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Quantification of triazine herbicides in soil by microwave-assisted extraction and high-performance liquid chromatography

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Abstract A method for the determination of herbicides residues, triazine (atrazine, metribuzin, ametryn, and terbutryn), in soil samples with high-performance liquid chromatography (HPLC)–UV detection is described. The proposed method is based on microwave-assisted extraction (MAE) of soil samples for 4 min at 80% of 850-W magnetron outputs in the presence of mixture of solvents (methanol/acetonitrile/ethylacetate). Related important factors influencing the MAE efficiency, such as the solvent type and volume, irradiation energy, and time, were optimized in detail. Calibration curve ranges established using HPLC for metribuzin, atrazine, ametryn, and terbutryn are 1.0–19.0, 0.9–18.0, 0.6–11.0, and 0.7–11.0 μg mL^{-1}, respectively. The limits of detection of metribuzin, atrazine, ametryn, and terbutryn are 0.30, 0.24, 0.16, and 0.20 μg mL^{-1} while limits of quantification are 1.0, 0.80, 0.50, and 0.60 μg mL^{-1}, respectively. A Plackett–Burman factorial design was used as a screening method in order to select the variables that influence MAE extraction. The recoveries of the method at three different spiked levels were assessed by analyzing real soil samples and were found to be in the range of 83.33 ± 0.12–96.33 ± 0.23 with good precision (<8%).

Keywords Ametryn · Atrazine · HPLC · Metribuzin · Microwave-assisted extraction · Terbutryn

Introduction

Determination of multiresidues of herbicides in soil is very important because it involves public health and environmental monitoring. The triazine creates pollution of soil, besides the environment of ecosystem. Triazines are important kinds of herbicides applied widely to soils for the control of weed in modern agriculture system. Triazines are applied as pre- and post-emergent weed control agents to improve crop yields. The half-lives of these herbicides vary from weeks to several months. The most important physicochemical properties of these herbicides are their solubility in water and the capacity to be retained by the organic matter of the soil (Frías et al. 2004).

Most of the reported methods for triazine determination include separation by solid phase extraction–liquid chromatography–UV (SPE–LC–UV; Beilstein et al. 1981; Lintelmann et al. 1993; Gennaro and Giacosa 1996; Pinto and Jardim 2000; Dopico et al. 2003; Zhou et al. 2006; Katsumata et al. 2006), LC–

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electrospray ionization tandem mass spectrometry (Pozzebon et al. 2003; Bichon et al. 2006), gas chromatography (GC; Beltran et al. 1997; Rodríguez-Plasencia et al. 1997; Dagnac et al. 2005), GC after liquid–liquid extraction (Nagaraju and Huang 2007) and GC with SPE (Shen and Lee 2003; Rocha et al. 2008), capillary electrophoresis (Carabias-Martínez et al. 2000; Khrolenko et al. 2002), immunoaffinity chromatography (Carrasco et al. 2001), and molecularly imprinted polymer (Sambe et al. 2007).

Recent studies have shown the repeated use of MAE for the extraction of triazine group of herbicides from various types of samples (Molins et al. 1996; Hoogerbrugge et al. 1997; Vryzas and Papadopoulou-Mourkidou 2002; Cheng et al. 2007; You et al. 2007). In comparison to other extraction procedures, MAE has the benefit of enhanced extraction in less times, low solvent consumption, and high extraction efficiencies (Afridi et al. 2006; Kolachi et al. 2010).

The aim of this investigation was to find out suitable high-performance liquid chromatography (HPLC) procedure for quantification and identification of selected triazine herbicides in the soil sample using microwave-assisted extraction with appropriate extraction solvent and cleanup procedure.

Experimental

Apparatus

An Acme 9000 Series HPLC equipped with SP930 isocratic pump, UV730D detector Young Lin (Korea) was used. All separations were carried out on HiQ SiL (C_{18} column HS 4.6 mm Ø × 150 mm KYATECH, Japan).

A KEN ST/SS25 KENWOOD (China) microwave was used for closed-vessel microwave-assisted extraction of triazines from soil samples. A ks300 KUM SUNG ultrasonic (Korea) sonicator was used as degasser. Centrifugation was carried out on a CL international clinical centrifuge (USA).

Reagents

All chemicals used were of analytical reagent grade or similar. Methanol was purchased from Merck (Darmstadt, Germany), ethylacetate from BDH (Poole, England), and acetonitrile from Scharlau Chemie (Italy). Atrazine, metribuzin, ametryn, and terbutryn standards were purchased from Dr Ehrenstorfer GmbH (Augsburg, Germany). Stock solutions (1,000 $\mu g\ mL^{-1}$) of all triazine herbicides were prepared by dissolving 0.01 g of standard in 10 mL methanol. Working standards of 100 and 10 $\mu g\ mL^{-1}$ solution were prepared by dilution of the stock solution with methanol.

Conditions of HPLC

A reverse-phase HPLC with HiQ SiL C_{18} column was used. Sample injection volume was 20 μL and the column was maintained at room temperature of about 25°C. The mobile phase used was methanol and water (70:30, *v*/*v*) and flow rate was kept at 1.0 $mL\ min^{-1}$. The absorbance was measured at 254 nm.

Sample preparation for analysis

Soil sample (20 g) was air-dried and homogenized using pestle and mortar. The homogenized sample was passed through a steel mesh of size 355 μm. There were no detectable levels of target analyte except atrazine in the soil before spiking. The soil sample (3 g) was spiked with different fortified level (2.0, 4.0, and 6.0 $\mu g\ g^{-1}$) of herbicides (atrazine, metribuzin, ametryn, and terbutryn). All these spiked samples were kept in dark for 24 h at room temperature.

Extraction procedure

A fortified soil samples (3 g) were transferred in a closed extraction vessel containing 50 mL of acetonitrile/ethylacetate/methanol (3:2:5) and mechanically shaken vigorously. Extraction in microwave was performed for 4 min at 80% of 850-W magnetron outputs of microwave. After extraction, the vessel was removed from the microwave and allowed to cool down to room temperature for about 10 min. The extract was filtered and the residue was washed three times with 3 mL of methanol. To reduce the volume of the extract, it was allowed to evaporate at room temperature

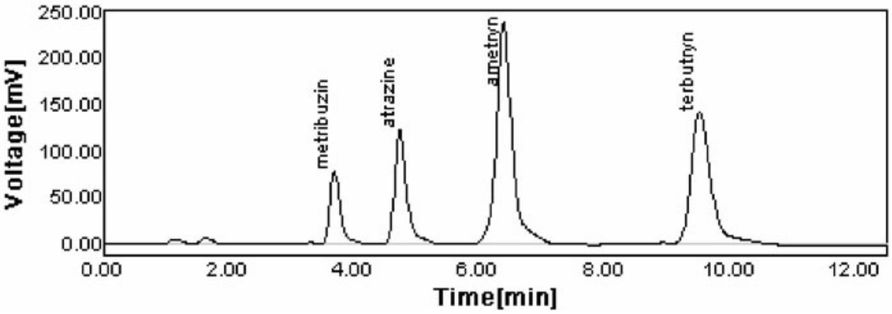

Fig. 1 HPLC separation of metribuzin, atrazine, ametryn, and terbutryn

for an hour followed by centrifugation (1,790×*g*) for 15 min and used for HPLC analysis.

Experimental design for screening of MAE

In order to find the most significant variables for MAE, Plackett–Burman design was built resulting in 12 experiments. Experimental design calculations were performed by using the software Stat-Ease Design-Expert trial Version 7.1.

Results and discussion

Optimization of HPLC conditions

A mobile phase flow rate in the range of 0.7–1.0 mL min^{-1} was evaluated using methanol/water in the ratio 60:40 and 70:30 for the separation of four triazine herbicides. There was no considerable difference in the resolution of the atrazine and ametryn peak in chromatograms obtained for all conditions mentioned above. A complete resolution of triazines mixture was achieved using 1.0 mL min^{-1} flow rate and mobile phase ratio of 70:30 in acceptable time of 11 min (Fig. 1). The retention times for metribuzin, atrazine, ametryn, and terbutryn are 3.68, 4.73, 6.38, and 9.50 min, respectively.

Screening phase

An experimental Plackett–Burman design was built resulting in 12 experiments for the determination of the main factors affecting the MAE. The analyzed factors influencing the extraction efficiency were solvent volume, irradiation energy, and equilibration time. These factors were evaluated at two levels each with a complete repetition performed in one run (Table 1). In each case, the percent recovery of metribuzin, atrazine, ametryn, and terbutryn was evaluated.

Using the data in Table 1, the effects of the main factors and their interactions with each other

Table 1 Plackett–Burman design built for factor selection

Standard run	A (% of 850 W)	B (min)	C (mL)	% Recovery			
				Metribuzin	Atrazine	Ametryn	Terbutryn
1	50	3.00	20	43	44	57	45
2	10	3.00	30	17	18	25	20
3	50	1.00	30	40	37	42	42
4	10	3.00	20	15	16	23	18
5	10	1.00	30	12	13	20	15
6	10	1.00	20	11	12	18	13
7	50	1.00	20	39	36	41	41
8	50	3.00	20	43	44	57	45
9	50	3.00	30	60	59	65	62
10	10	3.00	30	17	18	25	20
11	50	1.00	30	40	37	42	42
12	10	1.00	20	11	12	18	13

A irradiation energy, *B* time, *C* solvent volume

Table 2 Effect list of main factors and their interactions

Require in model or error	Term	% Contribution			
		Metribuzin	Atrazine	Ametryn	Terbutryn
Model	A	86.60	82.58	80.02	85.80
Model	B	5.84	9.17	13.56	6.45
Model	C	2.36	2.17	0.95	2.70
Model	AB	1.20	2.66	4.75	1.09
Model	AC	1.20	1.01	0.14	1.09
Model	BC	1.55	1.35	0.27	1.42
Model	ABC	1.20	1.01	0.27	1.42

A irradiation energy, *B* time, *C* solvent volume

were estimated for Plackett–Burman design. The results are given in Table 2. Among the three screened factors, all showed a positive effect but the most pronounced effect on the percent recovery of all four herbicide was found to be microwave irradiation energy (A) and equilibration time (B). Irradiation energy shows higher percent contribution for MAE extraction of metribuzin (86.60%) than for atrazine (82.58%), ametryn (80.02%), and terbutryn (85.80%). Time has prime impact on ametryn recovery (13.56%) that is more than its effect on all others. AB (4.75) is significant for ametryn and AC (1.20) and BC (1.55) for metribuzin among all possible combinations of interaction.

Optimization of MAE conditions

The effect of various experimental parameters of microwave-assisted extraction step was studied. Parameters, such as solvent type and volume, extraction time, and irradiation energy, were optimized.

Selection of solvent

Organic solvents such as methanol, acetonitrile, and ethylacetate were first studied for extraction of each triazine herbicide separately at 80% of 850-W output of microwave for 4 min using 30 mL of solvent in triplicate at three different concentrations of triazine herbicides in control

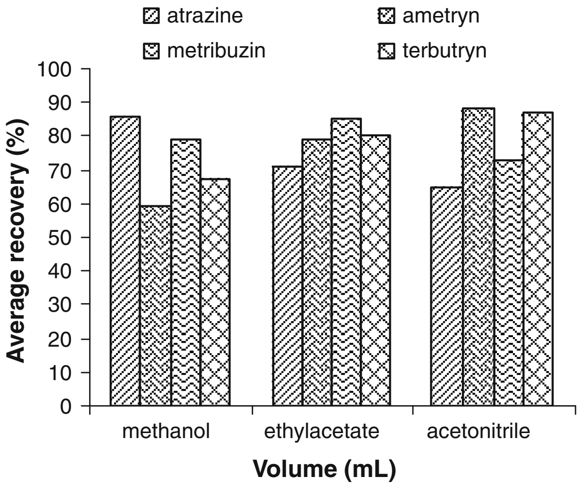

Fig. 2 The effect of solvent type on recoveries of triazines ($n = 3$). Irradiation energy = 80% of 850 W; solvent volume = 30 mL; irradiation time = 4 min

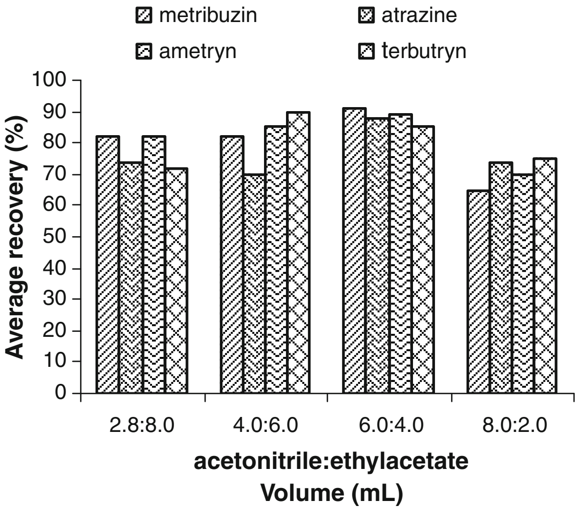

Fig. 3 Effect of solvent volume ratio on extraction average recoveries (percent) of triazines with acetonitrile/ethylacetate (v/v; $n = 3$). Irradiation energy = 80% of 850 W; solvent volume = 30 mL; irradiation time = 4 min

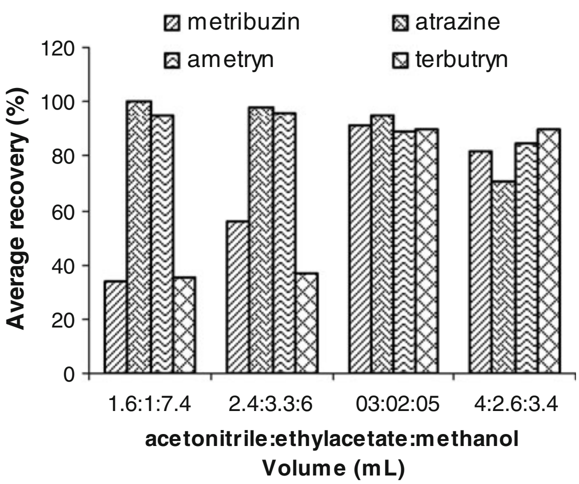

Fig. 4 Effect of solvent volume ratio on extraction average percent recoveries of triazines. With acetonitrile/ethylacetate/methanol (*v*/*v*; *n* = 3). Irradiation energy = 80% of 850 W; solvent volume = 30 mL; irradiation time = 4 min

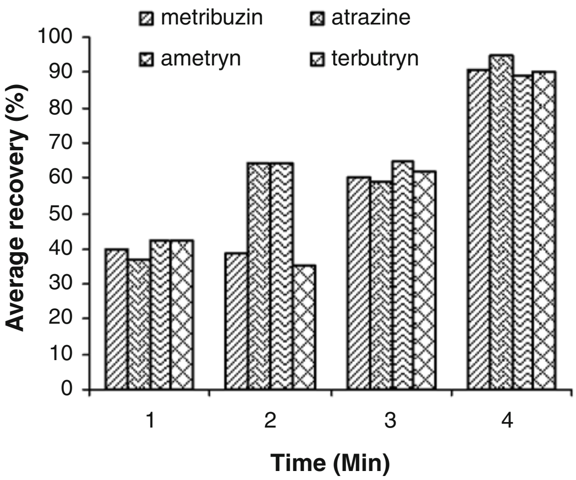

Fig. 6 The effect of extraction time on recoveries of triazines (*n* = 3). Extracting solvent = acetonitrile/ethylacetate/methanol (3:2:5); irradiation energy = 80% of 850 W; solvent volume = 30 mL

samples, and the average recoveries found are given in Fig. 2. Atrazine was found to be well extracted in methanol with average recoveries of 86%. Similarly, it was observed that metribuzin is 85% recovered in ethylacetate as compared to 79% and 59% average recovery in methanol and acetonitrile, respectively. Acetonitrile was found to be more effective as an extraction solvent for ametryn and terbutryn with average percent recovery of 88 and 87, respectively.

MAE for the mixture of the four triazines from soil samples was performed using acetonitrile: ethylacetate (Fig. 3), and acetonitrile/ethylacetate/methanol (Fig. 4). With 6:4

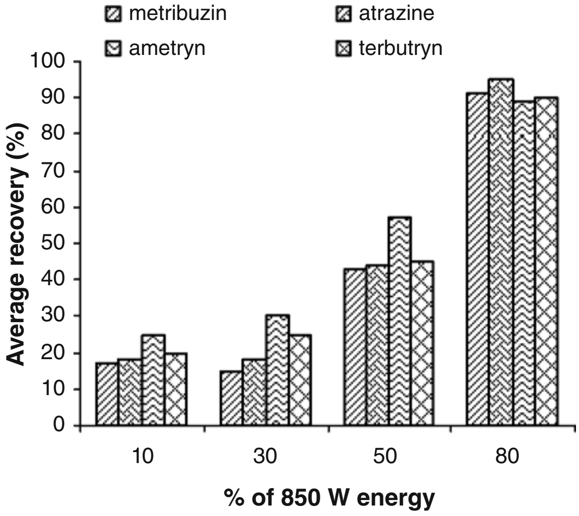

Fig. 5 The effect of irradiation temperature on recoveries of triazines (*n* = 3). Extracting solvent = acetonitrile/ethylacetate/methanol (3:2:5); solvent volume = 30 mL; irradiation time = 4 min

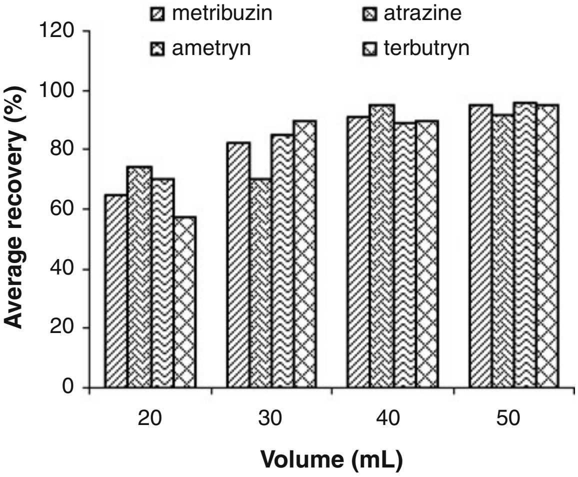

Fig. 7 The effect of extractant volume on recoveries of triazines (*n* = 3). Extracting solvent = acetonitrile/ethylacetate/methanol (3:2:5); irradiation energy = 80% of 850 W; irradiation time = 4 min

Table 3 Optical characteristics for HPLC–UV determination of four triazines

Analysis	Characteristics			
	Metribuzin	Atrazine	Ametryn	Terbutryn
Calibration curve range (μg mL^{-1})	1.0–19.0	0.9–18.0	0.6–11.0	0.7–11.0
Limit of detection (μg mL^{-1})	0.30	0.24	0.16	0.20
Limit of quantification (μg mL^{-1})	1.00	0.82	0.54	0.60
Standard deviation	0.10	0.08	0.06	0.05
Relative standard deviation	6.32	6.43	7.60	6.90
Regression equation	$1.40X + 0.00$	$1.135X + 0.00$	$0.185X + 0.00$	$1.183X + 0.00$
Correlation coefficient (r)	0.98289	0.98598	0.96454	0.98709

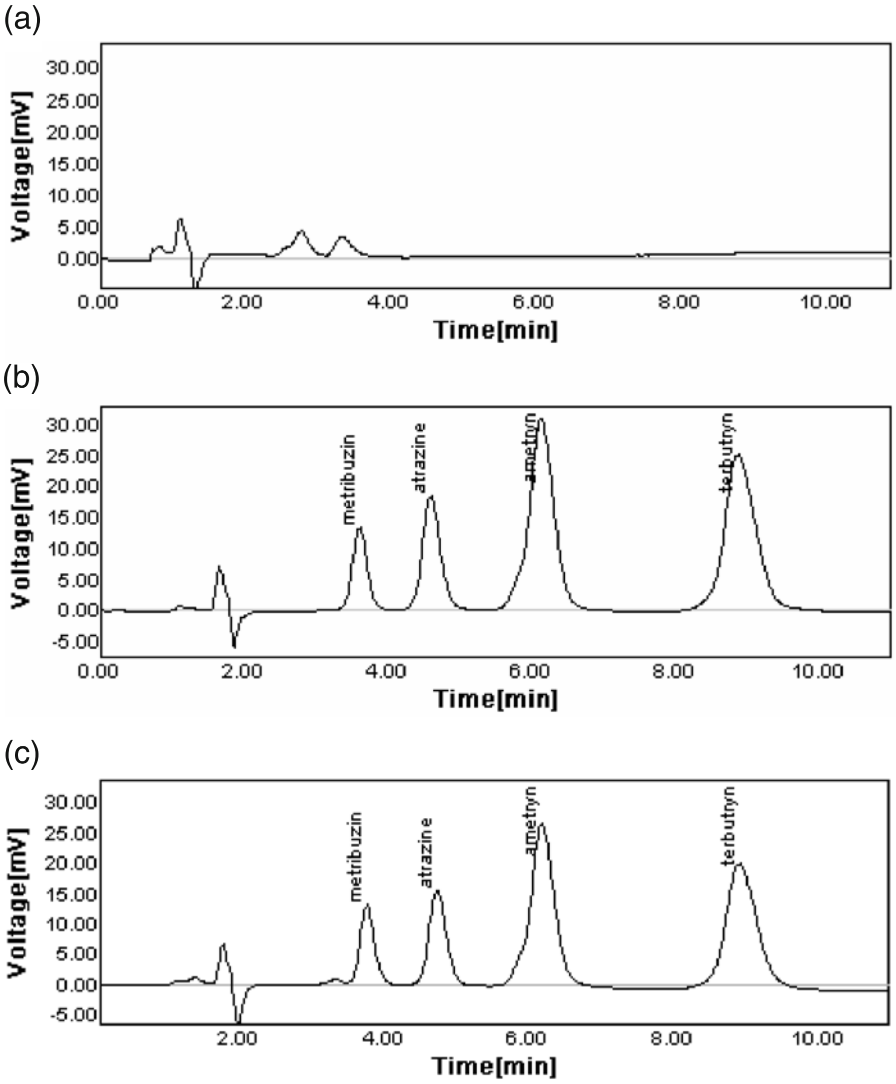

Fig. 8 Chromatograms of the extracts from soil obtained using MAE: **a** blank, **b** standard solution (18.00 μg mL^{-1}), and **c** spiked at fortified level (6.00 μg g^{-1})

combination of acetonitrile/ethylacetate, average recovery of metribuzin has improved significantly by 6%, where as that of atrazine, ametryn, and terbutryn remained unchanged. The recoveries of all four triazine herbicides were at their highest (metribuzin, atrazine, ametryn, and terbutryn was 91%, 95%, 89%, and 90%, respectively) level when methanol was introduced as an extraction solvent along with acetonitrile and ethylacetate shown in Fig. 4.

Effect of irradiation energy on extraction

To find out the effect of irradiation energy on the extraction efficiency, extractions were carried out at 10%, 30%, 50%, and 80% of 850-W output of microwave, respectively. The extraction recoveries are shown in Fig. 5. The recoveries increased clearly as the irradiation energy for extraction increased up to 80% of 850 W. Metribuzin, atrazine, ametryn, and terbutryn showed significant increase in percent recoveries (from 43%, 44%, 57%, and 45% to 91%, 95%, 89%, and 90%, respectively) when the irradiation energy was changed from 50% to 80% of 850 W.

Effect of time on extraction

Extraction time optimizations were carried out at 80% of 850 W for 1, 2, 3, and 4 min using acetonitrile/ethylacetate/methanol as the extraction solvent. Under the condition of changing the extraction time, the results of the MAE recoveries of four triazines from soil samples are shown in Fig. 6. When the extraction times were increased from 1 to 4 min, the recoveries of all four triazine herbicides increased.

Effect of extractant solvent volume

Four different volumes of extractant solvent, 20, 30, 40, and 50 mL, were studied, respectively (Fig. 7). The recoveries of metribuzin, atrazine, ametryn, and terbutryn increased when the solvent volume was increased from 20 to 30 mL and then 40 mL. Small significant increase in recoveries was observed when the extractant volume was increased up to 50 mL, except for atrazine where decrease in the recovery was observed. Thus, the optimum volume of extractant solvent was used as 50 mL.

Analytical characteristics

The statistical parameters calculated from the calibration graph are given in the Table 3. The linearity of calibration graphs was proved by the high value of correlation coefficient (*r*). The limits of detection and the limits of quantification were calculated using a signal-to-noise ratio of 3 and 10 times, respectively.

Accuracy and precision

The soil sample collected from corn field was analyzed. There were no detectable levels of the target analytes except for atrazine in the soil before spiking. Figure 8 shows the chromatograms of

Table 4 Percent recovery of four triazines from soil sample ($n = 3$)

Analyte	Concentration added (μg mL $^{-1}$)	Concentration found (μg mL^{-1})	% Recovery
Metribuzin	6.00	5.78	96.33 ± 0.23
	12.0	10.7	89.93 ± 0.10
	18.0	17.2	95.55 ± 0.08
Atrazine	6.00	5.54	92.33 ± 0.08
	12.0	10.1	84.25 ± 0.13
	18.0	16.0	88.88 ± 0.09
Ametryn	6.00	5.65	94.16 ± 0.16
	12.0	10.6	88.83 ± 0.11
	18.0	16.3	90.01 ± 0.12
Terbutryn	6.00	6.37	106.1 ± 0.25
	12.0	11.3	94.16 ± 0.08
	18.0	15.0	83.33 ± 0.12

triazines: (a) soil blank sample, (b) 18.00 μg mL^{-1} standard solution, and (c) 6.00 μg g^{-1} spiked soil sample. Recoveries of 83–96% for spiked samples indicated that the present method provides good recoveries and reasonable precision for triazines (Table 4).

Conclusion

MAE with extraction solvent acetonitrile/ethylacetate/methanol (3:2:5) followed by HPLC separation and UV detection has been used for recovery of atrazine, ametryn, metribuzin, and terbutryn from soil sample. MAE followed by HPLC separation provided high recoveries (83.33 ± 0.12–96.33 ± 0.23), minimum matrix effects, and good separation. The results obtained for recovery, precision, and accuracy show that the proposed MAE followed by HPLC separation and UV detection is an efficient and simple method for identification, determination, and quantification of these triazines in soil samples.

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