Membrane-Protected Molecularly Imprinted Polymer for the Microextraction of Indole-3-butyric Acid in Mung Bean Sprouts

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Supporting Information

ABSTRACT: Based on the hollow fiber protected molecularly imprinted polymer, a micro-solid-phase extraction (µ-SPE) method was developed and applied for the analysis of indole-3-butyric acid in mung bean sprouts by high-performance liquid chromatography. The extraction conditions of the µ-SPE method were optimized using L9(3⁴) orthogonal, and optimum conditions were found as follows: pH of sample solution was 2.0, chloroform was the organic solvent for embedding the µ-SPE bars, and acetonitrile was the desorption solvent. In addition, the extraction time was 80 min, desorption time was 5 min, stirring speed was 800 rpm, and concentration of NaCl was 10%. Under the optimum conditions, a standard curve was established for IBA, with a correlation coefficient of 0.9999. After extraction with phosphate buffer solution (pH = 9.0), successful pretreatment of mung bean sprouts was achieved by the µ-SPE method. The limit of detection was 0.075 mg/kg, and the recoveries were found to be in the range of 88.9–106.4%. This method is simple, environmentally friendly, and can be used for the determination of indole auxin contents in green bean sprouts quickly and accurately.

1. INTRODUCTION

Plant hormones are present at low concentrations in plant tissues but play an important role in regulating many aspects of plant growth.1 Auxins are the first of the major plant hormones to have been discovered. The auxins including indole-3-acetic acid (IAA), indole-3-propionic acid (IPA) and indole-3-butyric acid (IBA) are involved in many aspects of plant growth and development.2 IBA promotes rooting and is found to be highly effective compared to other auxins. Its concentration level in the presence of a template molecule.3,4 MIPs can selectively adsorb a target molecule in preference to other closely related compounds.5,6 MIPs was believed to be in SPE.7 Using µ-SPE, the amounts of sorbent, desorption solvent, and sample volume decreased, resulting in lower cost of the procedure.8–10 Since the solid sorbent is packed inside a membrane, which is often hydrophobic such as polypropylene hollow fibers,11–13 the direct contact of the sorbent with the sample matrix is avoided. Hollow fibers are widely used in liquid-phase microextraction (LPME). Its small volume (dozens of microliters) enables concentration of the target analytes. In addition, sample extraction and filtration are achieved simultaneously in a single step owing to the micropores (0.2 μm) on the fiber wall.14,15 Hence, complex samples can be easily treated without filtration.16 Moleculary imprinted polymers (MIPs) are synthetic materials obtained by copolymerization of functional and cross-linking monomers in the presence of a template molecule.17–19 MIPs can selectively adsorb a target molecule in preference to other closely related compounds.20,21 MIPs have superior specific recognition to other sorbents used in SPE. The most advanced application of MIPs was believed to be in SPE.22

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In this study, polypropylene hollow fibers were packed with MIP particles and formed microextraction bars. Tubular hollow fibers were used to protect the MIP rather than a bag made of polymeric membranes. The main purpose is to minimize the volume. A molecular imprinting μ-SPE (MI-μ-SPE) method based on the microextraction bars was proposed. This method takes advantages of both SPE and LPME, as shown in Figure 1. More importantly, it can overcome the poor recognition of MIPs in aqueous solutions. The proposed method integrated the processes of SPE and LPME in a single step and improved extraction efficiency. The microextraction bars were successfully used for the extraction of IBA in the mung bean sprouts and further determined by HPLC.

2. RESULTS AND DISCUSSION

2.1. SEM Characterization. Polypropylene hollow fibers, with an inner diameter, wall thickness, and pore size of 600, 200, and 0.2 μm, respectively, were cut into pieces of 2 cm length (Figure 2A,C). Subsequently, one end of the segment was sealed with flame, and the other end allowed packing of polymer particles. The MIP particles of 38−75 μm were sonicated for 5 min in ethanol and formed a slurry. Then the slurry was filled into the lumen of hollow fiber capillary, which was 2 cm in length, with the help of a hypodermic needle. The outer diameter of the needle is about 0.5 mm. The slurry was passed by a syringe attached to the needle and sieved by the tubular membranes. Ethanol passed through the pores of the membrane leaving the polymer particles inside the lumen, and subsequently, they filled up the hollow fibers. The microextraction bars were prepared after sealing other ends of the fibers by flame (Figure 2B,D), and each bar contains about 1.2 mg of polymer particles. Then the extraction bars were dried for 5 min and impregnated with a nonpolar organic solvent before use. Then two pieces of extraction bars were transferred into 4 mL of sample solution and magnetically stirred. After extraction, the bars were immersed in 2 mL of acetonitrile for elution of the analyte. The eluent solution was analyzed by HPLC. Finally, hollow fiber bars were cleaned and conditioned for the next extraction.

2.2. Imprinting Effectivity and Selectivity. For the preparation of microextraction bars, MIP for IBA was designed and synthesized. The MIP was prepared using vinylimidazole as the functional monomer and ethylene glycol dimethacrylate as the cross-linker. In our previous work,2 the recipe of the imprinted polymer was optimized concerning the functional monomer, cross-linker, and solvent in view of the molecular recognition of IBA. The MIP and nonimprinted polymer (NIP) obtained were investigated through breakthrough experiments using SPE cartridges, as shown in Figure 3. MIP presented higher binding capacity (1.13 mg/g) to IBA than NIP (0.87 mg/g) did, and the polymer showed an obvious imprinting effect in chloroform. The MIP and NIP particles were packed into an HPLC column (50 × 4.6 mm), and retentions of IBA and its analogues (IAA, IPA and tryptamine) were examined by HPLC. The selectivities of the polymers were also evaluated by comparing the retention times of IBA and analogue compound (Supporting Information, Table S1). It can be observed that the MIP has high selectivity to IBA.

2.3. Evaluation and Optimization of MI-μ-SPE Bars. In nonpolar solvents, MIPs usually show good imprinting effects but almost no efficiency in aqueous solutions. MIPs give the best recognition in polymerization solvents such as toluene, chloroform, and acetonitrile. So, microextraction bars prepared with MIP particles were immersed into nonpolar solvents before applying to the aqueous sample. Here, the different organic solvents were investigated for pretreatment of microextraction bars, as shown in Figure 4. The highest binding percentage was achieved with chloroform as shown in Figure 4A. Compared to other hydrophobic solvents, chloroform showed much higher binding to IBA. All these solvents can extract IBA from aqueous solution. Without organic solvents, the binding percentage was the lowest and much...
lower than that with organic solvents. This indicated that liquid-phase extraction has an important contribution to the overall extraction. MIPs usually have the best binding in the polymerization solvent. Chloroform was used as the solvent for preparation of the MIP against IBA. That is why the best binding was achieved in chloroform. It can be proven that there is the synergic extraction of LPME driven by chloroform and SPE driven by the MIPs. So, chloroform was chosen as the extraction solvent for further studies. Compared to liquid–liquid extraction, LPME and SPE, this method consumes much less organic solvent (about 50 μL). That is negligible and still can be regarded as a green extraction process, although chloroform was used here.

For quantitative analysis, it was required to desorb IBA from polymer extraction bars with a suitable solvent. Polar solvents such as methanol, methanol/acetic acid (9/1), acetonitrile, and ethanol were investigated, and the highest desorption rate (88%) was achieved with acetonitrile (as shown in Figure 4B). It was also found that 5 min of desorption time was enough to obtain satisfactory results. The extraction bars can be completely recovered with acetonitrile and reused. The results of repeated experiments showed that the extraction bars can be used over 30 times without any obvious loss of extraction efficiency.

2.4. Orthogonal Experiment Analysis. In order to effectively utilize imprinted polymer extraction bars for the clean-up of IBA from green bean sprouts, the extraction conditions were investigated by the L9(34) orthogonal experiment. The conditions include extraction time, rotation speed, pH, and concentration of salt (NaCl), as listed in Table 1. It can be seen from the analysis results (Supporting Information, Table S2) that contributions of the factors to the extraction are in the following order: extraction time (A) > rotation speed (B) > NaCl concentration (D) > pH (C). It was found that the optimum conditions were as follows: extraction time was 80 min, rotation speed was 800 rpm, sample pH was 2.0, and NaCl concentration was 10%. Originally, we expect to have better mass transfer due to the small diameter of the extraction bar. However, the result indicates that it needs a long time of extraction. That is because of poor mass transfer inside the fiber probably caused by restriction of very small space.

2.5. Analytical Performance and Application to Real Sample. Analytical performance and practical application of the proposed method were investigated using mung bean sprouts as a real sample. The auxin samples were collected and extracted using the method described in our previous work. A calibration curve was established using HPLC for the quantification of the eluents obtained from extraction bars (Supporting Information, Figure S1). The real samples were spiked within the range of 5–20 mg/L (as shown in Table 2).

Table 1. Orthogonal Layout Design

| level | extraction time (A) (min) | rotation speed (B) (rpm) | pH (C) | NaCl concentration (D) (%) |
|-------|--------------------------|--------------------------|--------|---------------------------|
| 1     | 40                       | 600                      | 2      | 0                         |
| 2     | 60                       | 800                      | 4      | 5                         |
| 3     | 80                       | 1000                     | 6      | 10                        |

Table 2. Recoveries of IBA for Green Bean Sprout Samples Spiked at Three Different Concentration Levels

| added (mg/L) | found (mg/L) | RSD (%) | recovery (%) |
|--------------|--------------|---------|--------------|
| 0            | 0.23         | 2.22    |              |
| 5            | 5.32         | 1.96    | 106.4        |
| 10           | 10.17        | 2.13    | 101.7        |
| 20           | 17.79        | 1.86    | 88.9         |

Table 3. Comparison of Analytical Performances of Different Methods for the Determination of IBA in Green Bean Sprout Samples

| method       | LOD (μg/kg) | RSD (%) | time (min) | recovery (%) | ref       |
|--------------|-------------|---------|------------|--------------|-----------|
| HF-LPMEa     | 0.570       | <4.8    | 60         | 88.6–100.7   | 29        |
| HILIC-SPE    | 0.010b      | <14.4   | 45         | 80.5–119.2   | 31        |
| MI-SPMEc     | 0.150       | <7.4    | 260        | 91.5–97.5    | 2         |
| MI-μ-SPE     | 0.075       | <2.13   | 80         | 88.9–106.4   | this work |

aHF: hollow fiber. bμg/kg. cMI-SPME: molecular imprinting solid-phase microextraction. dTime for extraction.
selective. In such work, pollen grains were used as hydrophilic sorbents for simultaneous determination of 16 plant growth regulators. However, the MIP not only has high specific binding to IBA but also has good selectivity to its analogues. Combined with HPLC, it can give high extraction of the auxins. In addition, this material is very useful for the fast measurement of individual IBA combined with the spectrophotometric method, typically where IBA is used alone for promoting plant growth.

3. CONCLUSIONS

In conclusion, in this work, molecular imprinting μ-SPE was developed that combines the excellent features of both liquid—liquid and solid-phase extractions. The new approach also effectively solved the poor recognition of MIPS in the aqueous phase. This method also allowed selective extraction of the auxin from green bean sprouts and sensitive quantification by HPLC. In addition, it has advantages of being simple, miniature, low consumption of organic solvents, and high reproducibility.

4. EXPERIMENTAL SECTIONS

4.1. Polymer Preparation. The rational design approach was applied for the preparation of MIPs. Selection processes of the functional monomer, cross-linker, and solvent were reported in our previous work. In brief, MIP and NIP were prepared according to the following procedure: 0.203 g (1 mmol) of template (IBA) and 0.376 g (4 mmol) of vinylimidazole (VI) were dissolved in 3.548 g of 5 N dimethylformamide (DMF) in a screw-capped glass vial. The mixture was sonicated for 5 min followed by the addition of 3.172 g (20 mmol) of ethylene glycol dimethacrylate (EGDMA) as the cross-linker and 0.071 g of initiator (AIBN). The polymer solution was sonicated for 5 min and purged with N2 for 10 min and then sealed. After that, the solution was polymerized at 80 °C for 24 h in an oil bath. The bulk polymer was ground and sieved to yield particles of 38–75 μm. Finally, the polymer particles were washed with methanol/acetic acid (9/1, v/v) and methanol. The same method was used to prepare NIP in the absence of templates (IBA).

4.2. Breakthrough Experiment. For the breakthrough experiments, MIP and NIP (100 mg) were packed in a 1 mL SPE column. The SPE column was activated with 5 mL of chloroform. The template IBA chloroform solution (60 mg/L) was loaded into the SPE column with a flow rate of 1 mL/min. Each extract was analyzed by UV–vis spectrophotometers at 280 nm. Finally, the adsorption amount of IBA was calculated by HPLC.

4.3. Preparation of MI-μ-SPE Bars. The hollow fibers were cut into pieces of 2 cm length and ultrasonicated with acetone for 20 min. Subsequently, one of the ends of the segment was sealed with flame, and the other end allowed packing of polymer particles in the form of slurry (carefully weighed, m1). The MIP particles were homogenized by sonication with ethanol for 5 min. Then the slurry was filled into the lumen of hollow fiber capillary, and the excess ethanol leaked through the pores of the membrane leaving the polymer particles inside the lumen. After being packed, the other end was also sealed by flame and dried for 5 min. Finally, hollow fiber bars were carefully weighed (m2), and the packing amount was calculated according to m1 and m2.

4.4. Pretreatment of Mung Bean Samples. One hundred grams of mung bean was put in a 200 mL beaker and washed with distilled water. Under ambient temperature and humidity, water was changed regularly 2–3 times per day. After three days, the grown bean sprouts were collected and stored at −20 °C. 20 mL of NaH2PO4·H2PO4 buffer solution (pH = 9.0) was added into 5 g of mung bean sprouts and kept in the dark overnight at 4 °C. The extraction solution was centrifuged (9000 rpm, 5 min) by high-speed centrifugation. The pH of the supernatant was adjusted to the desired value. Each μ-SPE device was conditioned by ultrasonication in chloroform for 30 s. Then, 4 mL of sample solution (0.1 mg/L NaCl, pH = 2) was added into the glass vial for extraction. Finally, the conditioned hollow fiber bars were transferred to 4 mL of sample solution and stirred for 80 min at 800 rpm. After extraction, the bars were washed with water twice. Then they were immersed in 2 mL of acetonitrile stirred for 5 min at 800 rpm to desorb the analyte. The desorption solution was analyzed by HPLC. Finally, hollow fiber bars were cleaned and conditioned for the next extraction.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b01550.

Table S1: Retention factors (k), imprinting factors (IFs), and selectivity factors (α) of IBA and its analogues on MIP and NIP; Table S2: Results of orthogonal experiment L9(34); Figure S1: HPLC chromatograms of IBA standard solutions λ = 281 nm; concentrations were 1, 2.5, 5, 10, 20, 50 mg/L; (inset) calibration curve: R² = 0.9999, y = 836.5356x − 5.8531; Figure S2: HPLC chromatograms of real samples were spiked with 5, 10, 20 mg/L IBA (PDF)

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Notes

The authors declare no competing financial interest.

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