

# Determination of Triazine Herbicides in Environmental Samples by Dispersive Liquid–Liquid Microextraction Coupled with High Performance Liquid Chromatography

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## Abstract

A simple, rapid, efficient, and environmentally friendly method for the determination of five triazine herbicides in water and soil samples was developed by using dispersive liquid–liquid microextraction (DLLME), coupled with high performance liquid chromatography–diode array detection (HPLC–DAD). The water samples were directly used for DLLME extraction. For soil samples, the target analytes were first extracted by water–methanol (99:1, v/v). In the DLLME extraction method, chloroform was used as an extraction solvent, and acetonitrile as a dispersive solvent. Under the optimum conditions, the enrichment factors of DLLME were in the range between 183–221. The linearity of the method was obtained in the range of 0.5–200 ng/mL for the water sample analysis, and 1–200 ng/g for the soil samples, respectively. The correlation coefficients ranged from 0.9968 to 0.9999. The limits of detection were 0.05–0.1 ng/mL for the water samples, and 0.1–0.2 ng/g for the soil samples. The proposed method has been successfully applied to the analysis of target triazine herbicides (simazin, atrazine, prometon, ametryn, and prometryn) in water and soil samples with satisfactory results.

## Introduction

Triazine herbicides are extensively used as selective herbicides for the control of broadleaf and grassy weeds in many agricultural crops over the past years. They are considered one of the most important classes of chemical pollutants due to their toxicity and high resistance. Moreover, atrazine has been classified as a human carcinogen (1,2). Consequently, for the sake of human health and environmental pollution control, sensitive and selective analytical methods are desirable for the determination of triazine herbicide residues in different sample matrices.

Different analytical methods, such as gas chromatography (GC) (3,4), gas chromatography–mass spectrometry (GC–MS) (5,6), high-performance liquid chromatography (HPLC) (7,8), and capillary electrophoresis (9), have been developed for the

separation and quantification of triazines residues. To achieve the necessary levels of sensitivity, an enrichment step is usually needed before the chromatographic analysis. For the determination of triazines residues, several sample preparation methods available have been developed, including liquid–liquid extraction (LLE) (10), solid-phase extraction (11), solid-phase microextraction (8,12), hollow fiber-based liquid-phase microextraction (HF–LPME) (13), microwave-assisted solvent extraction (MASE) (14), stir bar sorptive extraction (15), single-drop microextraction (5,16), supercritical fluid extraction (17), molecularly imprinted SPE (18,19), and ultrasound-assisted emulsification microextraction (20).

Recently, a novel microextraction technique, named the dispersive liquid–liquid microextraction (DLLME), based on the dispersion of tiny droplets of the extraction solvent within the aqueous solution has been developed by Assadi and coworkers (21). DLLME is a miniaturized LLE that uses microliter volumes of the extraction solvent. For DLLME, a water-immiscible extraction solvent dissolved in a water-miscible dispersive solvent was rapidly injected into an aqueous solution by a syringe. A cloudy solution containing fine droplets of the extraction solvent dispersed entirely into the aqueous phase was formed. The analytes in the sample were extracted into the fine droplets, which were further separated by centrifugation, and the enriched analytes in the sedimented phase were determined by either chromatographic or spectrometric methods. The advantages of the DLLME method are rapidity, low cost, simplicity of operation, and a high enrichment factor. DLLME has been applied for the analysis of a variety of trace organic pollutants and metal ions in environmental samples (22–27). Up to now, however, the reported applications of DLLME have been mainly focused on simple water samples. Therefore, the exploration of the potential applications of the DLLME technique in more complex matrix samples, such as soil and food, is very desirable.

In continuation to previous endeavors in LPME (20,28–31), a DLLME method coupled with HPLC–diode array detection (DAD) has been developed for the determination of triazine herbicides in water and soil samples, and described herein. Several parameters that could affect the extraction, including the kind and volume of the extraction solvent and dispersive solvent, pH of sample solution and salt addition, were studied and optimized.

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## Experimental

### Reagents and materials

All solvents (HPLC-grade) were supplied from the Beijing Chemical Reagents Co. (Beijing, China). Pesticide standards of triazine herbicides (simazine, atrazine, prometon, ametryn, and prometryn) were purchased from the Agricultural Environmental Protection Institution of Tianjin (Tianjin, China). Chloroform (CHCl<sub>3</sub>), tetrachloride ethylene (C<sub>2</sub>Cl<sub>4</sub>), carbon tetrachloride (CCl<sub>4</sub>), and chlorobenzene were obtained from the Beijing Chemical Reagents Co. Methanol was from Sinopharm Chemical Reagent Co. (Beijing, China). Sodium chloride (NaCl) was from the Tianjin Fuchen Chemical Reagent Factory (Tianjin, China). The water used throughout the work was double-distilled on a SZ-93 automatic double-distiller purchased from Shanghai Yarong Biochemistry Instrumental Factory (Shanghai, China).

River water samples were collected from Baoding (Baoding, China), reservoir water from Wangkuai reservoir (Baoding, China), well water from Xixiaozhuang (Baoding, China). Soil samples were collected from the plough layer of the mealie field at Xixiaozhuang and Beixinzhuang (Baoding, China), which were dried at room temperature, pulverized and passed through 250- $\mu$ m sieve.

A mixture stock solution containing simazine, atrazine, prometon, ametryn, and prometryn at 10.0  $\mu$ g/mL was prepared in methanol. A series of standard solutions were prepared by mixing an appropriate amount of the stock solution with double-distilled water in a 10-mL volumetric flask. All the standard solutions were stored at 4°C protected from light.

### Instruments

The HPLC system, assembled from modular components, consisted of an in-line degasser, a 600E pump, and a DAD detector. A Millennium<sup>32</sup> workstation was utilized to control the system and for the acquisition and analysis of the data. A Centurysil C<sub>18</sub> column (4.6 i.d.  $\times$  250 mm, 5.0  $\mu$ m) from Dalian Jiangshen Separation Science Company (Dalian, China) was used for separations. The mobile phase was a mixture of methanol–water (75:25, v/v) at a flow rate of 1 mL/min. DAD monitoring wavelengths were chosen at 222 nm for the five triazine herbicides.

The pH of the solution was measured with a PHS-3C digital pH meter (Hangzhou Dongxing Instrument Factory, Hangzhou, Zhejiang, China).

Extractions of the target triazine herbicides from soil samples were performed at 59 kHz of ultrasound frequency and 200 W of power on a KQ-2200DE ultrasonic water bath purchased from Kunshan Ultrasonic Instruments Co. Ltd. (Kunshan, China).

### Sample preparation before DLLME

Water samples were directly extracted by a DLLME procedure. The extraction of the target triazine herbicides from soil samples prior to DLLME was carried out according to the following procedures: soil samples were air-dried at room temperature, pulverized and passed through a 250- $\mu$ m sieve. 20.0 g of the soil sample was accurately weighed and put into a 100 mL centrifuge tube, to which 20.0 mL water containing 1% methanol was

added. The resultant sample mixture was first immersed into an ultrasonic bath at 25  $\pm$  2°C for 30 min of sonication, then filtrated under reduced pressure. A 5.0 mL aliquot of the above sample solution was extracted by the DLLME method.

### DLLME procedures

For the DLLME, a 5.00 mL aliquot of the sample solution was placed in a 10 mL screw cap glass tube with conic bottom and 0.5 g NaCl was added into the solution. A mixture of 1.0 mL of acetonitrile (as disperser solvent) and 100  $\mu$ L of CHCl<sub>3</sub> (as an extraction solvent) was injected into the sample solution by a syringe, and then the mixture was vortexed for 10 s. A cloudy solution that consists of very fine droplets of CHCl<sub>3</sub> dispersed into an aqueous sample was formed, and the analytes were extracted into the fine droplets. After centrifugation at 3500 rpm for 5 min, the CHCl<sub>3</sub> phase was sedimented at the bottom of the centrifuge tube. The sedimented phase was completely transferred to another test tube with a conical bottom using 100- $\mu$ L HPLC syringe and blown to dryness with a mild nitrogen stream. The residue was dissolved in 15  $\mu$ L methanol, and 10.0  $\mu$ L was injected into the HPLC system for analysis.

## Results and Discussion

In this experiment, 5.0 mL double-distilled water spiked with 50 ng/mL of each of the target triazine herbicides was used to study the extraction performance under different experimental conditions. All experiments were performed in triplicate, and the means of the results were used for optimization.

In order to obtain the optimum DLLME conditions, the influence of different experimental parameters, including the type and volume of the extraction solvents and dispersive solvent, salt addition, and sample pH were investigated. The enrichment factor (EF) and the extraction recovery (R) were introduced according to the Equations 1 and 2 to evaluate the effect of the previously mentioned factors:

$$EF = C_{inj}/C_0 \quad \text{Eq. 1}$$

where  $EF$ ,  $C_{inj}$ , and  $C_0$  are the enrichment factor, the analyte concentration in the injection solution and the initial analyte concentration in the aqueous samples, respectively.

$$R\% = (V_{rec}C_{inj}/C_0V_{aq}) \times 100 \quad \text{Eq. 2}$$

Where  $R\%$ ,  $V_{rec}$ , and  $V_{aq}$  are the extraction recovery, the volume of the reconstituted solution, and the volume of the aqueous sample, respectively.

### Selection of extraction and dispersive solvent

The selection of an appropriate extraction solvent is critical for the establishment of an efficient DLLME procedure. The extraction solvent has to meet some requirements, such as higher density than water, low water solubility, high extraction capability for the target analytes and form a stable cloudy system in the presence of an dispersive solvent. Based on these criteria, CCl<sub>4</sub>,

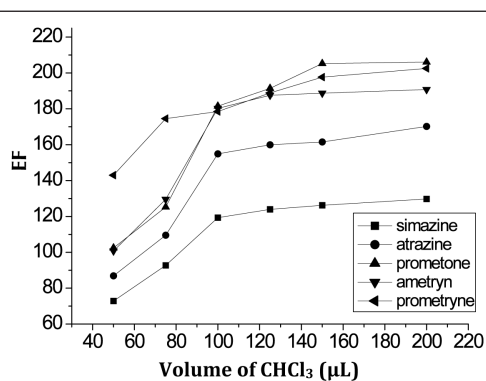
$\text{CHCl}_3$ ,  $\text{C}_2\text{H}_4\text{Cl}_2$ ,  $\text{CH}_2\text{Cl}_2$ ,  $\text{C}_2\text{Cl}_4$ , and  $\text{C}_6\text{H}_5\text{Cl}$  were selected for the study. On the other hand, the disperser solvent should be miscible with both water and the extraction solvent, and could form a cloudy state when injected with the organic extractant into water. The selection of a dispersive solvent is limited to solvents such as acetone, methanol, THF, ethanol, and acetonitrile. All combinations of using  $\text{CCl}_4$ ,  $\text{CHCl}_3$ ,  $\text{C}_2\text{H}_4\text{Cl}_2$ ,  $\text{CH}_2\text{Cl}_2$ ,  $\text{C}_2\text{Cl}_4$ , or  $\text{C}_6\text{H}_5\text{Cl}$  (100  $\mu\text{L}$ ) as an extractant with acetone, acetonitrile, methanol, THF, or ethanol (1.0 mL) as a dispersive solvent, were tried. In the case of  $\text{C}_2\text{H}_4\text{Cl}_2$  and  $\text{CH}_2\text{Cl}_2$  as an extraction solvents, a two-phase system was not observed with any dispersive solvents studied. For  $\text{CHCl}_3$ , a two-phase system was not observed either with methanol or ethanol as a dispersive solvent. Based on the experimental results,  $\text{CHCl}_3$  gave the highest overall extraction efficiency for the target analytes when acetonitrile was used as a disperser solvent. Therefore,  $\text{CHCl}_3$  was selected as the extraction solvent and acetonitrile as the disperser solvent for further study.

### Effect of extraction solvent volume

In order to examine the effect of the extraction solvent volume, water solutions containing different volumes of  $\text{CHCl}_3$  ranging from 50 to 200  $\mu\text{L}$ , were investigated with a constant volume of the dispersive solvent acetonitrile (1.0 mL). According to Figure 1, the extraction recoveries increased when the volume of  $\text{CHCl}_3$  was increased from 50 to 100  $\mu\text{L}$ , and then remained almost constant, or slightly increased when the extraction solvent volume was further increased until 200  $\mu\text{L}$ . Therefore, 100  $\mu\text{L}$   $\text{CHCl}_3$  was selected for further studies.

### Effect of disperser solvent volume

The influence of the volume of the disperser solvent acetonitrile was investigated by changing its volume from 0.5 to 0.75, 1.0, 1.25, and 1.5 mL, respectively. The results are shown in Figure 2. By increasing the volume of acetonitrile, the extraction recovery increased until 100  $\mu\text{L}$ . At higher volumes than 100  $\mu\text{L}$ , the recovery remained almost constant for the target analytes. The reason for this could be that at a low volume of acetonitrile, a cloudy state could not be formed well, therefore resulting in a low recovery. When the volume of acetonitrile reached or exceeded 100  $\mu\text{L}$ , the extraction solvent could be dispersed well in water, and the contact surface between the extraction solvent



**Figure 1.** Effect of the volume of the extraction solvent on the enrichment factor of the triazines. Extraction conditions: extraction solvent,  $\text{CHCl}_3$ ; dispersive solvent, 1.0 mL, acetonitrile; sample volume, 5.0 mL.

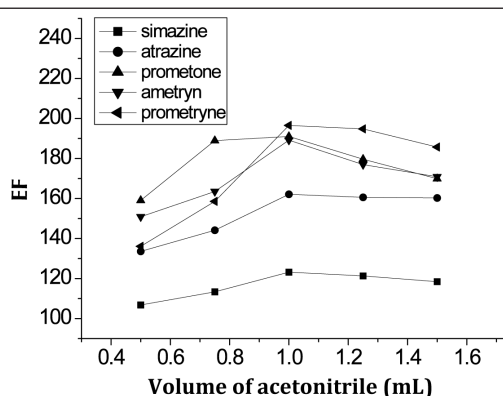
and the aqueous phase was very large, leading to the increased extraction efficiency because of an increase in the distribution coefficient. Based on the experimental results, 1.0 mL of acetonitrile was chosen.

### Effect of sample solution pH

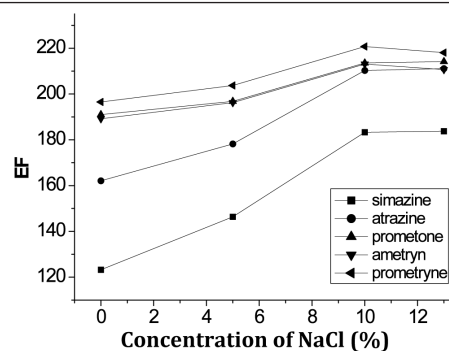
The pH of sample solution is another important parameter that may have an influence on the extraction performance. For basic triazines, to prevent the protonation of the weak bases in the acidic solution, the sample solution should not be rather acidic. In order to keep the analytes deionized, consequently reducing their solubility within the sample solution, the effect of the sample pH in a range of 5.0–12.0 on the extraction of the triazine herbicides was investigated. It was found that the sample pH does not have a significant effect on the extraction efficiency of triazines in the pH range investigated.

### Salt addition

The salting out effect plays an important role in the extraction performance. The effect of salting out was tested by adding different amounts of NaCl [0–15% (w/v)] into water samples, while the other conditions were kept constant. As shown in Figure 3, the extraction efficiency increases with the salt concentration increasing from 0–10%, and reaches a plateau at the salt concentration of 10–13%. However, at the salt concentration of 15%, the extraction solvent phase could not be sedimented at the



**Figure 2.** Effect of the volume of dispersive solvent (acetonitrile) on the enrichment factor of the triazines. Extraction conditions: extraction solvent, 100.0  $\mu\text{L}$   $\text{CHCl}_3$ ; sample volume, 5.0 mL.



**Figure 3.** Effect of salt addition on the enrichment factor of the triazines. Extraction conditions: extraction solvent, 100.0  $\mu\text{L}$   $\text{CHCl}_3$ ; dispersive solvent, 1.0 mL, acetonitrile; sample volume, 5.0 mL.

bottom of the centrifuge tube, but went to the upper layer in the tube. Based on such an observation, 10% (w/v) of NaCl was added in all the subsequent experiments.

Under the above optimized experimental conditions, the enrichment factors of DLLME for simazine, atrazine, prometon, ametryn, and prometryn were 183, 210, 214, 213, and 221, respectively.

## Water samples

### Linearity, repeatability, and limits of detection

To investigate the applicability of the proposed method for the determination of triazine herbicides in water samples, several factors including linear range, enrichment factors, repeatability, and limits of detection (LODs) were evaluated under optimum conditions. A series of working solutions containing each of simazine, atrazine, prometon, ametryn, and prometryn at six concentration levels of 0.5, 1.0, 5.0, 20.0, 50.0, 100.0, and 200.0 ng/mL were obtained for the establishment of the calibration curve. For each level, five replicate extractions were performed.

The characteristic calibration data listed in Table I were obtained under optimized conditions. The linearity was observed in the range from 0.5 to 200 ng/mL for the five target triazine herbicides. The correlation coefficient ( $r$ ) ranged from 0.9990 to 0.9999. The LODs, based on a signal-to-noise ratio (S/N) of 3, was 0.05 ng/mL for prometon and ametryn, and 0.1 ng/mL for the other target triazines (simazine, atrazine, and prometryn). The replicate experiments were carried out by extracting the spiked water samples at the concentration of each triazine at 10.0 ng/mL and the relative standard deviations (RSDs) for five replicate experiments varied from 2.9% to 5.2%. These results show that the proposed method has a high sensitivity and repeatability.

### Water samples analysis

The proposed method was applied to the analysis of the five triazines in the real water samples including well water, river, and reservoir water under the optimized procedure. As a result, no residues of the target triazines were found in well and river water; in reservoir water, however, 1.26 ng/mL simazine was detected. In order to validate the accuracy of this newly established method, these water samples were spiked with the standards of the target analytes at the concentrations of 5.0 and 50.0 ng/mL, respectively. The recoveries of the method were determined by expressing the mean concentrations found as a percentage of the spiked concentrations. For each concentration level, five replicate experiments were made and the results are given in Table II, which indicates that the recoveries for the studied triazines are between 84.2% and 102.0%. Figure 4 shows the typical chromatograms of the extracted triazines from the reservoir water sample before and after being spiked at 5 ng/mL each of the five triazines.

**Table I. Analytical Performance Data for the Triazines by the DLLME Technique**

Fungicides	Water sample				Soil sample			
	LR* (ng/mL)	$r$	LOD (ng/mL)	RSD (%) ( $n = 5$ )	LR* (ng/g)	$r$	LOD (ng/g)	RSD (%) ( $n = 5$ )
Simazine	0.5–200	0.9994	0.1	2.9	1–200	0.9993	0.2	4.0
Atrazine	0.5–200	0.9999	0.1	3.2	1–200	0.9997	0.2	3.5
Prometon	0.5–200	0.9990	0.05	3.6	1–200	0.9994	0.1	2.9
Ametryn	0.5–200	0.9998	0.05	4.5	1–200	0.9968	0.1	3.3
Prometryn	0.5–200	0.9996	0.1	5.2	1–200	0.9992	0.2	5.6

\*LR: linear range.

**Table II. Determination of Triazines Residues and Recoveries in Water Samples**

Triazines	Spiked (ng/mL)	Well water ( $n = 5$ )			River water ( $n = 5$ )			Reservoir water ( $n = 5$ )		
		Measured (ng/mL)	R <sup>†</sup> (%)	RSD (%)	Measured (ng/mL)	R <sup>†</sup> (%)	RSD (%)	Measured (ng/mL)	R <sup>†</sup> (%)	RSD (%)
Simazine	0	N.D.*			N.D.*			1.26		
	5	5.10	102.0	3.0	4.62	92.4	3.5	5.47	84.2	3.3
	50	47.8	95.6	3.4	48.3	96.6	4.6	48.9	95.3	4.5
Atrazine	0	N.D.*			N.D.*			N.D.*		
	5	4.91	98.2	2.8	4.93	98.6	4.3	4.68	93.6	4.2
	50	46.7	93.4	3.9	46.9	93.8	3.5	47.5	95.0	2.8
Prometon	0	N.D.*			N.D.*			N.D.*		
	5	4.77	95.4	4.1	4.27	85.4	5.3	5.10	102.0	4.8
	50	48.9	97.8	3.5	49.1	98.2	3.8	48.3	96.6	5.3
Ametryn	0	N.D.*			N.D.*			N.D.*		
	5	4.30	86.0	4.7	4.48	89.6	4.9	4.36	87.2	5.0
	50	48.4	96.8	4.3	48.4	96.8	5.0	48.9	97.8	3.7
Prometryn	0	N.D.*			N.D.*			N.D.*		
	5	4.62	92.4	5.2	4.75	95.0	4.7	4.70	94.0	4.5
	50	48.0	96.0	4.8	48.6	97.2	5.8	43.3	86.6	5.1

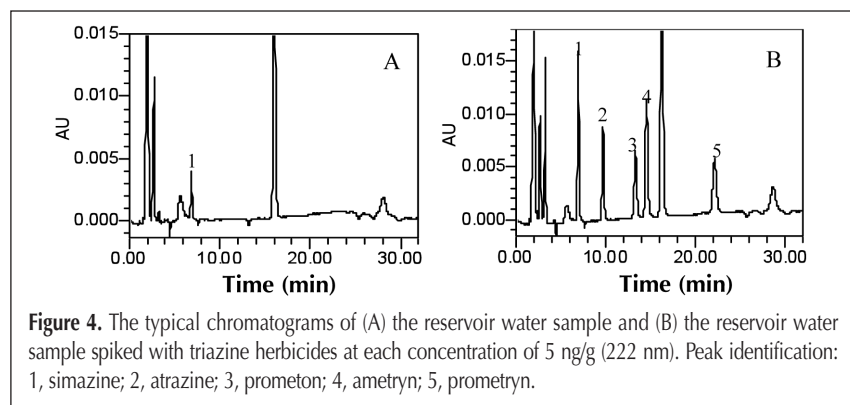
\* N.D. = not detected; <sup>†</sup>R = recovery of the method.

## Soil samples

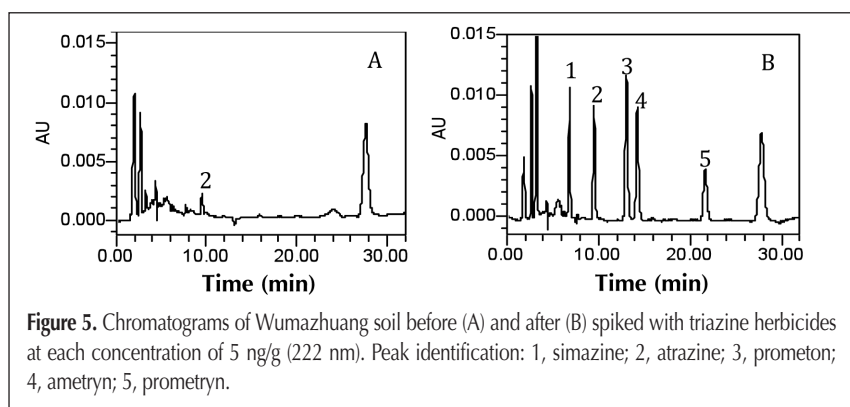
### Linearity, repeatability, and LOD

20.0 g of an air-dried soil sample, which was free of triazine herbicides, was accurately weighed and put into a 100 mL centrifuge tube. An appropriate amount of mixture standard solution of the target analytes was added into it. The mixtures were air-dried at room temperatures. A series of working solutions containing simazine, atrazine, prometon, ametryn,

and prometryn at six concentration levels of 0.5, 1.0, 5.0, 10.0, 50.0, 100, and 200.0 ng/g were obtained for the establishment of the calibration curve. The samples were then prepared and extracted with the DLLME procedures established above as described previously. For each level, five replicate extractions were performed. The characteristic calibration data were listed in Table I. The LOD (S/N = 3) was 0.1–0.2 ng/g for the target triazines. Linearity was observed in the range from 1.0 to 200.0 ng/g with  $r$  ranging from 0.9968 to 0.9997. The repeatability study was carried out by five parallel experiments at the concentration of 10.0 ng/g for each of the triazines under the optimal conditions. The resultant repeatabilities expressed as the RSDs varied from 2.9% to 5.6%. These results show that the proposed method has a high sensitivity and repeatability.



**Figure 4.** The typical chromatograms of (A) the reservoir water sample and (B) the reservoir water sample spiked with triazine herbicides at each concentration of 5 ng/g (222 nm). Peak identification: 1, simazine; 2, atrazine; 3, prometon; 4, ametryn; 5, prometryn.



**Figure 5.** Chromatograms of Wumazhuang soil before (A) and after (B) spiked with triazine herbicides at each concentration of 5 ng/g (222 nm). Peak identification: 1, simazine; 2, atrazine; 3, prometon; 4, ametryn; 5, prometryn.

### Soil samples analysis and recoveries of the method

To evaluate the applicability of the proposed method, the extraction and determination of the five triazines in different soil samples were performed according to the procedures described herein. As a result, no residues of the target triazines were found in the Biaobenyan soil sample. For the Wumazhuang soil sample, atrazine was found to be at 2.3 ng/g.

To test the accuracy of the method, these soil samples were spiked with the standards of the target analytes at the concentrations of 5.0 and 50.0 ng/g, respectively. For each concentration level, five replicate experiments for a whole analysis process were made. The recoveries of the method were expressed as the mean percentage between the amounts found and the ones added. The results are given in Table III. The recoveries for the triazines in soil samples were in the range from 82.0% to 98.0%. Figure 5 shows the typical chromatograms of the extracted triazines from the Wumazhuang soil sample before and after being spiked at 5 ng/g each of the five triazines.

**Table III. Determination of Triazines Residues and Recoveries in Soil Samples**

Triazines	Spiked (ng/g)	Wumazhuang (n = 5)			Biaobenyan (n = 5)		
		Measured (ng/g)	R <sup>†</sup> (%)	RSD (%)	Measured (ng/g)	R <sup>†</sup> (%)	RSD (%)
Simazine	0	N.D.*			N.D.*		
	5	4.58	91.6	4.9	4.74	94.8	5.0
	50	46.0	92.0	4.5	43.6	87.2	4.1
Atrazine	0	2.3			N.D.*		
	5	6.8	90.0	4.8	4.9	98.0	4.6
	50	44.2	83.8	4.3	45.2	90.4	3.8
Prometon	0	N.D.*			N.D.*		
	5	4.2	84.0	5.6	4.4	88.0	4.8
	50	47.4	94.8	3.2	43.2	86.4	4.1
Ametryn	0	N.D.*			N.D.*		
	5	4.6	92.0	5.1	4.5	90.0	4.3
	50	45.4	90.8	4.0	42.5	85.0	5.1
Prometryn	0	N.D.*			N.D.*		
	5	4.1	82.0	5.5	4.26	85.2	5.2
	50	44.6	89.2	3.1	45.2	90.4	4.3

\* ND = not detected; <sup>†</sup>R = recovery of the method.

## Conclusions

A novel, simple, and sensitive method based on DLLME in conjunction with HPLC–DAD detection has been developed for the extraction and determination of triazines in water and soil samples. Compared with other extraction methods, such as SPME and SPE, DLLME can offer advantages of low consumption of organic solvent, rapidity, simplicity, and ease of operation. It has been proven that the proposed method can provide a good repeatability, a high enrichment factor, and good recovery.

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