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Determination of Benzimidazoles Pesticides in Environmental Water Using Hollow Fiber Microextraction

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An extraction method for the analysis of benzimidazoles pesticides (carbendazim, tiabendazole and tiophanate-methyl) in environmental water was developed based on hollow fiber liquid-phase microextraction (HF-LPME) combined with HPLC. The influence of the different factors on the HF-LPME efficiency including the salt conentraction and extraction time were examined. The best HF-LPME conditions were as follows: 1-octanol impregnated in the pores of the hollow fiber, extraction time of 2 h and salt concentration of 0.25 g/mL. Under the optimized condition, provided very low concentrations 0.0001 μg/mL were detected, showing 0.2, 0.5 and 0.25 fold enrichment of carbendazim, thiabendazole and thiophanate-methy, respectively. This had higher selectivity and sensitivity than the other traditional methods.

Key Words: Liquid-phase microextraction, Hollow fiber, Benzimidazoles pesticides.

INTRODUCTION

Pesticides protect crops from diseases, insects and weeds *etc*., as well as increase production yields agricultural income. Therefore, they are essential for modern agriculture. On the other hand, pesticides are toxic to human beings and domestic animals and cause environmental degradation[1]. Recently, the need for evaluation to protect the environment. Pesticides are considered the cause of cancer, heredopathia and endocrine system diseases[2]. Benzimidazole pesticides are systemic fungicides composed benomyl, thiophanate-methyl, carbendazim and thiabendazole[3]. Fig. 1 shows the structure of three target compounds, such as carbendazim, thiabendazole and thiophanate-methyl. Pesticide extraction methods include solid-phase extraction and liquid-liquid extraction. Solid-phase extraction emplys a small amount of organic solvent and can extract pesticide compounds in a short time. Sorbents, such as silica, NH_2, C_{18} and cation exchange, can be applied in a range of fields[4]. On the other hand, an organic solvent removal process is requred after extraction. The sorbent is a very expensive consumable. Liquid-liquid extraction has been used for pesticide analysis for a long time for pesticide analysis. On the other hand, liquid-liquid extraction methods require lengthy sample preparation times and use large amounts of toxic organic solvents[5]. Solid-phase microextraction (SPME) was introduced by Pawlisyn[6]. solid-phase microextraction has several important advantages compared to established sample

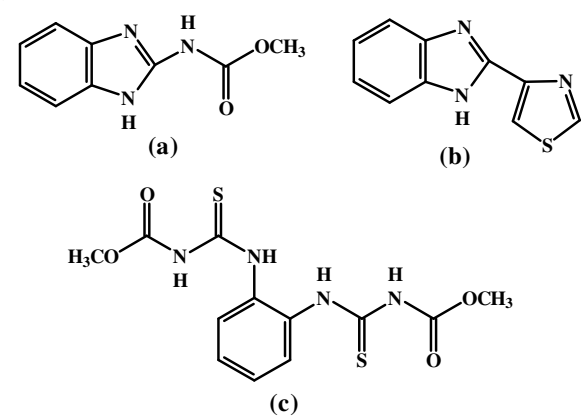


Fig. 1. Structures of (a) carbendazim, (b) thiabendazole and (c) thiophanate-methyl

preparation techniques. This method is fast and simple, employs a small amount of solvent,is effective adsorption/desorption technique and gives highly coherent, quantifiable results from very low concentrations[7,8]. Although solid-phase microextraction is popular, it have several drawbacks, such as unstable and swelling in organic solvents that limits the use of HPLC, fiber breakage and the bending of needles and expense[9]. A simple, inexpensive liquid-phase microextraction (LPME) technique was introduced recently to overcome these problems. liquid-phase microextraction is a supplement procedure of liquid-liquid extraction. In liquid-phase microextraction, extraction

normally takes place in a small amount of a water-immiscible solvent (acceptor phase) from an aqueous sample containing analytes (donor phase). There are several types of liquid-phase microextraction[10]. But the current study employed only hollow-fiber liquid-phase microextraction (HF-LPME). Pedersen-Bjergaard and Rasmussen introduced a different idea for liquid-phase microextraction based on the use of single, low cost, disposable, porous, hollow fibers, normally made of polypropylene[11-13]. Fig. 2 show schematic diagram of hollow fiber microextraction procedure. Hollow-fiber liquid-phase microextraction employs a microporous hollow fiber membrane, making it possible for extraction for sample cleanup and high enrichment using cheap and simple equipment. The major advantage of hollow-fiber liquid-phase microextraction is that the sample can be stirred or vibrated actively without the loss of extracting liquid becuse it is protected in hollow-fiber liquid-phase microextraction[14]. This study examined the extraction of benzimidazole pesticides from environmental water using hollow-fiber liquid-phase microextraction.

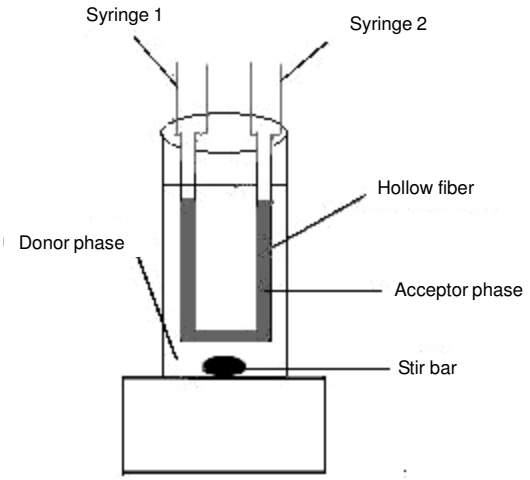


Fig. 2. Schematic diagram of hollow fiber liquid phase microextraction procedure

EXPERIMENTAL

The polypropylene hollow fibers used in all experiments were purchased from Membrana (GmbH, Wuppertal, Germany). The pore size, internal diameter and wall thickness were 0.2, 600 and 200 μm, respectively. Carbnedazim, thiabendazole and thiopanate- methyl were supplied Sigam-Aldrich (Milwaukee, WI, USA) acetonitrile (ACN) were obtained from Duksan Pure Chemical Co., Ltd. (Ansan, Korea). All other reagents used in the experiment were HPLC grade. Double distilled water was filtered using a vacuum pump (Division of Millipore, Waters, USA) and filter (HA-0.45, Division of Millipore, Waters, USA) prior to use.

Chromatographic condition: Chromatography was performed using a Waters 600s multisolvent delivery system, a Waters 616 liquid chromatography and waters 2487 variable wavelength, dual-channel UV detector (Waters Associates, Milford, MA, USA). The HPLC analysis was performed with a commercial C_{18} column (4.6 mm × 250 mm, 5 μm) purchased from RStech Co. (Daejeon, Korea). HPLC separation of benzimidazole pesticide was conducted using acetonitrile/water (40:60, v/v) as the mobile phase. The flow rate, UV wavelength and injection volume were set to 0.5 mL min^{-1}, 354 nm and 10 μL, respectively.

Preparation of the sample collection and standard solutions: The environmental water samples were collected from the Inha university lake. The sample was filtered through filter paper prior to extraction. A stock solution of three target compounds (0.01 mg/mL) was prepared in methanol.

Hollow-fiber liquid-phace microextraction: The hollow fiber membrane was sonicated in acetone for 10 min for decontamination prior to use. The fiber was dried completely and cut to the appropriate lengths. Prior to extraction, the tip of the micro syringe's needle was inserted into the hollow fiber, which was then immersed into the organic solvent to ensure that the pores had been filled with the extraction solvent. The organic solvent, normally 10-20 μm, forms a thin layer within the wall of the hollow fiber. The hollow fiber was then placed into a sample vial filled with the aqueous sample. The sample was stirred to speed up the extraction. The use of stands and clamps ensured reproducible and stable positioning of the hollow fiber. After extracting for a prescribed period of time at room temperature, the organic solvent was withdrawn into the microsyringe and analyzed by HPLC.

RESULTS AND DISCUSSION

The extraction recovery (R) and concentration enrichment factor (E) can be expressed by eqns. 1 and 2, respectively.

$$R = \frac{n_{a,\,final}}{n_{s,\,initial}} \times 100\,\% = \frac{v_a}{v_s} \times \frac{n_{a,\,final}}{n_{s,\,initial}} \times 100\,\% \quad (1)$$

$$E = \frac{C_{a,\,final}}{C_{s,\,initial}} \quad (2)$$

$n_{s,initial}$: Number of moles of analyte present in the donor phase. $n_{a,final}$: Number of moles finally saved in the acceptor phase. $C_{s,initial}$: Initial concentration of analyte in the donor phase. $C_{a,final}$: Final concentration of analyte in the acceptor phase. V_a: Sample volume of acceptor phase. V_s: Sample volume.

The factors affecting the hollow-fiber liquid-phase microextraction extraction efficiency including different organic extraction solvent, salt concentration, hollow fiber length and extraction time were optimized to obtain the highest enrichment of target compounds with high analytical sensitivity[15].

Linearity and reproducibility: A series of mixtures of standard solutions containing carbnedazim, thiabnedazole and thiopanate-methyl were diluted (0.001, 0.005, 0.01 and 0.1 mg/mL) with methanol. The linear regression equations of the three compounds were Y = 4.97685 + 107x – 4881.75 (r^2 = 0.999) for carbendazim, Y = 2.1931 + 107x – 9235.13 (r^2 = 0.999) for thiabendazole and Y = 9.41128 + 107x – 14318.38 (r^2 = 0.999) for thiopanate-methyl. Assays of repeatability calculated as the relative standard deviations (RSDs) were performed by injecting standard solutions 5 times in a 5-day period.

Extraction solvent: The selection of suitable extraction solvent is the most critical step in hollow-fiber liquid-phase microextraction. The organic solvents used must meet several

requirements. The target should provide high solubility and prevent solvent dissolution in water during extraction. The organic solvent should be easily fixed to the hollow fiber and have low volatility[16]. In this study, four different organic solvents, cyclohexane, chloroform, 1-octanol and toluene were examined. Cyclohexane and chloroform are highly volatile organic solvents that were not fixed to the hollow fibers. Fig. 3 shows the respective chromatogram extracted with 1-octanol and toluene. 1-Octanol and toluene was showed similar extraction. However, 1-octanol was low volatile. Therefore, 1-octanol was selected as the optimal extraction solvent.

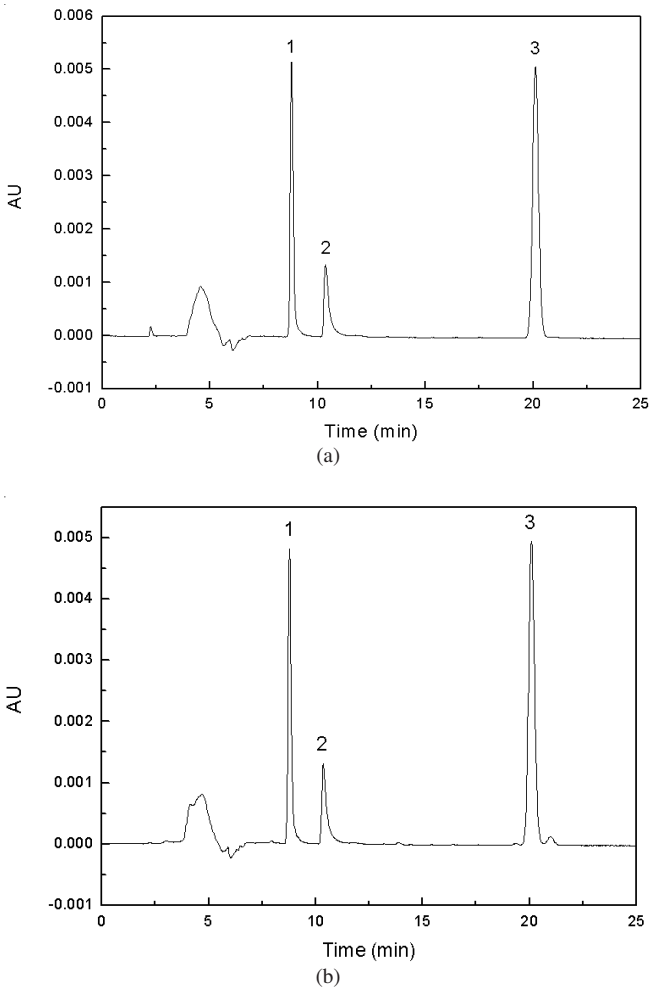


Fig. 3. Chromatograms of three compounds extraction by different solvent. (a) 1-Octanol, (b) toluene (mobile phase:acetonitrile-water = 40:60 (v/v), flow rate: 0.5 mL/min, column: C_{18} (250 mm × 4.5 mm, 5 µm), UV: 254 nm, injection volume: 10 µL, concentration: 1 µg/mL. 1: carbendazim, 2: thiabendazole 3: thiophanate-methyl)

Extraction time: The extraction efficiency was examined at different times. As shown in Fig. 4 the extraction increased with increasing exposure times, up to 2 h and decreased thereafter. The amount of extract was reduced when the long time extract in the hollow fiber extraction solvent had been expelled into the water sample. Therefore, 2 h was selected as the optimal extraction time.

Salt addition: Salt increaes the ionic strength of the water solution, which can have a significant effect on extraction can have some effects[17]. Normally, adding salt to the sample

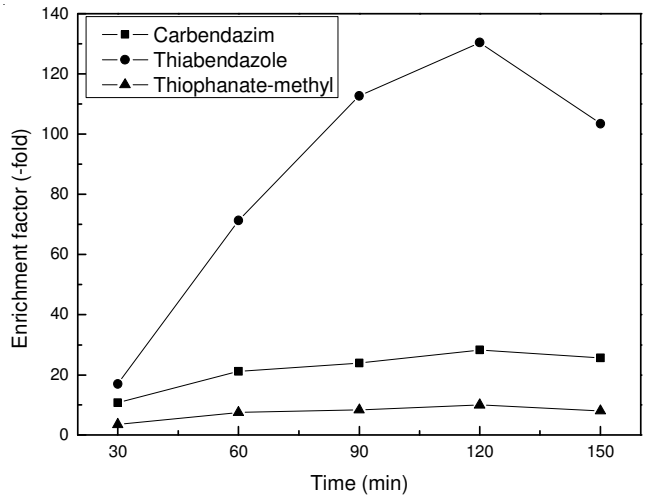


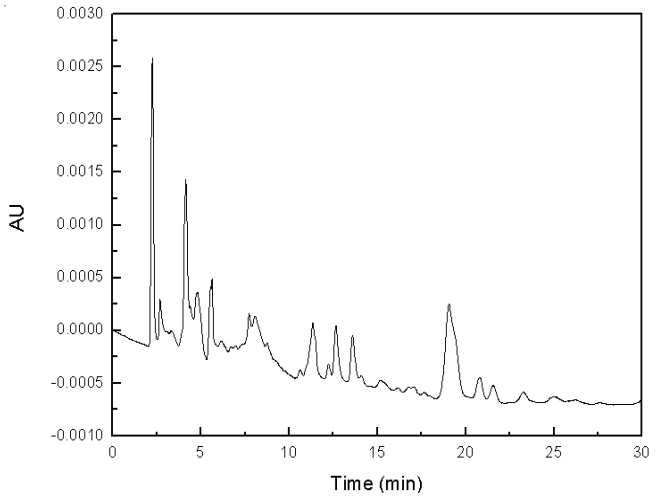
Fig. 4. Effect of extraction time on the enrichment factors of target compounds

improves the extraction of the more polar analytes depending on the solubility of the target compounds. This study examined, the effect of the NaCl concentration on the extraction efficiency[18]. Table-1 lists the enrichment factor of the target compounds extracted using different salt concentrations. The level of extraction increased with increasing NaCl concentration but when the salt concentration of up to 0.30 g/mL was not melting. Therefore, salt concentration of 0.25 g/mL was selected as the optimal NaCl concentration.

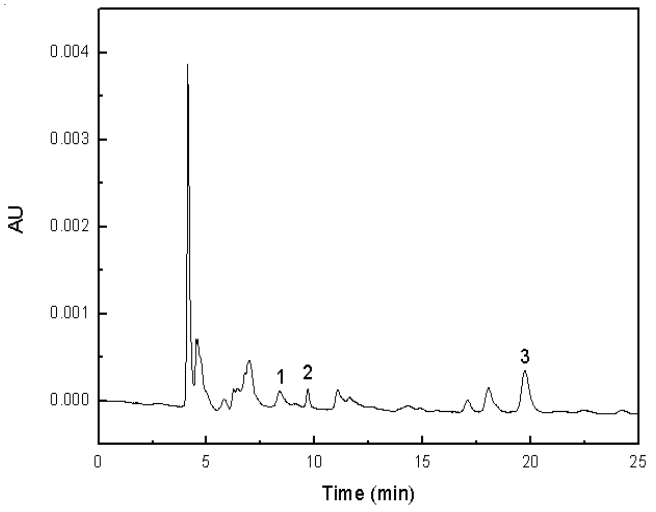
TABLE-1
ENRICHMENT FACTOR OF THE TARGET COMPOUNDS EXTRACTED BY DIFFERENT SALT CONCENTRATION

Salt conc. (g/mL)	Enrichment factor		
	Carbnedazim	Thiabnedazole	Thiophanatemethyl
0.00	15.71	130.45	9.96
0.05	32.58	273.56	28.14
0.10	53.10	361.36	35.73
0.15	49.83	322.91	53.88
0.20	53.49	320.46	61.97
0.25	66.04	376.07	76.00
0.30	Not melting	Not melting	Not melting

Hollow-fiber liquid-phase microextraction of environmental water sample: Real environmental water samples were analyzed using standard solution of hollow-fiber liquid-phase microextraction after determining the optimal extraction conditions. As shown in Fig. 5(a) with no hollow-fiber liquid-phase microextraction process, environmental water samples showed many other peaks not identified in the targeted compounds. On the other hand, with the hollow-fiber liquid-phase microextraction process, these other peaks were removed and small amounts of the target compounds were confirmed. In addition, carbendazim, thiabendazol and thiopanate-methyl added to the environment water samples were detected. The detection limits of the target compound concentrations were investigated. As shown in Table-2, 0.0001 µg/mL was found to be the lowest level detected. Table-3 lists the enrichment factor (-fold) and recovery of hollow-fiber liquid-phase microextraction under optimum conditions. enrichment factor of carbendazim, thiabendazole and thiopanate-methyl at very low concentrations was 0.2, 0.5 and 0.24 fold, respectively.



(a)



(b)

Fig. 5. Chromatograms of spiked environmental water sample. (a) Environmental water sample before hollow-fiber liquid-phase microextraction, (b) environmental water sample after hollow-fiber liquid-phase microextraction (mobile phase: acetonitrile-water = 40:60 (v/v), flow rate: 0.5 mL/min, column: C_{18} (250 mm × 4.5 mm, 5 μm), UV: 254 nm, injection vol: 10 μL, concentration: 1 μg mL^{-1}, 1: carbendazim, 2: thiabendazole, 3: thiophanate-methyl)

TABLE-2
ENRICHMENT FACTOR OF THE TARGET COMPOUNDS EXTRACTED BY LOW CONCENTRATION

Salt conc. (g/mL)	Enrichment factor		
	Carbnedazim	Thiabnedazole	Thiophanatemethyl
0.5	28.78	288.30	43.08
0.1	6.56	58.73	8.52
0.01	1.17	6.54	1.03
0.001	0.34	1.13	0.34
0.0001	0.10	0.74	0.21
0.00005	–	–	–

TABLE-3
EXTRACTION ENRICHMENT AND RECOVERIES OF CARBENDAZIM, THIABENDAZOLE AND THIOPHANATE-METHYL IN ENVIRONMENTAL WATER. (HF-LPME CONDITION: DONOR SOLUTION:(9 mL ENVIRONMENTAL WATER CONTAINING 0.0001 μg/mL CARBENDAZIM, THIABENDAZOLE AND THIOPHANATE-METHYL, THEN ADDED 0.25 mg/mL NaCl); HOLLOW FIBER LENGTH: 12 cm AND EXTRACTION TIME: 2 h)

Compounds	Enrichment (-fold)	Recovery (%)
Carbendazim	0.20	67
Thiabendazole	0.50	61
Thiophanate-methyl	0.24	60

Conclusion

Hollow-fiber liquid-phase microextraction was applied to the analysis of benzimidazoles pesticides in environmental water. The hollow-fiber liquid-phase microextraction technique involves a sample pre-concentration as well as an efficient sample clean-up effect. It showed that hollow-fiber liquid-phase microextraction is a promising technique for analyzing low concentrations of pesticides in environmental water samples.

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