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Preparation of 2,4-dichlorophenoxyacetic acid imprinted organic–inorganic hybrid monolithic column and application to selective solid-phase microextraction

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ABSTRACT

An organic–inorganic hybrid molecular imprinting monolith (HMIM) has been prepared, characterized and applied for the determination of 2,4-dichlorophenoxyacetic acid (2,4-D) in rice with high-performance liquid chromatography-photodiodes array detector (HPLC–PAD). By optimizing the polymerization conditions, such as the volume ratio of the inorganic alcoholysate and organic part, the 2,4-D-HMIM was synthesized in a micro pipette tip using acrylamide as the functional monomer, ethylene dimethacrylate as the cross-linker and methanol as the porogenic solvent. The morphology of the monolith was studied by scanning electronmicroscopy and Fourier transform infrared spectra. The imprinted factor of the monolith for 2,4-D reached 3.29. A simple, rapid and sensitive method for the determination of 2,4-D in rice using the HMIM microextraction combined with high-performance liquid chromatography-photodiodes array detector was developed. Some parameters affecting the sample pretreatment were investigated, including the type and volume of eluent, the flow rate and volume of sample solution. The assay exhibited a linear dynamic range of 167–4167 μg/kg with the correlation coefficient above 0.9972. The detection limit (at $S/N = 3$) was 50 μg/kg. The proposed method was successfully applied for the selective determination of 2,4-D in rice.

1. Introduction

2,4-dichlorophenoxyacetic acid (2,4-D) is one of the most applied herbicides around the world to control broad leave herbs in many crops. Large-scale application of 2,4-D in agriculture without proper withdrawal period led to accumulation of 2,4-D in cereals, vegetables, fruits, soils and environmental waters [1,2]. Because of continual overuse of 2,4-D, much attention has been paid to their potential carcinogenesis and teratogenic effects [3]. For these health concerns, the Joint Meeting of Pesticide Residues (JMMR) has adopted a maximum acceptable limit of residual 2,4-D in foods, for examples in rice (0.2 mg/kg), citrus (2 mg/kg), meat and eggs (0.05 mg/kg). Therefore, sensitive and selective methods are required for monitoring 2,4-D residues to guarantee the safety of food. Up to now, the main approaches for the detection of 2,4-D included gas chromatography (GC–MS) [4], high performance liquid chromatography (HPLC) [5], capillary electrophoresis (CE) [6] and liquid chromatography–mass spectrometry (LC–MS) [7].

The complexity of food matrices and contaminants presented in food at low concentration levels require performance analysis only after some clean-up and preconcentration steps. Some routine sample preparation technique, such as liquid–liquid extraction (LLE) [8], solid phase extraction (SPE) [9] and supercritical fluid extraction (SFE) [10] have been proposed for the extraction of 2,4-D. The commonly used solid phase extraction (SPE) and liquid–liquid extraction (LLE) processes are complex and low selectivity. SFE is carried out at elevated temperatures or pressures. Recently, researchers have been oriented towards the development of high selectivity, economical, and miniaturize sample preparation methods.

Molecular imprinting technology is routinely used for the preparation of synthetic polymers with a predetermined specificity [11]. In recent years, molecularly imprinted polymers (MIPs) have been increasingly used for preconcentration and efficient separation of trace compounds in complex matrices [12], due to their high selectivity, reusable, inexpensive to prepare, physiochemical stability and applicability in harsh chemical media. MIPs can be divided into three categories according to their properties. Organic MIPs have the excellent pH stability and the easy availability of various monomers [13]. However, these materials may shrink or swell when exposed to different mobile phases, and the various degrees

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of swelling in different solvents may considerably change the morphology of the polymer network [14,15]. On the other hand, the inorganic MIPs have good optical and mechanical properties and chemically inert, but the inorganic MIPs will inevitably present the cracking and shrinkage and the sol–gel process is difficult to control [16]. Organic–inorganic hybrid molecularly imprinted polymers (HMIPs) were developed in recent years. The HMIPs could successfully overcome the drawbacks of organic MIPs and inorganic MIPs [17]. HMIPs have received considerable attention due to the potential applications in the fields of chromatographic stationary phases [18,19], chiral separation [20,21], solid phase extraction [22,23]. Unfortunately, to the best of our knowledge, HMIPs almost have not been synthesized and applied for the SPME or SPE pretreatment of complex samples. Our team synthesized an organic-inorganic hybrid caffeine imprinted monolith in glass tube, which has been evaluated and applied as the enrichment material of caffeine in children's milk successfully [24].

In this work, a novel organic–inorganic hybrid molecularly imprinted monolith (HMIM) was synthesized in a micropipette tip using 2,4-D as the template for the first time. Several synthesis parameters were optimized. The characterization and selectivity of the monolithic column was investigated. The HMIM could be connected with syringes directly without any other treatment. A method for the determination of 2,4-D in rice using the HMIM microextraction combined with high-performance liquid chromatography-photodiodes array detector was developed. The experimental results indicated that the method can be applied for the rapid, selective and sensitive analysis of 2,4-D in rice samples.

2. Experimental

2.1. Reagents and standards

Ethylene dimethacrylate (EGDMA) purchased from Acros (New Jersey, USA) was extracted with 5% aqueous sodium hydroxide and water, then dried over using anhydrous magnesium sulfate. 2,2′-azobisisobutyronitrile (AIBN) was obtained from Shanghai No.4 Chemical Reagent Corp (Shanghai, China) and recrystallized in anhydrous ethanol before use. 2,4-dichlorophenoxyacetic acid (2,4-D), 4-chlorophenoxyacetic acid (4-CPA) and ibuprofen were purchased from Dikma Technology Inc (Beijing, China). Acrylamide (AM) purchased from Fuchen Chemical Reagent Company (Tianjin, China) was distilled under vacuum prior to use. 3-(methacryloxy) propyltrimethoxysilane (γ-MAPS) and methyltrimethoxysilane (MTMS) were purchased from Aladdin Reagent Inc (Shanghai, China). Methanol and acetonitrile (HPLC grade) were obtained from Tedia Company Inc (Ohio, USA). Phosphoric acid and other reagents used were all of analytical grade. The water used was purified on an Ultrapure Water System (Beijing, China).

2.2. Instrumentation

The chromatographic analysis was carried out on a Dionex Summit U3000 HPLC system equipped with a manual injector and a Photodiode Array Detector (PAD) (Dionex Technologies, USA). A personal computer equipped with a Chromeleon ChemStation program for LC was used to process chromatographic data. A amethyst-C18 column (4.6 mm × 250 mm, 5 μm) from Sepax Technologies Inc. (Newark, USA) was connected with a guard column (cartridge 2.1 mm × 12.5 mm, 5 μm, Agilent Technologies, PaloAlto, CA, USA) filled with the same packing material. The mobile phase was a mixture of acetonitrile –0.04% phosphoric acid solution (45:55, v/v) and the flow rate was 1.0 mL/min. The column temperature was set at 30 °C by a temperature controller (Nuohai Technologies, China). The UV detector was set at a wavelength of 285 nm for analytes. All injections were performed manually with a 20.0 μL sample loop. An LSP01-1A longer pump (Baoding Longer Precision Pump Co. Ltd., China) was used for pumping. 0.22 μm membrane was obtained from Xingya Scavenging Material Company (Shanghai, China). The microscopic morphology of the monolith was examined by a Model X-650 scanning electron microscope (Hitachi, Tokyo, Japan).

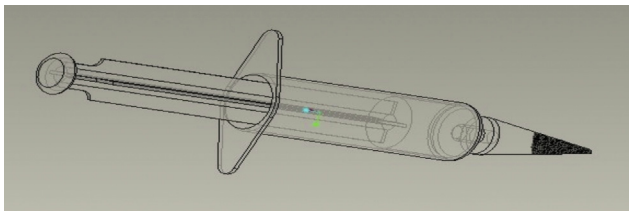

Fig. 1. Scheme of the novel HMIM microextraction device.

2.3. Preparation of molecularly imprinted monolith

Preparation of organic pre-polymer solution: The pre-polymer solution for molecularly imprinted polymer was prepared by dissolving 0.1 mmol of 2,4-D in 1.0 mL of methanol. The solution was mixed thoroughly on a vortex mixer. Then, 0.4 mmol of AM was added and kept at room temperature for 4 h using an ultrasonic method. Then, 2 mmol of cross-linker EGDMA and 12 mg of initiator AIBN were added, and degassed by ultrasonication for about 10 min.

Preparation of inorganic solution: 800 μL of MTMS was dissolved in 300 μL of methanol. Then, 200 μL of HNO_3 was added. The mixture was stirred at 40 °C for 1 h. Next, 150 μL of γ-MAPS was added, and stirred at 40 °C for another 1 h.

The inorganic solution was added to above organic pre-polymer solution as the ratio of 1:3 (v/v). Next, 100 μL of the mixture was transferred in a micro pipette tip which had been sealed at one end with silicon rubber. Subsequently, the other end of the pipette tip was sealed as the same way. After polymerization at 60 °C for 24 h, the silicon rubber at the two sealed-ends was removed. The resultant HMIM was washed with methanol to remove the template molecules.

An organic–inorganic hybrid non-imprinted polymer monolithic column (HNIM) was prepared following the same procedures without 2,4-D in the synthesis.

2.4. Preparation of the extraction device

As shown in Fig. 1, the HMIM could be connected with syringes in different sizes simply without any other treatment. This extraction device was simple and convenient for extraction operation. A syringe infusion pump (Baoding Longer Precision Pump) was employed for the delivery of simple solution, washing solution and desorption solvent.

2.5. Sample preparation

The stock standard solution was prepared in methanol at a concentration of 1.0 mg/mL and stored at 4 °C in refrigerator. Working standard solutions of 2,4-D were prepared by appropriate dilution of the stock solution using deionized water.

3.0 g of chopped rice sample were weighed into a 50 mL glass beaker. Then, 15 mL of acetonitrile was added. Briefly, extraction was performed by sonication, followed by washing with acetonitrile (twice, 10 mL each) during filtration in Buchner funnel. The three extraction fractions were collected to concentrate in rotator-evaporator at 40 °C until dryness. Then, it was reconstituted in