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*-resveratol 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). Isopropyl 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.

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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 on-line 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 mixture was sonicated again for 20 min and filtrated. The percolate was concentrated with rotary evaporation at 45°C to approximately 20 mL. The extract was then fixed to 25.0 mL with MeOH (concentrated extract solution). The samples tested in the extraction experiment were a 10-time dilution of the concentrated extract solution. The concentrations of *trans*-resveratrol and emodin in the test samples were determined by reversed-phase HPLC analysis.

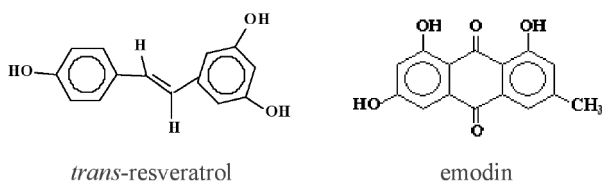

Figure 1. Molecular structures of trans-resveratrol and emodin.

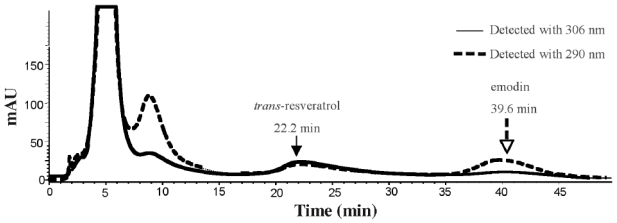

Figure 2. Elution chromatograms of *P. cuspidatum* extract on MIP column with MeOH–water (80/20, v/v) as the mobile phases. Flow rate, 1.0 mL/min; column temperature, 30°C; detection wavelength, 306 nm and 290 nm; injection volume, 20 µL.

Table I. The Linearity Range Used in the Experiment and Limits of Determination for Trans-Resveratrol and Emodin in the RP-HPLC Analysis

Compound	Trans-resveratrol	Emodin
Calibration curve	$y = 17.75 + 132.045x$ ($r = 0.9993$)	$y = 351.4 + 59.631x$ ($r = 0.9994$)
Linearity range (µg/mL)	5.00–80.00	5.98–95.68
LOD (µg/mL)	0.01	0.01
LOQ (µg/mL)	0.04	0.04

On-line extraction experiment

An MIP column with *trans*-resveratrol imprinted polymer as the packing material was used in the on-line 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 on-line extraction. A 150 × 4.6 mm column with Inertsil ODS-2 C_{18} 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 30ºC 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

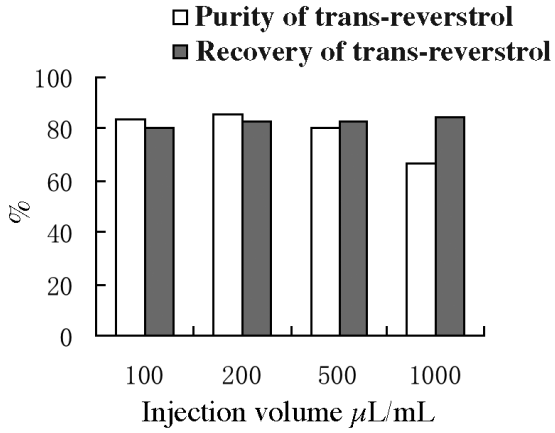

Figure 3. The purity of *trans*-resveratrol in the fraction and recovery of extraction process with different loading amount.*

* The concentration of *trans*-resveratrol in the *P. cuspidatum* extract loaded was 140 µg/mL.

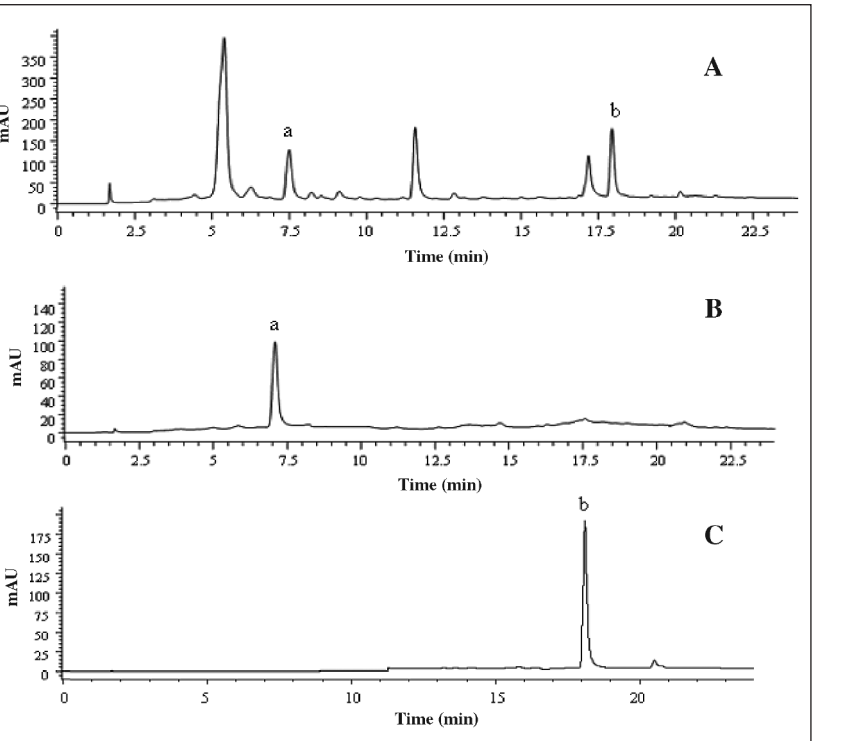

Figure 4. Chromatograms of P. cuspidatum extract (A) and trans-resveratrol fraction (B), emodin fraction (C) from MIP extraction. In the chromatogram, peak a: trans-resveratrol; peak b: emodin. Chromatographic conditions are described in the "Quantitative analysis with reversed-phase HPLC" section.

Table II. The Recovery of the Extraction Process and Purity of the *trans*-Resveratrol and Emodin in the Fractions after the MIP Column Extraction (n = 5)

Component	Recovery % (RSD %)	Purity % (RSD %)
trans-Resveratrol	82.9, (1.2)	80.1, (0.6)
Emodin	98.6, (0.7)	90.7, (1.1)

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.

Acknowledgments

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