Selective On-Line Extraction of Trans-Resveratrol and Emodin from *Polygonum cuspidatum* Using Molecularly Imprinted Polymer

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Abstract

High-performance liquid chromatographic separation is performed to extract active components from the traditional Chinese medicine *Polygonum cuspidatum* using a trans-resveratrol imprinted polymer. Good separation and purification of trans-resveratrol and emodin from the *Polygonum cuspidatum* extract are achieved after condition optimization. The extraction recoveries are 83% and 99% for trans-resveratrol and emodin, respectively. The results show that the molecularly imprinted polymer can be used as a selective extraction material for the extraction and purification of trans-resveratrol and emodin from *Polygonum cuspidatum*.

Introduction

Trans-resveratrol and emodin are two active components in *Polygonum cuspidatum*, an herbal polygonum which is used in traditional Chinese medicine for the treatment of suppurative dermatitis, gonorrhea, arthralgia, jaundice, amenorrhea, and chronic bronchitis (1). In recent years, a great amount of research has been carried out on the pharmacology of resveratrol. It is reported that resveratrol is a preventive agent against some pathologies [e.g., vascular disease, cancer, viral infection, or neurodegenerative processes (2–6)]. Because resveratrol-mediated cardioprotection is achieved through the preconditioning effect (the best yet devised method of cardioprotection), resveratrol likely fulfills the definition of a pharmacological preconditioning compound (7). Researchers have also reported that emodin is a medicine against cancer, mutation, bacterial, and prokinetic actions on gastrointestinal smooth muscles (8–10). To study the pharmacological mechanism or to develop new pharmaceuticals, trans-resveratrol and emodin have to be extracted from *Polygonum cuspidatum*. However, multiple steps, including preliminary extraction such as solvent extraction, and further purification such as chromatography (11–12), were involved with conventional extraction methods. Method development for a more efficient process is desirable.

Molecularly imprinted polymer (MIP), with pre-determined selectivity for a particular molecule or group of structural analogues, has been applied in many analytical areas such as liquid chromatography, capillary electrophoresis, solid phase extraction, immunoassay, and chemical sensors (13–17). Among the separation applications, molecularly imprinted solid phase extraction (MISPE) is the one that has very attractive practical potential for the clean-up of environmental and biological samples (13,18). MISPE is also used to extract and analyze active components from natural plants including traditional Chinese herbal medicines (19).

To extract trans-resveratrol from *P. cuspidatum*, MIP with trans-resveratrol as template was synthesized and evaluated (20). In the experiment, it was found that both trans-resveratrol and emodin could be separated from the *P. cuspidatum* extract using the MIP column. To establish a method for the on-line extraction of trans-resveratrol and emodin with MIP as the stationary phase, the chromatographic conditions were selected and optimized. The research work is presented in this paper.

Experimental

Chemicals

Trans-resveratrol was purchased from Linjing Scientific and Trading Co. Ltd. (Shanxi, China). Methanol (MeOH) and acetonitrile (ACN) were from Xingke Chemical Company (Tianjin, China). Isopropanol alcohol was from Beifang Tianyi Chemical Company (Tianjin, China). Emodin standard solution with concentration of 11.96 mg/mL was provided by Tianjin Institute for Drug Control (Tianjin, China). All chemicals are of analytical grade.

Standard solutions of trans-resveratrol and emodin were prepared in a series of concentrations with MeOH as the solvent to obtain the calibration curve in quantitative analysis and for the peak identification.
Equipment
An Agilent 1100 HPLC system (Palo Alto, CA) equipped with a quaternary pump, thermostatted column compartment, multiple wavelength UV-Vis detector, and manual injector was used in the experiment.

Preparation of trans-resveratrol imprinted polymer
Bulk trans-resveratrol imprinted polymer was synthesized with a non-covalent method, using trans-resveratrol as the template, 4-vinylpyridine as the functional monomer, ethylene dimethacrylate as the cross-linker, acetone as the porogenic agent, and 2,2’-azobisisobutyronitrile as initiator (20). The bulk polymer obtained was ground and sieved to obtain polymer particles with diameters less than 45 µm. After sedimentation and removal of template molecules, the polymer particles were dry-packed (0.9 g) into a stainless steel column (150 × 4.6 mm) for the online extraction.

Preparation of P. cuspidatum extract
Dry P. cuspidatum rootstock were chopped with an herb grinder and sieved with a 60-mesh sieve. P. cuspidatum powder (2.5 g) was mixed with 35 mL MeOH and sonicated. A soaking (24 h) was subsequently performed for full lixiviation. Then the bulk polymer obtained was ground and sieved to obtain polymer particles with diameters less than 45 µm. After sedimentation and removal of template molecules, the polymer particles were dry-packed (0.9 g) into a stainless steel column (150 × 4.6 mm) for the online extraction.

On-line extraction experiment
An MIP column with trans-resveratrol imprinted polymer as the packing material was used in the online high-performance liquid chromatography (HPLC) extraction. Different solutions were used as the mobile phases to separate trans-resveratrol and emodin from the matrix of the extract. The extract (test samples) was injected without any other pretreatment. The respective fractions of trans-resveratrol and emodin were collected and then concentrated to dryness by rotary evaporation at 45°C. Then the residue was re-dissolved with MeOH and used for the reversed-phase HPLC analysis.

Quantitative analysis with reversed-phase HPLC
Reversed-phase HPLC analysis was performed for the quantitative determination of trans-resveratrol and emodin in the original extract and in the fractions collected from the online extraction. A 150 × 4.6 mm column with Inertsil ODS-2 C18 packing material (GL Science, 5 µm) was used in the analysis. The mobile phase consisted of solvent A [MeOH–water (3/7, v/v)] and solvent B (MeOH) with the gradient elution (%B): 0 min, 0%; 18 min, 100%; 25 min, 100%. The column temperature was 30°C and flow rate of mobile phase was 1.0 mL/min. The detection wavelength was 290 nm for emodin and 306 nm for trans-resveratrol, respectively. The external standard method (ESTD) was used in the quantitative analysis. The calibration curve was determined by the standard solution prepared by trans-resveratrol and emodin in MeOH.

Results and Discussion
P. cuspidatum rootstock contains many components, including polydatin, trans-resveratrol, emodin, physcion, rhein, chrysophanol, and anthraglycoside A, etc. (21–23). Molecular structures of trans-resveratrol and emodin are shown in Figure 1. Our previous work demonstrated that trans-resveratrol imprinted polymer can selectively extract trans-resveratrol (20). Based on the result of the study, trans-resveratrol imprinted polymer was used for the online HPLC extraction of trans-resveratrol from P. cuspidatum. The conditions for the extraction were selected and optimized.

Elution solvent selection
In the online extraction study, the elution solvent selection is the most important step. On line extraction can be separated into two steps: washing and eluting; or with one step using one mobile phase. The better mobile phase should stabilize the trans-resveratrol-MIP interaction and also facilitate the separation between the
trans-resveratrol and matrix in *P. cuspidatum* extract. The result of our previous study indicated that binding of trans-resveratrol on MIP can be enhanced in ACN and in water. A polar solvent with H-bonding ability such as MeOH interrupts the interaction between the MIP and trans-resveratrol molecule (20). Meanwhile, because MeOH was used for the *P. cuspidatum* extraction, it is possible that polar organic solvent is the strong eluting solvent for the matrix components. To choose an elution solution, the combinations of water and three polar solvents were evaluated: MeOH, isopropyl alcohol, and ACN.

The experimental results demonstrated that trans-resveratrol could not be separated from emodin when using mobile phases containing isopropyl alcohol or ACN. On the other hand, using MeOH–water (80/20, v/v) as the mobile phase, trans-resveratrol and emodin can be separated from the matrix and from each other (Figure 2). MeOH–water (80/20, v/v) was used for further experiments.

**Quantitative determination of trans-resveratrol and emodin in the fraction from on-line extraction with reversed-phase HPLC**

To have more accurate determinations of trans-resveratrol and emodin, fractions from the on-line extraction were analyzed with reversed-phase HPLC. The external standard method was used for the quantitative determination of trans-resveratrol and emodin. The linearity ranges used in the experiment, LOD (limit of determination, determined by 3 times of noise), and LOQ (limit of quantitation, determined by 10 times of noise) in the analysis are listed in Table I.

**Loading capacity of the MIP column**

To find out the loading capacity of the MIP column, different injection volumes (from 100 µL to 1 mL) of *P. cuspidatum* extract containing approximately 140 µg/mL trans-resveratrol were used for the MIP extraction process. The fractions collected from MIP column were treated with the procedure described in the “On-line extraction experiment” section. The amount of trans-resveratrol in the *P. cuspidatum* extract and in the fraction from MIP column was determined with the reversed-phase HPLC. The recovery was calculated by a comparison of the amount of trans-resveratrol loaded onto the MIP (the amount of trans-resveratrol in the *P. cuspidatum* extract) and that found in the fraction after extraction. The purity of trans-resveratrol obtained from the online extraction was analyzed with reversed-phase HPLC by the peak area normalization calculation. Both the recovery and purity of trans-resveratrol were more than 80% when the injection volume was 500 µL, whereas the purity was below 70% when injection volume was increased to 1 mL (Figure 3). Injections of 500 µL of concentrated *P. cuspidatum* extract (containing approximately 280 µg/mL trans-resveratrol) resulted in lower purity. Finally, 500 µL of *P. cuspidatum* extract containing 140 µg/mL trans-resveratrol (70 µg of trans-resveratrol totally in the extract) was chosen as the injection amount for the extraction process.

**Extraction and purification efficiency**

The efficiency and recovery of the extraction process was examined after the determination of the chromatographic conditions for on-line extraction. A comparison of the chromatograms of the extract and fractions after the extraction process are shown in Figure 4.
process (Figure 4) demonstrated that the on-line extraction had good purification efficiency. The recoveries of the extraction process for trans-resveratrol and emodin are shown in Table II. The recovery rate was calculated by the mass of trans-resveratrol (or emodin) in the extraction fraction divided by its mass in the P. cuspidatum extract injected into MIP column. The data indicated that the condition developed in this research was good for extracting trans-resveratrol and emodin from P. cuspidatum.

After being used for 6 months, the selectivity and retention ability of the MIP column were almost unchanged, which demonstrates that the MIP column has a stable structure to meet the practice requirements.

Conclusion

A method of on-line extraction of trans-resveratrol from P. cuspidatum by MIP was developed in the research. The method has good efficiency for the extraction and separation of trans-resveratrol and emodin from P. cuspidatum extract. The established chromatographic conditions can be scaled up when applied for a larger amount of extraction. The experiment results demonstrated that trans-resveratrol imprinted polymer can be used as the stationary phase to selectively retain template molecules and separate them from complex matrices, which provides a new method of refining traditional Chinese medicine by chromatography.

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References