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A molecularly imprinted polymer for the selective solid-phase extraction of dimethomorph from ginseng samples

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ABSTRACT

A molecularly imprinted polymer (MIP) was synthesized and evaluated to selectively extract dimethomorph from ginseng samples. Dimethomorph molecularly imprinted polymers with template to monomer molar ratios were contrived and developed via precipitation polymerization employing methacrylic acid as functional monomer, ethylene dimethacrylate as cross-linker and butanone:N-heptane (7:3, v:v)as porogen. The LOD (limit of detection) of this method was 0.002 mg kg^{-1}, and the LOQ (limit of quantification) was 0.005 mg kg^{-1}. The different spiked level of ginseng was 0.1 mg kg^{-1}, 1.0 mg kg^{-1}, 5.0 mg kg^{-1}, and the average recovery of dimethomrph was 89.2–91.6%. Under the optimized condition, good linearity was obtained from 0.01 to 5 mg kg^{-1} ($r^2 \geq 0.9997$) with the relative standard deviations of less than 3.20%. This proposed MISPE-GC procedure eliminated the effect of template leakage on quantitative analysis and could be applied to direct determination of dimethomrph in ginseng samples.

1. Introduction

Dimethomrph (4-[3-(4-chlorophenyl)-3-(3, 4-dimethyloxyphenyl) propenyl] morpholine) is a kind of morpholine fungicide developed by BASF. [1] It is cinnamic acid analogs, concentrated fungicide. It is mainly used for preventing and curing plant disease like Plasmopara viticola, Phytophthora infestans and Pseudoperonospora cubensis. Dimethomrph is mainly applied on grapevines, apples, ginsengs, tomatoes, potatoes, cucumbers, Chinese cabbage and other crops [2].

Molecular imprinting is a versatile and facile method for preparing synthetic polymers with predetermined molecular recognition properties and is presently attracting widespread interest, especially as the technological potential of molecularly imprinted polymers (MIPs) in chromatographic separations [3], solid-phase extraction [4] and catalysis [5] has now been established. Among these methods, precipitation polymerization is the simplest and the most efficient because it does not require any surfactant or interfering additives to be used. Molecular imprinting is considered as an elegant and convenient technology that can introduce special recognition and binding sites in imprinted materials, which are chemically and geometrically complementary to the template [6]. Compared to biological counterparts, molecularly imprinted polymers (MIPs) are more stable, less costly, and easier to produce [7]. Their use as sorbent material for SPE is one of the most exciting applications of MIPs because it would provide a simple and effective pretreatment method for complex samples. The traditional MIPs for compound analysis are prepared by using one kind of compound as template [8–11], which may be influenced by template leaking when using MIPs as SPE sorbents. As the filler of solid phase extraction, the MIP was get into solid phase extraction column [12,13]. Its application was evaluated.

The aim of the present work was to demonstrate the feasibility of using a molecular imprinting solid phase extraction (MISPE) cartridge for the selective clean-up and quantification of trace amounts of dimethomrph in ginseng samples. Dimethomorph molecularly imprinted polymers with template to monomer molar ratios were contrived and developed via precipitation polymerization employing methacrylic acid as functional monomer, ethylene dimethacrylate as cross-linker and butanone and N-heptane as porogen. The synthesized MIP enabled direct determination of the target compound. Combination of gas chromatography with MIP-SPE could be successfully used for quality control of pesticide residues. The experimental results indicated that: the extracts of ginseng could be effectively separated by MISPE, a high degree of cleaning up and acceptable recoveries were obtained. The

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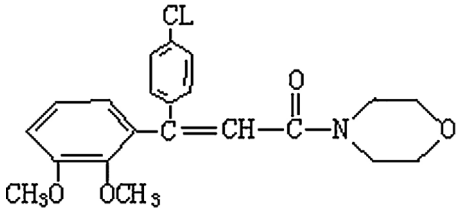

Fig. 1. Chemical structure of dimethomorph.

application of MISPE in pre-treatment of dimethomrph was further developed in the thesis and provided a new way in samples analysis and detection. Following a conventional non-covalent imprinting protocol, several binding rebinding parameters have been evaluated in an attempt to extract selectively dimethomrph from ginseng samples.

2. Experimental

2.1. Materials and chemicals

The active compound of dimethomorph (97.6%) was purchased from BASF SE (Fig. 1). Chromatographic grade acetonitrile (ACN), Azobiisobutyronitrile (AIBN), Methacrylic acid (MAA) and Ethylene dimethacrylate (EDMA) were purchased from Beijing Chemical Factory (Beijing, China). Inhibitor in MAA was removed by cleanup on activated alumina columns. EDMA was extracted with 25% aqueous sodium hydroxide three times to remove the inhibitor prior to use. AIBN was recrystallized from methanol before use. Methanol, Acetic acid, Butanone, N-heptane and Tetrahydrofuran (THF) were all analytical grades and obtained from Beijing Chemical Factory (Beijing, China). Water was doubly distilled.

2.2. Apparatus and chromatographic conditions

Gas chromatographic analysis was performed on an Aglient GC system, model Aglient 6890 N, equipped with micro-electron capture detector (μ-ECD) and HP-1 capillary column (30 m × 0.25 mm i.d. × 0.25 μm). A 1 μL aliquot of the standard/sample was injected in the splitless mode and analyzed under the following conditions. The initial temperature of the column was maintained at 120 °C for 1 min, raised to 260 °C at 20 °C min^{-1} and maintained at 260 °C for 22 min. The injector, detector temperatures were 280 °C and 280 °C. The pre-polymerizations were detected by Ultraviolet spectrometry (UV-2450, SHIMADZU, Japan) and Fourier-transform infrared spectrometry (FTIR-IRAffinity-1, SHIMADZU, Japan). The morphology of MIPs was characterized by scanning electron microscopy (SSX-550, SHIMADZU, Japan).

2.3. Preparation of pre-polymerisation

0.017 mmol L^{-1} of dimethomorph and a series of various concentrations of MAA (0.034 mmol L^{-1}, 0.068 mmol L^{-1}, 0.102 mmol L^{-1}, 0.136 mmol L^{-1}) were prepared in butanone:N-heptane (7:3, v:v) solution, then dimethomorph solution mixed in identical volumes respectively. The pre-polymerisations were kept in water bath at 30 °C for 5 h and then placed in the refrigerator (°C) overnight. The pre-polymerisations were scanned by UV spectroscopy. Dimethomorph were prepared separately in THF. Then dimethomorph solution mixed with equivalent volumes of various concentration of MAA for preparation of different pre-polymerisations. The pre-polymerisations were treated in the same manner as described above. The IR spectra of pre-polymerisations in THF were detected by a KBr crystal demountable assembly.

2.4. Preparation of polymers

The pre-polymerisations were incubated in a bath at 30 °C for 5 h to prearrange dimethomorph and MAA. EDMA and AIBN were added sequentially to the solutions, the solutions were oscillated to make them uniform. The pre-polymerisations then purged with a gentle flow of nitrogen for 10 min. The polymerization was initiated at 60 °C for 24 h. The generated polymeric particles were gathered by centrifugation at 4000 rpm for 10 min. To remove the template bound within the polymer matrix, the resulting powder was washed for 30 min with a mixture of methanol and acetic acid (9:1, V: V). The washing procedure was repeated until no more dimethomorph could be detected in the washing solution. These MIPs were then washed with methanol to eliminate residual acetic followed by acetonitrile three times. And corresponding nonimprinted polymers were synthesized at the same experimental conditions by omitting the template and treated in an identical manner as described above. Finally, the MIPs and NIPs were dried under vacuum at 40 °C overnight. Before use, each cartridge was activated by treatment with the same solvent used in the loading step.

2.5. Characterization of polymers

The morphology of the produced polymer particles was analyzed by scanning electron microscopy. The samples were prepared in methanol and sputtered a thin gold film prior to measurement in SEM. The structure of the polymers was characterization by FTIR spectroscopy in the range of 4000–500 cm^{-1} by KBr pellet method.

2.6. Equilibrium adsorption experiment

For adsorption isotherm studies, 20 mg of polymer was placed in an Erlenmeyer flask containing 50 mL of dimethomorph (20–200 mg L^{-1}) prepared in acetonitrile/water (7:3, V:V). The solution was shaken for 24 h at room temperature at a speed of 150 rpm. Upon equilibration, all samples were filtered through a 0.22 μm filter to minimize the interference of particles during analysis. The residual concentration of dimethomorph was analyzed by GC. The amount of dimethomorph was determined from the difference in concentrations at the beginning and at the end of each batch test. Before analysis, 10 mL of rinse solution was loaded onto the conditioned MIP cartridge. After a washing step with 1 mL acetonitrile, dimethomorph was recovered with 1 mL
methanol.

2.7. Solid phase extraction (SPE) studies

In order to establish the optimum conditions under which the template can be recognized by the corresponding MIP, a standard solution of dimethomorph was initially prepared in various proportions (10–100%) of ACN/H_2O and DMF/H_2O mixture. To a 3 mL empty polypropylene solid phase extraction cartridges, 200 mg corresponding control polymer NIP was packed between two polypropylene frits. Before analyte loading, the polymer was conditioned with 1 mL methanol, 1 mL acetonitrile and 1 mL H_2O. In the loading step, 2 mL of dimethomorph solution (20 mg L^{-1}) prepared in a mixture of ACN/H_2O (7:3, V:V) was passed through the cartridge. Finally, dimethomorph was eluted with 1 mL of methanol. Ginseng powder samples were diluted with acetonitrile and were then filtered through a 0.22 μm syringe filter and were kept in the freezer until their use. Before analysis, 10 mL of ginseng samples were loaded onto the conditioned MIP cartridge. After a washing step, dimethomorph was recovered with methanol.