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Dispersive liquid–liquid microextraction combined with high performance liquid chromatography–fluorescence detection for the determination of carbendazim and thiabendazole in environmental samples

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ABSTRACT

A rapid and sensitive method for the determination of carbendazim (methyl benzimidazole-2-ylcarbamate, MBC) and thiabendazole (TBZ) in water and soil samples was developed by using dispersive liquid–liquid microextraction (DLLME) coupled with high performance liquid chromatography with fluorescence detection. The water samples were directly used for the DLLME extraction. For soil samples, the target analytes were first extracted by 0.1 mol L^{-1} HCl. Then, the pH of the extract was adjusted to 7.0 with 2 mol L^{-1} NaOH before the DLLME extraction. In the DLLME extraction method, chloroform ($CHCl_3$) was used as extraction solvent and tetrahydrofuran (THF) as dispersive solvent. Under the optimum conditions, the enrichment factors for MBC and TBZ were ranged between 149 and 210, and the extraction recoveries were between 50.8 and 70.9%, respectively. The linearity of the method was obtained in the range of 5–800 ng mL^{-1} for water sample analysis, and 10–1000 ng g^{-1} for soil samples, respectively. The correlation coefficients (r) ranged from 0.9987 to 0.9997. The limits of detection were 0.5–1.0 ng mL^{-1} for water samples, and 1.0–1.6 ng g^{-1} for soil samples. The relative standard deviations (RSDs) varied from 3.5 to 6.8% (n = 5). The recoveries of the method for MBC and TBZ from water samples at spiking levels of 5 and 20 ng mL^{-1} were 84.0–94.0% and 86.0–92.5%, respectively. The recoveries for soil samples at spiking levels of 10 and 100 ng g^{-1} varied between 82.0 and 93.4%.

1. Introduction

Sample preparation is one of the most important and crucial procedures in a whole analytical process. It is often also the bottleneck for rapidly obtaining the desired results, especially for the determination of trace analytes in a complex matrix sample. The objective of the sample preparation is not only to isolate the target analytes from the samples, thus reducing or even eliminating the interferences originally present in the sample, but also simultaneously to concentrate the analytes to facilitate their determinations at low levels. A variety of methods for the separation and preconcentration of the analytes from sample matrix have been developed, such as liquid–liquid extraction (LLE) [1], solid-phase extraction (SPE) [2], solid-phase microextraction (SPME) [3] and liquid-phase microextraction (LPME) [4–9].

LLE and SPE are the most commonly used techniques for the preconcentration and cleanup of the selected analytes. However, LLE suffers from the disadvantages of being time-consuming, expensive, and requiring large volumes of both samples and toxic organic solvents. SPE techniques typically require reduced amounts of organic solvents relative to LLE, but SPE can still be tedious, time-consuming, relatively expensive, and sometimes suffers from analytes breakthrough when large sample volumes are analyzed. SPME, a more recent procedure, is a simple, organic solvent-free and efficient extraction technique [3]. However, SPME also suffers from some problems such as sample carry-over, relatively high cost and fiber fragility.

Recently, LPME has emerged as an attractive alternative for sample preparations because of its simplicity, effectiveness, low cost, minimum use of solvents and excellent sample cleanup ability. Different configurations of this technique have recently emerged, including static LPME, dynamic LPME, single-drop LPME and hollow fiber-based liquid-phase microextraction (HF-LPME) [4–9]. However, several disadvantages, such as the instability of liquid drop in single-drop LPME, air bubbles formation in HF-LPME, long analysis time and relatively low precisions, are often encountered for such techniques.

Very recently, a novel microextraction technique, named dispersive liquid–liquid microextraction (DLLME), based on dispersion of tiny droplets of the extraction solvent within the aqueous solution has been developed by Assadi and co-workers [10]. DLLME is a miniaturized LLE that uses microliter volumes of the extraction

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solvent. For DLLME, water-immiscible extraction solvent dissolved in a water-miscible dispersive solvent was rapidly injected into an aqueous solution by syringe. A cloudy solution containing fine droplets of extraction solvent dispersed entirely in 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 high enrichment factor. DLLME has been applied for the analysis of a variety of trace organic pollutants and metal ions in the environmental samples [11–17]. But until now, 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.

Because of the widespread use of agricultural pesticides for different applications, the pesticide residues may present a main source of pollution, which poses risks to plant, animal and human health. Benzimidazole fungicides are widely used pesticides in agriculture for pre- and post-harvest treatment for the control of a wide range of pathogens. They are either applied directly to the soil, or sprayed over crop fields. Most of these compounds persist in the environment after their application, with some even remaining for many years [18]. Carbendazim (methyl benzimidazole-2-ylcarbamate, MBC) and thiabendazole (TBZ) are the widely used benzimidazole fungicides. The thermal instability of MBC and TBZ does not permit their analysis directly by gas chromatography unless they are derived into thermally stable derivatives. The most frequently used methods for the determination of these benzimidazol fungicides are fluorescence spectroscopy [19] and high performance liquid chromatography (HPLC) with UV, fluorescence or mass spectrometric detection [1–2]. The different pretreatment methods, such as LLE [1], SPE [2], SPME [20], microwave-assistant extraction [21], supercritical fluid extraction [22], and cloud point extraction [23] have been used for the preconcentration and cleanup procedures of these fungicides from different samples.

In continuation to our previous endeavors in the exploration of novel sample pretreatment techniques [24–28], herein, a DLLME method in combination with HPLC-fluorescence detection was developed for the determination of MBC and TBZ in water and soil samples. To the best of our knowledge, this may be the first report about the application of the DLLME method for the determination of these fungicides. The effects of various experimental parameters, such as the kind and volume of extraction and disperser solvent, extraction time and salt effect have been studied. The applicability of the presented method for the analysis of real water and soil samples has also been investigated.

2. Experimental

2.1. Reagents and materials

MBC (99%) and TBZ (99%) were purchased from the Eighth Chemical Factory of Baoding (Baoding, Hebei, China). Chloroform ($CHCl_3$), carbon tetrachloride (CCl_4) and chlorobenzene (C_6H_5Cl) were purchased from Beijing Chemical Reagents Company (Beijing, China). Acetone, tetrahydrofuran (THF), acetonitrile, ethanol and HPLC-grade methanol were from Sinopharm Chemical Reagent Co. Ltd (Tianjin, China). Sodium chloride (NaCl), sodium hydroxide (NaOH) and hydrochloric acid (HCl) were from Tianjin Fuchen Chemical Reagent Factory (Tianjin, China). All the reagents were analytical reagent grade unless otherwise stated. Double-distilled water was produced by a SZ-93 automatic double-distiller (Shanghai Yarong Biochemistry Instrumental Factory, Shanghai, China), which was used for preparation of aqueous solutions.

Rain water, well water and lake water samples were collected from Baoding (Baoding, China). Soil samples were collected from the plough layer of the field at Ximachi and Wumazhuang (Baoding, China), which were dried at room temperature, pulverized and passed through 250-μm sieve. All the solvents and water samples were filtered through a 0.45-μm membrane to eliminate particulate matter before analysis.

A mixture stock solution containing each of MBC and TBZ at 1.0 mg mL^{-1} 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 in the dark.

2.2. Instrument

The HPLC system, assembled from modular components (Waters, Milford, MA, USA), consisted of an in-line degasser, a 600E pump, and a fluorescence detector. A Millennium^{32} workstation (Waters) was utilized to control the system and for the acquisition and analysis of the data. The injection loop volume is 20.0 μL. A Centurysil C_{18} column (4.6 i.d. × 250 mm, 5.0 μm) from Dalian Jiangshen Separation Science Company (Dalian, China) was used for separations. The mobile phase was a mixture of methanol–water (60:40, v/v) and the flow rate was 1.0 mL min^{-1}. For detection, the fluorescence excitation and emission wavelengths were set at 280 and 315 nm, respectively.

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

2.3. Sample preparation before DLLME

Water samples were filtered through 0.45 μm filter prior to extraction by DLLME.

The extraction of MBC and TBZ from soil samples before DLLME was carried out according to the following procedures reported in the documents [29,30]. Soil samples were air-dried at room temperature, pulverized and passed through 250-μm sieve. 20.0 g of the soil sample was accurately weighed and put into a 100 mL centrifuge tube, to which 40.0 mL 0.1 mol L^{-1} HCl was added. The resultant sample mixture was first vigorously shaken on a vibrator for 30 min and then filtrated under reduced pressure. The pH of the filtrate was adjusted to 7.0 by 2 mol L^{-1} NaOH. A 5.0 mL aliquot of the above sample solution was used for DLLME.

2.4. DLLME procedures

For the DLLME, a 5.00 mL aliquot of water sample 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 0.75 mL of THF (as disperser solvent) and 80.0 μL $CHCl_3$ (as extraction solvent) was injected into the sample solution by 1.00 mL syringe, and then the mixture was vortexed for 10 s. A cloudy solution that consists of very fine droplets of $CHCl_3$ dispersed into aqueous sample was formed, and the analytes were extracted into the fine droplets. After centrifugation at 3500 rpm for 5 min, the $CHCl_3$ phase was sedimented at the bottom of the centrifuge tube. The sedimented phase was completely transferred to another test tube with conical bottom using 100-μL HPLC syringe and blown to dryness with a mild nitrogen stream. The residue was dissolved in 15 μL methanol and 10.0 μL was injected into the HPLC system for analysis.