. *Sulfonylurea herbicides are widely used for control for most broad-leaves weeds and common grasses in agricultural crops in quite low application rate (less than 100 g/ha). Their persistence in the soil environment is mainly dependent on several site specific factors, such as rainfall, soil properties (especially pH and organic matter), climate and combination of factors. The fate of sulfonylurea herbicides in soil ranges from few weeks to three or more years. Generally, sulfonylurea herbicides represent potential environment pollutants in soil and water. One of the most applicable SU herbicide is nicosulfuron (1-(4.6-dimethoxypyrimidin-2-yl)-3-(3-dimethylcarbamoyl-2-pyridylsulfonyl) urea). In extreme weather conditions, nicosulfuron residue in soil can influence phytotoxicity symptoms in some sensitive crops, such as sugar beet or soybean. In order to develop simple and rapid, but enough sensitive method for determination of nicosulfuron residues in soil sample, this study was conducted. Method validation was performed by analyzing spiked uncontaminated soil samples (10 g) with nicosulfuron at three levels 2.5, 5.0 and 7.5 μg/ml. Extraction was done with phosphate buffer pH 7.4/methanol (80/20, v/v) solution. The mixture was shaken for 1 min using Vortex and liquid and solid phases were separated by centrifugation at 3000 rpm for 5 min. The extraction process was repeated two times, followed by cleaning up on C18 cartridges. Analysis was carried out using an HPLC-UV diode array detection system (Agilent 1100, USA), with an Agilent Zorbax Eclipse C18 column (50 mm × 4.6 mm, 1.8 μm) and mobile phase consisting of ultrapure water pH 2.5 with H_3PO_4 and acetonitrile (70/30, v/v). Presence of nicosulfuron was confirmed by overlapping spectra (240 nm) from standard solution and from soil sample spiked with nicosulfuron. The accuracy of the defined method was confirmed by the good results of recovery assay (89%). The limit of detection and limit of quantification were found to be 0.01 mg/kg and 0.05 mg/kg, respectively. Described method is applicable for analysis of this herbicide in real soil samples.*

Key words: *sulfonylurea herbicide, nicosulfuron, residue, soil, HPLC*

INTRODUCTION

Sulfonylurea herbicides (SUs) are widely used as control chemicals for most broad-leaves weeds and common grasses in agricultural crops (Figure 1). Sulfonylurea herbicides have become very important in agricultural production since 1982 (BEYER et al., 1988). These herbicides are extremely active in quite low application rate (less than 100 g of active ingredient per hectare).

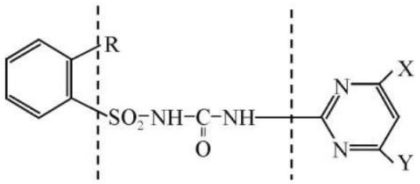


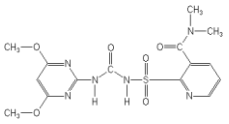
Figure 1. Structure of sulfonilurea herbicides

SUs herbicides are effective inhibitors of the enzyme acetolactate synthase (ALS), an important enzyme necessary for biosynthesis of branched-chain amino acids isoleucine, leucine and valine with both soil and foliar activity (VENCILL, 2002). ALS inhibitors display very high herbicidal activity in soil (SARMAH AND SABADIE, 2002).

One of the most applicable SU herbicides is nicosulfuron (Table 1). Nicosulfuron is used for successfully control of annual and perennial weeds in corn in amount of 40 g active substance per hectare. Persistence of SU herbicides in the soil environment is mainly dependent on several site specific factors, such as rainfall, soil properties (especially pH and organic matter), climate and combination of factors. The fate of sulfonylurea herbicides in soil ranges from few weeks to three or more years (SOLTANI et al., 2002). Generally, sulfonylurea herbicides represent potential environment pollutants in soil and water.

Table 1.

Nicosulfuron

Common name	Nicosulfuron
IUPAC name	(1-(4.6-dimethoxypyrimidin-2-yl)-3-(3-dimethylcarbamoyl-2-pyridylsulfonyl) urea)
Empirical formula	$C_{15}H_{18}N_6O_6S$
CAS No.	(111991-09-4)
Structural formula	
Log K_{ow}	-o.36 (pH 5), -1.77 (pH 7), -2.0 (pH 9)
Relativ molecular mass	410.4 g/mol
pK_a	4.6 (25°C)

With increasing public concerns for agrochemicals and their potential movement and persistence in the ecosystem, pesticide residues in our environment need to be more effectively documented. Information about persistence and mobility of pesticides in soil is required, considering that presence of nicosulfuron herbicide in soil can affect phytotoxic symptoms in sensitive crops, such as sugar beet or soybean (GREENLAND, 2003; SOZERI, 1996). Extreme weather conditions, such as waterless and reduced soil quality, can strongly influence presence of pesticide residue in soil.

The trace determination of nicosulfuron and sulfonylurea herbicides residues generally in environmental samples presents a challenging analytical problem. The low dosage used requires the application of highly sensitive analytical techniques to detect trace concentrations of residues in soil (YE et al., 2006). Due to the low level present and complexity of sample, clean-up and enrichment before analysis is necessary and become a crucial step for the determination of nicosulfuron in environmental samples. In particular the isolation, identification, and quantitation of polar, labile analytes such as SUs in water have been shown to be difficult. Developing analytical methods for SUs has been particularly problematic for chemists because of the wide range in polarity and the chemical instability of these compounds (GRAHOVAC et al., 2013). Methods based on reversed-phase and normal phase liquid chromatographic (LC) techniques with adequate sensitivity from ultraviolet detectors have been widely reported (LIU et al., 2003).

In order to develop simple, rapid and sensitive method for determination of nicosulfuron residues in soil sample, this study was conducted, using liquid chromatography in combination with diode array detection.

MATERIAL AND METHODS

Chemicals and solutions

Certified standard of nicosulfuron (purity 97%) was purchased from Dr Ehrenstorfer (Augsburg, Germany). The extraction solvent, acetonitrile (ACN), was of a suitable grade (HPLC) for pesticide residue analysis and H_3PO_4 were purchased from J.T. Baker (Germany). The water was purified with a water purification system (TKA, Germany).

A stock solution of nicosulfuron was prepared in acetonitrile at a concentration of 0.1 mg/ml and stored at -10 °C, in the dark. Calibration solutions for HPLC analysis were prepared by further dilution with acetonitrile, achieving concentrations in a range from 2.5 to 20 μg/ml.

The HPLC/DAD system and operating conditions

Nicosulfuron determination and quantification were performed by HPLC with diode-array detection (Agilent 1100 Series LC system, United States). An Agilent Zorbax Eclipse C18 column (50 mm × 4.6 mm internal diameter, 1.8 μm particle size) was used. Analysis was done using isocratic elution of mobile phase ultrapure water pH 2.5 with H_3PO_4 and acetonitrile in ratio 70/30, v/v, flow rate 1.0 ml/min, column temperature 40 °C, wavelength 254 nm, injected volume 5 μl.

Sample preparation

After the fresh samples were collected, they were air-dried at 35 °C for up to 96 h, sieved through a 2 mm sieve and stored at 4 °C. The procedure is consists of extracting nicosulfuron from homogenised soil samples (10g) with 10 ml of phosphate buffer pH 7.4/methanol (80/20, v/v) solution. The mixture was shaken for 1 min using Vortex and liquid and solid phases were separated by centrifugation at 3000 rpm for 5 min. The extraction process with 10 ml of mixture solution was repeated two times. Clean-up is necessary in order to reduce the detection limits of methods and/or to avoid interferences from the matrix (Ostojić et al., 2012). Clean-up procedure was done using C18 column packed with 500 mg of highly cross-linked octadecyl sorbent. The SPE cartridges were conditioned with 5 ml of methanol and 5 ml of phosphate buffer pH 7.4/methanol (80/20, v/v) solution. The sorbent was never allowed to dry during the conditioning and sample loading steps. The extract of soil was transferred through the column. The residues of nicosulfuron were eluted from the column by using a 5 ml of acetonitrile. The eluant was collected in a 50 ml round-bottom flask and then concentrated to near dryness using nitrogen stream. The residue was dissolved in 1 ml of acetonitrile, degassed in an ultrasonic bath and filtered through a 0.45 μm nylon membrane filter (HP 9301-0895).

Validation of the analytical method

Method validation was performed by analyzing spiked uncontaminated soil samples (10 g) with nicosulfuron at three levels 2.5, 5.0 and 7.5 μg/ml. The developed and optimized method for quantitative analysis of nicosulfuron in soil was validated in terms of linearity, precision, recovery, limit of detection and limit of quantification.

RESULTS AND DISCUSSION

Chromatographic determination of nicosulfuron was carried out on a HPLC using reversed phase procedure and UV detection at 245 nm. HPLC/DAD chromatogram of nicosulfuron standard in acetonitrile is shown in figure 2. The UV spectrums of nicosulfuron in ACN and in soil matrix are illustrated in Figure 3.

The calibration curve for nicosulfuron in pure solvent was obtained by plotting the peak areas against the concentrations of the corresponding calibration standards at five calibration levels ranging from 2.5 and 20 µg/ml. The linearity of calibration curves was expressed by regression equation and the correlation coefficient (R^2). Results showed that the calibration curves were linear with correlation coefficient of 0.999 (Figure 4). The analytical parameters for method determination of nicosulfuron in soil samples are presented in Table 2.

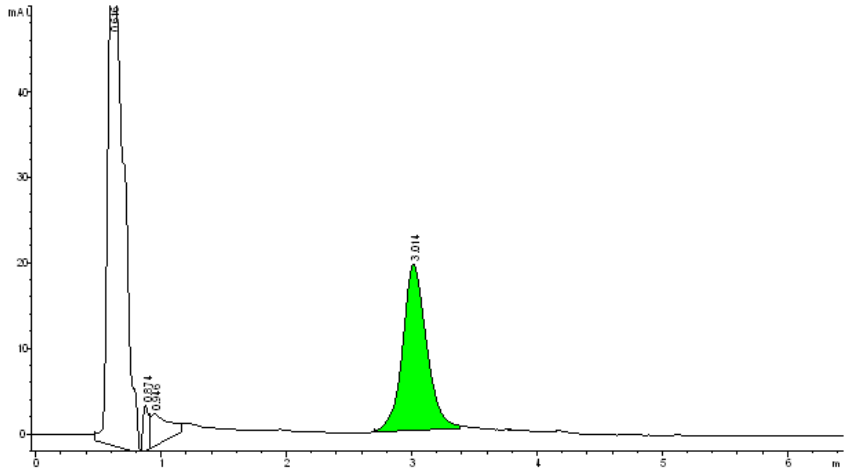


Figure 2. HPLC/DAD chromatogram of nicosulfuron standard at a concentration of 5 µg/ml

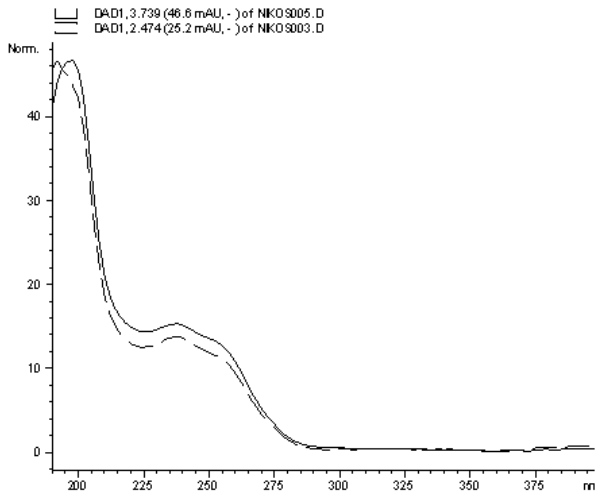


Figure 3. UV spectrum of nicosulfuron standard in acetonitrile and soil matrix

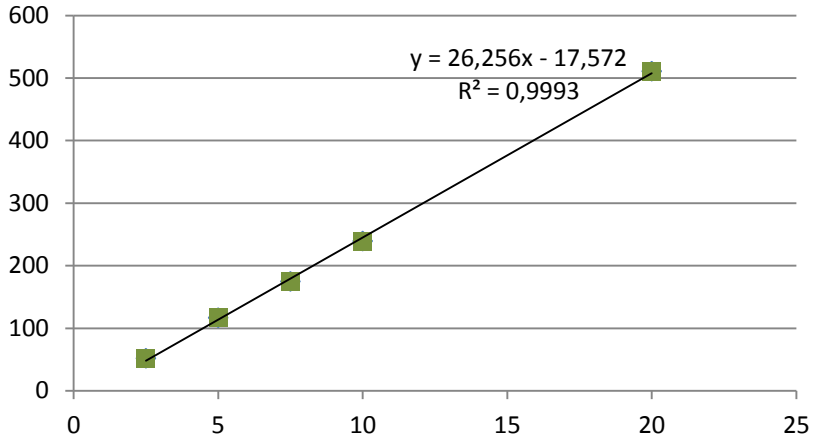


Figure 4. Calibration curve for nicosulfuron standard in acetonitrile

Table 2.

Analytical parameters for HPLC/DAD determination of nicosulfuron

Parameter	Concentration interval (µg/ml)	Slope[a]	Correlation coefficient[a]
Nicosulfuron	2.5-20	26.25x	0.999

[a]Y = ax + b

Limit of detection (LOD) and limit of quantification (LOQ) for nicosulfuron was estimated from the fortified samples. LOD established as 0.010 mg/kg and LOQ at 0.050 mg/kg.

The precision of measurement of an analyte can be evaluated as repeatability or reproducibility. In this study, precision is expressed as repeatability. Precision was checked by matrix matched nicosulfuron standard (5.0 µg/ml) five times on the same day. The retention time of nicosulfuron was 3.002 min. Relative standard deviations (*RSD)* of the retention times and of the peak areas were 0.79 and 1.06%, respectively, fulfilling with criteria for of chromatographic measurements of RSD≤2%.

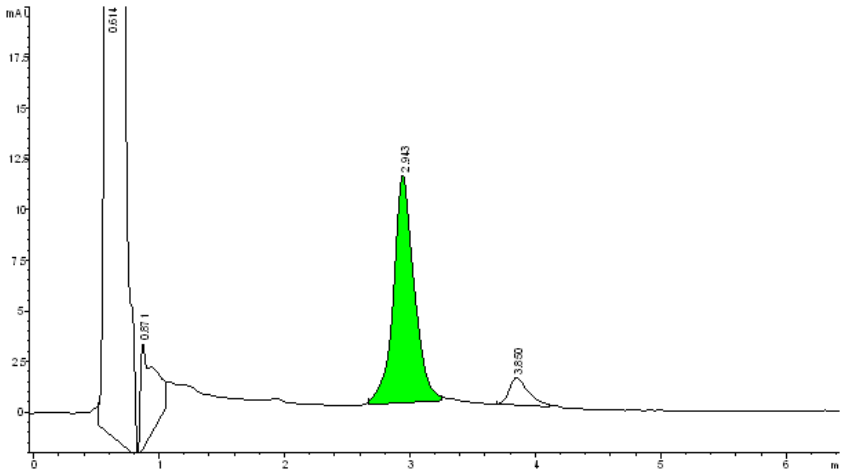


Figure 5. HPLC/DAD chromatogram of spiked soil sample

The accuracy of the method was evaluated by recovery studies. For recovery studies, a soil sample was spiked before the extraction procedure with nicosulfuron herbicide at three levels (2.5-7.5 mg/kg). HPLC/DAD chromatogram of nicosulfuron in soil matrix is illustrated in Figure 5. The mean recoveries were in the range of 89.1% at these three spiking levels with associated relative standard deviations (RSDs) of 1.9%.

CONCLUSIONS

In this study, a method for determination of nicosulfuron residues in soil samples was described. Reverse phase and isocratic elution based liquid chromatographic conditions are used for determination of nicosulfuron. The obtained calibration curve displayed good linearity. The good recovery and low *RSD* confirm the suitability of the proposed SPE-HPLC method. Considering the obtained values of analytical parameters, the proposed method proved to be an efficient and sensitive method for determination of nicosulfuron herbicide residues in soil samples.

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