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Dedicated to the memory of
Professor Candin Liteanu on his 100th anniversary

DETERMINATION OF TRIAZINE HERBICIDES IN SOIL SAMPLES BY ULTRASOUND-ASSISTED EXTRACTION FOLLOWED BY DISPERSIVE LIQUID-LIQUID MICROEXTRACTION BASED ON SOLIDIFICATION OF FLOATING ORGANIC DROPLET AND HPLC-UV ANALYSIS

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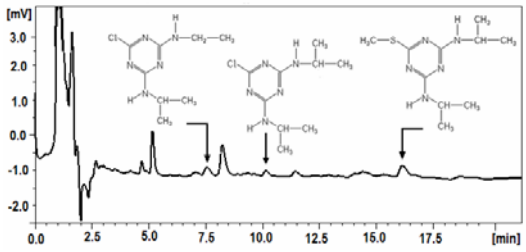
Nowadays, the miniaturized extraction techniques are increasingly used due to their environmentally friendly features, efficiency and short extraction time. In this study, a procedure for the extraction of seven triazines (simazine, prometon, atrazine, ametryn, propazine, prometryn, terbutryn) from soil samples which combines the Ultrasound-Assisted-Extraction (USAE) as an effective extraction method and the Dispersive Liquid-Liquid MicroExtraction based on Solidification of Floating Organic droplet (DLLME-SFO) as a method for the extract clean-up and the concentration of target triazines has been developed. The influence of several parameters (pH, ionic strenght and extraction solvent) over the triazine extraction from the soil matrix has been investigated. The determination of triazines was carried out by HPLC with UV detection at 220 nm. The developed USAE-DLLME-SFO-HPLC-UV procedure provided high enrichment factors (176-247), good linearity (R^2, 0.992-0.999), low limits of detection/quantification (0.19-0.68/0.62-2.06 µg/kg), and it was applied to analyse some real soil samples.



INTRODUCTION

Triazines are herbicides with a heterocyclic aromatic structure containing three nitrogen atoms. The first triazine discovery in 1952 at the chemical company J.R. Geigy, in Switzerland, has revolutionized agriculture by the development of a new family of herbicides.[1] The triazines are applied pre- and post-emergence acting in plants by inhibiting the Hill reaction which plays an important role during photosynthesis.[2] Their fate in the environment is influenced by many different factors, for example, in soil by pH, texture, temperature, and differences in tillage practices.[3]

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Moreover, sunlight, microbial activity, and moisture also influence the triazine transport and its degradation.[4] The data concerning the triazine degradation are divided with literature studies showing a half-life time between 30 and 245 days,[5, 6] depending on soil type. Studies regarding the toxicological effects of triazines have shown feminizing effects on amphibians and fish and reproductive toxicity on rats and hatching female quails.[7-9] As a consequence of their harmful effects over the ecosystems, these herbicides have drawn a special attention.

Recently, the European Commission introduced atrazine and simazine on the list of priority substances[10] and established the maximum allowable concentration in surface waters, recommending to the EU member states to monitor these compounds in various environmental factors. Taking into account this fact it is important to develop reliable methods to analyze triazines at low levels with a minimal expenditure of solvents and time.

Gas and liquid chromatography are the most frequently employed methods for triazine analysis.[11-14] Other methods used for the determination of these compounds are micellar electrokinetic chromatography (MEKC)[15] and capillary electrophoresis (CE).[16]

Usually in the environmental factors the triazine herbicides are found at trace levels. For this reason, their determination involves different extraction methods that depend on the matrices in which these compounds are enclosed. For the extraction of triazines from soil samples the most commonly used methods are Soxhlet extraction, supercritical fluid extraction (SFE)[17] and pressurized liquid extraction (PLE).[18] While Soxhlet extraction is time consuming and requires a large volume of solvent, PLE and SFE are rather expensive. Another method, like microwave assisted extraction (MAE), offers high efficiency and shorter extraction time, but still uses a large volume of extraction solvent.[19] During the last years ultrasound-assisted extraction (USAE) has been developed for triazine extraction from solid samples.[20] This technique uses the ultrasonic energy to obtain an efficient recovery of the analyte due to a better contact between the solid and the extraction solvent. Sonication causes an effect known as cavitation which generates numerous tiny bubbles in the liquid medium and a mechanical erosion of solid, including solid particle rupture, having as a result a better penetration of the extraction solvent into the solid matrix. Even if by this technique the extraction time and the organic solvent volume are reduced, a further clean-up step to eliminate the co-extracted interferences along with the target compounds is frequently necessary.[21]

The usual extraction methods for liquid samples, like liquid-liquid extraction (LLE) and solid phase extraction (SPE)[22,23] exhibit certain disadvantages, such as being time consuming and requiring significant volumes of organic solvents. Modern approaches focused on the miniaturization of the extraction process which offer a higher sensitivity have been developed, the well-known being the solid-phase microextraction (SPME)[14] and the stir bar sorptive extraction (SBSE).[24] Recently, other new microextraction methods like the ultrasound assisted emulsification microextraction (USAEME),[25] ionic liquid dispersive liquid-liquid microextraction (IL-DLLME)[13] and dispersive liquid-liquid microextraction based on the solidification of floating organic droplet (DLLME-SFO)[26] have been proposed. The main disadvantages of USAEME and IL-DLLME methods are the emulsification, the obstruction regarding mass transfer between immiscible phases, and the difficulty to handle the frozen droplet.[27,28] DLLME-SFO avoids such drawbacks, offering an extraction equilibrium which is rapidly achieved as a result of the ample surface contact between the fine droplets of the extraction solvent and the sample solution.[29] Consequently, the development of some alternative methods able to reduce to a minimum the solvent volume and the analysis time have been a great concern in the last years.

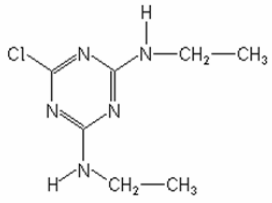
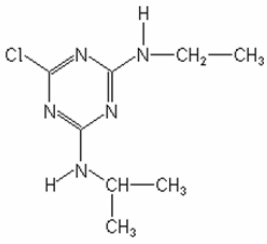
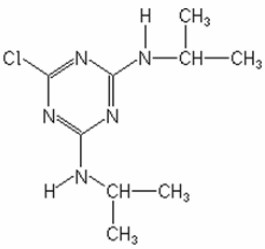
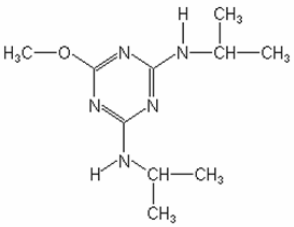
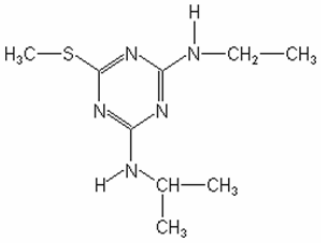
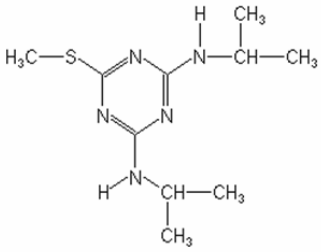
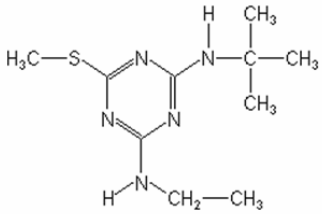
In this study, a procedure for the extraction of seven triazines (simazine, prometon, atrazine, ametryn, propazine, prometryn, terbutryn) from soil samples which combines the ultrasound-assisted-extraction as an effective extraction method and the dispersive liquid-liquid microextraction based on the solidification of floating organic droplet as a method for the extract clean-up and the concentration of the target triazines is proposed. Some factors which influence USAE, *i.e.* extraction solvent type, and DLLME-SFO, *i.e.* the solution ionic strength and pH value, were studied. High performance liquid chromatography with UV detection (HPLC-UV) was used for the identification and the quantification of the selected triazines. The developed procedure USAE-DLLME-SFO-HPLC-UV was applied to extract and to analyze these triazine herbicides in real soil samples.

RESULTS AND DISCUSSION

The structural formulas of the seven studied triazines are presented in Table 1.

Table 1

Structural formulas and acid dissociation constants (pKa) of the studied triazines

			
Simazine	Atrazine	Propazine	Prometon
pKa 1.62, very weak base	pKa 1.7, very weak base	pKa 1.7, very weak base	pKa 9.73, weak acid
			
Ametryn	Prometryn	Terbutryn	
pKa 4.1, weak base	pKa 4.1, weak base	pKa 4.3, weak base	

The development of USAE-DLLME-SFO-HPLC-UV procedure involves the finding of the best parameters for DLLME-SFO and USAE in order to obtain the maximum efficiency of the extraction and clean-up for triazine extract.

DLLME-SFO Optimization

For the USAE extract clean-up and the concentration of studied triazines from aqueous matrices, the DLLME-SFO method of Sanagi *et al.*[26] was optimized, establishing the optimal pH value and the sodium chloride amount for the salt-out effect. The DLLME-SFO performance was expressed by the enrichment factor (EF) and the extraction recovery (ER).

The enrichment factor was calculated based of the Eq. (1):[11]

$$EF = C_{org}/C_0 \quad (1)$$

where C_{org} is the analyte concentration in the floating phase and C_0 the initial analyte concentration in the aqueous sample. The obtained EF values are given in Table 1 and are ranged between 176 and 247.

The extraction recovery was defined as the ratio between the analyte amount in the floating phase (n_{org}) and the initial analyte amount (n_0) within the sample:[11]

$$ER = (n_{org}/n_0) \times 100 = [(C_{org}V_{org}) / (C_0V_0)] \times 100 = EF \times (V_{org}/V_0) \times 100 \quad (2)$$

where V_{org} is the volume of the floating phase and V_0 is the volume of the aqueous sample.

Eq. (2) was used for the calculation of the analyte recovery by DLLME-SFO method (Figs. 1-3).

Influence of pH

The results obtained for the pH values ranged between 2 and 9 show that the extraction efficiency (recovery) of studied triazines increases with the increasing of pH value until 8 value (Fig. 1). The reduced recovery at low pH can be explained by the easy hydrolysis of these triazines at an acidic pH.[30]

Moreover, taking into account the pKa values of the target triazines which are situated between 1.7 and 4.3, excepting prometon with 9.73 (Table 1), this means that these compounds are weak acid which are protonated in acidic medium. Therefore, a pH around the neutral value gives the

best recoveries can.[31] Consequently a pH of 8 value was selected to be used for the further experiments.

Solution ionic strength

The main role of adding salt in the aquatic samples is to improve the extraction efficiency. Such a situation is attained by decreasing the solubility of analytes in the aqueous phase by the salting-out effect and increasing their solubility in the organic phase. For the evaluation of this effect, sodium chloride in concentrations ranging from 5 to 25% was added to the aqueous solution. In Fig. 2 one can observe an increase of the triazine extraction efficiency up to 15-20% sodium chloride and afterward a significant decrease can be noted. For further experiments, a concentration of 20% sodium chloride was selected to obtain the salting-out effect in the triazine aqueous phase.

USAE Optimisation

The selection of the extraction solvent plays an important role in the extraction efficiency. The solvent should satisfy the following demands: good selectivity for the target compounds, good penetration rate into the soil matrix and easily separation from the sample matrix. For the triazine extraction from soil, three solvents like acetone, acetonitrile and methanol were investigated. As it can be seen in Fig. 3, methanol provides the best recovery over 95% for all selected triazines, excepting simazine with 80%. Therefore, methanol was chosen as triazine extraction solvent from soil samples for the further studies.

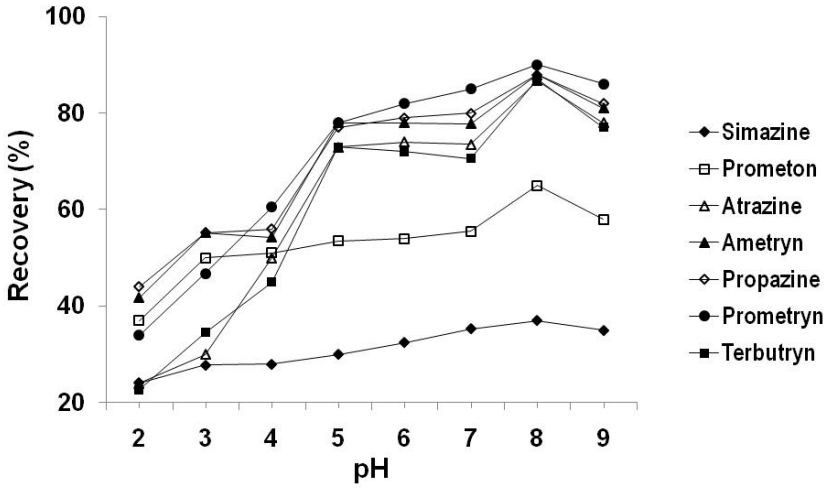


Fig. 1 – Influence of pH on the triazines extraction efficiency.

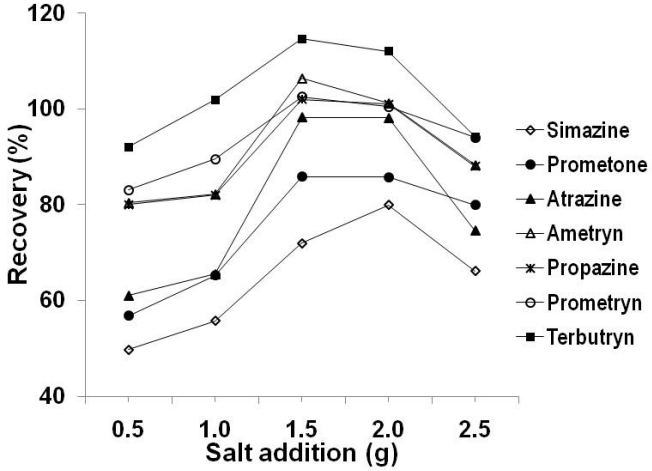


Fig. 2 – Influence of the solution ionic strength
on the triazine extraction efficiency (10 mL aquatic sample volume).

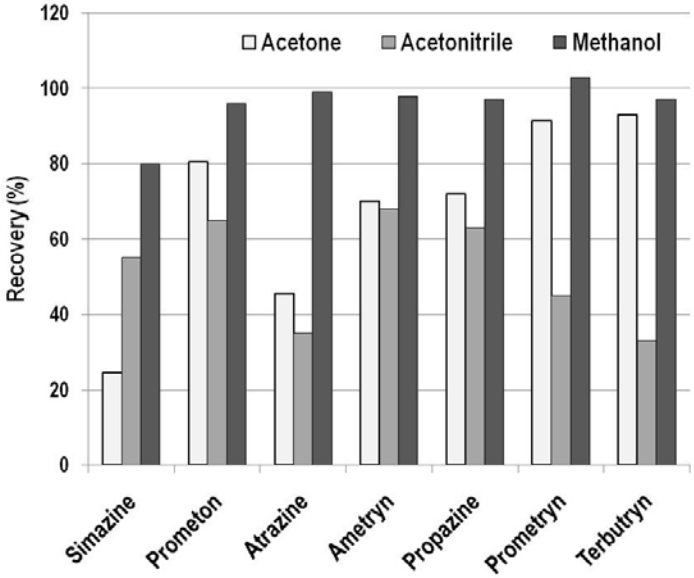


Fig. 3 – Influence of the extraction solvent on the triazine extraction efficiency from soil samples.

Table 2

Analytical performances obtained for the triazine extraction from soil samples by the proposed USAE-DLLME-SFO-HPLC procedure

Herbicide	Linear curve equation (liniar range 50-400 ng)	R^2	Slope	SD	LOD (µg/kg)	LOQ (µg/kg)	RSD (%)	EF
Simazine	y = 496.73 x + 1403.7	0.996	496.73	0.08	0.54	1.63	10.42	176
Prometon	y = 562.79 x – 800.65	0.992	562.79	0.10	0.59	1.79	9.51	189
Atrazine	y = 548.82 x – 408.91	0.999	548.82	0.05	0.30	0.91	6.74	216
Ametryn	y = 753.71 x – 9423.5	0.998	753.71	0.04	0.19	0.57	5.91	218
Propazine	y = 752 x – 8618.7	0.999	752	0.06	0.25	0.74	6.68	220
Prometryn	y = 771.79 x – 8691.1	0.996	771.79	0.05	0.21	0.62	5.23	221
Terbutryn	y = 694.68 x – 1235.2	0.999	694.68	0.14	0.68	2.06	7.31	247

R^2, coefficient of determination; SD, standard deviation; RSD, relative standard deviation for (n = 5); LOD, limit of detection; LOQ, limit of quantification, EF, enrichment factor.

Analytical performances of the proposed extraction procedure

The best experimental conditions obtained for the triazine extraction from soil samples were: methanol as extraction solvent for USAE, a value of 8 for pH and 20% NaCl in aqueous phase for DLLME-SFO.

The performances of the proposed USAE-DLLME-SFO-HPLC-UV procedure expressed by the repeatability (RSD, %), linearity (R^2), limit of detection (LOD), limit of quantification (LOQ), and enrichment factor (EF) are given in Table 2.

Precision was expressed as intra-day precision (repeatability) by means of five replicates (n = 5) of spiked soil samples with 300 ng each triazine (3 µL standard mixture). RSD was ranged between 5.23 and 10.42 %.

The quantification of the target compounds in real samples was made by means of the calibration curves which were constructed using peak area *versus* the concentration of each analyte. The data of Table 2 show a good linearity for all target triazines and the R^2 values ranged from 0.992 to 0.999. LOD and LOQ of studied triazines were determined using the standard deviation and the slope of each calibration curve. LODs were situated in the range of 0.19-0.68 µg/kg and LOQs in the range of 0.57-2.06 µg/kg, respectively.

In Table 3 are presented the results obtained for accuracy which was expressed as recovery of triazines from spiked real soil samples.

As can be seen from the data of Table 3, the developed USAE-DLLME-SFO-HPLC-UV procedure provides a good accuracy, the recovery exceeding 95% for all compounds, excepting simazine which has 60% recovery. The lower recovery for simazine could be caused by unsatisfied extraction efficiency appeared both in USAE and DLLME-SFO.

Table 3

Extraction recovery of the studied triazines

Herbicide	Concentration (µg/kg)			Recovery (%)
	Initial	Added	Found	
Simazine	nd	1.33	0.798	60.00
Prometon	nd	1.33	1.286	96.69
Atrazine	0.32	1.33	1.681	102.33
Ametryn	nd	1.33	1.304	98.05
Propazine	0.55	1.33	1.871	99.32
Prometryn	1.53	1.33	2.889	102.18
Terbutryn	nd	1.33	1.416	106.46

nd - not detected

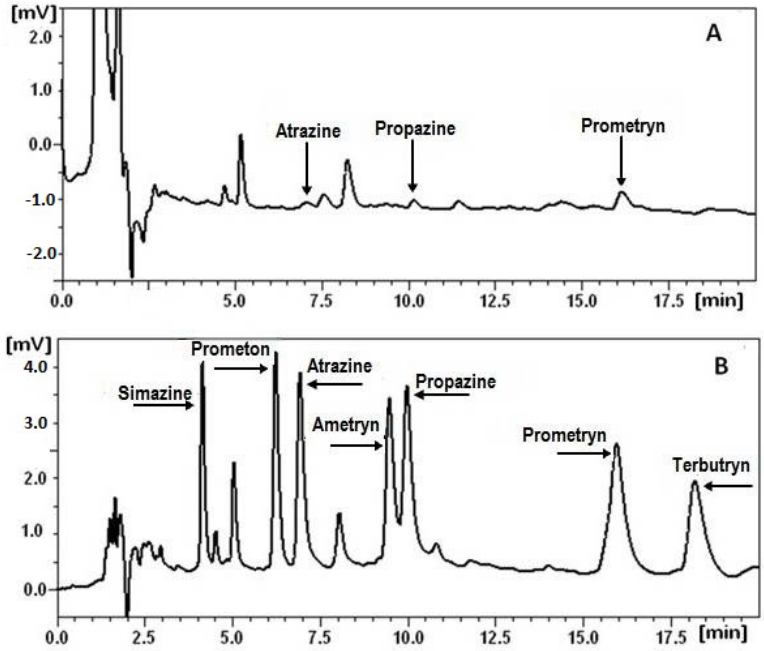


Fig. 4 – Chromatograms of a real soil sample (A) and its spiked soil sample (B).

Analysis of real soil samples

In order to test the developed USAE-DLLME-SFO-HPLC-UV procedure, a few soil samples were collected from an agricultural area from Transylvania, Roumania. The chromatogram of a soil sample collected from a wheat culture and its spiked soil sample are shown in Fig. 4. According to the presented chromatogram of a real soil sample three triazines, namely atrazine, propazine and prometryn were found. These results demonstrate the efficacy of the proposed USAE-DLLME-SFO-HPLC-UV procedure for real soil samples.

EXPERIMENTAL

Chemicals

A standard mixture containing seven triazines (simazine, prometon, atrazine, ametryn, propazine, prometryn, terbutryn) in concentration of 1 mg/mL of each triazine was purchased from Sigma-Aldrich. A stock solution in concentration of 100 µg/mL of each compound was prepared in methanol by dilution of standard mixture. The used extraction solvents (1-undecanol and acetone of 99.89% purity; acetonitrile and methanol of HPLC grade) were acquired from Merck, Germany. Milli-Q water was prepared using a Milli-Q-Plus ultra-pure water system from Millipore, USA.

Instruments and methods

The analyses of the target triazines were performed using a Shimadzu HPLC system, equipped with 10LC module pump, a 10LSD UV/Vis detector and a manual injection valve of 5 µL loop. The separation of compounds was performed on a reverse-phase column Nova-Pak C18 (3.9 × 300 mm, 4 µm particle size) purchased from Waters Corporation, USA, by isocratic elution with the 25 mM monopotassium phosphate:acetonitrile (60:40, *v/v*) mobile phase at 1.2 mL/min flow rate. The detection was carried out at a 220 nm wavelength. The sample centrifugation was done using a centrifuge Eppendorf Model 5804 R (Austria).

DLLME-SFO method

In a 15 mL screw cap vial with conical bottom, to 10 mL Milli-Q water spiked with 100 ng of each triazine (1 µL

triazine standard mixture) were added 1 mL of methanol and 200 µL of extraction mixture containing 150 μL^{-1} acetonitrile (disperser solvent) and 50 µL 1-undecanol (extraction solvent), obtaining a cloudy solution due to the dispersion of the 1-undecanol fine droplets in the aqueous sample. In order to separate the extraction solvent containing triazines (extract), the sample from vial was centrifuged for 5 min at 4500 rpm. Then, the vial was placed in an ice-water bath for 10 min for the extract solidification. By means of a spatula the resulted solidified extract was transferred into a 1 mL conical vial. After its melting at room temperature, a volume of 5 µL extract was injected into HPLC for further analysis.

Influence of pH

Milli-Q water samples at different pH values (2, 3, 4, 5, 6, 7, 8, 9) were prepared using 0.1 M sodium hydroxide solution for alkaline pH and 1% *ortho*-phosphoric acid for acidic pH. A volume of 10 mL of each prepared sample was spiked with 100 ng of each triazine (1 µL standard mixture). To each spiked sample 0.5 g sodium chloride were added and then the triazines were extracted according to the DLLME-SFO protocol described above.

Solution Ionic strength

Five sodium chloride solutions in concentrations of 5, 10, 15, 20 and 25% respectively were prepared. Each NaCl solution was alkalinized at pH 8 with 0.1 M sodium hydroxide solution and then spiked with 100 ng of each triazine. Further the DLLME-SFO method described above was used to extract the studied triazines.

USAE method

Three grams of soil sample were introduced in a 15 mL screw cap vial with conical bottom and spiked with 300 ng of each triazine (3 µL standard mixture). A volume of 3 mL of organic solvent was added into the vial, vigorously shaken for 15 min and subjected to ultrasonication for 15 min and then to centrifugation for 5 min at 4500 rpm. The extraction efficiency of three organic solvents (acetone, methanol and acetonitrile) was studied. Subsequently, 1 mL of clear triazine organic solvent extract was added to an another vial of 15 mL which contains 10 mL of water alkalinized at pH 8 with 0.1 M sodium hydroxide solution and 2 grams NaCl. The studied triazines were extracted by DLLME-SFO presented above.

Soil samples preparation

The soil samples were collected from an agricultural area of Transylvania, Roumania, where triazine herbicides were used for plant treatment. After the soil samples were dried in air at room temperature, they were crushed and passed through a 250 µm sieve.

Three grams of the fine ground soil sample were weighed and introduced in a 15 mL screw cap vial with conical bottom and extracted with 3 mL of methanol. The sample was further processed according to the above described USAE and DLLME-SFO methods.

The quantification of the target compounds in real samples was made by means of the calibration curve. For this purpose five soil samples were spiked with 50, 100, 200, 300 and 400 ng of each triazine and then extracted under the procedure described above and analyzed by HPLC-UV.

CONCLUSIONS

In the present paper, a miniaturized extraction procedure USAE-DLLME-SFO-HPLC-UV for the analysis of seven triazine herbicides in soil samples was proposed. The developed procedure has good accuracy over 95%, except simazine with 60%; good repeatability (RSD ranged between 5.23 and 10.42%), linearity (R^2>0.991), low LODs (0.19-0.68 µg/kg) and LOQs (0.57-2.06 µg/kg), and good EF values (176-247).

The procedure developed for the extraction and analysis of triazines from soil samples offers some advantages like rapidity and simplicity, low organic solvent consumption, and easy operation.

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