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Ultrasound Assisted Emulsification Microextraction as a Simple Preconcentration Method for Determination of Atrazine in Environmental Samples

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Abstract

We represent an environmentally friendly sample of pre-treatment method, ultrasound assisted emulsification microextraction (USAEME) followed by gas chromatography nitrogen phosphorous detector (GC-NPD), to specify atrazine residues in environmental samples. Some parameters affecting the extraction efficiency such as the type and volume of the extraction solvent, emulsification time and addition of salt are optimized. According to the results, 30 μL of chlorobenzene was chosen as the extraction solvent and the required time for quantitative analysis was 5 minutes without ionic strength and pH adjustment. Under the optimum conditions, limit of detection (LOD) is 0.02 μgL^{-1} and the percentage of mean extraction efficiency for 3 μgL^{-1} of analyte is 93.3% with good precision about 2.5% for triplet analysis. The calibration curve is linear at the range of 0.1-600 μgL^{-1}. The procedure is applied successfully for assessing a matrix effect on agricultural water samples and lettuce with relative recovery of 100.8-102.5% with precision in the range of 2.5-3.2%. The results have demonstrated a successful robustness of the method for rapid and quantitative determination of trace amounts of atrazine in environmental samples.

***Key words**: Ultrasound assisted emulsification microextraction, Environmental samples, Atrazine, Gas Chromatography.*

Introduction

Atrazine,1-Chloro-3-ethylamino-5-isopropyl-amino-2,4,6-triazine (Figure 1), as a triazine herbicide is in the list of chemical pollutants that need to be more heavily monitored due to the toxicity, persistence, accumulation in the

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environment [1,2]. According to the European Union Directive, the concentration of each pesticide in drinking water must not exceed $0.1\mu gL^{-1}$ for an individual compound and some of its degradation products, and $0.5\mu gL^{-1}$ for the sum of all compounds [3]. Due to the low concentration of herbicides in water samples, a suitable enrichment procedure should be performed prior to instrumental analysis for sufficient selectivity and sensitivity.

Chromatographic techniques are the commonly used methods for determination of triazine herbicides such as high performance liquid chromatography and gas chromatography [4-12]. Solid phase extraction is the most widely method used for preconcenration of herbicides in environmental water samples [13-16]. Single drop microextraction (SDME) and solid phase microextraction (SPME) are sample preparation methods reported for determination of atrazine too [17, 18].

In 2006, Asadi and co-workers have developed an interesting mode of microextraction method named dispersive liquid-liquid microextraction (DLLME), which showed many advantages such as rapidity, low cost, simplicity and high enrichment factor for determination of wide range of compounds [19-24]. It is based on a ternary component solvent system such as homogeneous liquid extraction and cloud point extraction [25, 26]. In this microextraction mode, the extraction solvent is dispersed in sample solution by the assistance of a water miscible organic solvent. Ultrasound assisted emulsification microextraction (UAEME) was used for the first time by Garcia-Jares and co-workers for the extraction of synthetic musk fragrances, phthalate esters and lindane in environmental waters [27]. In this microextarction procedure, a microvolume of water immiscible acceptor phase is emulsified in sample solution by the assistance of ultrasound energy.

By formation of tiny droplets of an organic solvent, the target analytes are extracted to the extraction solvent and after centrifuging, the sediment phase is determined by analysis methods. Hence there is no necessity to use a polar solvent such as methanol or acetonitrile to disperse the extraction solvent into the sample solution which is the most important defect in DLLME. Using a disperser solvent decreases the partition coefficient of analyte between the sample solution and extraction solvent which may lead to lower extraction efficiency. The approach of ultrasonic radiation facilitates the emulsification and mass transfer phenomenon between two immiscible phases.

The combination of microextraction and ultrasound radiation causes the enlargement of the contact surface between two phases for determination of analytes at trace levels with increment in extraction efficiency. In this method the same as SPME and SDME, preconcentration and extraction are performed in one step prior to analysis. Minimizing

the extraction time and the volume of organic solvent are the best advantages of this microextraction method. USAEME has been also used for the determination of polybrominated diphenyl ethers, polycyclic aromatic hydrocarbons, phenolic preservatives, polychlorinated biphenyls organochlorine, and organophosphorous pesticides in water samples [28-32]. The purpose of the present study is to applying an environmental friendly technique (USAEME) for preconcentration and extraction of atrazine from environmental water samples and further determination by GC-NPD. The results revealed that the microextraction process is progressive successfully in a short time with high efficiency and precision for determination of atrazine.

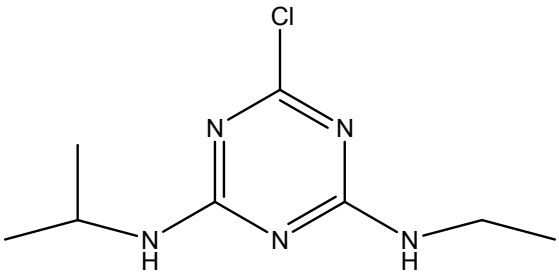

Figure 1. Chemical structure of the atrazine.

Experimental

Reagents and materials

All chemicals used in this research were of analytical-reagent grade. Chlorobenzene ($C6H_5Cl$), chloroform ($CHCl_3$), carbon tetrachloride (CCl_4), tetra chloride ethylene (C_2Cl_4), sodium hydroxide (NaOH) and sodium chloride (NaCl) were obtained from Merk chemical company (Darmstadt, Germany). A stock standard solution containing 1000 mgL-1 of atrazine was prepared in methanol and stored in the dark at 5ºC. Other working solutions with lower concentrations were prepared daily prior to analysis. In order to develop the described method, water samples were collected from agricultural fields in north of Iran-Babol and stored in dark at 4ºC. In addition, lettuce samples were selected as real ones to evaluate the compatibility of the method. An amount of 5 gr of lettuce sample was digested with 5mL of 14 mol L^{-1} HNO_3 in a covered beaker to near dryness. In order to ensure a complete digestion, 2mL of 0.5 mol L^{-1} HCl was added, too. After cooling, the digested solution was diluted to 50 mL with deionized water [33]. 6 mL of the obtained clear solution was used for real sample analysis as procedure.

Equipment

A gas chromatograph (Agilent technologies, CA, USA), was used to determine the

atrazine after preconcentration by ultrasound emulsification microextraction. The GC was equipped with a HP-5, (5% phenyl, methyl polysiloxane), fused silica capillary column (50 m length, 0.32 mm i.d and 0.25μm film thickness) and split/splitless injection system. Ultra pure helium (99.9999%, Air products, UK) passes through a molecular sieve trap and oxygen trap (Chromatography Research Supplies, USA) is used as the carrier gas at constant linear velocity of 5 mL/min.

The injection port was held at 250 ◦C and used in the splitless mode with a splitless time of 0.5 min. For decreasing the products degradation, deactivated glass liner was used. The oven temperature was programmed as follows: there was an initial column temperature of 100 ºC held for 1 minute then it was raised to 250 ºC at a rate of 10 ºC min^{-1} and held for 2 min. The NPD temperature was maintained at 300 ºC, while hydrogen gas was generated with hydrogen generator for NPD at a flow of 3 mL/min. The flow of zero air (99.999%, Air Products) for NPD was 60 mL/min.

An ultrasonic cleaning system from Hettich (Tuttlingen, Germany) with a voltage line of 230 V and frequency of 50-60 HZ was used for dispersion of organic solvent in an aqueous sample solution. Centrifuges were performed by a centrifuge system from Hettich (Tuttlingen, Germany). The pH-meter model 731 (Herisau, Switzerland) supplied with a glass combined electrode and universal pH indicator (pH 0-14) was used for pH measurements. A 100 μL syringe was purchased from Hamilton (USA) for injection of organic phase in the sample solution and measuring the volume of sedimented phase. All 10 mL screw cap glass test tubes with conic bottom (as the extraction vessels) were remained in nitric acid (1molL^{-1}) for 24 hours and maintained at 250 ◦C, for cleaning.

Recommended USAEME procedure

Aliquots of 6.00 mL sample solution containing 3 μg L^{-1} of atrazine was placed in a 10 mL screw cap glass tube with a conical bottom. 30 μL of chlorobenzene (extraction solvent) was added into the sample solution with the 100-μL syringe. The tube was immersed into an ultrasonic water bath. Dispersion of very fine droplets of chlorobenzene in sample solution caused high turbidity and cloudy state in aqueous phase. The procedure was performed for 5 minutes at 25°C. To disrupt the cloudy solution, a three-minute centrifuging at 5000 rpm was performed and the organic phase was sedimented at the bottom of the conical tube. 0.5 μL of the sediment phase was removed using a 1 μL syringe and injected into GC. The volume of sedimented phase was determined using a microsyringe which was about 28 μL.

Results and discussion

In this study, the applicability of USAEME with GC/NPD was explored as a simple and fast

method for the preconcentration, extraction, and determination of atrazine in environmental samples. The variables affecting the extraction recovery were studied and optimized. In the optimum conditions extraction efficiency, and enrichment factor were calculated by using the equations (a) and (b) as follows:

$$(a)\quad ER = \frac{C_p V_p}{C_i V_i} \times 100$$

$$(b)\quad EF = \frac{C_p}{C_i}$$

Where ER and EF are extraction recovery and enrichment factor, Cp and Ci are atrazine concentrations after preconcentration found by GC/NPD and initial concentration, respectively. V_p is the volume of organic acceptor phase after preconcentration and V_i is the volume of aqueous initial solution. For determination of final concentration of atrazine after extraction, direct injection of atrazine standard solutions in chlorobenzene with different concentrations was performed in the range of 0.1–2 mgL^{-1}. In this research, a maximum extraction recovery of 93.3±2.5% (n=3) was obtained. The enrichment factor was found to be 200 with 6.00 mL of an initial sample solution.

Selection of extraction solvent

Selection of a suitable extraction solvent is critical to achieve an efficient USAEME procedure. The desired characteristics for appropriate extraction solvent are low water solubility, high extraction capacity for the target analyte, ability to form a stable emulsion system under ultrasound energy and also compatibility with gas chromatography system. Based on mentioned considerations, five solvents, $CHCl_3$, CCl_4, $C2Cl_4$, C_6H_5Cl, CH_2Cl_2 were contemplated to be appropriate in this work. Preliminary experiments were performed by using 100 µL of each solvent. The stable emulsion solution was obtained by each of these solvents except CH_2Cl_2 which showed high solubility in water. The results are shown in Figure 2. All the experiments were repeated three times. As it can be seen among the tested solvents, C_6H_5Cl acted most quantitatively and effectively. So in further experiments C_6H_5Cl was used as the extraction solvent.

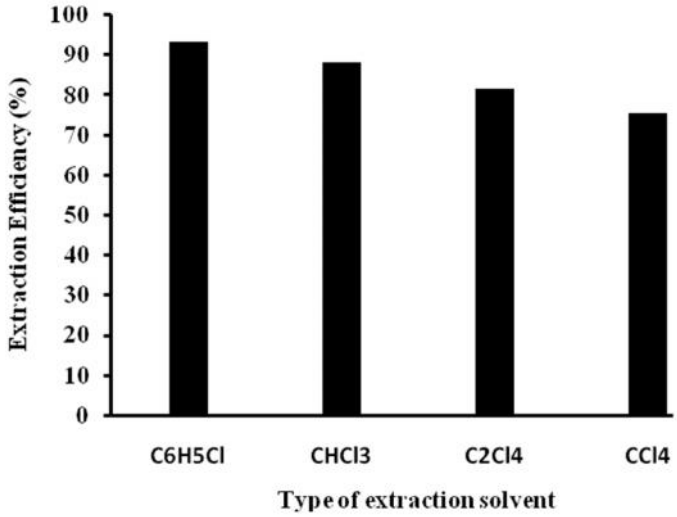

Figure 2. Effect of type of extraction solvent on extraction efficiency.

Effect of extraction solvent volume

Different volumes of chlorobenzene ranging from 10-80 µL were examined with the same USAEME procedures. The results obtained from three times analysis are offered in Figure 3. According to the figure, as the volume of the extraction solvent increases to 30 µL, the extraction efficiency increases and then remains almost constant up to 80 µL. It indicates the high distribution coefficient of atrazine and quantitative analysis. In addition, the amount of enrichment factors decrease because of increase in the volume of sedimented phase (from 8-75µL). Therefore, the amount of 30 µL of chlorobenzene was selected as the optimum volume of the extraction solvent to achieve a good recovery and enrichment factor.

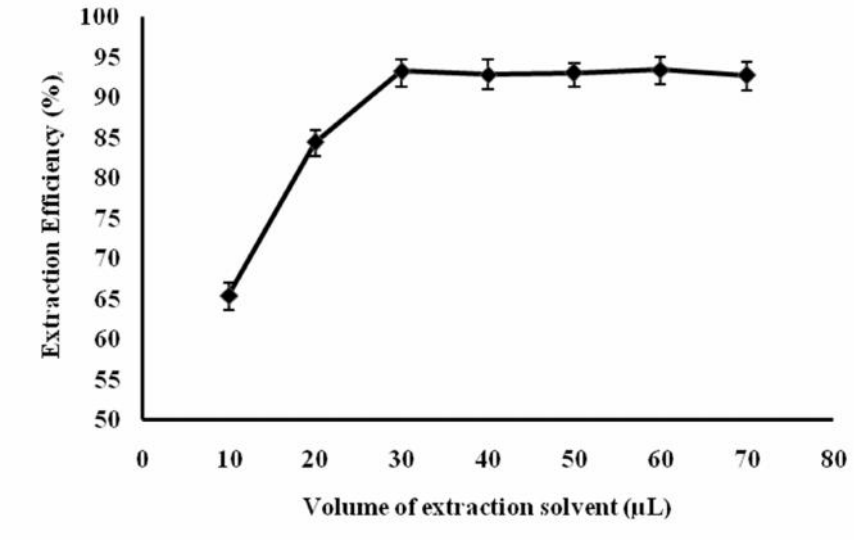

Figure 3. Correlation between the volume of added extraction solvent volume and extraction efficiency.

Effect of ionic strength

The influence of the ionic strength on the performance of USAEME was investigated by using various concentrations of NaCl (0-10% W/V) in sample solution. The results (Figure 4) state that increasing ionic strength has no special impact on extraction efficiency but the volume of deposited phase increases due to the reduction in solubility of organic solvent in water. Overall, salt addition decreases the solubility of analyte and promotes both mass transfer and extraction efficiency. On the other hand, presence of salt in a sample solution increases the density and viscosity of the solution, which can prevent formation of fine droplets of organic phase. It is worth showing that even at high and variable levels of ionic strength, the responses are reproducible. Regarding above results, salt addition was not considered in this study.

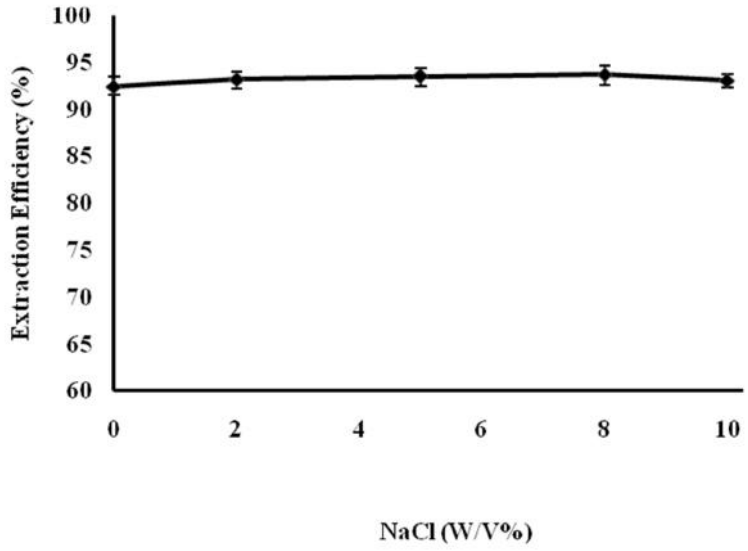

Figure 4. Ionic strength effect on the extraction efficiency.

Effect of sample pH

In order to establish an efficient UASEME procedure, pH of aqueous solution should be adjusted. It is demonstrated that the pH of sample solution determines the existing state of analyte and thus influence the extraction efficiency, especially for acidic or basic analytes. The experimental results (repeated three times) are shown in Figure 5. It is obvious that the extraction efficiency is affected by pH. The recovery of atrazine increased by increasing pH from 2 to 5, and remained constant up to 7. Due to the molecular structure of atrazine as a weak base (pka=1.65), it is clear that in acidic pHs, atrazine exists in its protonated form so its transfer to organic acceptor solvent is declined. In pH > 7, the obtained recoveries are lower than others

which show the hydrolysis of atrazine in basic solutions [32]. Therefore, the pH=6 of sample solutions was adjusted in the experiments.

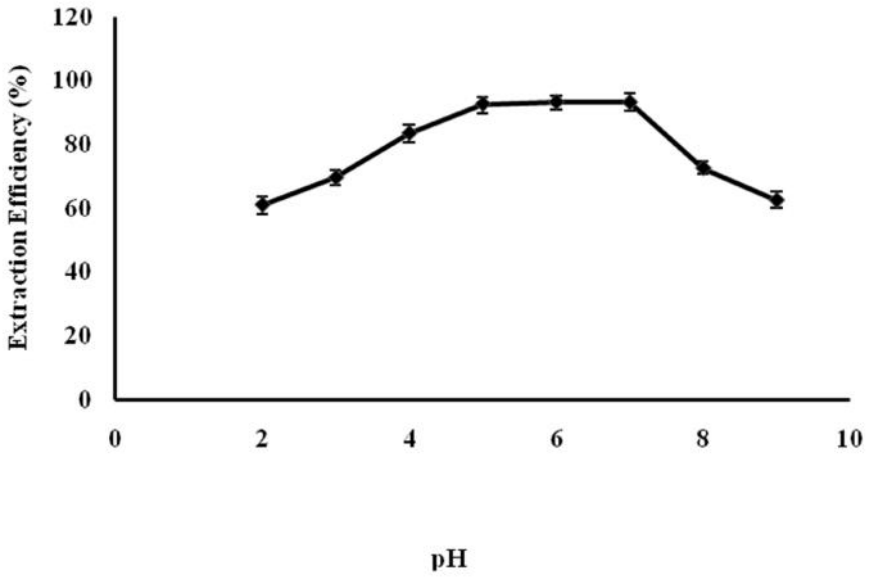

Figure 5. Effect of pH on the extraction efficiency of atrazine.

Effect of extraction and centrifuging time

Time of extraction is described as the time interval between the moment at which the extraction solvent is added and the time when the sonication ends just before centrifuging onset. Since time duration can affect emulsification and mass transfer process, it should be studied to achieve the best response in a minimum time. In the present study, extraction time was investigated in the range 0-15 minutes. Figure 6 shows the extraction efficiency of extracted analyte versus extraction time. It can be seen that the recovery increased up to maximum value in the first 5 minutes, and then remained almost constant. It shows that a homogeneous and invariant emulsion is achieved after 5 minutes because of large surface area between two immiscible phases. As a result, a period of 5 minutes was the selected as extraction time for USAEME procedure. After preconcentration step in ultrasonic bath, centrifuging was carried out for disrupting the emulsion solution and phase separation. A different centrifuging time period was examined at 4000 rpm ranging from 3 min to 15 min. The mentioned results were obtained during the whole study time. According to the results, a period of 3 minutes was the minimum required period to centrifuge and achieve a completely biphasic system.

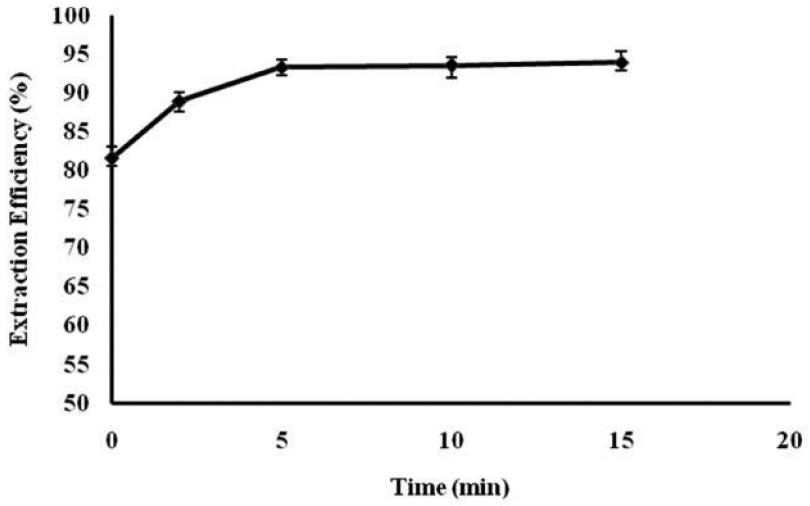

Figure 6. Effect of time on the extraction efficiency.

Sonication in comparison with vigorously stirring

Sonication in ultrasonic bath was compared with the vigorously stirring of the solution. Since sonication by ultrasound waves produces smaller droplets of organic phase, the contact surface between two phases increases and mass transfer improves impressively. Therefore, the efficiencies obtained by sonication were higher and better in reproducibility (RSD =2.5%) in comparison with RSD obtained by vigorously stirring which was about 9%. In conclusion, USAEME was carried out by sonication assistance.

Performance of USAEME in water samples.

Analytical figures of merit

The analytical performance of the proposed method was validated under optimum conditions. Calibration graphs were constructed by using solutions of atrazine of known concentrations. Linear dynamic range (LDR) of the method was obtained over the range of 0.1- 600 μgL^{-1} with the regression coefficient (R2) 0.9989. Limit of detection (LOD), calculated as 3 signal to noise) was 0.02 μgL^{-1}. Precision, expressed as relative standard deviation (RSD%), was evaluated as 2.5% in terms of repeatability based on the peak area for 5 replicates. A comparison between USAEME-GC-NPD and other analysis method is reported in Table 1. It can be seen that such analysis method offers a good linear range and detection limit in comparison with other techniques. In addition, use of small volume of organic solvent makes it an environmental friendly sample pre-treatment method.

Table 1. Comparison of the results obtained by USAEME-GC-NPD with other reported methods.
[a]head space-solid phase microextraction-ion mobility spectrometr

Methods	LOD (μgL^{-1})	LDR (μgL^{-1})	Volume of organic solvent	RSD%	Sample	Reference
SPE-HPLC	0.1	0.5-30	10 mL	8.3	water	[14]
SPE-GC-MS	0.002	0.1-1	5 mL	6.9	water	[13]
HS-SPME-IMS[a]	15	50-2800	-	<10	water	[34]
SPE-HPLC	9	1120	1mL	2.3	water	[35]
SPME-GC-FID	56	100-5500	-	9.3	water	[36]
SPE-HPLC	0.05	10-100	10 mL	<2.8	water	[37]
SDME-HPLC	0.04	0.15-37.5	20 µL	5	water	[17]
USAEME-GC-NPD	0.02	0.1-600	30 µL	2.5	water	Present research

Real sample analysis

In order to evaluate the accuracy and precision of the mentioned method, the procedure was performed to the analysis of atrazine in agricultural water and lettuce samples. For assessing matrice effect, the samples were spiked with different levels of atrazine and analysed as reported. Table 2 shows the results and Figure 7 represents the typical chromatogram obtained by USAEME-GC-NPD for spiked and non-spiked agricultural water samples. It is revealed that the matrice has no adverse effect on method efficiency.

Table 2. Determination of atrazine in different water samples by USAEME-GC-NPD.

Real sample	Spiked levels (μgL^{-1})	Found (μgL^{-1})	Recovery %	RSD (%), n=3
Agricultural water	-	n.d.[a]	-	-
	5	5.04	100.8	3.2
	8	8.1	101.2	2.9
Lettuce	-	n.d.	-	-
	4	3.8	95	2.5
	10	9.2	92	3.2

a: not detected

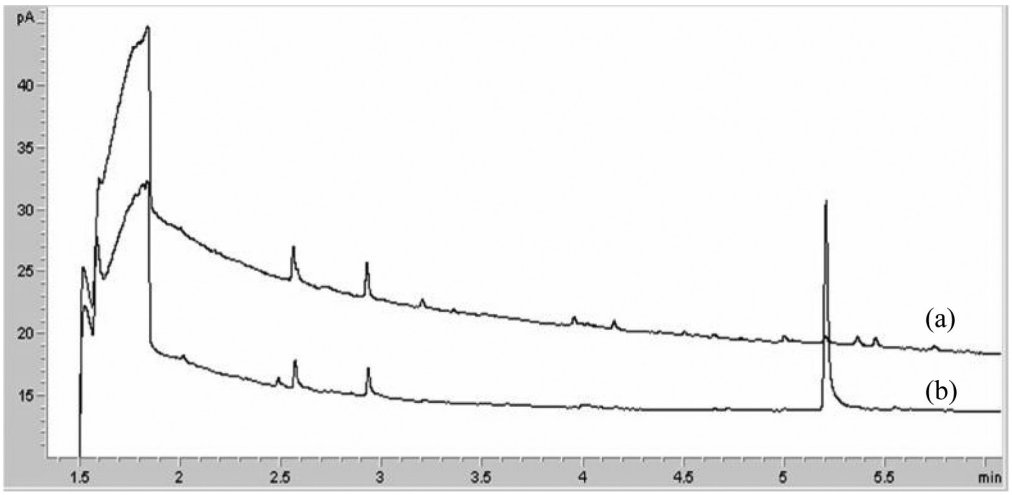

Figure 7. GC chromatograms obtained from agricultural water and spiked agricultural water samples. (a) agricultural water and (b) agricultural water spiked with atrazine at concentration of 8 $ngmL^{-1}$.

Conclusion

In this paper, USAEME coupled with GC-NPD has been outlined for determination of atrazine in environmental samples. The method provided low detection limit, appropriate repeatability, good extraction recovery and wide linear dynamic range. Consuming low volume of organic solvent which is expected in sample preparation techniques is another significant property of the method. Application of ultrasonic waves prompted and accelerated mass transfer and emulsification phenomenon. Totally, the reported method as a viable, inexpensive, rapid, easy and environmental friendly method could be used for quantitative analysis of atrazine with satisfactory results.

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