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An in situ immobilized pipette tip solid phase microextraction method based on molecularly imprinted polymer monolith for the selective determination of difenoconazole in tap water and grape juice

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ABSTRACT

A pipette tip-based molecularly imprinted polymer monolith microextraction (PT–MIPMME) method was developed for the selective extraction of difenoconazole in tap water and grape juice. In this method, molecularly imprinted polymer (MIP) monolith used as the sorbent was synthesized at the tip of a micropipette. This in situ polymerization reaction used difenoconazole as the template and methacrylic acid (MAA) as the functional monomer, ethylene glycol dimethacrylate (EGDMA) as the cross-linker and the mixture of toluene–dodecanol as the porogenic solvent. The pipette tip containing MIP monolith was matched to a syringe for performing the polymer monolith microextraction (PMME). Several parameters affecting the proposed PT–MIPMME method were investigated, including the flow rate, sample volume, pH and salt concentration of sample, the type and volume of eluent. Under the optimal conditions, the PT–MIPMME method showed a low limit of detection of 0.5 $\mu g L^{-1}$. The recoveries were in the range of 87.6–95.4% with relative standard deviations less than 4.9%. The results showed that difenoconazole was selectively enriched from tap water and grape juice samples.

1. Introduction

Difenoconazole is a triazole fungicide which has a good ability to interfere with ergosterol biosynthesis and inhibit of steroid demethylation. So difenoconazole is widely used for the control of fungal diseases on fruits, vegetables, cereals, and other field crops [1]. Some fruits were made into juice beverage to meet different people's flavor and difenoconazole sprayed on croplands can easily migrate to environmental water. Hence, extensive use of difenoconazole may cause residues in juice beverage and environmental water, which have negative effect on human health. It has been reported that this fungicide was associated with an increase in the incidence of hepatocellular adenomas and carcinomas in the group of male and female mice following long-term dietary exposure [2]. However, the matrix of juice beverage is very complicated, containing large amount of pigment and endogenous components and the determination of the target analytes at trace level is also difficult. Preparation of the sample is the most critical step in the determination of pesticide residues in environmental and food samples. Conventional methods for the determination of pesticide residues in environmental and food samples are laborious and time-consuming, requiring considerable amounts of organic solvents and extracting undesirable interferants from the matrix [3]. The most commonly used methods are based on liquid–liquid extraction (LLE), solid-phase extraction (SPE) [4–7], matrix solid-phase dispersion (MSPD) [8–10], and stir bar sorptive extraction (SBSE) [11]. Solid-phase microextraction (SPME) emerged as a versatile alternative method of analyte extraction and preconcentration, which requires little or no organic solvents, is easily automated, and can also improve the limits of detection [12]. However, these methods could just solve some certain matrix interferences problems in the determination of pesticide residues of environmental and food samples. Since contaminants are present in low concentrations and the matrices are complex, extraction techniques with high selectivity to enrich the target analytes from complicated matrix are desirable before chromatography analysis.

One of the technique methods to make highly selective extraction is the immune affinity extraction (IAE) which incorporated biomolecules such as enzyme and antibody or antigen in the procedures. However, although good specificity can be achieved, it

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still has some disadvantages such as lack of stability and high costs of antibody preparation. Molecular imprinting is a technique which can create the artificial receptor-like binding sites with a "memory" for the shape and functional group positions of the template molecule. So, molecularly imprinted polymers (MIPs) are good alternatives to biological substances. MIPs can be synthesized conveniently by a mixture solution containing template molecule, porogenic reagent, functional monomer, cross-linker and initiator. After polymerization, template molecules are removed and polyporous materials with selectively functional binding sites are obtained. Due to the high stability, ease of preparation, and high sensitivity, MIPs have been used widely in different application, such as chromatographic stationary phases [13,14], solid phase extraction (SPE) [15–18], catalysis and sensing [19–24]. MIPs-based SPE is one of the most successful and useful application. MIPs-based SPE combines both the advantages of MIPs and SPE, and exhibits good extraction efficiency, reusability and selectivity to certain kinds of analytes [25], which is promising to selectively and effectively extract drugs in complicated matrix. The most widely used technique for preparing MIP materials is by conventional free-radical solution polymerization. In order to acquire particles with the appropriate size for HPLC and SPE, the bulk MIPs have to be crushed, grounded and sieved. The particles produced in this time-consuming process are irregular in size and shape, resulting in significant loss in chromatographic performance [26]. In addition, some active sites are destroyed during the grinding process leading to lower MIP loading capacity. To overcome these disadvantages, the MIP monolithic columns were prepared by in situ polymerization directly inside a capillary or stainless steel or the tip of a micropipette. This method could avoid the tedious grinding and sieving procedures as well as the problems of costly particle loss, particles in homogeneity, and molecular imprinted spots loss and could easily obtained a MIP monolith with the ideal porous structure and low back pressure at high flow rate.

To use the synthesized MIP monolith directly as SPE sorbents is a promising method. Recently, Zheng et al. [27] prepared a MIP monolith inside a fused-silica capillary and applied it in the extraction of fluoroquinolones from milk samples. Monoliths in a capillary and other microtubes can be synthesized quickly since some steps such as crushing, sieving and packing can be omitted [28]. Besides, the amount of template molecule required during monolith preparation is much less than that of other methods [29]. Compared with solid phase extraction, the volume of sorbents and eluting solvents could be reduced greatly, so the extraction efficiency could be increased as a result [30]. As one type of solid phase microextracion, polymer monolith microextraction (PMME) integrates sample extraction, enrichment and injection into a single step. The combination of molecular imprinting technique, PMME could exhibit excellent extraction selectivity in dealing with complicated samples. However, the MIP monolith that synthesized in a capillary was fragile, and required tedious pretreatment process.

To the best of our knowledge, MIP sorbents using difenoconazole as the template have not been reported, and little attention has been paid to make use of MIP monolith and PMME for high selective extraction of difenoconazole from complex matrices. In this work, difenoconazole-imprinted polymer monolith was synthesized in a pipette tip for the first time. The pipette tip could match to a syringe without any other treatment to perform the molecularly imprinted polymer monolith microextraction (PT–MIPMME). The MIP monolith was applied for the selective extraction of difenoconazole. Various experimental parameters affecting the difenoconazole–MIP monolith were optimized. The optimized method based on PT–MIPMME combined with high-performance liquid chromatography (HPLC) was established and applied for the determination of difenoconazole in tap water and grape juice samples.

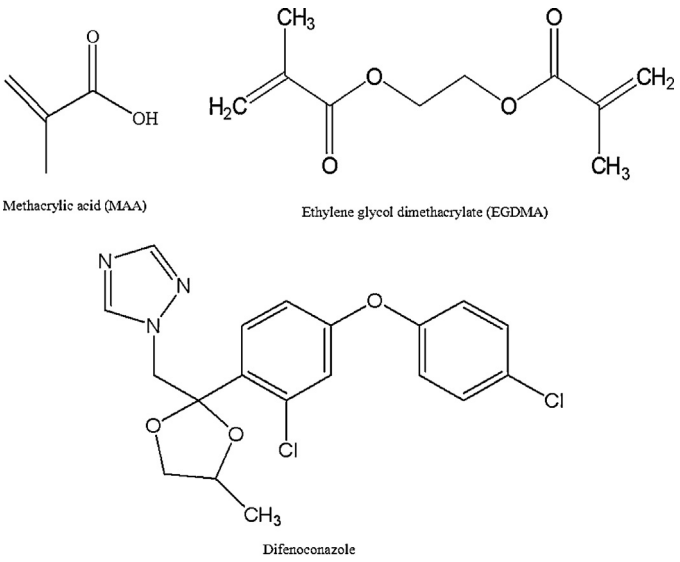

Fig. 1. The molecule structures of difenoconazole, MAA and EGDMA.

2. Experimental

2.1. Instruments

The chromatographic analysis was carried out on an Agilent 1200 HPLC system (Agilent Technologies, Palo Alto, CA, USA), equipped with an auto injector and a diode array detector (DAD). A reversed phase Agilent SB-C18 column (250 mm × 4.6 mm i.d., 5 μm) was used for the separation of analytes. The mobile phase was methanol–water (95:5, v/v) at a flow rate of 0.6 mL min^{-1}. The column temperature was 30 °C and the detection wavelength was set at 210 nm. The injection volume was 10 μL. Ultrasonic instrument KQ-100DE was purchased from Kunshan Ultrasonic Instrument Co., Ltd. (Jiangsu, China) and a pHS-3C digital pH meter (Shanghai Rex Instruments Factory, China) was employed for pH measurements.

2.2. Chemicals and materials

Ethylene glycol dimethacrylate (EGDMA, 98% pure) was purchased from Acros (New Jersey, USA). Methacrylic acid (MAA), 2,2′-bisisobutyronitrile (AIBN, AR), toluene (AR), dodecanol (AR), sodium hydroxide (AR) and hydrochloric acid (AR) were obtained from Tianjin Kermel chemical reagents development centre (Tianjin, China). Difenoconazole was purchased from Sigma-Aldirich (St. Louis, MO, USA), its chemical structure was shown in Fig. 1. Methanol (HPLC grade) and acetonitrile (HPLC grade) were ordered from Tedia (Fair Lawn, New Jersey, USA). Sodium chloride was procured from Zhanyun Chemical Co, Ltd. (Shanghai, China). Ultrapure water was purified on a Mill-Q water purification system (Millipore Corporation, Billerica, MA, USA).

The stock standard solution of difenoconazole was prepared by weighing 2.5 mg difenoconazole dissolved in 50 mL HPLC-grade methanol. Then the stock standard solution at a concentration of 50 μg mL^{-1} was made and stored at 4 °C in a refrigerator. A series of standard solutions were daily prepared by appropriate dilution from the stock solution with ultrapure water.

2.3. Sample preparation

Tap water samples were collected from Central China Normal University (Wuhan, China). Grape juice was purchased from a local supermarket (Wuhan, China), and all of them were kept at 4 °C.