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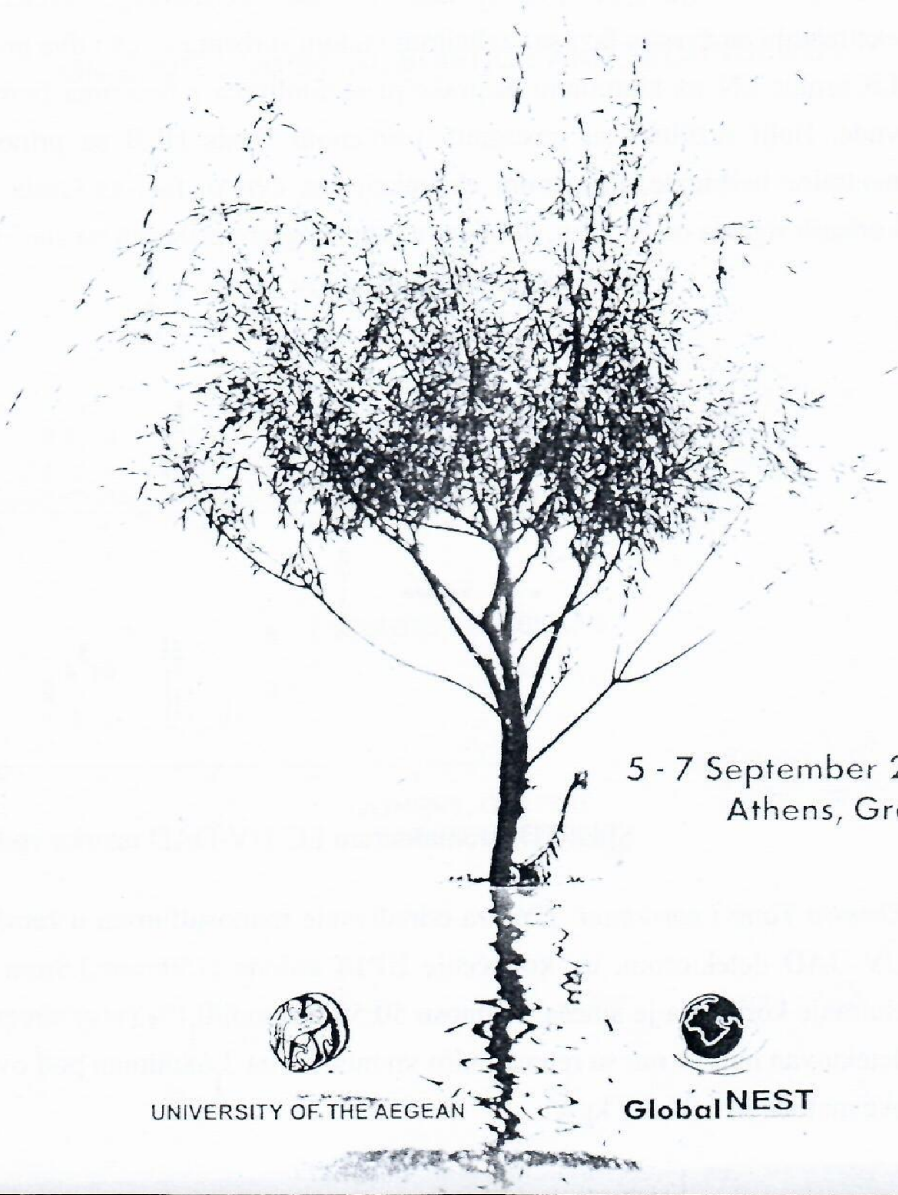

5 - 7 September 2013
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ANALYTICAL VALIDATION METHOD FOR THE DETERMINATION OF SULFONYLUREA HERBICIDES IN WATER SAMPLES BY SOLID-PHASE EXTRACTION AND HPLC WITH DIODE-ARRAY DETECTION

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EXTENDED ABSTRACT

Pesticides having different structures and biological activities are widely used for agricultural and non-agricultural purposes. Due to their widespread use, pesticides need to be determined in various environmental matrices. A wide range of analytical techniques have been developed in order to identify the organic contaminants often present at trace levels in environmental samples. The aim of this work was to develop a reliable, rapid, robust and cost-effective method for the determination of five sulfonylurea herbicides (nicosulfuron, oxasulfuron, tribenuron methyl, triasulfuron and tritosulfuron) in spiked river water samples. The samples were diluted with 0.5% acetic acid (1:1) and purified by solid-phase extraction (SPE) on Oasis MAX cartridges. After the cartridges were sequentially washed with water, methanol, ethyl acetate, and acetonitrile, they were eluted with 0.1% formic acid in acetonitrile. Elutes were evaporated to dryness and reconstituted in acetonitrile. The purified extracts were analyzed by reversed-phase high-performance liquid chromatography with diode-array detection (HPLC-DAD) system. The best separation was achieved on a Zorbax Eclipse XDB-C_{18} (50mm x 4.6mm x 1.8μm) analytical column with gradient elution at a flow rate of 1 mL/min. The analyte was monitored at 230 nm. The linearity of calibration curve within the tested concentration range exhibited correlation coefficients (r^2) higher than 0.998. The accuracy of the method was acceptable since the average recoveries measured at two fortification levels were in the range of 92.5-97.5% (n=3). The precision of the developed procedure expressed as the relative standard deviations (RSDs) were lower than 4.76% in all cases. The repeatability of the retention time and peak area was checked by injecting standard solution ten times. The relative standard deviation of the retention time and the peak area was found to be less than 0.2% and 0.6%, respectively. The method was also validated by analyzing freshly spiked river water samples. The proposed analytical method might be successfully applied in monitoring of sulfonylurea herbicides (nicosulfuron, oxasulfuron, tribenuron methyl, triasulfuron and tritosulfuron) in surface water and groundwater samples.

KEYWORDS: sulfonylurea herbicides; determination; surface water; HPLC-DAD.

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Keywords: sulfonylurea herbicides; determination; surface water; HPLC-DAD

1. INTRODUCTION

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 because of the presence of pesticides in the aquatic system as a result of their common use. Control and monitoring of the pesticide residues content in water (groundwater and surface water) is extremely important since the groundwater is one of

the main sources of drinking water in Vojvodina (Dalmacija, 2007). It is essential that contamination by pesticide residues is prevented in order to safeguard drinking water supplies and to protect fish and other aquatic life (Environment Agency, 1999). According to the restrictive European Union (EU) regulations, which limit the maximum amount allowed for a single pesticide in drinking water to 0.1 μg/L and for the sum of the pesticides to 0.5 μg/L including toxic transformation products, very sensitive analytical methods for monitoring drinking and surface water samples are required (European Council Directive 98/83/EC; Directive 2000/60/EC; Directive 2008/105/EC).

Sulfonylurea herbicides (SUs) have become very important in agricultural production since the registration of chlorsulfuron in 1982 (Beyer et al., 1988). These herbicides are extremely active against a wide spectrum of weeds and some grasses by using relatively low application rates, typically less than 100 g of active ingredient per hectare, which consequently, makes their detection and analysis difficult compared to that of traditional herbicides (Dinelli & Brandolini, 1995; Brown, 1990). SUs exhibit herbicidal effects because they are potent inhibitors of the enzyme acetolactate synthase (ALS), involved in branched chain amino acid biosynthesis in plants. ALS inhibitors are regarded as the most active group of herbicidal compounds found to date and they display very high herbicidal activity in soil (Sarmah & Sabadie, 2002). Despite relatively low applications rates, the increased usage of SUs has allowed for certain species to develop a limited resistance on traditional SUs mixtures (Mazur et.al., 1987). To combat this behavior, SUs have to be applied in greater quantity to achieve the desired effect (Kudsk et.al., 1995). Consequently, an increased concentration of SUs residues is expected. With the increase of the application scope of SUs, the concern for the effects of SUs in the environment and human health has increased. Despite the beneficial impact of SUs on agricultural productivity, concern has been raised by the public and regulatory authorities regarding the potential for their adverse impact on groundwater and environmental quality. Since the SUs are water soluble, they may be transported to surface and groundwater with the possibility of environmental contamination (Biziuk et.al., 1996; Fung & Ikesaki, 1991). The trace determination of sulfonylurea residues in environmental samples such as surface water presents a challenging analytical problem and the low dosage used requires the application of highly sensitive analytical techniques to detect trace concentrations of residues in water. Due to the low level present and complexity in sample, clean-up and enrichment before analysis is necessary and become a crucial step for the determination of SUs 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. Most sulfonylurea compounds lack the thermal stability and volatility required for gas chromatographic analysis. Derivatization is required and although successful for some analytes (Klaffenbach et.al., 1993), it is not practical for the group as a whole. Single-analyte methods based on reversed-phase and normal phase liquid chromatographic (LC) techniques with adequate sensitivity from ultraviolet detectors have been widely reported (Morrica et.al., 2002, Seccia et.al., 2011). The purpose of the present work was to develop a rapid, selective, sensitive and reliable method for the simultaneous determination of the selected five sulfonylurea herbicides from water samples, using liquid chromatography in combination with diode array detection.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Methanol (MeOH) and acetonitrile (ACN) of chromatography grade were obtained from J.T. Baker (USA). Formic acid (purity 98%, w/w) was purchased from Sigma-Aldrich (Steinheim, Germany). Certificated analytical standards of nicosulfuron (93.5% purity), oxasulfuron (98.0%), tribenuron methyl (99.0%), triasulfuron (99.5%) and tritosulfuron

(98.0%) were purchased from Dr. Ehrenstorfer GmbH (Germany). The water used was purified with Ultra Clear 2002-D UV (SG Water, Germany) (conductivity at 25 ^{0}C, 0.055 µS/cm). All other chemicals were of analytical grade. Structures of nicosulfuron, oxasulfuron, tribenuron methyl, triasulfuron and tritosulfuron are shown in Figure 1.

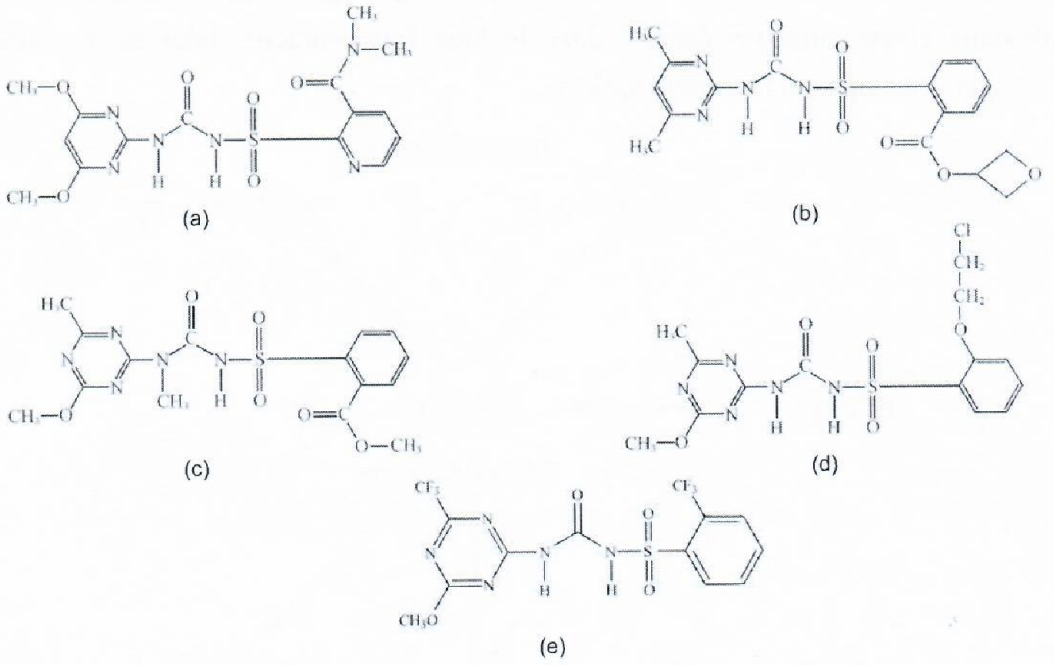

Figure 1. Structures of sulfonylurea herbicides: (a) nicosulfuron, (b) oxasulfuron, (c) tribenuron methyl, (d) triasulfuron and (e) tritosulfuron

2.2. Apparatus

The target compounds were extracted from water samples using Waters Oasis MAX 6cc/500mg SPE Extraction Cartridge (Waters Corp., Milford, MA) and SPE Manifold (Supelco, Inc., Bellefonte, PA). The high-performance liquid chromatography (HPLC) analyses during the method development and validation procedures were carried out using a Agilent 1100 (Agilent Technologies Inc., USA) with DAD G1315A diode array detector (DAD), G1312A pump, G1322A degasser and G1313A autosampler was used. The detector was set at 230 nm, and peak areas were integrated automatically by a computer. Chromatographic data were collected and recorded using a ChemStation software program. Separations were carried out on a rapid resolution HT cartridge Zorbax Eclipse XDB-C18 (50mm x 4.6mm i.d., 1.8µm particle size, Agilent Technologies).

2.3. Preparation of Reference Solutions

The stock solutions of nicosulfuron, oxasulfuron, tribenuron methyl, triasulfuron and tritosulfuron were prepared by weighing 10 mg of the reference substance, transferring to a 10 mL volumetric flask and diluting to volume with acetonitrile to obtain a concentration of 1 mg/mL. The stock solutions were stored at - 18°C protected from light and diluted daily with the acetonitrile to give final concentrations of 0.01, 0.05, 0.5, 1.0 and 5.0 µg/mL for the working standards.

2.4. Chromatographic Conditions

The HPLC analyses were performed at ambient temperature. An appropriate aliquot (10 µL) was injected. The mobile phase was composed of ACN (solvent A) and 0.1% acetic

acid in water (solvent B) with flow rate at 1 mL/min. The following gradient profile was used: 0–2 min: linear from 52% to 47% A; 2–2.5 min: linear from 47% to 45% A; 2.5–5 min: linear from 45% to 52% A and then re-equilibrium of the column. Before using, the mobile phase was degassed in an ultrasonic bath and filtered by through a 0.45 µm nylon membrane filter (HP 9301-0895).

2.5. Source of samples and preparation of fortified samples

River water from the Danube was used for this method validation. Water samples of 500 mL were diluted with 0.5% acetic acid (1:1) immediately prior to the extraction procedure, then fortified and filtered. To validate the method, a set of validation water samples were prepared: two control samples in triplicate (50 and 80 ng/mL) and five level standards for calibration curves in the range of 10–5000 ng/mL by spiking the extracts with appropriate volume of combined working standard solutions. For recovery studies, a water sample was spiked before the SPE extraction procedure with the mixture of the studied SUs.

2.6. Principle of the Analytical Method

The sulfonylurea compounds were extracted using solid-phase extraction with Bond Elut PPL, Oasis HLB and Oasis MAX cartridges. The cartridges were first conditioned with MeOH (5 mL), followed by water (5 mL). After the conditioning step, aliquots of 250 mL of sample, filtered and diluted with 0.5% acetic acid (1:1), were slowly passed through the column. The analytes were eluted with 0.1% formic acid in acetonitrile after the cartridges were sequentially washed with water, methanol, ethyl acetate, and acetonitrile. Eluates were evaporated to dryness at 30-35°C and reconstituted in acetonitrile. The purified extracts were analyzed by reversed-phase HPLC.

2.7. Validation of the analytical method

The developed and optimized method for quantitative analysis of the five investigated pesticides in water was validated in terms of linearity, LOD, reproducibility and recovery. All results are expressed as percent and n represents the number of replicates.

3. RESULTS AND DISCUSSION

In this study, different pH values of water samples were tested prior to applying on SPE columns. The reason for applying acidic or basic water sample before extraction process is that SUs weak acidic compounds can be ionized in it. The ionization extent of SUs is affected by its pKa and pH value of water samples which determines the extraction efficiency on applied SPE column. In this study, to analyze the extraction efficiency of SPE cartridges, different pH values (such as pH 5.0, 7.0 and 8.5) of water samples were tested. It was detected that the average recoveries of water sample fortified at 50 and 80 ng/ml ranged from 30 to 59% at pH 5.0 of water sample, from 85 to 99% at pH 7.0 and 45 to 77% at pH 8.5 for applied to SPE cartridges (table 1). Therefore the pH 7.0 value of water sample was adopted. Under this condition the SUs is in anion form which is easily combined with the adsorbent of SPE and consequently retained in SPE cartridges. The results indicated that the recoveries of the water extract were higher for water samples adjusted on pH 7.0 that of other adjusted extract (pH 5.0 and pH 8.5).

Sample amount (volume) is also a critical parameter that affects the determination of SUs in water samples. In this study, we investigated the recoveries of the analytes from the SPE cartridges using three different volumes of water sample (0.25, 0.5 and 1.0 L). As the sample volume was over 0.5 L, the recoveries were decreased, indicating that cartridges could be used for analysis of 0.25–0.5 L of water sample and the volume of 0.25 L was chosen for further tests.

Cartridges with different sorbent materials were applied for optimization of the SPE parameters. In order to optimize extraction of SUs, three types of sorbents were tested

and compared for the evaluation of extraction efficiency of five sulfonylurea pesticides (table 1).

Table 1. The properties of used sorbents

Sorbent	Retention mechanism	Surface area (m^2g^{-1})	Particle size (μm)	Pore size (Å)
Bond Elut PPL	polar exchange reversed phase	100	600	150
Oasis HLB	hydrophilic-lipophilic-balanced reversed phase	60	30	80
Oasis MAX	mixed mode anion-exchange reversed phase	150	60	80

In each case a volume of 250 mL river water was spiked with the five SUs at concentrations of 50 and 80 ng/ml. Spiked samples were passed through the cartridges followed by elution of the herbicides with different eluents. In order to optimize the SPE extraction of SUs for each type of sorbent the following eluents were tested: acetonitrile, methanol and ethylacetate with the addition of formic acid (0.1%). The efficiency of the tested eluents was estimated by comparing the recovery levels of the eluted SUs (table 2).

Table 2. The SUs recovery by using different eluents

Eluent	SUs recovery from different sorbents (%)± RSD*			
		Bond Elut PPL	Oasis HLB	Oasis MAX
0.1% formic acid in methanol	Nicosulfuron	63.1±3.3	76.9±3.0	87.7±4.8
	Oxasulfuron	62.6±2.5	75.0±4.1	86.2±2.6
	Tribenuron methyl	60.9±1.7	75.3±3.7	86.0±3.2
	Triasulfuron	62.2±1.9	75.7±1.8	86.7±1.2
	Tritosulfuron	61.2±2.3	62.2±1.2	84.2±4.3
0.1% formic acid in acetonitrile	Nicosulfuron	58.5±4.1	75.8±3.2	93.2±2.4
	Oxasulfuron	61.5±3.8	77.6±6.4	92.5±2.7
	Tribenuron methyl	58.0±2.6	77.3±5.1	96.2±2.9
	Triasulfuron	59.3±1.8	76.9±2.0	95.3± 3.7
	Tritosulfuron	60.1±2.4	78.1±1.5	96.1± 1.6
0.1% formic acid in ethylacetate	Nicosulfuron	70.3±4.0	85.2±3.1	91.1±1.1
	Oxasulfuron	68.4±2.3	82.0±2.3	86.6±2.4
	Tribenuron methyl	69.1±3.1	84.7±1.7	88.7±1.7
	Triasulfuron	69.3±1.3	83.9± 1.0	90.1± 2.8
	Tritosulfuron	71.5±2.7	81.7± 2.5	92.5± 0.8

*Average ± RSD (relative standard deviation)

Each experiment was made in triplicate and the relative standard deviation (RSD) was calculated. The results showed that the use of 0.1% formic acid in methanol and ethylacetate, leads to a poor recovery for the sorbent Bond Elut PPL for analyzed SUs (table 2). For the other two type of sorbents the SUs recovery ranged between 75 and 97.5%. SUs eluted with 0.1% formic acid in ethylacetate and methanol had similar recoveries for Oasis HLB and Oasis MAX. This might be because the mixed-mode ion-exchange sorbents are specifically designed to combine a polymeric skeleton with ion-exchange groups, so they can mix two types of interaction mechanisms reversed-phase and ionic-exchange, which leads to rich recoveries when a polar compound, such as SUs are extracted. It can be assumed that recovery of SUs on Bond Elut PPL decreased in

the analysis of complex aqueous samples, such as river water, because the salts and ionic species are present in the complex matrix. Present salts and ionic species interfered with SUs during the extraction process and they influenced to decrease recoveries of SUs.

Oasis MAX showed the best recoveries and peak shapes in developed HPLC–UV/DAD analysis (figure 2). The improvement in the peak shape might be also attributed to the elution solvent used for Oasis MAX (0.1% formic acid in acetonitrile). Besides the regularly shaped and well defined peaks of the SUs pesticides investigated, SPE-HPLC chromatograms contained group of peaks likely to orginate from the river water matrix. There were no significant interfering peaks present in the elution region of SUs pesticides.

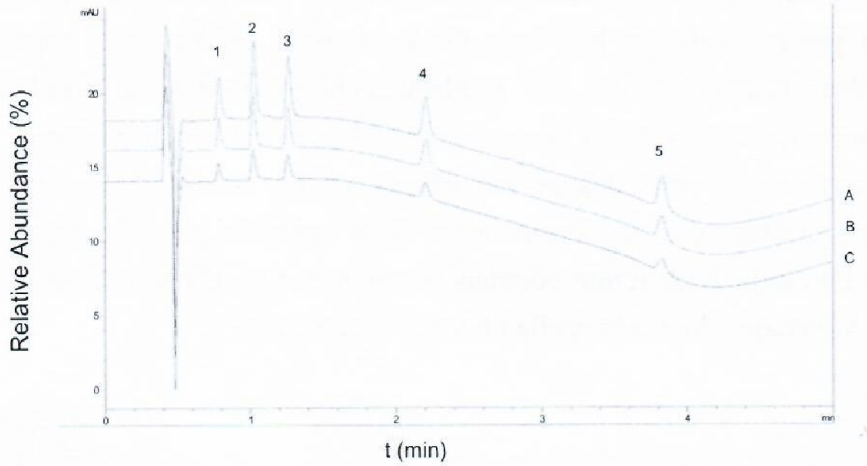

Figure 2. Chromatograms of spiked river water samples (50 ug/ml of each substance) with different sorbents. (A) Oasis MAX; (B) Oasis HLB; (C) Bond Elut PPL. Peak designation: (1) Nicosulfuron; (2) Oxasulfuron; (3) Triasulfuron; (4) Tribenuron methyl; (5) Tritosulfuron.

The linearity of the method was evaluated by a calibration curve in the range 0.01–1.0 µg/mL (n=3). Calibration curves were prepared by the analysis of samples spiked with various volumes of each working standard SUs solution. The samples were then submitted to the processes of extraction, chromatographic separation and detection described above. The linearity of the analytical response across the studied range was excellent with correlation coefficients higher than 0.998. Precision was expressed as the repeatability and was examined by analysis of river water samples (n=5) at two different concentrations on the same day. The RSD values were within the range of 1.54-4.76% for all SUs pesticides. The repeatability of the retention time and peak area was checked by injecting standard solution ten times and the relative standard deviation of the retention time and that of the peak area was found less than 0.2% and 0.6%, respectively.

The LOD was calculated as 3.3α/b, where α is the standard deviation of the y-intercept and b is the slope of the calibration curve. This parameter was determined by analyzing a series of decreasing concentrations of the spiked river water samples (table 3). Very low detection limits can be obtained for sulfonylurea herbicides in water. The LODs were 12.5-35.1 ng/L for tested river samples. The lowest LOD level was observed for tritosulfurone (12.5 ng/L) and the highest for triasulfurone (35.1 ng/L). T

Table 3. LODs of five sulfonylurea herbicides in river water samples

Herbicides	LOD (ng/L)
Nicosulfuron	28.1
Oxasulfuron	21.2
Tribenuron methyl	34.0
Triasulfuron	35.1
Tritosulfuron	12.5

5. CONCLUSIONS

A rapid and sensitive method described in this paper provides reliable, simultaneous quantitative analysis of five sulfonylurea pesticides in fortified river water. The optimized SPE-HPLC-UV/DAD procedure provided significant advantages including simplicity, employment of usual laboratory equipment and high extraction efficiency. Reverse phase and gradient elution based liquid chromatographic conditions provided efficient separation of investigated SUs.

The results of the present work showed that the Oasis MAX cartridge was the best alternative for the SUs extraction in the river water. The main advantages applied Oasis MAX cartridge was high recovery and enrichment factor. The recoveries of the method for applied cartridge ranged between 92.5% and 97.5% for all target analytes with associated relative standard deviations (RSDs) between 1.6 and 3.7%. The developed method was rapid and had high sensitivity, specificity, and accuracy. The obtained calibration curves displayed good linearity. Therefore, the proposed analytical procedure could be satisfactorily used in regular monitoring of sulfonylurea herbicides (nicosulfuron, oxasulfuron, triasulfuron, tribenuron methyl and tritosulfuron) residues on a large number of surface water and groundwater samples.

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