

# Proceedings of the 7th Congress on Plant Protection

## Доклады 7-ого Конгресса по защите растений



Plant Protection Society of Serbia  
Общество по защите растений Сербии



International Organization for Biological Control

-East Palearctic Regional Section (IOBC-EPRS)

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Международная организация по биологической борьбе

- Восточно палеарктическая региональная секция (МОББ-ВПРС)

- Западно палеарктическая региональная секция (МОББ-ЗПРС)

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## NICOSULFURON RESIDUES IN AGRICULTURAL SOIL

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### ABSTRACT

Nicosulfuron is one of the most applicable sulfonylurea herbicides for control of annual and perennial weeds in maize. Its residues can persist in soil at phytotoxic concentrations and affect sensitive succeeding crops more than one season after treatment. Therefore, monitoring the trace levels of this herbicide in soil is a challenging task and demands highly efficient, selective, and sensitive analytical technique. This study was conducted for the purpose of investigating nicosulfuron residues presence in soil. Soil samples were collected before crop seeding, from two soil layers, surface (0-30 cm) and sub-surface (30-60 cm). Nicosulfuron determination and quantification were performed by HPLC with diode-array detection, using isocratic elution of mobile phase. Nicosulfuron was extracted from the soil sample with mixture of phosphate buffer (pH 7.4)/methanol (80/20, v/v) solution with clean-up on C18 extraction cartridge. The linearity of detector response showed that the calibration curves were linear with correlation coefficient ( $R^2$ ) of 0.999. Relative standard deviations (RSD) of the retention times and of the peak areas were 0.79 and 1.06%, respectively and fulfilled the criteria of chromatographic measurements. The accuracy of the defined method was confirmed by the good results of recovery assay, while achieved limit of detection (LOD) and limit of quantification (LOQ) for nicosulfuron in soil were in accordance with SANCO/825/00 (SANCO, 2010). The proposed analytical procedure was applied for monitoring of nicosulfuron herbicide in soil. Average values of nicosulfuron residues in soil samples from surface and sub-surface horizon were 0.05 mg/kg.

**Key words:** nicosulfuron, residue, soil

### INTRODUCTION

Modern agriculture depends to a large extent on herbicides used for control of weeds that compete with the crops. Herbicides represent about 50% of the demand for agricultural chemicals and are applied directly or indirectly to increase crop yields. Furthermore, herbicides protect crops from undue competition from weeds and enhance food nutrition quality of food. Their prolonged use involves the risk of their retention and accumulation in the environment, so the analysis of these compounds has become an important part of the monitoring program.

Stability of herbicides in the soil is also dependent on degradation manners. A very important indicator, which defines potential persistence of the herbicide active ingredient in the soil, is the half-life period

(DT<sub>50</sub>). The value of DT<sub>50</sub> is a characteristic feature of individual active ingredients of herbicides and it may range from several days (e.g. quizalofop-P, mesotrione, MCPA) to as long as several months (e.g. trifluralin, ethofumesate, pendimethalin) (Sekutowski, 2011). It is only a rough indication of the potential persistence of herbicide active substances in soil. Herbicide degradation and translocation under field conditions may occur faster or much slower, since it is result of interactions between chemical properties of the active ingredient itself and moisture content, temperature, absorbing capacity of soil, pH and soil microorganisms. Thus the risk of persistence and translocation of herbicide active ingredients in soil may not be considered only on the basis of one of the parameters e.g. DT<sub>50</sub>, K<sub>oc</sub>, R<sub>f</sub> as under field conditions the interactions of all these factors affect



the rate of chemical and biological processes, which in turn determine the behavior of active ingredients of herbicides in the soil environment.

One of the most important herbicide classes are sulfonylureas. These herbicides are extremely active in quite low application rates (less than 100 g of active ingredient per hectare). However, even at low rates, these herbicides can persist in the soil throughout more than one growing season (Moyer, 1995; cit. Bedmar et al., 2006). Sulfonylurea herbicides show a wide range of persistence in both laboratory and field conditions, depending upon soil pH, temperature, and soil moisture. Several authors reported that persistence of sulfonylurea herbicides increased with increasing rate of application, increasing soil pH and decreasing organic matter content (Smith and Hsiao, 1985; Goetz et al., 1989; Castro et al., 2002).

Some sulfonylurea herbicides exhibit longer residual soil activity and may injure following crops. Phytotoxicity to sensitive crops may occur at very low residue concentrations in soil and phytotoxic effects to susceptible plants can be seen for a period several times longer than the DT<sub>50</sub>. Concentrations at tenths of a nM can cause inhibition of the target enzyme ALS in susceptible crops (Hock et al., 1995). Particularly sensitive are crops from cruciferous family (yellow mustard, oil seed rape), but also other crops such as sugar beet and sunflower are very susceptible (Soukup et al., 2002).

Nicosulfuron is one of the most applicable sulfonylurea herbicides for control of annual and perennial weeds in maize, one of the most frequent pre-crops of sunflower, soybean and sugar beet. Weak volatility and longer persistence of this herbicide have effect to possibility of increased contamination risk to crops in rotation and water systems (Grahovac et al., 2013), more than one season after treatment.

The research in this paper was carried out with the aim of checking presence of nicosulfuron residues in soil.

## MATERIAL AND METHODS

### Chemicals and solutions

Certificated analytical standard of nicosulfuron (99.1%) was obtained from Dr Ehrenstorfer (Augsburg, Germany). Acetonitrile and methanol, HPLC grade solvents, and H<sub>3</sub>PO<sub>4</sub> were purchased from J.T. Baker, Germany. Ultra pure water for HPLC analysis (TKA, Germany) was used. The sulfonylurea herbicide standard stock solution was prepared in acetonitrile at a concentration level of 100 µg/ml, while suitable concentrations of working standards were prepared

from the stock solutions by dilution with acetonitrile, achieving concentrations in a range from 2.5 to 20 µg/ml. For matrix-matched calibration, standards were prepared in the same concentrations, by adding standard stock solutions in blank matrix extracts.

### Extraction and determination

The extraction and determination procedures had been optimized in our previous study (Lazić and Šunjka, 2014). Nicosulfuron was extracted from homogenized 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. Clean-up procedure was done using C18 column. The extract of soil was passed through the column. Nicosulfuron residues were eluted from the column by using a 5 ml of acetonitrile. The eluant was collected in a 10 ml kivet and then concentrated to dryness in nitrogen stream. The residue was dissolved in 1 ml of acetonitrile, ultrasonically homogenized and filtered through a 0.45 µm nylon membrane filter prior to the analysis.

The HPLC-DAD system used consisted of Agilent 1100 Series LC system, equipped with a reversed phase Zorbax Eclipse C18 analytical column of 50 mm × 4.6 mm and particle size 1.8 µm. The external standard method was used for the quantification of nicosulfuron residues.

### Sampling

Soil sampling was carried out nine months after nicosulfuron application. Commercial formulation of nicosulfuron was used at the recommended dose, according to the manufacturer's instructions. Samples were collected from surface (0–30 cm) and sub-surface (30–60 cm) soil horizons. In order to ensure representative sample, from a plot on a cultivated field, 5 sub-samples were taken diagonally. The samples were mixed and the average sample of 500 g was formed. In this manner prepared samples were placed into plastic bags, marked and identified, and then transferred to the laboratory. In laboratory prepared average samples that 100 g were air-dried, milled, sieved and analyzed.

## RESULTS AND DISCUSSION

Monitoring the trace levels of nicosulfuron herbicide in soil is a challenging task and demands highly efficient, selective and sensitive analytical technique. For selection



of a detection technique the most important criteria in the evaluation is the concentration, at which a given analyte may be found in the tested sample. This problem appears when the herbicide is used once or several times during vegetation in small doses of <50 g/ha (Sadowski et al., 2002, cit. Sekutowski, 2011), as it is the case with products based on nicosulfuron. The method applied in this study is based on reversed-phase liquid chromatography with diode array detection. Method validation ensures analysis credibility. In this study, the parameters accuracy, precision, linearity, limits of detection (LOD) and quantification (LOQ) were tested. The analytical parameters for method determination of nicosulfuron in soil are presented in Table 1 (Lazić and Šunjka, 2014).

The accuracy of the method was evaluated by recovery tests, using the spiked blank soil samples, whereas linearity was determined at five concentrations ranging from 2.5 µg/ml to 20 µg/ml and expressed by correlation coefficient.

The limit of detection (LOD) was determined as the lowest concentration giving a response of 3 times the baseline noise defined from the analysis of control (untreated) sample. The limit of quantification (LOQ) was determined as the lowest concentration of a given herbicide with a response of 10 times the baseline noise. In accordance with SANCO/825/00 rev. 8.1 (SANCO, 2010), the limit of quantification for the determination of pesticides residues in soil should be 0.05 mg/kg.

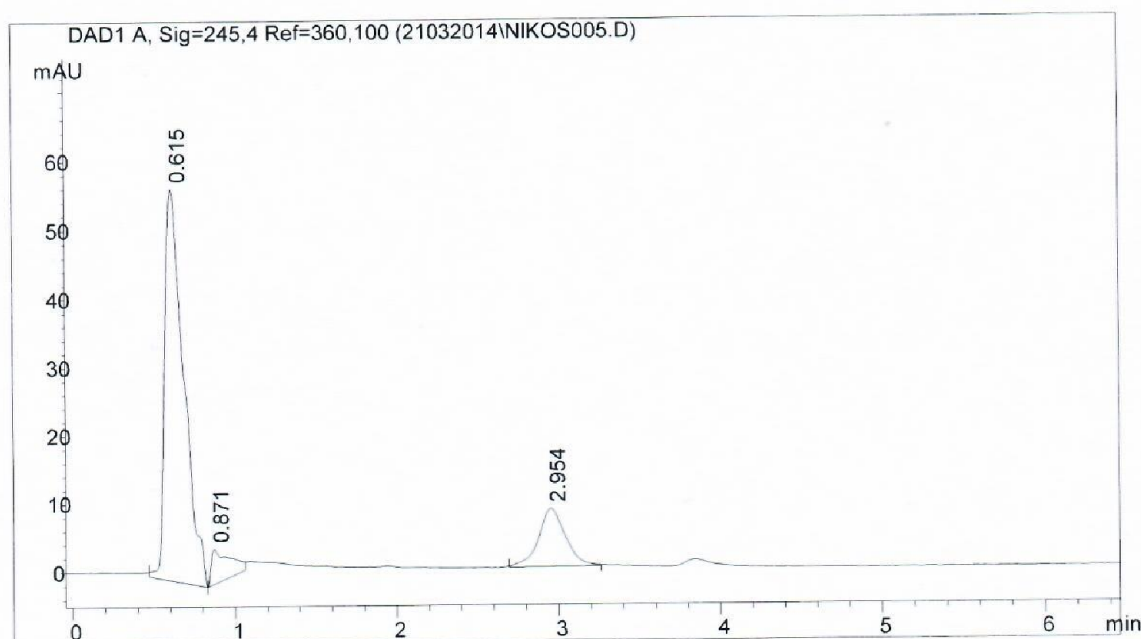
**Table 1.** Analytical parameters for HPLC/DAD determination of nicosulfuron in soil

Parameter	Correlation coefficient <sup>a</sup>	LOD mg/kg	LOQ mg/kg	Mean recovery %	Precision RSD%
Nicosulfuron	0.999	0.01	0.05	89.10	<1.06%

<sup>a</sup> $Y = ax + b$

Presence of matrix effect (SS/E, signal suppression/enhancement) represents influence of soil matrix to nicosulfuron signal. Influence of matrix was determined for whole linear measurement range, from 2.5 to 20.0 µg/ml. Matrix effect was evaluated comparing slope ratio of MMC (matrix matched calibration) and SSC (solvent standard calibration) calibration curves. Regression equation obtained from MMC and SSC were  $y=25.43x+19.52$  and  $y=26.25x+17.57$ , respectively. Achieved matrix effect was 96.87%.

Check of the extraction yield was preceded by check of nicosulfuron presence in soil samples that were used as untreated control. The recoveries were calculated by solvent calibration and matrix-matched calibration curves of nicosulfuron. The average value of nicosulfuron extract value from the soil achieved by this method was 89.10% and 86.73%, based on solvent and on matrix-matched calibration curves, respectively, with RSDs less than 2.5%. Obtained values completely fulfil the criteria SANCO/825/00 rev. 8.1 (SANCO, 2010) for pesticide residues in soil (70-120%).



**Figure 1.** Chromatogram of nicosulfuron in soil sample

The validated method was applied for the analysis of real soil samples. Soil samples were collected from two soil layers, surface (0-30 cm) and sub-surface (30-60 cm) and prepared according to previously described procedure. MMC was used in order to avoid matrix-effect. Average values of nicosulfuron residues in soil samples from surface and sub-surface horizon were  $0.05 \pm 0.003$  mg/kg and  $0.05 \pm 0.002$  mg/kg. According to the Sekutowski (2011), determined content of nicosulfuron residues could be harmful for sensitive following crops. Furthermore, the same amount of nicosulfuron found in the both investigated soil layers could be the consequence of agricultural practices applied during autumn.

## CONCLUSION

Analytical method described in this study was successfully applied for the determination of nicosulfuron residues in soil samples from surface and subsurface layers. Average values of nicosulfuron residues in soil samples from both horizons were 0.05 mg/kg. The same amount of nicosulfuron found in the both investigated soil layers could be the consequence of cultural practices applied during autumn. Determined content of nicosulfuron residues could be harmful for sensitive following crops. The obtained results indicate that nicosulfuron residues in soil should be monitored to avoid toxicity occurrence in the succeeding crops in crop rotation, especially in years with low amount of precipitation and lower average temperatures.

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