

Determination of residues of sulfonylurea herbicides in soil by using microwave-assisted extraction and high performance liquid chromatographic method

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Abstract

A modified method for the analysis of nicosulfuron, rimsulfuron and prosulfuron was developed and validated by using microwave-assisted extraction (MAE) and ultra-performance liquid chromatography with diode array detection in the ultraviolet region (HPLC-UV-DAD). The most important experimental parameters of extraction procedure and HPLC-UV-DAD technique were optimised in respect to those sulfonylurea herbicides. High recoveries of the microwave-assisted extraction were obtained by using a dichloromethane–acetonitrile mixture (2:1 volume ratio) acidified with acetic acid (0.8 vol.%) with the addition of urea. The mean recoveries at three spiking levels ranged from 97.47 to 98.76% for nicosulfuron, 97.88 to 99.17% for rimsulfuron and from 97.91 to 99.83% for prosulfuron. The limits of detection of nicosulfuron, rimsulfuron and prosulfuron were 0.95, 0.91 and 0.89 $\mu\text{g kg}^{-1}$, respectively. The accuracy of the developed method was confirmed by HPLC coupled with tandem mass spectrometry parallel analyses. The developed method was used to investigate the dissipation dynamics of sulfonylurea herbicides in the real field trials in Vojvodina Province, Serbia. The obtained half-lives were 0.05, 0.23 and 0.15 days for recommended dose application of nicosulfuron, rimsulfuron and prosulfuron, respectively. Low residues and short half-life in soil suggested that the risk to sensitive rotational crops after application of those sulfonylurea herbicides is low when they are used in the appropriate dosages.

Keywords: sulfonylurea herbicide, microwave-assisted extraction, HPLC-UV-DAD, soil, dissipation.

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Sulfonylurea herbicides (SUs) are a class of highly selective agents which are used to control broad-leaf weeds and grasses in corn, soy, wheat, barley, etc. They are very effective inhibitors of acetolactate synthetase, the key enzyme in the biosynthesis of essential amino acids, by stopping the division of the plant cells [1]. However, increased application of SUs, even at low levels (10–50 g ha^{-1}), caused an increased concern about herbicides residues as the consequence of their prolonged persistence in some soils and a rather high solubility due to the polar nature of SUs molecules [2–4]. Hence, they can be potential contaminants of

water, and thus present a risk to aquatic plants and microorganisms [5]. Another problem is that herbicide residues in soil may have a lasting, unwanted effect on rotational crops [6]. Residual phytotoxicity has become a significant problem in the cultivation of subsequent plants. In this regard, continuous control of residues of SUs in soil from one growing season to another is of great importance in agriculture management since remaining even very low concentrations of these substances can still affect the growth of post-rotating susceptible plants.

Sample preparation is one of the most important step in the complete analytical procedure. Several sample preparation procedures have been proposed for the determination of SUs in soil samples, including liquid–liquid extraction (LLE) [7], supercritical fluid extraction (SFE) [8], solid-phase extraction (SPE) [9–11], cloud point extraction [12], multi-walled carbon nano-

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tubes extraction SPE [13], dispersive solid-phase extraction followed by dispersive liquid–liquid microextraction (DSPE-DLLME) [14], molecularly imprinted SPE (MISPE) [15] and microwave-assisted extraction (MAE) [16]. To date, a number of instrumental methods have been established for determination of SUs residues in different samples, including enzyme linked immune-assay [17], high performance liquid chromatography (HPLC) [8,11,12,16,18,19] capillary electrophoresis [7,20], gas chromatography [21] and high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS-MS) [9,10,22–24]. HPLC with UV detection or diode array detection (DAD) has been the most frequently used technique for the analysis of these polar and thermally labile herbicides [13–15,25–27].

Nicosulfuron, rimsulfuron and prosulfuron (Fig. 1) belong to the group of SU herbicides which are used mainly in corn cultivation. Although dissipation and analysis of residues of those herbicides have been reported in the literature, presented data are focused on the analysis of soil from the specific region in the world [22,28,29]. Further, despite to a large number of defined analytical methods for SUs determinations, transfer of the method to the certain laboratory and specific soil samples imposes modification in sample preparation and analysis conditions due to the differences in pH, contents of total C, sand and clay of various types of soils, etc. Consequently, since dissimilar interactions between soil and SUs are expected, different sample preparation procedure should be applied to extract herbicides from the soil.

In this work, a modified microwave-assisted extraction and HPLC with diode array detection in the ultraviolet region was established to determine nicosulfuron, rimsulfuron, and prosulfuron in soil samples. A field research was designed to investigate the dissipation dynamics and final residues of chosen herbicides in soil from the area of Vojvodina, the north region of Serbia, under the real field conditions. To the best of our knowledge, no information is available on the dissipation of those individual SUs herbicides in the soil of this region. In addition, presented work aimed to provide the basic information for safe and controlled usage of some SUs, as well as the proper data about the risk to sensitive post-rotating crops after applic-

ation of mentioned herbicides in order to achieve optimal exploitation of soil in this region.

EXPERIMENTAL

Chemicals and reagents

Reference standards for sulfonylurea herbicides were obtained from Dr. Ehrenstorfer GmbH (purity >99%, Augsburg, Germany). Standard stock solutions ($1000 \mu\text{g L}^{-1}$) were prepared in acetonitrile and stored at $-20 \text{ }^\circ\text{C}$. Acetonitrile, dichloromethane, methanol, ethyl acetate and acetic acid were of HPLC grade, purchased from J.T. Baker (Deventer, The Netherlands). The purified water ($\text{TOC} < 3 \text{ ppb}$) was prepared using an SG Ultra Clear water system (SG Water GmbH, Barsbüttel, Germany). Anhydrous sodium sulphate and urea were purchased from Sigma–Aldrich. The silica solid phase extraction (SPE) cartridges (DSC-Si, 100 mg mL^{-1}) were obtained from Supelco (Bellefonte, PA, USA). Commercial formulations used in the field experiment were purchased from the domestic market.

Microwave-assisted extraction of herbicides

The SUs from the soil samples were extracted using a Milestone ETHOS 1 microwave system (Shelton, CT, USA), equipped with a 24-piece rotor and 75 mL TFM Teflon closed vessels, and an ATC-FO (Automatic Fiber Optic) temperature control system. An optic fibre probe within the control system was used for the temperature monitoring of the sample inside the vessel. The soil samples that served as model systems were taken from the experimental plots immediately before the treatment with herbicides (known to contain no analysed herbicides) dried at $105 \text{ }^\circ\text{C}$. The soil samples without the presence of water were spiked with the selected herbicides at the levels of 5, 50 and $100 \mu\text{g kg}^{-1}$ soil, left overnight and used in the optimization of the extraction procedure.

The soil samples (10 g) were transferred to the TFM vessels, and 10 mL of the extraction solvent (the mixture of dichloromethane–acetonitrile (2:1 volume ratio) acidified with acetic acid (0.8 vol.%) and 0.3 g of urea was added. The extraction program was as follows: 2 min of preheating at 150 W; 2 min of preheating at 300 W, and 10 min of extraction at 450 W. Two extraction

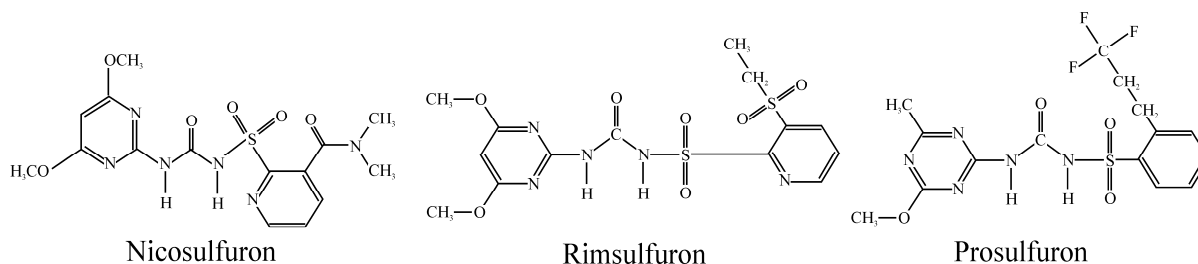


Figure 1. The structural formulas of examined sulfonylurea herbicides.

cycles were carried out with fresh solvent. After that, the sample was centrifuged at 4000 rpm for 15 min and dried over anhydrous sodium sulphate. The extract was evaporated to dryness at 40 °C in a rotary vacuum evaporator and the dry residue was dissolved in dichloromethane (2 mL) using ultrasound. The obtained solution was filtered into the vial through 0.22 µm syringe filter and then analysed by HPLC-UV-DAD without further clean-up. In the case of HPLC-MS-MS analysis, additional SPE step were required for the sample cleaning-up purpose. The samples solutions were loaded on SPE cartridges (DSC-Si, 100 mg mL⁻¹) which were previously preconditioned with 2 mL of acetone, followed by 1 mL of dichloromethane. After passing the sample, the cartridges were rinsed with 1 mL of *n*-hexane, then the analytes were eluted with a 3 mL of the mixture of dichloromethane:acetone (75:25 volume ratio) [30]. After solvent evaporation using water bath (40 °C) the residue was redissolved in acetonitrile for further HPLC-MS-MS analysis.

Chromatographic analysis

An Agilent Technologies 1100 series HPLC system (Agilent Technologies Inc., USA), equipped with a diode array detector (DAD) was used. The HPLC system consisted of a G 1312A binary pump, G 1313A autosampler, G 1322A degasser, and the column compartment. The column, a reversed-phase Zorbax Eclipse XDB-C₁₈ analytical column (50 mm×4.6 mm, 1.8 µm) (Agilent Technologies Inc., USA), was thermostated at 25 °C. The mobile phase (acetonitrile (A) and 0.1% aqueous acetic acid (B)) was delivered at a flow rate of 1 mL min⁻¹ in gradient mode (0 min 52% A, 2 min 47% A, 2.5 min 45% A; 5 min 52% A, re-equilibrium time 3 min). The injection volume was 10 µL and the UV wavelength was 240 nm.

Comparative analyses were performed on an Agilent Technologies 1200 Series HPLC coupled with a 6410A Triple Quad MS-MS detection with electrospray ion source, controlled by a MassHunter software version B.01.03. (Agilent Technologies Inc., USA). The column (the same type as above) was held at 30 °C, and the injection volume was 5 µL. Mobile phase solvents, A (0.1% aqueous acetic acid) and B (acetonitrile), were delivered at a flow rate of 1 mL min⁻¹ in isocratic conditions (70% A and 30% B). The herbicides were detected by MS-MS using the ion parameters as follows: drying gas (N₂) flow 9 L min⁻¹, nebulization gas (N₂) pressure 40 psi, and temperature 350 °C, capillary

voltage 4 kV, positive polarity. The MS-MS acquisition was performed in the dynamic multiple reaction monitoring (MRM) mode using the specific parameters for the each compound (precursor ion, product ion, fragmentor voltage, collision voltage) given in Table 1.

Field trials

The field trial was performed on the location of Rimski Šančevi (latitude 45°19'26.46"N, longitude 19°51'5.94"E), Vojvodina Province, Serbia, on the experimental field of the Institute of Field and Vegetable Crops, Novi Sad.

Soils were characterized by using standard assay procedures in our laboratory. The soil samples were air-dried and milled to the particle size <2 mm by using stainless sieve [31]. Mechanical characteristics of soil were determined in the <2 mm fraction by the internationally recognized pipette method. The size fractions were defined as clay (<0.002 mm), silt (0.02–0.002 mm), fine sand (0.2–0.02 mm) and coarse sand (2–0.2 mm). The soil pH value was measured pH meter with standard glass electrode [32]. The total organic carbon, cation exchange capacity, total carbon and nitrogen were measured in accordance with methods for soil quality [33–35].

The chemical and mechanical properties of the soil were as follows: total carbon 2.70%, total organic carbon 1.51%, total nitrogen 0.186 g kg⁻¹, pH 6.90, cation exchange capacity 23.2 meq 100 g⁻¹, clay 28%, fine sand 37.54%, coarse sand 0.50% and silt 33.96%. According to specified characteristics, the soil is Calcic Chernozem Clayic, Pachic (CH-cc-ce.ph) type according to the FAO-WRB classification [36].

The experimental plots were set up by using the randomized complete block design [37] with 4 treatments including three formulation treatments and one control for each of the investigated herbicide. The treatment and control plots were 15 m² and the buffer zone was set up between plots. Each treatment for a particular herbicide was carried in four replicates. The corn hybrid NS 640 Ultra was sown in the middle of April 2014. The herbicides were applied by using an R&D CO₂ knapsack sprayer, with the wing width of 3 m (6 TeeJet TR11003 nozzles) and pressure of 2 bar, at the stage of 4–5 leaves.

In order to investigate their dissipation, the three selected herbicides were applied as aqueous solutions of their commercial formulations in order to investigate their dissipation: nicosulfuron (Kelvin®, 40 g L⁻¹), rim-

Table 1. Mass spectrometry parameters for the determination of sulfonylurea herbicides

Compound	Precursor, <i>m/z</i>	Product, <i>m/z</i>	<i>V</i> _{fragmentor} / V	<i>V</i> _{collision} / V
Nicosulfuron	411	182	120	16
Rimsulfuron	432	182	120	20
Prosulfuron	469	254	120	16

sulfuron (Rimex®, 250 g kg⁻¹) and prosulfuron (Peak®, 750 g kg⁻¹). The recommended dosage of which were 50, 50 and 20 g active ingredient (a.i.) per ha, respectively. The dissipation experiments of their dissipation were carried out on different plots at three dosages, namely the recommended dosage and two higher than the recommended dose. The neutral plots were sprayed with water, and served as the control.

Representative soil samples were collected at intervals of 2 h, 2, 6, 15, 30 and 50 days after the application of herbicides in the top soil layer (0–15 cm). The soil samples (about 2 kg) were taken randomly from 5 sites of each plot using a stainless steel hand auger. After drying at 40 °C, the samples were milled, passed through 2 mm sieves and stored at –20 °C for further analysis.

RESULTS AND DISCUSSION

Optimization of extraction procedure

Influence of mixed solvents on MAE

The choice of optimal extraction solvent for MAE is a very important factor in determining the effectiveness of extraction procedure. In this study, the pure polar solvents for extraction of thermolabile SUs could not be included due to the intense absorption of microwave energy and energetic heating which can induce local heating effects and degradation of SUs. On the other hand, pure non-polar solvents do not absorb microwave energy. Hence, to achieve heating of the extraction mixture it is necessary to use a mixture of non-polar and polar solvents.

In order to obtain maximum recovery of the selected herbicides from the soil, several solvent mixtures, *viz.* dichloromethane–methanol, dichloromethane–acetonitrile, hexane–methanol, hexane–acetonitrile and ethyl acetate–acetonitrile, prepared in different ratios (2:1, 2:5, 2:8 volume ratio) for each mixture, were investigated. These mixtures have different dielectric properties in MAE, and they will expectedly have

different effects on the extraction of SUs from the soil.

To achieve uniform heating, *i.e.*, avoid the occurrence of the so-called “hot points” in the case of less polar reaction mixtures, the procedure was carried out by using a teflon stir bar. With the same aim, a lower microwave power and longer heating ramps were used. The microwave power pre-heating steps were 150 and 300 W, lasting 2 min and the extraction was performed at 450 W for 10 min, two times, each time with a new portion of fresh solvents, and the obtained extracts were collected together. The recoveries of selected herbicides with the mixture of the dichloromethane–acetonitrile (2:1 volume ratio) were the highest and varied in the range of 47 to 56% whereas the recoveries with the other solvent mixtures were below 30%. A further examination was required to maximise the recovery of selected herbicides from the soil by using other compounds.

It is known that SUs (weak acidic compound) in the process of extraction from the soil can be ionized in the solvent and thus affect the extraction efficiency. The extent of ionization of SUs depends on their pK_a and the acidity of the extraction solvent can influence the efficiency of the extraction [23]. Probably, the absence of water in the soil [38], beside its characteristics, can have a noticeable impact on the stronger interaction between the soil and the selected herbicides. Hence, the influence of the content of acetic acid and urea in the dichloromethane–acetonitrile (2:1 volume ratio) mixture was further tested by varying the proportion in the range of 0.1–1.0% for acetic acid and 0.1–0.5 g for urea. Thus, it was important to find an appropriate balance between the solvent acidity and loss of the sorption affinity of the herbicides to the soil surface. The dichloromethane–acetonitrile (2:1 volume ratio) mixture, acidified with acetic acid (0.8 vol.%) and the addition of urea (0.3 g) showed to be the most efficient extraction solvent for the extraction of nicosulfuron, rimsulfuron and prosulfuron from the soil (Fig. 2) and it was used in the further study.

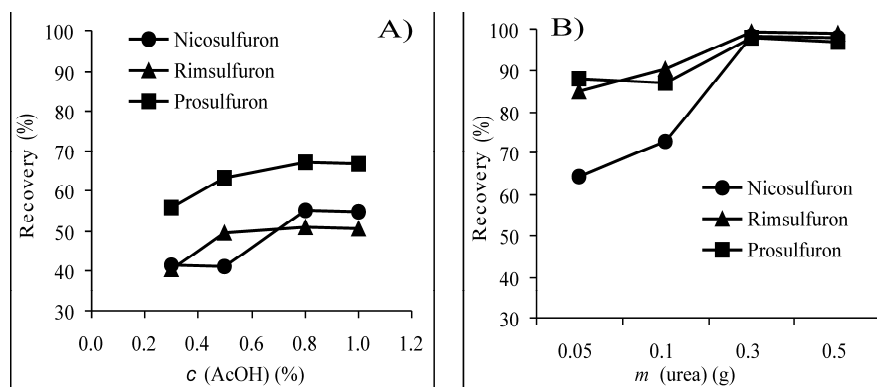


Figure 2. Recovery of the selected SUs from soil using: A) dichloromethane–acetonitrile mixture (2:1 volume ratio) acidified with different contents of the acetic acid (%); B) dichloromethane–acetonitrile mixture (2:1 volume ratio) acidified with acetic acid (0.8 vol.%) and different contents of urea (g).

Influence of extraction temperature on MAE

The selection of optimal extraction temperature is essential in MAE due to the possible temperature-dependent degradation of the thermolabile SUs in micro-waves field. In order to evaluate the temperature effect on the recovery of the selected herbicides, extractions were evaluated in the range of temperature from 40 to 70 °C by using dichloromethane–acetonitrile (2:1 volume ratio) mixture acidified with acetic acid (0.8 vol.%) and the addition of urea (0.3 g) as extraction solvent. The highest recoveries of the herbicides (97.4–99.44%) were achieved at the temperature of 50 °C. Extraction temperatures higher or lower than 50 °C resulted in the decrease of the recovery. Higher extraction temperatures probably contributed to the transformation of SUs, as it was reported previously [16,39]. Therefore, the temperature of 50 °C was chosen as optimum for the extraction.

Influence of solvent to material ratio on MAE efficiency

Influence of solvent to material ratio on MAE recovery was investigated from 0.8 to 3 mL g⁻¹, and recovery of selected herbicides was varied from 65.1–99.5%. Maximal recoveries were obtained at the solvent to the material ratio of 1 mL g⁻¹ and it was adopted as the most suitable in the experiments.

Influence of extraction time on MAE efficiency

The extraction recoveries of selected herbicides by using different time were investigated under conditions described in previous sections. The recovery of selected herbicides from soil increased with the increase of extraction time (1–15 min) and varied in the range of 74.53–99.76%. The highest recoveries were achieved when the extraction time was 10 min, so that this time was taken as the optimum.

In Table 2 are summarised conditions for MAE of SUs from soils.

Table 2. Optimal conditions for MAE of SUs from soils

Parameter	Optimal conditions
Solvent mixture and the addition of urea	Dichloromethane–acetonitrile (2:1 volume ratio) acidified with acetic acid (0.8 vol.%), urea (0.3 g)
Temperature	50 °C
Solvent to material ratio	1 mL g ⁻¹
Time	10 min

Method optimization of HPLC-UV-DAD conditions

The high molar absorptivity of SUs allows their precise detection in the UV region. Hence, HPLC-UV-DAD is one of the frequently applied techniques used in the analysis of the sulfonylureas. Preliminary experiments were performed with the objective of finding the best

instrumental conditions to enable good separation with a high sensitivity. The optimum chromatographic conditions were selected based on the analysis of the mixture of standard solutions of nicosulfuron, rimsulfuron and prosulfuron. Different chromatographic conditions such as the temperature, detection wavelength, eluent composition and elution conditions were investigated in order to achieve adequate selectivity and separation of the analytes. The temperature of the column was varied in the range from 20 to 30 °C and the detection wavelength ranged from 230 to 245 nm. Based on results obtained, 25 °C was adopted as column temperature. The analysed SUs showed the optimal UV absorption at 240 nm. The gradient elution (0 min 45% A; 17 min 53% A; 25 min, 60% A) was tested using the mobile phase composed of acetonitrile (A) and aqueous acetic acid (0.05 vol.%) (B), as reported for the simultaneous determination of SUs in surface water [18]. Therefore, due to the differences in the performance of the applied C₁₈ columns it was necessary to optimize the SUs separation. The retention of ionizable compounds such as the SUs depends of acidity and ionic strength of the mobile phase. Hence, experiments involving modification of the elution gradient and different percentages of aqueous acetic acid (0.05–0.2%) were carried out in order to establish favourable conditions for the separation. The chromatographic conditions with mobile phase of acetonitrile (A) and 0.1% aqueous acetic acid (B) at a flow rate of 1 mL min⁻¹ in gradient mode (0 min 52% A, 2 min 47% A, 2.5 min 45% A; 5 min 52% A, re-equilibrium time 3 min) were adopted as the most suitable for separation of selected SUs within a short time. Therefore, these chromatographic conditions, optimized by applying a mixture of standard solutions, allowed a reliable determination of nicosulfuron, rimsulfuron and prosulfuron in real soil samples. Chromatograms of blank, spiked soil sample and standard solution of investigated SUs are shown in Fig. 3. Retention times for nicosulfuron, rimsulfuron and prosulfuron were 0.81, 1.26 and 4.19 min, respectively.

The effects of the co-eluted matrix compounds can significantly influence the precision and accuracy of the method. In this investigation, the signal suppression due to the matrix effect was assessed by the method of post-extraction spike, by comparing the slope of the matrix-matched calibration curve with that of the standard calibration curve at the same concentration level. The matrix effect was in the range of 0.841–0.937 for each individual herbicide, indicating an effect of the matrix. A good linearity of the matrix-matched calibration, with the coefficient of correlation (r^2) higher than 0.99, was obtained in the range of 5–100 mg L⁻¹ (Table 3).

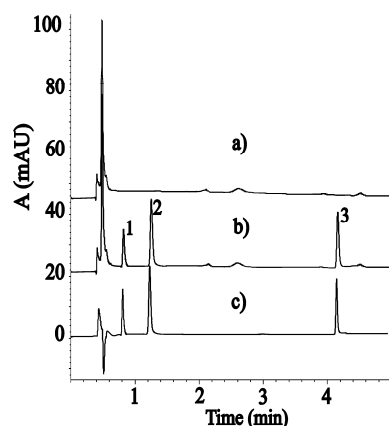


Figure 3. Chromatograms of blank (a), spiked soil sample (b) and standard solution of investigated SUs (c). Peak 1: nicosulfuron; peak 2: rimsulfuron; peak 3: prosulfuron.

The accuracy of the method expressed as the recovery (R in %) of SUs from the spiked blanks were studied at three concentration levels (5, 50, 100 $\mu\text{g kg}^{-1}$), with five repetitions. The recovery results presented in Table 4 confirmed that the optimal recovery (97.47–99.83% at each spiking level with RSD of <5%) and indicated good accuracy and repeatability of the method [39]. The limits of detection ($LODs$) and quantification ($LOQs$) were determined for investigated analyte as the lowest concentration yielding a signal-to-noise ratio (S/N) of 3 and 10, respectively.

The sensitivity of the modified method was good, sufficient to provide a reliable determination of residues of nicosulfuron, rimsulfuron and rimsulfuron at the level of 3.16, 3.04 and 2.97 $\mu\text{g kg}^{-1}$, respectively.

Evaluation of the proposed HPLC-UV-DAD method accuracy was done by using of parallel HPLC-MS-MS analysis. The MRM chromatograms (Fig. 4) of quantifier ions at standard solution of investigated herbicides ($\sim 3 \mu\text{g mL}^{-1}$) and soil sample (collected 0th day, 2 h after the treatment at recommended dosage) were obtained under the conditions described above.

The results residues of nicosulfuron, rimsulfuron and prosulfuron from soil real samples by HPLC-UV-DAD are in good agreement with results obtained using HPLC-MS-MS as a comparative method. Further, the paired t -test was performed to compare the means and does not detect significant difference between the obtained herbicide residues in the soil sample analysis by the two methods at 95% confidence level:

$$|t_{\text{nicosulfuron}}| = 1.96 < t_{8,0.05} = 2.31, |t_{\text{rimsulfuron}}| = 2.09 < t_{5,0.05} = 2.57, |t_{\text{prosulfuron}}| = 2.01 < t_{8,0.05} = 2.31$$

Dissipation of the investigated herbicides in soil under field conditions

The applicability of the optimised MAE technique was evaluated by extracting nicosulfuron, rimsulfuron and prosulfuron from soil samples after their application in the real field trials at different times. The extracts were analysed by developed HPLC-UV-DAD method, and the obtained results were used for the dissipation assessment of selected herbicides.

The starting concentrations (measured in the samples taken on the 0th day, 2 h after the treatment) in the top soil layer (0–15 cm) were 427.42, 620.27 and 703.47 $\mu\text{g kg}^{-1}$ of nicosulfuron and concentrations of

Table 3. Matrix-matched calibration curve of nicosulfuron, rimsulfuron and prosulfuron in soil pure solvent and samples

Compound	Sample	Linear equation	Correlation coefficient (r^2)	Matrix effect ^a
Nicosulfuron	Pure solvent	$y = 3.108x + 23.714$	0.995	0.841
	Soil	$y = 2.614x + 19.002$	0.994	
Rimsulfuron	Pure solvent	$y = 2.214x + 17.729$	0.993	0.937
	Soil	$y = 2.075x + 8.070$	0.990	
Prosulfuron	Pure solvent	$y = 3.655x + 27.604$	0.995	0.915
	Soil	$y = 3.344x + 15.106$	0.994	

^aSlope matrix/slope solvent

Table 4. Accuracy, precision, limits of detection ($LODs$) and limits of quantification ($LOQs$) of selected sulfonylurea herbicides using MAE sample preparation procedures

Compound	Spiking level, $\mu\text{g kg}^{-1}$	Mean R , %	Repeatability, RSD / %	LOD / $\mu\text{g kg}^{-1}$	LOQ / $\mu\text{g kg}^{-1}$
Nicosulfuron	100.0	97.47	1.43	0.95	3.16
	50.0	98.02	1.05		
	5.0	98.76	1.72		
Rimsulfuron	100.0	99.17	1.37	0.91	3.04
	50.0	97.88	1.43		
	5.0	99.10	2.67		
Prosulfuron	100.0	97.91	1.02	0.89	2.97
	50.0	98.04	1.52		
	5.0	99.83	2.13		

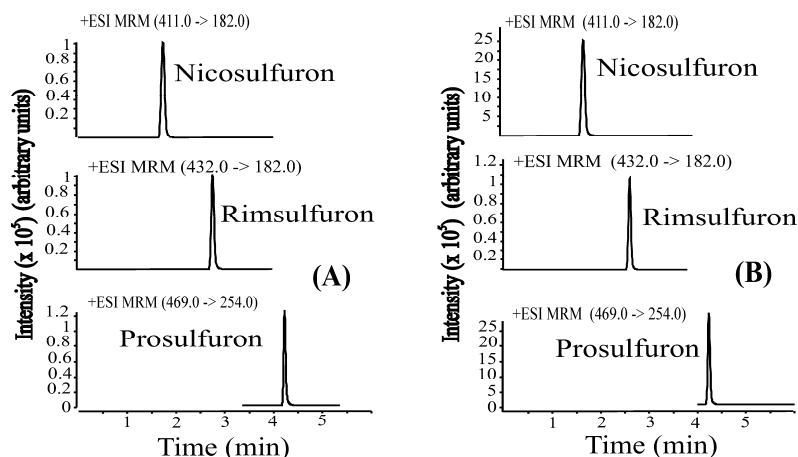


Figure 4. MRM traces of nicosulfuron, rimsulfuron and prosulfuron. A) Standard compounds ($\sim 3 \mu\text{g mL}^{-1}$); B) soil samples collected 0th day, 2 h after the treatment at recommended dosage.

rimsulfuron were 13.67, 30.20 and 33.59 $\mu\text{g kg}^{-1}$ after the treatment with 50, 80 and 100 g a.i. ha^{-1} , respectively. The corresponding results for prosulfuron were 84.02, 176.84 and 248.81 $\mu\text{g kg}^{-1}$ for the treatment with 20, 30 and 50 g a.i. ha^{-1} , respectively. After two days concentrations of nicosulfuron and prosulfuron were lower by more than 90% while residues of rimsulfuron were lower more than 75% compared to the initial concentration. Fifteen days after the treatment the amount of residues were below the LOQ for nicosulfuron (3.16 $\mu\text{g kg}^{-1}$) and prosulfuron (2.97 $\mu\text{g kg}^{-1}$), whereas the concentrations of rimsulfuron were below the method LOQ (3.04 $\mu\text{g kg}^{-1}$) 6 days after the application of all doses.

The dissipation of nicosulfuron, rimsulfuron and prosulfuron were described by using the Mittag–Leffler function $cE_a(-bt)$. This function is a direct generalization of the exponential function and represents the solution of fractional order differential or fractional order integral equation [40–42]. Model coefficients a , b and c were obtained from the experimental data, by using a fitting procedure. The corresponding dissipation kinetic equations, described by the Mittag–Leffler function are

given in Table 5. The obtained half-time (DT_{50}) values of nicosulfuron and prosulfuron were in the range of 0.05 (50 g a.i. ha^{-1}) to 0.35 d (80 g a.i. ha^{-1}) and 0.12 (50 g a.i. ha^{-1}) to 0.34 d (30 g a.i. ha^{-1}), respectively. For rimsulfuron obtained DT_{50} values were from 0.23 (50 g a.i. ha^{-1}) to 0.78 d (100 g a.i. ha^{-1}).

The dissipation kinetic of nicosulfuron was the highest, followed by prosulfuron and rimsulfuron for the recommended application dose.

These half-times values were lower compared to those obtained by Poppell *et al.* [29] for nicosulfuron (1.3–5.3 d) and rimsulfuron (1.2–3.1 d) presumably due to different the soil characteristics. Namely, the soil analysed in our research had higher pH and cation exchange capacity as well as higher percentage of clay. In addition to the differences in the soil characteristics, experimental conditions may have also influenced faster dissipation of investigated SUs in comparison to the other DT_{50} values [22,43]. Another possible explanation for fast dissipation could be leaching to the depths below the sampled layer, although no similar observations were reported in some previous investigations [44,45]. Afyuni *et al.* [46] made a precipitation

Table 5. Regression equations and other statistical parameters for dissipation of SU herbicides under field conditions

Compound	Application rate, g a.i. ha^{-1}	Regression equation, $y(t) = cE_a(-bt)$			Correlation coefficient (r^2)	DT_{50}^a / d
		a	b	c		
Nicosulfuron	50	0.35	15.01	101.77	0.999	0.05
	80	0.84	2.00	100.21	0.999	0.35
	100	0.46	4.12	100.47	0.999	0.19
Rimsulfuron	50	0.83	3.01	100.32	0.999	0.23
	80	0.90	1.77	100.18	0.999	0.39
	100	0.99	0.87	98.31	0.982	0.78
Prosulfuron	20	0.31	5.57	100.62	0.999	0.15
	30	0.81	2.09	100.22	0.999	0.34
	50	2×10^{-8}	8.75	100.87	0.999	0.12

^aThe time required to dissipate 50% of the initially applied dose

simulation experiment and concluded that there was no migration of nicosulfuron in the soil, and thus, to some extent, excluded the possibility of its leaching to the zone below the sampling layer.

Dissipation rates of investigated SUs under field conditions probably were influenced by non-biotic processes in the tested soil type. The relative significance of these processes changed with the properties of soil and chemical structures herbicides.

CONCLUSIONS

In this work, a HPLC-UV-DAD method with modified microwave-assisted extraction sample preparation was established for simultaneous determination of three sulfonylurea herbicides in soil including nicosulfuron, rimsulfuron and prosulfuron. The most efficient microwave-assisted extraction solvent was dichloromethane–acetonitrile mixture (2:1 volume ratio) acidified with acetic acid (0.8 vol.%) with addition of urea (0.3 g). The developed method showed satisfactory validation parameters in terms of selectivity, linearity, recovery, sensitivity and repeatability. Matrix-matched calibration method was used to eliminate changeable matrix effect of soil and to provide precise quantification. The recovery of the method was in the range of 97.4–99.8%, and all analyzed herbicides showed good linearity ($r^2 > 0.990$) in a relatively wide concentration range (5–100 mg L⁻¹). The proposed method yielded results that were in good agreement with those obtained by HPLC–MS–MS method as comparative reference method. Based on it, the dissipation and residues of nicosulfuron, rimsulfuron and prosulfuron in soil of Vojvodina Province were investigated. Initial residues of nicosulfuron and prosulfuron were higher than for rimsulfuron and they were degraded faster after 2 days. The residues of rimsulfuron for all the tested doses were less than LOQ after 6 and 15 days for other analysed herbicides. The results showed that the half-lives of nicosulfuron, rimsulfuron and prosulfuron after their application at the recommended doses were 0.05, 0.23 and 0.15 days which indicates that the adverse effects of their residues on the soil and rotational crops are low. Therefore, dissipation study of investigated SUs herbicides in the soil was useful for not only sensitive post-rotating crops but also for their controlled usage in order to realize optimal exploitation of soil in this region.

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IZVOD

ODREĐIVANJE OSTATAKA SULFONILUREA HERBICIDA U ZEMLJIŠTU PRIMENOM MIKROTALASNE EKSTRAKCIJE I TEČNE HROMATOGRAFIJE VISOKIH PERFORMANSI

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(Naučni rad)

Modifikovana metoda za analizu nikosulfurona, rimsulfurona i prosulfurona razvijena je i validovana uz pomoć mikrotalasne ekstrakcije (MAE) i tečne hromatografije visokih performansi sa ultravioletnim detektorom sa nizom dioda (HPLC-UV-DAD). Najvažniji eksperimentalni parametri ekstrakcione procedure i HPLC-UV-DAD tehnike su optimizirani za ispitivanje sulfonylurea herbicide. Visoki prinosi mikrotalasne ekstrakcije dobijeni su primenom mešavine dihlormetan–acetonitril (zapreminski odnos 2:1) zakišeljene sa sirćetnom kiselinom (0,8 zapr.%) uz dodatak uree. Prosečan prinos na tri spajkovana nivoa se kretao u opsegu od 97,47 do 98,76% za nikosulfuron, 97,88 do 99,17% za rimsulfuron i od 97,91 do 99,83% za prosulfuron. Granice određivanja za nikosulfuron, rimsulfuron i prosulfuron su bile 0,95, 0,91 i 0,89 $\mu\text{g kg}^{-1}$, redom. Tačnost razvijene metode je potvrđena uporednom analizom uz pomoć HPLC u kombinaciji sa masenom detekcijom. Razvijena metoda je korišćena za ispitivanje dinamike rasipanja sulfonylurea herbicida u realnom poljskom ogledu na području Vojvodine. Dobijena vremena poluraspada za preporučenu dozu primene nikosulfurona, rimsulfurona i prosulfurona su bila 0,05, 0,23 i 0,15 dana, redom. Nizak sadržaj ostataka ispitivanih herbicida i kratko vreme poluraspada u zemljištu sugerišu na smanjen rizik za osetljive rotirajuće useve nakon primene u odgovarajućim dozama.

Ključne reči: Sulfonylurea herbicid • Mikrotalasna ekstrakcija • HPLC-UV-DAD • Zemljište