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Analytical Methods

Novel restricted access materials combined to molecularly imprinted polymers for selective solid-phase extraction of organophosphorus pesticides from honey

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ABSTRACT

A novel restricted access materials (RAM) combined to molecularly imprinted polymers (MIPs), using malathion as template molecule and glycidilmethacrylate (GMA) as pro-hydrophilic co-monomer, were prepared for the first time. RAM-MIPs with hydrophilic external layer were characterized by scanning electron microscopy and recognition and selectivity properties were compared with the restricted access materials-non-molecularly imprinted polymers (RAM-NIPs) and unmodified MIPs. RAM-MIPs were used as the adsorbent enclosed in solid phase extraction column and several important extraction parameters were comprehensively optimized to evaluate the extraction performance. Under the optimum extraction conditions, RAM-MIPs exhibited comparable or even higher selectivity with greater extraction capacity toward six kinds of organophosphorus pesticides (including malathion, ethoprophos, phorate, terbufos, dimethoate, and fenamiphos) compared with the MIPs and commercial solid phase extraction columns. The RAM-MIPs solid phase extraction coupled with gas chromatography was successfully applied to simultaneously determine six kinds of organophosphorus pesticides from honey sample. The new established method showed good linearity in the range of 0.01–1.0 μg mL^{-1}, low limits of detection (0.0005–0.0019 μg mL^{-1}), acceptable reproducibility (RSD, 2.26–4.81%, $n = 6$), and satisfactory relative recoveries (90.9–97.6%). It was demonstrated that RAM-MIPs solid phase extraction with excellent selectivity and restricted access function was a simple, rapid, selective, and effective sample pretreatment method.

1. Introduction

Organophosphorus pesticides (OPPs) are an important source of environmental and food contamination due to their extensive use for fighting the enormous number of crop-eating insects in agriculture (Zhao et al., 2011). The majority of OPPs and their residues have high acute toxicity when absorbed by human organisms because they are acetylcholinesterase inhibitors and exposure to high levels can lead to acute food poisoning (Patel, Fussell, Macarthur, Goodall, & Keely, 2004). In order to avoid potential human exposure to OPPs residues via drinking water and food, the effective method to degrade and remove OPPs residues for health purposes is badly needed. Many methods have been developed in the last few years for the determination of OPPs. Liquid chromatography (LC) (Topuz, Ozhan, & Alpertunga, 2005), LC–mass spectrometry (LC–MS) (Liu, Hashi, Song, & Lin, 2005), gas chromatography (GC) (Guan, Wang, Xu, & Guan, 2008), and GC–MS (Rodrigues et al., 2011) are among the most common determination techniques following solid-phase extraction (SPE) (Ballesteros & Parrado, 2004), solid-phase microextraction (SPME) (Lambropoulou & Albanis, 2003), liquid-phase microextraction (LPME) (Bidari, Ganjali, Norouzi, Hosseini, & Assadi, 2011; Khalili, Yamini, Yazdanfar, & Shariati, 2008) or liquid–liquid microextraction (LLME) (Naeeni, Yamini, & Rezaee, 2011) of the sample. However, these pretreatment methods have no selectivity for the target in the complex matrices.

In recent decades, the molecularly imprinted technique has been known as an accurate, selective and sensitive pretreatment method for detecting trace amounts of chemicals. The

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development and application of molecularly imprinted polymers (MIPs), with highly specific recognition ability for target molecules, have attracted significant attention. Although the MIPs exhibit efficient extraction and high selectivity for a compound or class of compounds, they cannot simultaneously discard macromolecules, such as proteins and lipids which are strongly adsorbed to the surfaces of MIPs by hydrophobic interaction, adversely interfering with recognition properties of MIPs (Bures, Huang, Oral, & Peppas, 2001; Mullett & Pawliszyn, 2003).

Restricted access material (RAM) is porous chromatographic supports specifically designed for the removal of macromolecules, partially based on the size-exclusion mechanism that is a hydrophilic barrier enables the small molecules to permeate into the hydrophobic pores of the stationary phase, while it excludes the macromolecules (Sadilek, Satinsky, & Solich, 2007).

MIPs have specific recognition sites for the small molecule, while RAM can exclude large molecules. So it is ideal to combine the characteristics of MIPs with RAM for extraction of trace target molecules with low molecular mass in real samples. The RAM-MIPs has applied in the biological, environment and food sample analysis (Haginaka, Takehira, Hosoya, & Tanaka, 1999; Paula, Pietro, & Cass, 2008; Sambe, Hoshina, & Haginaka, 2007; Xu et al., 2010). Puoci et al. reported on synthesis of RAM-MIPs for the selective recognition and the controlled/sustained release of *p*-acetaminophenol and the applicability of this kind of materials as Drug Delivery System (Puoci et al., 2009).

To the best of our knowledge, there have been no reports on the use of RAM-MIPs for the determination of OPPs as applied in food samples.

In the present study, the purpose was to develop a simple, rapid, efficient and sensitive sample pretreatment method for enriching, separating, and determining OPPs (ethoprophos, phorate, terbufos, dimethoate, malathion, and fenamiphos) using a novel prepared RAM-MIPs with both high selectivity and restricted access function as a sorbent in SPE of food samples through GC-FPD.

2. Experimental

2.1. Chemicals and standards

All the reagents used were of analytical grade or HPLC-grade. Standard solutions of malathion, ethoprophos, phorate, terbufos, dimethoate, and fenamiphos were provided by Agro Environmental Protection Institute of the Ministry of Agriculture (Tianjin, China). Methacrylic acid (MAA) was purchased from the Sinopharm Chemical Reagent Company (Shanghai, China). MAA was purified by distillation to remove inhibitors. Ethylene glycol dimethacrylate (EDMA) and Glycidilmethacrylate (GMA) was purchased from J&K Chemical, Chemical Reagent Company (Beijing, China). The initiator, 2,2-azoisobutyronitrile (AIBN), was ordered from Kemiou Chemical Reagent Company (Tianjin, China) and recrystallized using methanol prior to use. Double distilled water was used in the current study. Methanol, alcohol, acetonitrile (Fisher Scientific, U.S.A.), n-hexane (TEDIA Company, U.S.A.) and acetone (Mallinckrodt Baker, Inc., U.S.A.) were HPLC-grade. C18 and Florisil SPE were purchased from Waters Associates. Inc. (U.S.A.).

Standard solutions (10.0 mg mL^{-1}) for each compound were stored in a freezer. Diluted solutions were prepared daily from the stock solutions.

2.2. GC analysis

The GC analyses were carried out using an Agilent 7890 GC (Agilent Technologies, U.S.A.) equipped with a flame photometric detector (FPD). Separations were performed on a 30 m × 0.25 mm i.d., 1.4 μm film thickness HP-5 column (Agilent Technologies, U.S.A.). The conditions of the instrument were as follows. Injector temperature was at 250 °C. Nitrogen was used as carrier gas at a constant flow of 3.0 mL min^{-1}. Column temperature program was set at an initial temperature at 150 °C, then increased at an increment of 10 °C min^{-1} until the temperature reached 240 °C, held for 11 min. The total running time was 20 min. The sample was injected at the splitless mode. The separated species were measured by FPD held at 250 °C.

2.3. Synthesis of molecularly imprinted microspheres (MIMs) and non-imprinted microspheres (NIMs)

The MIMs were prepared by precipitation polymerization. For a general polymerizing procedure, template molecule malathion (0.268 mL, 1.0 mmol), functional monomer MAA (0.509 mL, 6.0 mmol) were dissolved in 40 mL chloroform in a 250 mL round bottomed flask. After pre-polymerization for 5 h, the cross-linking monomer EDMA (5.642 mL, 30.0 mmol) and initiator AIBN (0.096 g) and 120 mL chloroform were added into the flask. The solution was degassed in an ultrasonic bath for 10 min and purged with nitrogen in an ice bath for 5 min to remove the oxygen. The flask was then attached to the oil bath and the mixture was continuously stirred during the polymerization with a magnetic stirrer at about 150 rpm. The polymerization reaction was carried out at 70 °C for 10 h. After that, the template was removed via Soxhlet extraction with methanol–acetic acid (9:1, v/v) until no template molecule was detected, followed by a final wash in methanol for 8 h to remove the print molecule and then dried in vacuo overnight at 25 °C. Corresponding NIMs were prepared using the same protocol but with no template.

2.4. Synthesis of restricted access material-molecularly imprinted microspheres (RAM-MIMs)

RAM-MIMs were prepared by two steps. The first step was as exactly the same synthetic routes as the MIMs and NIMs, including the addition of the co-monomer GMA (0.199 mL, 6.0 mmol). The molar ratio of Malathion, MAA, GMA and EDMA was 1:6:6:30, with adding AIBN (0.146 g) in chloroform. The second step was breaking the epoxide ring. In a 150 mL round bottom flask, 800 mg of RAM-MIMs and RAM-NIMs were added to 50 mL of a perchloric acid water solution (1:9, v/v). The flask was then agitated (150 rpm) for 24 h at room temperature. After reaction, the particles were filtered, washed with 200 mL of ethanol, 200 mL of acetone, 200 mL of diethyl ether and then dried under vacuum overnight at 25 °C. RAM-NIMs were also prepared in the similar manner described as above, except for the absence of template.

2.5. Binding experiments

To evaluate the binding capacity of the MIMs, NIMs, RAM-MIMs and RAM-NIMs, static adsorption tests were carried out. In a centrifuge tube, particles (20 mg) were incubated with 4 mL malathion solution of various concentrations (0–50 μg mL^{-1}) at room temperature for 20 h. After centrifugation, the amounts of the template bound to the particles at binding equilibrium (Q) were determined by GC. The adsorption capacity was calculated using the following equation: $Q = (C_0 - C)V/M$, where C_0 and C (μg mL^{-1}) was the initial concentration and the residual concentration of the malathion, respectively, V (mL) was the volume of the initial solution, and M (g) was the amount of the particles.