

# SENSITIVE DETERMINATION OF DDT AND ITS METABOLITES WITH IONIC LIQUID DISPERSIVE LIQUID-PHASE MICROEXTRACTION PRIOR TO HPLC

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## Abstract

DDT and its main metabolites are important environmental pollutants. It is of great value to develop simple, rapid, sensitive and easy to operate method for monitoring them. Present work established a novel temperature-controlled ionic liquid dispersive liquid-phase microextraction method for the enrichment and determination of DDT and its metabolites. Proposed method used only ionic liquid 1-hexyl-3-methylimidazolium hexafluorophosphate ([C<sub>6</sub>MIM][PF<sub>6</sub>]) for the enrichment. A series of factors that would affect the extraction performance were systematically investigated and optimized. The experimental results indicated that the detection limits obtained for *p,p'*-DDD, *p,p'*-DDT, *o,p'*-DDT and *p,p'*-DDE were 0.24, 0.24, 0.45, 0.24 ng mL<sup>-1</sup> respectively. The linear ranges for them were from 1.0 ng mL<sup>-1</sup> to 100 ng mL<sup>-1</sup>, and the precisions were between 3.8-6.7% (n=6). The proposed method was validated with four real-world samples and excellent results were achieved.

## Introduction

Organochlorine pesticides (OCs) have been used extensively and worldwide in agriculture and other control programmes in the past years. Due to their large consumption, they have posed important impact on the animals and human health. For these reasons, many countries had forbidden the production and application of DDT for many years. However the residue and its main metabolites could still be found in a variety of environmental samples. Recently, high levels of DDT and its main metabolites have been frequently detected in various media such as soils, water, sediments and plants in China, as well as in other countries<sup>1-7</sup>. Up to now, many methods have been developed for the analysis of DDT and its metabolites, and the often-used methods were gas chromatography (GC) and high performance liquid chromatography (HPLC). Chromatographic methods were widely applied as the preferred technique for the identification and quantification of DDT and its metabolites due to its intrinsic good merits. However, a sample pretreatment and clean-up procedure is often used for the determination of such pollutants because they are present at very low level in the environment and the sample matrices are usually complex.

Recently, many important contributions have been reported for the sample pretreatment. Among them, liquid phase microextraction has been developed successfully and achieved much more attention due its advantages. Single drop microextraction (SDME)<sup>8</sup> is a mode of LPME and has been applied for separation/preconcentration of organic pollutants. Assadi have developed a novel liquid phase microextraction named as dispersive liquid-liquid microextraction (DLLME)<sup>9</sup>. DLLME has many merits, yet toxic solvents such as benzene, chlorobenzene or chloroform have been often used as extraction phase. In order to reduce the effect of toxic solvent on the environment, environmental friendly solvents are expected for use.

Ionic liquids (ILs) have been considered as green solvent and have been applied in many fields. On the other hand, ionic liquids are designable according to the need for use. Liu has provided a review on the application of ionic liquids in analytical chemistry<sup>10</sup>. Manzoori et al. established a new single-drop LPME method with ILs for the determination of manganese in water samples prior to electrothermal atomic absorption spectrometry<sup>11</sup>. However, the instability of the suspending drop and the small dimension of the drop of SDME results in the difficulty of operation and low enrichment factor. Recently, our group developed a new LPME method termed temperature controlled ionic liquid dispersive liquid phase microextraction using single solvent ionic liquid and which has been successfully applied to determine the organophosphorus pesticides and pyrethroid pesticides<sup>12,13</sup>.

This new method avoided the instability of suspending of single drop liquid phase microextraction and used no toxic organic solvents as disperse solvents. Meanwhile, it earned many merits such as simplicity, easy to operate, and low cost etc and is a valuable and environmental friendly method.

The aim of this study is to develop a simple and sensitive and suitable temperature controlled ionic liquid dispersive liquid phase microextraction method for the preconcentration of DDT and its main metabolites in water samples. The effect of various experiment conditions on the extraction of DDT and its main metabolites is investigated and discussed in detail.

## Materials and Methods

1-Hexyl-3-methylimidazolium hexafluorophosphate ( $[C_6MIM][PF_6]$ ) was purchased from Acros Organics (Geel, Belgium). *p,p'*-DDD, *p,p'*-DDT, *o,p'*-DDT and *p,p'*-DDE were purchased from Ying Tian Yi standard solution company(Beijing, China). Ultra-pure water was prepared in the lab using Ultra-Clear (Barsbittel, Germany) and all the other solvents were analytical reagent grade unless otherwise stated. 0.5 M of sodium hydroxide and concentrated hydrochloric acid were used for adjusting the pH value of the water samples.

A high performance liquid chromatography system, which consisted of two LC-10ATvp pumps and an SPD-10Avp, ultraviolet detector (Shimadzu, Kyoto, Japan) was used for the analysis and separation. A reversed-phase Agilent Zorbax C18 column (150 mm×2.1 mm I.D., particle size 5  $\mu$ m) was used for separation at ambient temperature and Chromato Solution Light Chemstation for LC system was employed to acquire and process chromatographic data.

In the extraction procedure, 50  $\mu$ L  $[C_6MIM][PF_6]$  was added into a 10 mL glass marked conical tube, and ultra-pure water was added to the 10 mL mark. This solution was spiked with a concentration of 20 ng mL<sup>-1</sup> for the four DDTs. Then the conical tubes were heated with the temperature controlled at 75 °C.  $[C_6MIM][PF_6]$  was then dissolved completely in the aqueous solution and would mix with the solution entirely. The analytes would migrate into the IL phase based on the higher solubility of analytes in IL. The tube was thereafter cooled with ice water and the solution became turbid. Then the solution was centrifuged for 15 min. The upper aqueous phase was removed with a syringe, the residue was dissolved in 200  $\mu$ L methanol and 20  $\mu$ L was injected into the HPLC system for analysis.

## Results and Discussion

In the experimental procedure, some important parameters that would affect the extraction performance were optimized.

The volume of extraction solvent was a crucial parameter that seriously influenced the extraction performance in liquid phase microextraction. In present study, the volume of extraction solvent may be enlarged according to the needed enrichment. Under the described procedure above, the extraction efficiency of different volume of ionic liquid was investigated respectively. The results indicated that along with the increasing of volume of ionic liquid, the peak area of DDT and its main metabolites increased when the ionic liquid volume was lower than 50  $\mu$ L and then kept a higher level when the volume of ionic liquid was more than 50  $\mu$ L. Hence 50  $\mu$ L was selected for use in the subsequent experiment.

In this experiment, effect of pH on the extraction performance within the range of pH 2 ~ pH 10 was investigated. It could be seen that the peak areas of DDT and its main metabolites increased at the range of pH 2 ~ pH 6. When the pH further increased, the peak areas of *p,p'*-DDD, *p,p'*-DDT and *o,p'*-DDT decreased markedly. Meanwhile, the peak width of *p,p'*-DDT, *o,p'*-DDT were enlarged and then split peak of *p,p'*-DDT appeared at pH 10. All these would influence the accuracy and reproduction of the proposed method. Based on comprehensively consideration, pH 6 was utilized in the following experiments.

In the proposed method, temperature was the key factor and also was the driving force for the complete dispersion of  $[C_6MIM][PF_6]$  into the aqueous solution. A series of experiments were designed for the optimization of temperature between 65 ~ 85 °C. It could be found that an excellent extraction performance was achieved at 75 °C.

Centrifugation was a crucial step in this proposed method. However it was very difficult to separate  $[C_6MIM][PF_6]$  from the aqueous solution. After centrifugation, the  $[C_6MIM][PF_6]$  drops were coated with a filmy layer of water.  $[C_6MIM][PF_6]$  drops were very small when the centrifuging time was too short, and longer centrifuging time resulted in heat generation which leading to the dissolving of parts of the  $[C_6MIM][PF_6]$  phase and the loss of sensitivity. So the effect of centrifuging time was investigated from 5 min to 25 min. The

experimental results showed that the peak areas of DDTs increased along with the increase of the centrifuging time, but the increase was in a very limited extent. For the sake of time, 15 min was used in the final.

Important parameters such as linear range, precisions and detection limits were determined to evaluate the method performance. Under the optimal conditions, a series of experiments were designed for investigating such factors. It was found that for the DDTs, good linearity with correlation coefficients over a range of 0.9987–0.9994 was achieved. The precisions were obtained in the range of 3.8–6.7% (RSD,  $n = 6$ ) by six reduplicate extractions of spiked water samples with a concentration of  $5 \mu\text{gL}^{-1}$ . The sensitivity of this proposed method was determined based on the ratio of signal to noise ( $S/N = 3$ ), and the detection limits were over the range of  $0.24 \sim 0.45 \mu\text{gL}^{-1}$ . All these results were very satisfactory. These merits indicated that this novel approach would be a creative development in analytical and environmental fields. Four real water samples from local regions were used to validate the applicability of the established method. The experimental results indicated that DDTs could not be found in the blank and spiked recoveries were satisfied in the range of 87.4–110.0% with a very low level ( $5 \mu\text{gL}^{-1}$ ).

### Conclusion

This paper has developed a simple, rapid, sensitive and cheap method for the determination of DDTs in environmental water samples. The proposed method overcame the shortcomings of conventional single drop microextraction such as the instability of extraction solvent suspending, small volume of extraction solvent and so on. The proposed method using ionic liquid as extraction solvent, substituting for the toxic solvents, avoided the secondary pollution. Meanwhile, proposed method provided excellent sensitivity and would be very competitive in the routine analysis of environment pollutants.

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