Journal of Chromatography A, 1217 (2010) 6455–6461

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Preparation of styrene-co-4-vinylpyridine magnetic polymer beads by microwave irradiation for analysis of trace 24-epibrassinolide in plant samples using high performance liquid chromatography

Zhuomin Zhang, Yi Zhang, Wei Tan, Gongke Li*, Yuling Hu

School of Chemistry and Chemical Engineering, Sun Yat-sen University, Guangzhou 510275, China

ARTICLE INFO

Article history:
Received 13 April 2010
Received in revised form 8 August 2010
Accepted 23 August 2010

Keywords:
24-Epibrassinolide
Styrene-co-4-vinylpyridine
Magnetic polymer bead
Microwave irradiation
High performance liquid chromatography
Plant

ABSTRACT

In the study, a kind of novel styrene-co-4-vinylpyridine (St-co-4-VP) porous magnetic polymer beads was prepared by microwave irradiation using suspension polymerization. Microwave heating preparation greatly reduced the polymerization time to 1 h. Physical characteristic tests suggested that these beads were cross-linking and possessed spherical shape, good magnetic response and porous morphologies with a narrow diameter distribution of 70–180 μm. Therefore, these beads displayed the long-term stability after undergoing 100-time extractions. Then, an analytical method for the determination of trace 24-epiBR in plant samples was developed by magnetic polymer bead extraction coupled with high performance liquid chromatography–fluorescence detection. St-co-4-VP magnetic polymer beads demonstrated the higher extraction selectivity for 24-epiBR than other reference compounds. Linear range was 10.00–100.0 μg/L with a relative standard deviation (RSD) of 6.7%, and the detection limit was 6.5 μg/kg. This analytical method was successfully applied to analyze the trace 24-epiBR in cole and breaking-wall rape pollen samples with recoveries of 77.2–90.0% and 72.3–83.4%, respectively, and RSDs were less than 4.1%. The amount of 24-epiBR in real breaking-wall rape pollen samples was found to be 26.2 μg/kg finally. This work proposed a sensitive, rapid, reliable and convenient analytical method for the determination of trace brassinosteroids in complicated plant samples by the use of St-co-4-VP magnetic polymer bead extraction coupled with chromatographic method.

1. Introduction

Brassinosteroids (BRs) have been considered as a new sixth class of plant hormones with wide occurrence in plant samples [1,2]. More than 50 BRs have been found from 61 plants since 1972 [3]. BRs have crucial biological effects on plant growth and development [4] and the ability to protect plants from various environmental stresses [5]. Trace 24-epibrassinolide (24-epiBR) in plant samples is one of crucial BRs and displays the high activity compared to natural BRs in several bioassays [6]. Recently, 24-epiBR has been proved to possess the effect on growth parameters, metal uptake and accumulation in Brassica juncea L. seedlings [7].

Owing to trace amounts of BRs in the complicated plant matrix and their non-volatile and polar properties, it is imperative to develop a sensitive method for the analysis of BRs including efficient sample preparation and detection technique. Until now only simple solvent distillation is commonly used in many previous reports for the analysis of BRs [8]. Conventional solvent extraction always requires long extraction time, large amounts of solvent and multiple steps. Magnetic polymer beads are one of the most popular materials and have been applied to bio-separation of proteomics [9,10], catalysis [11], immobilizing bioactive agents [12], drug delivery [13], resonance imaging contrast enhancement [14], diagnostic analysis [15] and so on. After extraction, magnetic polymer beads can be quickly separated from the solution by a simple and cheap magnet [16,17].

Various methods have been developed to prepare magnetic polymer beads, such as grafting method, emulsion polymerization, precipitation polymerization, suspension polymerization and dispersion polymerization [18–20]. However, these methods have some natural drawbacks, including broad size distribution, rough surface, low magnetism and low mechanical strength [21] due to the uneven heating and secondary nucleation during preparation [22]. It is well known that conventional methods for polymerization of magnetic polymer beads are mainly based on conventional heating and UV light [23]. To date there have been still few reports focusing on the application of microwave irradiation in bulk poly-

* Corresponding author at: Sun Yat-sen University, Institute of Analytical Sciences, School of Chemistry and Chemical Engineering, Xingang West Road 135, Guangzhou, Guangdong 510275, China. Tel.: +86 20 84110922; fax: +86 20 84115107.
E-mail address: cesgkl@mail.sysu.edu.cn (G. Li).

0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.08.052

merization. The use of microwave energy for rapid synthesis of polymer beads was firstly reported in 1994 [24]. Microwave irradiation polymerization utilizes the microwave energy to arouse molecular movement and liquid rotation with a permanent dipole leading to a very rapid inner heating of solvent and sample. Therefore, compared to conventional heating methods, microwave irradiation can greatly accelerate the reaction rate, reduce the polymerization time and achieve the higher yield [25–27]. Although the mechanism of microwave heating is still under debate, microwave irradiation method for polymer synthesis develops rapidly and is considered a replacement of conventional method [28–30].

Conventional detection methods of BRs are mainly based on bioassays such as the wheat leafunrolling test [31] and rice lamina inclination test [32]. However, these bioassays cannot meet the requirement of quantitative analysis of trace BRs in plant samples. Nowadays chromatographic techniques, mainly including high performance liquid chromatography (HPLC) [33] and gas chromatography–mass spectrometry (GC–MS) [34], are used for the determination of BRs.

Until now, there is still no report focusing on the quantitative analysis of 24-epiBR in plant samples by chromatographic methods combined with the efficient sample preparation technique. In this study, a new suspension polymerization method was developed for the preparation of novel styrene-co-4-vinylpyridine (St-co-4-VP) porous magnetic polymer beads by microwave irradiation. A new method for the determination of trace 24-epiBR in the fresh cole and breaking-wall rape pollen samples was developed by the use of St-co-4-VP magnetic polymer bead extraction coupled with HPLC.

2. Experimental

2.1. Chemicals and plant samples

24-EpiBR standard was purchased from Sigma–Aldrich (St. Louis, MO). Stock solution of 24-epiBR was prepared at a concentration of 100 mg/L in methanol and stored at −18 °C in dark. Working solutions were prepared by appropriate dilution of the stock standard solution with water and were stored at 4 °C in dark. Water was doubly distilled. Homobrassinolide (homoBR) was obtained from Sanland Chemical Company (Xiamen, China). Estrone, β-estradiol and estriol with the purity > 99% were purchased from Zizhu Tiangong Tech. (Beijing, China). All other reagents were of analytical grade. All solutions used for HPLC were filtered through a nylon 0.45 μm filter before use.

Breaking-wall rape pollen was obtained from Bai Caotang Chinese Traditional Medicine Corporation (Luzhou, China). Cole samples were purchased from Guangzhou local markets.

2.2. Preparation of St-co-4-VP magnetic polymer beads

2.2.1. Preparation of Fe_3O_4 nanoparticles

According to the previous work in our group [17], the diameter of the Fe_3O_4 particles synthesized by the same method was only 30–50 nm as cores of the magnetic beads. The process of synthesis of magnetite particles by co-precipitation was conducted according to the previous work [35] with small modification as follows. $NH_3{\cdot}H_2O$ (28 wt.%) was quickly dropped into a solution containing $FeCl_3$ (1.0 mol/L) and $FeSO_4$ (0.5 mol/L) by vigorously stirring under N_2 protection. The pH value was maintained approximately at 12 during reaction process. The resultant black mixture was aged by an MAS-I microwave synthesizer from Sineo Microwave Chemistry Technology Company (Shanghai, China) at 80 °C for 1 h with deoxygenating and fierce agitation. Finally, the precipitate was collected by a magnet and washed three times with 10% acetic acid (v:v) and doubly distilled water. And then surface modification of Fe_3O_4 nanoparticles was carried out by the reaction of Fe_3O_4 (2.0 g) and polyethylene glycol (PEG)-6000 (10.0 g) dissolved in doubly distilled water (30 mL). After sonicating for 20 min, a homogeneously dispersed solution was obtained.

2.2.2. Suspension polymerization

A pre-polymerization mixture was prepared as follows. The functional monomer 4-vinylpyridine (4-VP) (1.29 mL, 12 mmol) was dissolved in dimethyl sulfoxide (DMSO) (5.00 mL, 70.4 mmol). The solution was sparged with oxygen-free nitrogen and then stored in dark for 12 h. The pre-polymerization solution, PEG-Fe_3O_4 particles, dispersing media (doubly distilled water, 100 mL), Styrene (St) (8.00 mL, 69.7 mmol), cross-linker Trimethylolpropane trimethacrylate (TRIM) (1.50 mL, 4.70 mmol) and initiator azo (bis)-isobutyronitrile (AIBN) (0.10 g, 0.60 mmol) were well mixed in a 300 mL single-necked flask and dispersed by vigorous agitation (600 rpm) and bubbled with nitrogen throughout the whole reaction. Microwave irradiation was carried out with a programmed temperature control as follows: initial from room temperature to 40 °C within 2 min, from 40 °C to 60 °C within 2 min, from 60 °C to 70 °C within 2 min and finally keeping at 70 °C for 60 min. Fig. 1 demonstrates the polymerization procedures for these magnetic polymer beads. Magnetic polymer beads were washed extensively with distilled water, 10% (v:v) acetic acid in methanol and methanol under ultrasonic agitation respectively until no leakage and residue of polymerization were observed.

2.3. Study of physical characterization and extraction capability

Magnetic polymer beads obtained were placed on aluminum pegs and sputter coated with 15 nm of gold. Scanning electron microscopy (SEM) was conducted by a Philips XL-30 scanning electron microscopy from Philips (Eindhoven, Netherlands). Bead size distribution was examined by a Malvern MasterSizer 2000 particle size analyzer from Malvern (Malvern, Britain). Infrared absorption spectrum of these beads between 400 and 4000 cm^{-1} was obtained by the use of an IR-prespige-21 FTIR spectrometer (Shimadzu, Japan). Thermogravimetric analysis was performed under inert atmosphere (N_2) in an STA-409 PC thermogravimetric analyzer (Netzsch, Selb/Bavaria, Germany), over the temperature range of 20–800 °C. The resulting particles were characterized by magnetic analysis using a SQUID-based magnetometer form Quantum Design (San Diego, CA).

Extraction capability of St-co-4-VP magnetic polymer beads was evaluated from two aspects including extraction capacity and selectivity. Extraction capacity was investigated with a series of 24-epiBR standard solutions in the range of 0.1000–600.0 μg/L. Extraction selectivity was studied by the use of the mixed standard solution containing 24-epiBR and four reference compounds homoBR, β-estradiol, estriol and estrone at a concentration of 50.00 μg/L.

2.4. Extraction performance and HPLC analysis

2.4.1. Sample preparation

Fresh cole and breaking-wall rape pollen were selected as plant samples for method validation. Fifty grams of whole cole sample mixed with 24-epiBR standard solutions was smashed, well balanced and then dissolved in 50 mL methanol. The solution was filtrated through a funnel, dried with vacuum distillation, and then dissolved in 25 mL water–acetonitrile (v:v, 1:1). Twenty-five grams of breaking-wall rape pollen mixed with 24-epiBR standard solutions was smashed, well balanced and then dissolved in 150 mL methanol. After that, samples were distilled by microwave assisted extraction (MAE) under extraction temperature of 55 °C for 20 min.

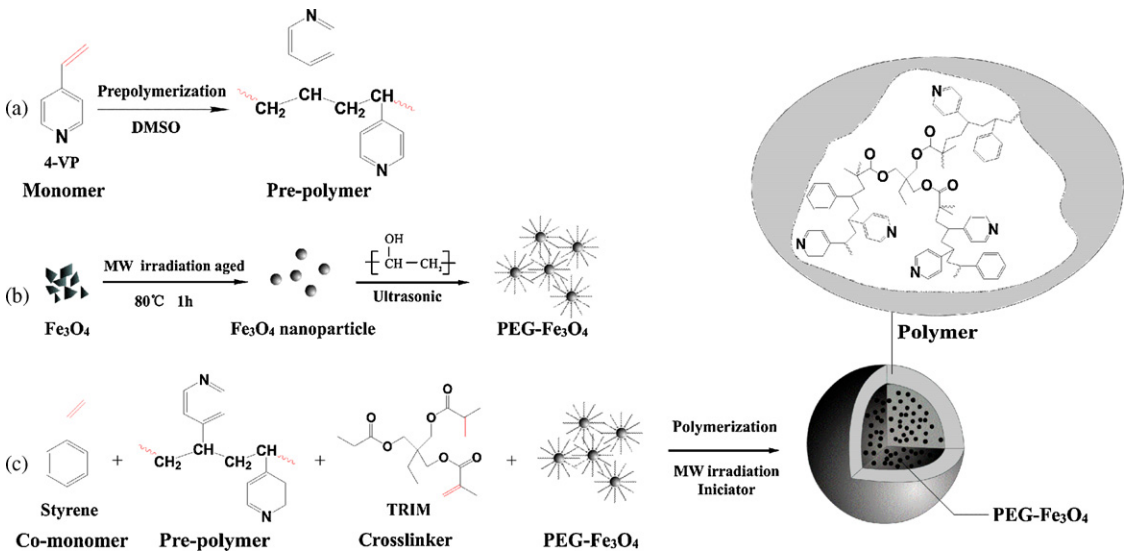

Fig. 1. Schematic representation of pre-polymerization of 4-VP in DMSO (A), PEG modification of Fe_3O_4 particles (B) and polymerization of St-co-4-VP magnetic polymer beads (C).

Then the sample was filtrated, and extraction solutions were dried with vacuum distillation and dissolved with 30 mL distilled water. After being partitioned by ethyl acetate for three times, the organic layer was concentrated by vacuum distillation and dissolved in 25 mL water–acetonitrile (v:v, 1:1). Finally, the homogenized sample was centrifuged for 20 min at 3000 rpm. Three milliliters of supernatant liquid was extracted by St-co-4-VP magnetic polymer beads.

2.4.2. Magnetic polymer bead extraction

The standard 24-epiBR solutions and plant sample extraction solutions were extracted by magnetic polymer beads, and extraction procedures were similar to the previous works [22,36]. Before each use, the recycled beads were revived at 120 °C for 12 h. Known mass beads were added into a 50 mL conical flask and immersed in 3.0 mL standard or sample extraction solution under a reciprocating shaking-table at room temperature. After being incubated for 60 min with the shaking rate of 120 rpm, 0.12 g beads were magnetically separated and then eluted for 20 min in 1.0 mL acetic acid–acetonitrile (1%, v:v) as desorption solvent. The analyte elution was dealt with a nitrogen drying step and then derivatizated with 60 μL of 9-phenanthreneboronic acid (10 mg/L) in 1% (v:v) pyridine–acetonitrile. The mixture was heated at 70 °C for 20 min. After cooling, the derivatization solution was dried with a nitrogen stream and redissolved in 100 μL of acetonitrile [37]. 20 μL of sample solution was for HPLC injection and consequent analysis.

The validation for analysis of cole and breaking-wall rape pollen samples was investigated by spiked experiments, and the amounts of 24-epiBR standard added to cole and breaking-wall rape pollen samples were set at 15.0, 25.0 and 35.0 μg/kg; and 30.0, 50.0 and 70.0 μg/kg, respectively.

2.4.3. HPLC analysis

A Shimadzu LC-20A chromatography equipped with a fluorimetric detector (Shimadzu RF-10Axl) was used in the study (excitation 307 nm, emission 371 nm). A reversed-phase column of C_{18} (250 mm × 4.6 mm i.d., 5 μm) (Dikma, Beijing, China) was used at 30 °C. The injection volume was 20 μL. The optimum mobile phase for the separation of 24-epiBR bisphenanthreneboronates was acetonitrile–water (v:v, 90:10) at a flow rate of 1.0 mL/min.

3. Results and discussion

3.1. Preparation of magnetic polymer beads by microwave irradiation

The whole polymerization process consisted of two steps. First, Fe_3O_4 nanoparticles were modified with surfactants. Thus, the surfactant amounts used during polymerization were optimized based on different surfactants including PEG, polyvinyl alcohol (PVA) and oleic acid. Oleic acid could not disperse in water and modify Fe_3O_4 well. Homogeneously dispersed solutions were obtained by the use of PEG or PVA. However, when PVA was used as modification surfactant, complicated subsequent procedures were needed during microwave irradiation. The solubility of PVA was not as well as PEG in water, so modification of Fe_3O_4 with PVA should be set at 90 °C for 5 min under continuous stirring. Therefore, PEG was chosen as optimum surfactant finally.

For attaining considerable superficial area for extraction capability, the selection of polymerization solvent, cross-linker and monomer was very important. Initially, the recognition capability was extremely dependent on the functional monomer. Thus, acrylamide (AM), methacrylicacid (MAA) and 4-VP were used for the selection of functional monomer, and the amount of each functional monomer was also optimized. PEG–Fe_3O_4 particles could not merge into beads well when using AM or MAA as functional monomer. When using 4-VP, the better homogeneous morphology of the resultant beads was achieved. And the productive yield, homogeneity and morphological structure did not change much with 4-VP amounts varying from 4 to 12 mmol. However, the use of 12 mmol 4-VP resulted in a narrower diameter distribution of 70–150 μm, so 12 mmol 4-VP was chosen as the optimum functional monomer in the study. And then, 4-VP was prepolymerized through the C–C double-bonded interaction in DMSO. After PEG–Fe_3O_4 suspension, cross-linker, copolymer monomer, initiator, prepolymer solution and water were added to the suspension and mixed well, followed by microwave irradiation polymerization. To avoid the leakage of Fe_3O_4 nanoparticles and fragility of the resultant beads, styrene was utilized as a copolymer monomer, which was attributed to its unsaturated bonds to form the cross-linking main chain within the polymeric network and proved to make the reaction equilibrium shifting to the complex formation side [38].

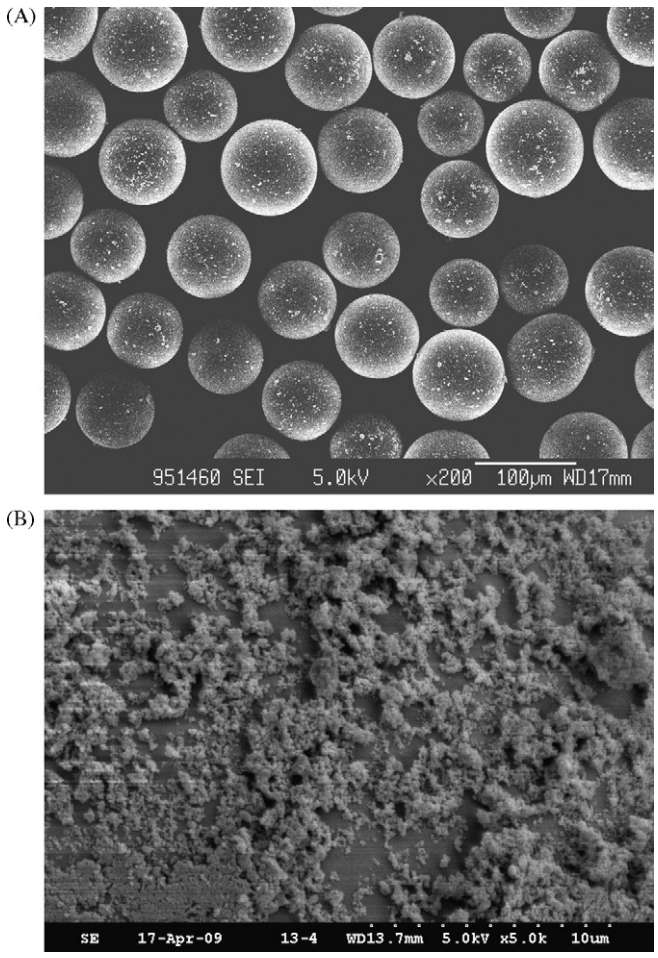

Fig. 2. Scanning electron micrographs of St-co-4-VP magnetic polymer beads prepared by microwave irradiation. Magnifications: (A) 200× and (B) 5000×.

Water-to-monomer ratio during polymerization affects the morphology and size distribution of the resultant beads. Thus, the water-to-monomer ratio was optimized to achieve the good morphology and size distribution. The results indicated that better uniformity and narrower size range could be obtained with the water-to-monomer ratio of 9:1.

Polymerization time is a crucial factor influencing the polymerization degree. Thus, the polymerization time in the study was optimized at 30, 60, 90 and 120 min. Polymerization time of 60 min could ensure the satisfied productive yield, spherical structure and good magnetic property. The shorter polymerization time (<60 min) resulted in low yields of the resultant beads due to the insufficient polymerization, while the longer polymerization time (>60 min) caused a broad size distribution due to a secondary polymerization.

3.2. Physical characterization of magnetic polymer beads

3.2.1. Surface morphology

The particle size and morphology of St-co-4-VP magnetic polymer beads were observed by SEM (Fig. 2) under the different magnifications of 200-fold and 5000-fold, respectively. It can be seen from Fig. 2(A) that the majority of the beads are well-shaped spherical particles. And Fig. 2(B) demonstrates the rough and porous surface morphology of these beads, which would be suitable for rebinding and releasing target molecules by the use of magnetic polymer beads. On the other hand, porous structure would enhance surface area and guarantee good extraction efficiency for trace BRs. Particle size distribution was further studied by particle size analysis (see supplementary materials Fig. 1s). These magnetic polymer beads are homogeneous and possess a narrow size distribution of 70–180 μm. This result is consistent with SEM analysis.

3.2.2. Infrared absorption test and thermal stability

Infrared absorption spectrum of these magnetic polymer beads (see supplementary materials Fig. 2s) shows that an absorption band at 540 cm^{-1} corresponds to the Fe–O bond. Stretching vibration of the C=C bond at 1610 cm^{-1} is attributed to ethylene groups of 4-VP molecule. The typical bands at 3020 and 2920 cm^{-1} are due to the C–H aromatic stretching vibration of styrene units.

Thermal stability of the beads was tested by thermogravimetric analysis. The result suggests that there is no obvious weight loss below 310 °C (see supplementary materials Fig. 3s). Dramatic weight loss occurs from 310 to 520 °C, and the fastest mass loss occurs at 490.5 °C. Thermogravimetric analysis results suggested that these magnetic polymer beads were suitable for the subsequent HPLC analysis. The remaining mass was attributed to the thermal resistance of Fe_3O_4 particles, and the quantity of Fe_3O_4 particles in the beads was 0.84%. This result agreed with the magnetic hysteresis loops analysis.

3.2.3. Magnetization characteristic and solvent-resistant property

Saturation magnetization of magnetic polymer beads was investigated by magnetic hysteresis loop analysis (see supplementary materials Fig. 4s). The symmetrical general shape curves suggest that there is no magnetic retentivity, so St-co-4-VP magnetic polymer beads show superparamagnetism and attain a saturation magnetization value of 1.25 emu/g. Although the magnetic encapsulation is not very high, the magnetic response can fully satisfy the request of magnetic separation.

In order to investigate the solvent-resistant property of the beads, these magnetic polymer beads were immersed in methanol, acetonitrile, acetone, chloroform, ethyl acetate, benzene, tetrahydrofuran, toluene, 10% (v:v) acetic acid in methanol and 10% (v:v) acetic acid in acetonitrile followed by ultrasound for 30 min. The beads showed no desquamation or crack phenomenon in these solvents, which proved the excellent chemical stability of St-co-4-VP magnetic polymer beads. Therefore, the beads under the optimum preparation conditions could remain the good surface morphology and extraction efficiency after the use of more than 100 times.

3.3. Evaluation of extraction capability of magnetic polymer beads

3.3.1. Extraction capacity

Extraction capability of St-co-4-VP magnetic polymer beads was evaluated from extraction capacity and selectivity. Extraction capacity of magnetic polymer beads was investigated via extraction of a series of 24-epiBR standard solutions in the range of 0.1000–600.0 μg/L with the optimum amounts of magnetic polymer beads of 120 mg. As seen from Fig. 3, extraction yields increase gradually with the increasing concentrations of 24-epiBR standard solutions in the range of 0.1000–400.0 μg/L and reach the equilibrium when the concentration is up to 400.0 μg/L. Accordingly, extraction capacity of St-co-4-VP magnetic polymer beads could be calculated to around 90 pmol for 24-epiBR. The proposal method provided a high enrichment factor, 4–5-fold for 24-epiBR.

3.3.2. Extraction selectivity

Extraction selectivity of St-co-4-VP magnetic polymer beads was studied via extraction of the mixed standard solutions containing 24-epiBR and four reference compounds including homoBR, β-estradiol, estriol and estrone at the concentration of 50.00 μg/L.

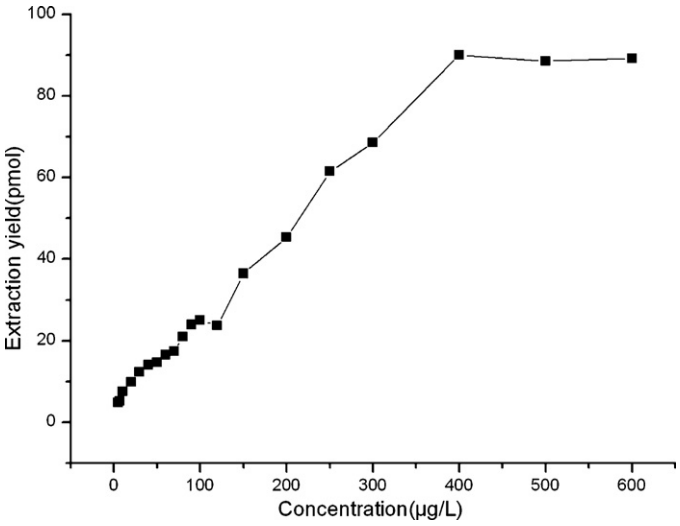

Fig. 3. Extraction yield curve by St-co-4-VP magnetic polymer bead extraction for 24-epiBR in the range of 0.500–400.0 μg/L.

Four reference compounds have the similar structures as 24-epiBR. Especially, homoBR is an artificially external plant pheromone and has almost the same molecular structure as 24-epiBR. It can be seen from Fig. 4 that extraction yields of 24-epiBR, homoBR, β-estradiol, estriol and estrone in water by magnetic polymer bead extraction are 78, 56, 20, 12, and 5 pmol, respectively. It is clear that the extraction yield of 24-epiBR is much higher than those of reference compounds by St-co-4-VP magnetic polymer bead extraction. This result can be tentatively explained as follows. In the structure of St-co-4-VP, there are lone pair electrons in the N atom of 4-VP which do not join the saturation system of pyridine ring. Therefore, in the hybrid orbital of the molecule nonbonded electrons possess the nucleophilicity and are easy for the interaction with hydroxyl groups in the target molecules. Since 24-epiBR has two hydroxyl groups, more than β-estradiol, estriol and estrone, 24-epiBR exhibits the higher molecular polarity than these three reference compounds, which is likely to be bonded with N atom of 4-VP. On the other hand, 24-epiBR and these three reference compounds (β-estradiol, estriol and estrone) are of hydrophobicity, but 24-epiBR molecule possesses the hydrophobic group in the side chain, which would make the greater contribution to the molecular hydrophobocity. Therefore, even in the water phase the extraction yield of 24-epiBR by St-co-4-VP magnetic polymer beads is higher than that of reference compounds. 24-epiBR and homoBR have the

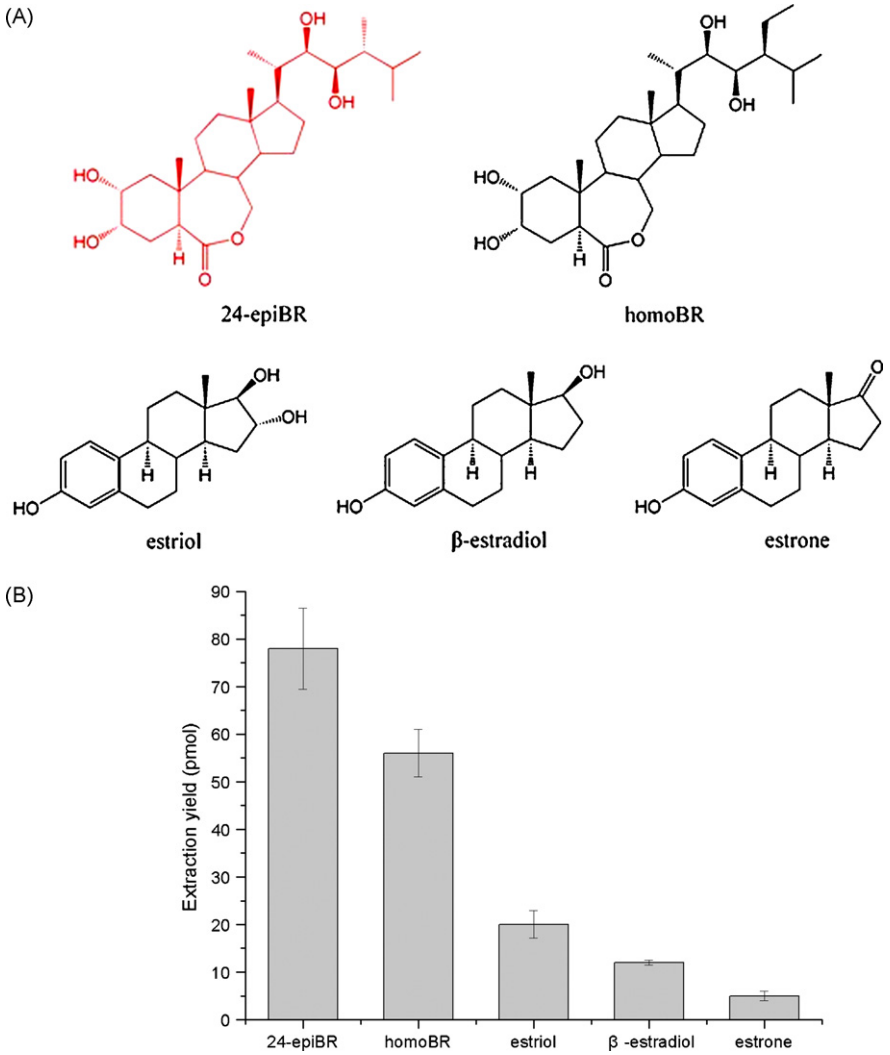

Fig. 4. Extraction yields of 24-epiBR, homoBR, estriol, β-estradiol and estrone by magnetic polymer beads at 50.0 μg/L ($n = 3$). (A) Structure of 24-epiBR and four reference compounds; (B) Extraction selectivity for 24-epiBR by magnetic polymer beads based on extraction yields.

same steroid ring structure, but there is a methyl group in C-24 of 24-epiBR while in that of homoBR there is an ethyl group. Alkyl group is a weak electron-donating group and it would reduce the molecular polarity. Therefore, polarity of 24-epiBR is a little higher than that of homoBR, which leads the higher extraction yield of 24-epiBR by St-co-4-VP magnetic polymer beads than that of homoBR.

3.4. Development of analytical method

3.4.1. Optimization of extraction conditions

In order to attain the optimum extraction conditions and best extraction efficiency, the factors influencing the extraction by the use of St-co-4-VP magnetic polymer beads, including the amounts of magnetic polymer beads, shaking rate, extraction solvent, extraction time, desorption solvent and desorption time, were optimized based on the HPLC peak area of 24-epiBR (see supplementary materials Fig. 5s).

In the study, the amounts of magnetic polymer beads were optimized in the range of 20–300 mg at room temperature with constant shaking, and 120 mg of magnetic polymer beads was selected for extraction under the optimum shaking rate of 120 rpm. Adsorption and desorption kinetic parameters during extraction including extraction solvent, extraction time, desorption solvent and desorption time are another important factors influencing the extraction efficiency. Solvent polarity greatly influences the absorption procedure. Thus, different polar solvents including methanol, acetonitrile, water, acetone, tetrahydrofuran (THF), hexane, acetic ester and DMSO were applied for selection of the optimal extraction solvent by magnetic polymer bead extraction of 50.00 μg/L 24-epiBR standard solution. Finally, water was chosen as extraction solvent in the study with the optimum extraction time of 60 min, at which the response of the analyte reached the highest level and extraction equilibrium was obtained. On the other hand, the optimization of desorption solvent and desorption time was conducted. Desorption solvent was optimized by the use of methanol, acetonitrile, acetic acid in methanol (1% or 10%, v:v) and acetic acid in acetonitrile (1% or 10%, v:v). The results showed that 1% acetic acid in acetonitrile could achieve the best desorption efficiency, and was selected as optimal desorption solvent with the optimal desorption time of 20 min, which was needed to obtain desorption equilibrium.

3.4.2. Analytical method

HPLC is a useful tool for the separation and determination of BRs. BRs have no suitable chromophore for detection, so they have to be derived with pre-labeling reagents in order to make them responsible to ultraviolet (UV), FLU or electrochemical detector. Boronic acid as derivative reagent has high selectivity and reactivity for the *cis*-1,2-diols of BRs, which usually have four hydroxyl groups as two sets of vicinal diols, in the A-ring (2α, 3α-position) and in the side-chain (22*R*, 23*R*-position) [39,40]. In this study, 9-phenanthreneboronic acid as derivative reagent was used prior to FLU detection [37].

The theoretic molar ratio of BRs to 9-phenanthreneboronic acid was 1:2 according to the reaction equation. However, in order to make 24-epiBR derivated absolutely, the amount of 9-phenanthreneboronic acid was optimized in the study. Considering peak shape and protection of chromatographic column, 60 μL of 9-phenanthreneboronic acid (10 mg/L) in 1% (v:v) pyridine–acetonitrile was selected. A series of 24-epiBR standard solutions were prepared and analyzed under the optimal magnetic polymer bead extraction conditions coupled with HPLC–FLU detection. The linear range of this analytical method was 10.00–100.0 μg/L with a relative standard deviation (RSD) of 6.7% achieved for extraction of 50.00 μg/L 24-epiBR standard solution by five batches of magnetic polymer beads. The detection limit was 6.5 μg/kg based on a signal-to-noise ratio of 3 and calculated to be 0.12 ng.

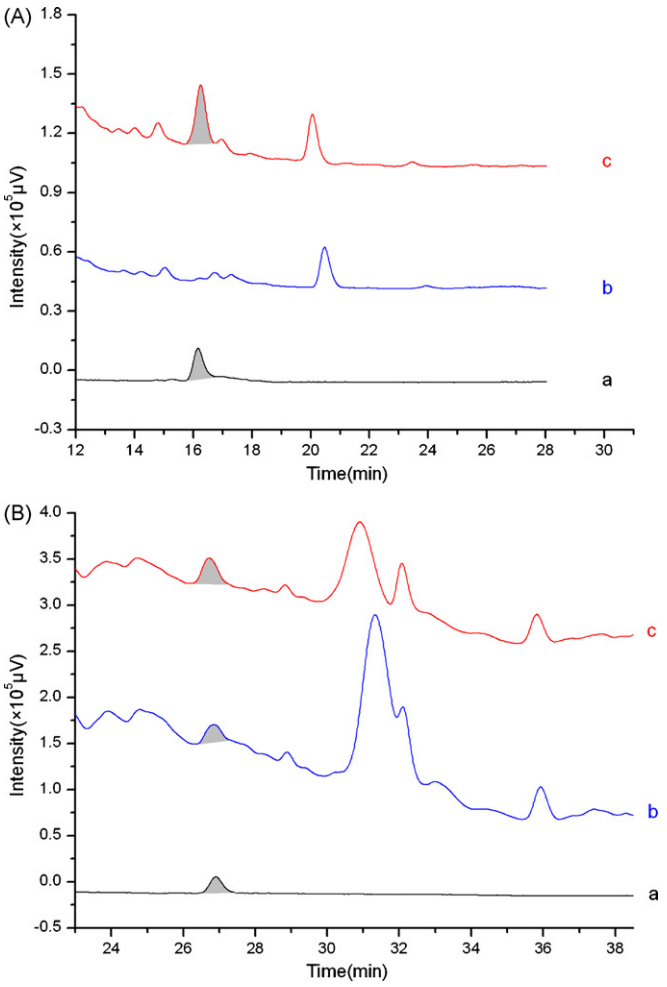

Fig. 5. Chromatograms of the cole (A) and breaking-wall rape pollen sample (B) extracted by St-co-4-VP magnetic polymer beads. Curve a is the chromatogram of direct injection of standard 24-epiBR (50.00 μg/L); Curve b is the chromatogram of sample solutions extracted by magnetic polymer beads; Curve *c* is the chromatogram of spiked sample solutions (25.00 μg/kg for cole spiked samples and 50.00 μg/kg for breaking-wall rape pollen spiked samples) extracted by magnetic polymer beads. The peaks labeled in gray represent 24-epiBR.

3.5. Analysis of 24-epiBR in plant samples

After the analytical method was established, two plant samples including cole and breaking-wall rape pollen were selected for method validation. First, spiked experiments were conducted. For fresh cole sample, 50 g samples were spiked with 24-epiBR standard solution and resulted in the spiked samples with the 24-epiBR amounts of 15, 25 and 35 μg/kg. Then the spiked samples were extracted by organic solvent and resulted in the pre-extraction solution for the sequent magnetic polymer bead extraction. And then the beads were subjected to pre-extraction solution followed by extraction procedure coupled with HPLC–FLU detection. For breaking-wall rape pollen samples, 25 g samples were spiked with 24-epiBR standard and resulted in the spiked samples with the 24-epiBR amounts of 30, 50 and 70 μg/kg. Then, the spiked samples were extracted by MAE followed by filtration, distillation and centrifugation. Then the solution samples were extracted by the beads coupled with HPLC–FLU detection. Typical chromatograms of the spiked cole and breaking-wall rape pollen samples by St-co-

Table 1
Recoveries of 24-epiBR in spiked plant samples extracted by magnetic polymer beads ($n = 3$).

Plant sample	Added (μg/kg)	Found (μg/kg)	Recovery (%)	RSD (%)
Fresh cole	15.0	13.0	86.6	1.0
	25.0	22.5	90.0	4.1
	35.0	26.4	77.2	3.0
Breaking-wall rape pollen	30.0	47.9	72.3	3.1
	50.0	65.1	77.8	0.7
	70.0	84.6	83.4	1.8

4-VP magnetic polymer bead extraction are illustrated in Fig. 5. It can be seen that compared to the chromatograms of direct HPLC analysis of 24-epiBR standard solution (curve a), the chromatographic responses of 24-epiBR in two spiked samples (curve c) are greatly enhanced by the proposed magnetic bead extraction and analytical method, which suggested the improvement of analytical sensitivity. The recoveries of spiked cole and breaking-wall pollen samples were found to be 77.2–90.0% and 72.3–83.4%, and RSDs were 1.0–4.1% and 0.7–3.1% in Table 1, respectively.

Another interesting point in the study is that the extract of real breaking-wall rape pollen samples after magnetic polymer bead extraction can be sensitively detected by HPLC–FLU. As shown in Fig. 5(B), a high degree of sensitivity is achieved by the proposed magnetic polymer bead extraction (curve b). The amount of 24-epiBR in real breaking-wall rape pollen sample was calculated to be 26.2 μg/kg. From all the results mentioned above, it is clear that the proposed method is applicable for the analysis of trace 24-epiBR in plant samples.

4. Conclusion

In this work, novel St-co-4-VP magnetic polymer beads for the extraction of trace 24-epiBR in plant samples were prepared by microwave irradiation using the suspension polymerization method. A series of surface and physical tests showed that St-co-4-VP magnetic polymer beads possessed the narrow size distribution, uniform morphology and porous structure. Extraction capability experiments showed that St-co-4-VP magnetic polymer beads had high enrichment capability (4–5-fold) and good extraction selectivity for 24-epiBR in comparison with other reference compounds. Then, an analytical method for the determination of 24-epiBR was developed by the use of St-co-4-VP magnetic polymer bead extraction coupled with HPLC–FLU detection. The detection limit was 6.5 μg/kg with the RSD of 6.7%. During the analysis of plant samples, the recoveries of the spiked cole and breaking-wall pollen samples were found to be 77.2–90.0% and 72.3–83.4% respectively with RSDs less than 4.1%. It was very interesting that the amount of 24-epiBR in real breaking-wall rape pollen samples could be determined and calculated to be 26.2 μg/kg based on this method.

Acknowledgement

The authors would like to thank the National Natural Science Foundation of China for financially supporting this research under grant numbers 90817012, 20775095 and 20705042, and by Key Program of Guangdong Provincial Natural Science Foundation of China under grant number 9251027501000004.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.08.052.

References

[1] A. Bajguz, A. Tretyn, Phytochemistry 62 (2003) 1027.
[2] B.K. Kim, S. Fujioka, S. Takatsuto, M. Tsujimoto, S. Choe, Biochem. Biophys. Res. Commun. 374 (2008) 614.
[3] H. Seto, S. Fujioka, H. Koshino, S. Yoshida, Tetrahedron 55 (1999) 8341.
[4] A. Bajguz, Plant Physiol. Biochem. 45 (2007) 95.
[5] C. Mussig, Plant Biol. 7 (2005) 110.
[6] N. Ikekawa, F. Nishiyama, Y. Fujimoto, Chem. Pharm. Bull. 36 (1988) 405.
[7] P. Sharma, R. Bhardwaj, Gen. Appl. Plant Physiol. 33 (2007) 59.
[8] T. Yokota, T. Matsuoka, T. Koarai, M. Nakayama, Phytochemistry 42 (1996) 509.
[9] H.M. Chen, C.H. Deng, X.M. Zhang, Angew. Chem. Int. Ed. 49 (2010) 607.
[10] S. Lin, G.P. Yao, D.W. Qi, Y. Li, C.H. Deng, P.Y. Yang, X.M. Zhang, Anal. Chem. 80 (2008) 3655.
[11] H.H. Yang, S.Q. Zhang, X.L. Chen, Z.X. Zhuang, J.G. Xu, X.R. Wang, Anal. Chem. 76 (2004) 1316.
[12] J.S. Huang, B.Z. Han, W. Yue, H.S. Yan, J. Mater. Chem. 17 (2007) 3812.
[13] S.S. Huang, Y. Fan, Z.Y. Cheng, D.Y. Kong, P.P. Yang, Z.W. Quan, C.M. Zhang, J. Lin, J. Phys. Chem. C 113 (2009) 1775.
[14] J.F. Kuntz, P. Palmas, D. Canet, J. Magn. Reson. 188 (2007) 322.
[15] L. Levy, Y. Sahoo, K.S. Kim, E.J. Bergey, P.N. Prasad, Chem. Mater. 14 (2002) 3715.
[16] F. Patolsky, Y. Weizmann, E. Katz, I. Willner, Angew. Chem. Int. Ed. 42 (2003) 2372.
[17] M. Tudorache, M. Co, H. Lifgren, J. Emneus, Anal. Chem. 77 (2005) 7156.
[18] S.D. Plunkett, F.H. Arnold, J. Chromatogr. A 708 (1995) 19.
[19] E. Turiel, J.L. Tadeo, P.A.G. Cormack, A.E. Martin, Analyst 130 (2005) 1601.
[20] F.G. Tamayo, E. Turiel, A.E. Martin, J. Chromatogr. A 1152 (2007) 32.
[21] Y. Zhang, R.J. Liu, Y.L. Hu, G.K. Li, Anal. Chem. 81 (2009) 967.
[22] P.J. Dowding, B. Vincent, Colloids Surfaces A: Physicochem. Eng. Aspects 161 (2000) 259.
[23] R.J. Ansell, K. Mosbach, Analyst 123 (1998) 1611.
[24] M. Murray, D. Charlesworth, L. Swires, P. Riby, J. Cook, B.Z. Chowdhry, J. Martin, J. Chem. Soc. Faraday Trans. 90 (1994) 1999.
[25] C. Zhang, L.Q. Liao, S.Q. Gong, Green Chem. 9 (2007) 303.
[26] D. Bogdal, P. Penczek, J. Pielichowski, A. Prociak, Adv. Polym. Sci. 163 (2003) 51.
[27] F. Wiesbrock, R. Hoogenboom, U.S. Schubert, Macromol. Rapid Commun. 25 (2004) 1739.
[28] D.R. Baghurst, D.M.P. Mingos, J. Chem. Soc. Chem. Commun. (1992) 674.
[29] F. Wiesbrock, R. Hoogenboom, M.A.M. Leenen, M.A.R. Meier, U.S. Schubert, Macromolecules 38 (2005) 5025.
[30] E. Marand, K.R. Baker, J.D. Graybeal, Macromolecules 25 (1992) 2243.
[31] K. Wada, H. Kondo, S. Marumo, Agric. Biol. Chem. 49 (1985) 2249.
[32] H. Abe, T. Morishita, M. Uchiyama, S. Takatsuto, N. Ikekawa, M. Ikeda, T. Sassa, T. Kitsuwa, S. Marumo, Experientia 39 (1983) 351.
[33] K. Gamoh, T. Krrsuwa, S. Takatsuto, Y. Fujimoto, N. Ikekawa, Anal. Sci. 4 (1988) 533.
[34] S. Takatsuto, N. Ikekawa, Chem. Pharm. Bull. 34 (1986) 3435.
[35] R.Y. Hong, T.T. Pan, H.Z. Li, J. Magn. Magn. Mater. 303 (2006) 60.
[36] Y.L. Hu, R.J. Liu, Y. Zhang, G.K. Li, Talanta 79 (2009) 576.
[37] K. Gamoh, K. Omote, N. Okamoto, S. Takatsuto, J. Chromatogr. 469 (1989) 424.
[38] J. Matsui, S. Goji, T. Murashima, D. Miyoshi, S. Komai, A. Shigeyasu, T. Kushida, T. Miyazawa, T. Yamada, K. Tamaki, N. Sugimoto, Anal. Chem. 79 (2007) 1749.
[39] K. Gamoh, H. Sawamoto, S. Kakatsuto, Y. Watabe, H. Arimoto, J. Chromatogr. 515 (1990) 227.
[40] K. Gamoh, S. Takatsuto, J. Chromatogr. A 658 (1994) 17.