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Ionic liquid-based ultrasound-assisted emulsification microextraction coupled with high performance liquid chromatography for the determination of four fungicides in environmental water samples

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ABSTRACT

A highly efficient and environmentally friendly sample preparation method termed ionic liquid-based ultrasound-assisted emulsification microextraction (IL-USAEME) combined with high performance liquid chromatography has been developed for the determination of four fungicides (azoxystrobin, diethofencarb, pyrimethanil and kresoxim-methyl) in water samples. In this novel approach, ionic liquid (IL) was used as extraction solvent in place of the organic solvent used in conventional USAEME assay, and there is no need for using organic dispersive solvent which is typically required in the common dispersive liquid–liquid microextraction method. Various parameters that affect the extraction efficiency, such as the kind and volume of IL, ultrasound emulsification time, extraction temperature and salt addition were investigated and optimized. Under the optimum extraction condition, the linearities of calibration curves were in the range from 3 to 5000 ng mL^{-1} for target analytes with the correlation coefficient higher than 0.9992. The enrichment factors and the limits of detection were in the range of 88–137 and 0.73–2.2 ng mL^{-1}, depending on the analytes. The environmental water samples were successfully analyzed using the proposed method, and the relative recoveries at fortified levels of 50 and 100 ng mL^{-1} were in the range of 83.9%–116.2%.

1. Introduction

Azoxystrobin, diethofencarb, pyrimethanil and kresoxim-methyl are new fungicides which have a different mode of action compared to traditional fungicides. Azoxystrobin and kresoxim-methyl are strobilurines that act on the respiration process by blocking the transport of electrons within the mitochondria from cytochrome b to cytochrome c_1 by binding a specific site [1]. Diethofencarb is a phenylcarbamate which inhibits phospholipid and fatty acid biosynthesis, and pyrimethanil is an anilinopyrimidine that acts by inhibiting the biosynthesis of methionine by the pathogen [2]. They can be used to protect against gray molds, fungi and other agents that may affect crop yield. However, the residues of these fungicides may enter into the environment through various routes, such as spraying, the discharge of waste water and soil seepage, leading to possible surface and ground water contamination as well as cause public health problems due to their toxicity and persistence. According to the European Union Directive, a maximum allowed concentration of 0.1 μg L^{-1} for individual pesticide and of 0.5 μg L^{-1} for total pesticides in drinking water has been established [3]. The high requirements on water quality have resulted in an increasing need for the reliable, sensitive and rapid analytical technique to monitor the fungicides at trace levels.

Gas chromatography (GC) [4,5] and high performance liquid chromatography (HPLC) [6,7] are the common used techniques for the separation and quantification of fungicides residues in different matrices. Moreover, a previous sample preparation process is required in order to decrease the complexity of the matrix and increase the concentration of target compounds. Application of conventional liquid–liquid extraction (LLE) and solid-phase extraction (SPE) methods was limited with the disadvantages such as time-consuming, labor-intensive and need a large amount of organic solvents [8,9]. Solid-phase microextraction (SPME) and stir bar sorptive extraction (SBSE) have also been proposed for fungicides extraction. However, the routine application of SPME and SBSE in fungicide monitoring studies still requires solving some practical drawbacks, such as the limited stability of some SPME coatings, cross-contamination risks and the cost of SPME fibers and polydimethylsiloxane coated bars [10,11].

Recent efforts are being placed on the development of miniaturized, efficient and environment-friendly extraction techniques for the analysis of fungicides in complex matrix. Ultrasound-assisted

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emulsification microextraction (USAEME) is an efficient, simple, rapid and cheap extraction technique which was first introduced by Regueiro and coworkers in 2008 [12]. This approach is based on the emulsification of a microvolume of organic extractant in an aqueous sample by ultrasound radiation, and further separation of both liquid phases by centrifugation. In the technique, the application of ultrasonic radiation facilitates the emulsification phenomenon and accelerates the mass-transfer process between two immiscible phases. This leads to an increment in the extraction efficiency in a minimum amount of time. USAEME has been widely used for the extraction of organic compounds and metal ions in environmental samples [13–16]. However, organic extraction solvents tend to volatilize under the ultrasonic radiation.

Ionic liquids (ILs) are salts with low melting points that are composed of organic cations and various types of anions, and have been considered as environmental benign solvents [17]. There are aroused increasing interests for their promising role as alternative solvents in organic synthesis, catalysis and electrochemistry [18,19]. ILs have negligible vapor pressure and non-flammability as well as good solubility for inorganic and organic compounds, and have been successfully applied in various areas of analytical chemistry, especially in separation sciences [20–23]. The application of ILs in sample preparation technologies, such as LLE [24], liquid-phase microextraction (LPME) [25,26] and dispersive liquid–liquid microextraction (DLLME) [27,28] has been reported.

In the present study, the application of IL as extraction solvent instead of organic solvent in USAEME was explored, and a novel method of ionic liquid-based ultrasound-assisted emulsification microextraction (IL-USAEME) coupled with HPLC was developed for the determination of four fungicides (azoxystrobin, diethofencarb, pyrimethanil and kresoxim-methyl, the chemical characteristics of these compounds were compiled in Table 1) in environmental water samples. The effects of various experimental parameters, such as the kind and volume of IL, ultrasound emulsification time, extraction temperature and salt addition were investigated and optimized.

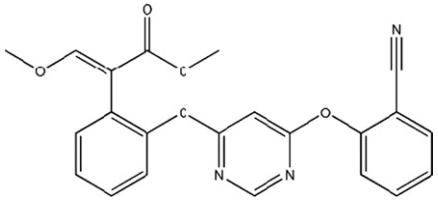
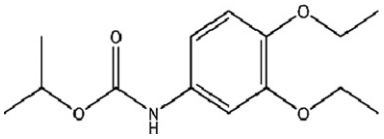
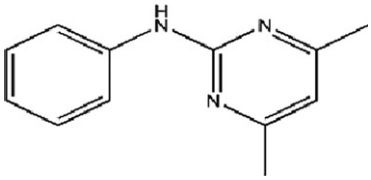
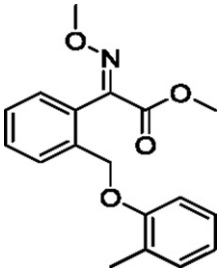
2. Experimental

2.1. Reagents and standards

Azoxystrobin (94%), diethofencarb (98%), pyrimethanil (98%) and kresoxim-methyl (99.5%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The individual stock standard solution were prepared in methanol at a concentration of 100 μg mL^{-1} and stored at 4 °C. The standard working solutions were daily prepared by dilution of stock standard solution with deionized water to the required concentrations.

1-Butyl-3-methylimidazolium hexafluorophosphate ($[C_4MIM][PF_6]$), 1-hexyl-3-methylimidazolium hexafluorophosphate ($[C_6MIM][PF_6]$) and 1-octoyl-3-methylimidazolium hexafluorophosphate ($[C_8MIM][PF_6]$) were purchased from Shanghai Chengjie Chemical Co., Ltd. (Shanghai, China). The HPLC-grade methanol was obtained from TEDIA Company (Fair lawn, NJ, USA). The water used in the work was purified on a Milli-Q water purification system (Millipore Corporation, Billerica, MA, USA). All chemicals used in this work were of analytical reagent grade or better. All the solvents and water samples were filtered through a 0.45 μm membrane to eliminate particulate matter before analysis.

Table 1
Chemical characteristics of the studied compounds.

Name	Chemical structure	Molecular weight	Molecular formula
Azoxystrobin		403.4	$C_{22}H_{17}N_3O_5$
Diethofencarb		267.3	$C_{14}H_{21}NO_4$
Pyrimethanil		199.3	$C_{12}H_{13}N_3$
Kresoxim-methyl		313.4	$C_{18}H_{19}NO_4$

2.2. Instrumentation

The chromatographic analysis was performed on an Agilent 1100 HPLC system equipped with a manual injector and a variable wavelength detector (VWD). A personal computer equipped with an Agilent ChemStation program for LC was used to process chromatographic data. A Venusil MP C18 column (5 μm, 4.6 mm × 250 mm) from Bonna-Agela Technologies (Tianjing, China) was used for separation. The mobile phase was a mixture of methanol–water (0.045% acetic acid, v/v) (63:37, v:v) and the flow rate was 1.0 mL min^{-1}. The column temperature was set at 30 °C and the VWD detector was set at a wavelength of 210 nm. A 60-kHz ultrasonic water bath with temperature control (Kunshan Ultrasound Instruments Company, Jiangsu, China) was used for assisting the emulsification of IL. An 80-2 centrifuge (Changzhou Guohua Electric Appliance Co. Ltd., PR China) was used for centrifuging.

2.3. IL-USAEME procedure

A 5.00 mL aliquot of aqueous sample containing 1% (w/v) NaCl was placed in a 10-mL glass centrifuge tube, and then 40 μL [C_8MIM][PF_6] were added into the sample solution as extraction solvent. The resulting mixture was immersed in an ultrasonic bath at 60 kHz frequency and 25 °C for 15 min. During the sonication, a turbid cloud solution was formed due to the dispersion of IL into the aqueous bulk, and the analytes in aqueous sample were extracted into the fine droplets. The emulsion was disrupted by centrifugation at 3000 rpm for 5 min, and the dispersed fine droplets of IL were sedimented at the bottom of the centrifuge tube. After removal of the upper aqueous phase, 20 μL of the sedimented phase was filtrated through a 0.45 μm filter prior to HPLC analysis.

3. Results and discussions

In order to obtain the optimized extraction conditions, enrichment factor (*EF*) was used to evaluate the extraction efficiency under different conditions. The enrichment factor was defined as the ratio between the analyte concentration in the sedimented phase (C_{sed}) and the initial concentration of analyte (C_0) within the sample

$$EF = C_{sed}/C_0$$

3.1. Selection of extraction solvent

The structure of ILs has significant influence on its physicochemical properties, such as solubility in water, the viscosity and extraction capacity which might greatly affect the extraction efficiency of target analytes. Three imidazolium-ILs with PF_6^- as anion, including [C_4MIM][PF_6], [C_6MIM][PF_6] and [C_8MIM][PF_6], were accessed in this work. It was found that the cloud solution could not be formed under ultrasound and no sedimented phase appeared at the bottom of the tube after centrifugation when using [C_4MIM][PF_6] as extraction solvent. The main reason is the higher solubility of [C_4MIM][PF_6] (18.8 g L^{-1}) in water compared to [C_6MIM][PF_6] (7.5 g L^{-1}) and [C_8MIM][PF_6] (2.0 g L^{-1}) [29]. The extraction efficiencies for [C_6MIM][PF_6] and [C_8MIM][PF_6] were investigated and shown in Fig. 1. As can be seen, a higher EF was obtained with [C_8MIM][PF_6] as extraction solvent. Thus, [C_8MIM][PF_6] was chosen as the extraction solvent for the subsequent experiments.

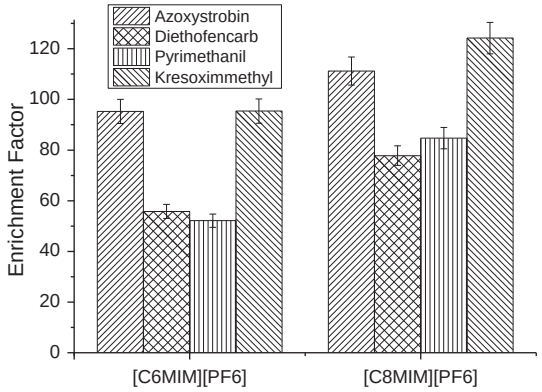


Fig. 1. Effect of different extraction solvents on the extraction efficiency of the fungicides.
Extraction conditions: sample volume: 5.0 mL; extraction solvent volume: 40 μL; ultrasonic time: 10 min; extraction temperature: 25 °C.

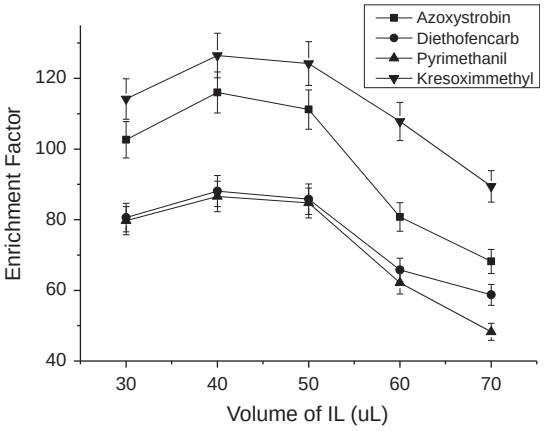


Fig. 2. Effect of the volume of extraction solvent on the extraction efficiency of the fungicides.
Extraction conditions: sample volume: 5.0 mL; extraction solvent: [C_8MIM][PF_6]; ultrasonic time: 10 min; extraction temperature: 25 °C.

3.2. Effect of extraction solvent volume

The volume of extraction solvent was another crucial parameter that could affect the extraction efficiency. To investigate the effect of extraction solvent volume on the extraction efficiency, the volume of [C_8MIM][PF_6] varied in the range from 30 to 70 μL, and subjected to the same IL-USAEME procedure. Fig. 2 depicts the EF versus volume of IL for the target analytes. As can be seen, the EF increased with the increase of volume of IL from 30 to 40 μL, and decreased above 40 μL. The main reason maybe that appropriate volume of [C_8MIM][PF_6] could be dispersed sufficiently in aqueous solution under ultrasound, but with the volume of [C_8MIM][PF_6] increased, the analytes could not be transferred into the IL droplets sufficiently for its high viscosity and density. Therefore, 40 μL [C_8MIM][PF_6] was used in the following experiments.

3.3. Effect of ultrasound emulsification time

Owing to the high viscosity and density of IL, adequate sonication time is needed to ensure equilibrium extraction and mass transfer of the analytes in IL-USAEME process. Nevertheless, prolonging sonication time could result in descent of extraction efficiency because of volatilization of the analytes and dissolution of IL in aqueous solution for the abundant sonication heat

generated. The effect of ultrasound emulsification time on extraction efficiency was evaluated in the range of 5–30 min, and the results were shown in Fig. 3. The results indicated that the extraction equilibrium could be attained within 15 min. Thereby, 15 min was chosen as the extraction time.

3.4. Effect of extraction temperature

Temperature can affect the mass transfer rate and the contact area between sample solution and the extraction solvent, thus influencing the extraction efficiency. It has also a significant effect on the solubility of IL. The effect of different extraction temperature on extraction efficiency was investigated in the range from 20 to 40 °C, and the results were shown in Fig. 4. The extraction efficiency increased with the increase of temperature from 20 to 25 °C, and then decreased slightly when the temperature exceeds 25 °C. The main reason maybe that high temperature induces the dissolution of IL and analytes in aqueous solution. Therefore, the extraction temperature of 25 °C was chosen in this study.

3.5. Effect of salt addition

The effect of salt addition on the performance of IL-USAEME was investigated by adding different amount of sodium chloride (0–5%, w/v) in aqueous solution, and the results were shown in Fig. 5. The results revealed that the extraction efficiency increased with the increase of NaCl concentration to 1%. At higher NaCl concentration, a decrease in extraction efficiency occurred. This could be considered as the result of two major competitive effects: salting-out effect and viscous resistance effect [30]. Based on the experimental results, 1% NaCl was added in sample solution.

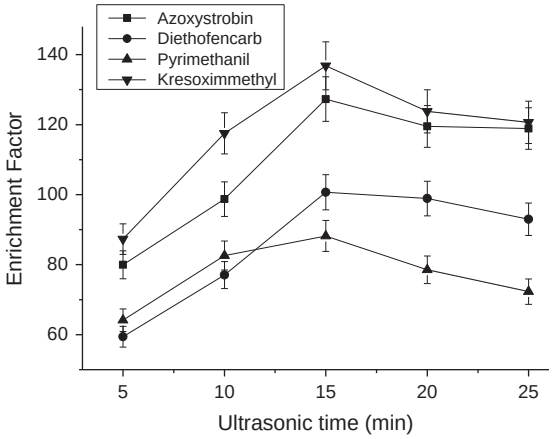


Fig. 3. Effect of ultrasonic time on the extraction efficiency of the fungicides. Extraction conditions: sample volume: 5.0 mL; extraction solvent: 40 μL [C_8MIM]-[PF_6]; extraction temperature: 25 °C.

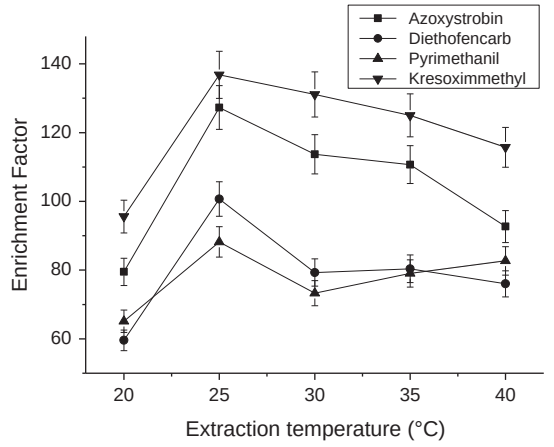


Fig. 4. Effect of extraction temperature on the extraction efficiency of the fungicides.
Extraction conditions: sample volume: 5.0 mL; extraction solvent: 40 μL [C_8MIM][PF_6]; ultrasonic time: 15 min.

3.6. Evaluation of the method

Chromatograms obtained for a real sample (lake water) after IL-USAEME extraction under the optimum conditions were shown in Fig. 6. The chromatograms were characterized by symmetrical peak shape.

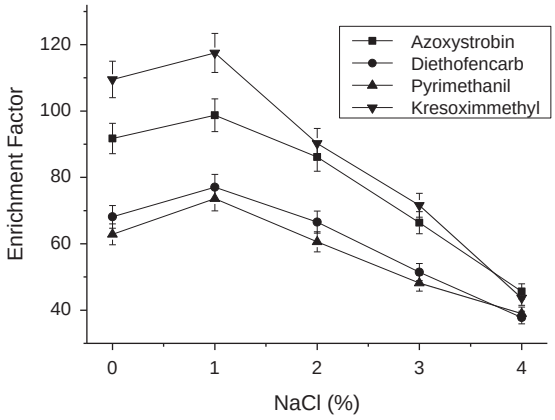


Fig. 5. Effect of salt addition on the extraction efficiency of the fungicides. Extraction conditions: sample volume: 5.0 mL; extraction solvent: 40 μL [C_8MIM][PF_6]; ultrasonic time: 15 min.

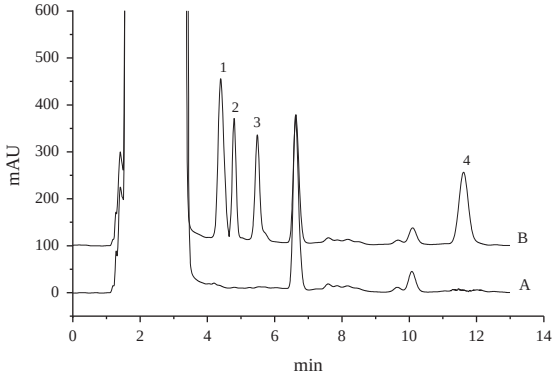


Fig. 6. Chromatograms obtained for lake water after IL-USAEME procedure under the optimal conditions: (A) lake water; (b) lake water fortified with 50 ng mL^{-1} targets. Peak identification: 1-azoxystrobin, 2-diethofencarb, 3-pyrimethanil, and 4-kresoxim-methyl.

Table 2
Analytical performance data of the IL-USAEME method.

Compound	Linear range (ng mL^{-1})	R	RSD (%)	LOD (ng mL^{-1})	EF
Azoxystrobin	5–5000	0.9998	5.4	2.2	128
Diethofencarb	3–5000	0.9994	3.1	0.73	101
Pyrimethanil	5–5000	0.9992	4.6	1.8	88
Kresoxim-methyl	5–5000	0.9996	5.2	1.3	137

Table 3
Comparison of the proposed IL-USAEME method with other methods.

Method	Linear range	LOD	RSD (%)	Reference
LLE-HPLC-DAD	0.10–2.50 mg L^{-1}	0.2–0.8 mg L^{-1}	8.9–11.4	[8]
SPE-LC-MS	0.9–1000 ng mL^{-1}	0.9 ng mL^{-1}	1.2–4.2	[9]
SPME-GC-MS	12.5–250 ng g^{-1}	1.8–3.1 ng g^{-1}	7.4–15.0	[10]
SBSE-HPLC-DAD	–	0.3–2.0 ng g^{-1}	6.5–10.7	[11]
LPME-GC-MS	0.5–300 ng mL^{-1}	0.028–0.04 ng mL^{-1}	4.1–9.8	[13]
IL-USAEME-HPLC	5–5000 ng mL^{-1}	0.73–2.2 ng mL^{-1}	3.1–5.4	This method

Table 4
Analytical results of four fungicides residues and recoveries in water samples (with confidence limit of 95%).

Fungicides	Spiked (ng mL^{-1})	River water			Lake water			Tap water		
		Found (ng mL^{-1})	Recovery (%)	RSD (%)	Found (ng mL^{-1})	Recovery (%)	RSD (%)	Found (ng mL^{-1})	Recovery (%)	RSD (%)
Azoxystrobin	0	nd			nd			nd		
	50	57.3	114.6	5.4	57.1	114.2	6.4	57.4	114.8	5.2
	100	110.2	110.2	8.3	105.9	105.9	9.2	112.2	112.2	5.4
Diethofencarb	0	nd			nd			nd		
	50	56.8	113.6	3.1	56.4	112.8	4.6	54.6	109.2	6.5
	100	93.5	93.5	9.1	88.0	88.0	8.0	98.3	98.3	4.7
Pyrimethanil	0	nd			nd			nd		
	50	55.1	110.2	3.4	58.1	116.2	5.6	52.8	105.6	4.4
	100	94.9	94.9	8.5	92.8	92.8	9.5	96.4	96.4	4.7
Kresoxim-methyl	0	nd			nd			nd		
	50	43.7	87.4	8.4	46.6	93.2	6.3	47.8	95.6	6.1
	100	88.7	88.7	9.7	83.9	83.9	8.4	98.2	98.2	7.2

3.6.1. Features of the method

Under the optimum conditions, linear range, precision (RSDs), enrichment factor (EF) and limits of detection (LODs) of the IL-USAEME method for all target compounds were obtained and shown in Table 2. As can be seen, good linearities were observed for all analytes with the correlation coefficient (R) ranging from 0.9992 to 0.9999. The LODs were calculated at a signal-to-noise (S/N) ratio of 3. The precisions of this method, determined by consecutively extracting seven water samples spiked with the analytes at concentration of 50 ng mL^{-1}, varied from 3.1–5.4%.

The analytical performance of the proposed method was compared with the corresponding performance of other methods [8–11,13] for the extraction and determination of the target fungicides. As can be seen from Table 3, the IL-USAEME method has comparable LODs with other extraction methods, good linear ranges and precisions. Moreover, Ionic liquid $[C_8MIM][PF_6]$ is used as the extraction solvent, thus preventing the use of toxic organic solvents. Therefore, the present method can be used as a green analytical method for the determination of fungicides in environmental water samples.

3.6.2. Real samples analysis

The proposed method was applied to the determination of the four fungicides in river water, lake water and tap water under the optimum conditions. None of the target analytes was detected in all the three matrices, indicating the analytes were not present or were below the LODs of the method. All the water samples were fortified with the target analytes at concentration levels of 50 ng mL^{-1} and 100 ng mL^{-1} to study the matrix effects on the extraction recovery. Each treatment was done in triplicate, and the results are shown in Table 4. The relative recoveries for the four fungicides ranged from 87.4 to 114.6% for river water, 83.9 to 116.2% for lake water and 95.6 to 114.8% for tap water. The results suggest that the matrices of environmental water samples have little effect on the IL-USAEME process, and the proposed method could be a reliable method for the determination of fungicides in real water samples.

4. Conclusion

In this study, a novel, simple and sensitive IL-USAEME technique coupled with HPLC-VWD was developed and successfully applied for the determination of four fungicides in environmental water samples. The method provides low LODs, good repeatability, high enrichment factors and good recoveries, and uses ionic liquid instead of organic solvent as extraction solvent. Therefore, the proposed method is a green and environment friendly analytical technique.

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