Simultaneous Determination of Dihydromyricetin and Resveratrol in *Ampelopsis sinica* (Miq.) W.T. Wang by High-Performance Liquid Chromatography Coupled with a Diode Array Detection Method

Min-Yi Jin¹, Yue Ding¹, Tong Zhang¹*, Zhen-Zhen Cai² and Jian-Sheng Tao³

¹Experiment Center For Teaching and Learning, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China, ²Experiment Center For Science and Technology, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China, and ³School of Pharmacy, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China

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Yeputaoteng is the dried ground part of *Ampelopsis sinica* (Miq.) W.T. Wang, which is widely used in traditional Chinese medicine for preventing and treating tumors, chronic nephritis, hepatitis, rubella, traumatic bleeding, stomach heat and vomiting. A simple and reliable method using high-performance liquid chromatography with diode array detection (HPLC–DAD) was developed for the simultaneous determination of dihydromyricetin and resveratrol in Yeputaoteng. The chromatographic analysis was performed on a Dikma C18 column (250 × 4.6 mm, 5 µm) at 30°C with a gradient elution of acetonitrile and 0.1% phosphoric acid at a flow rate of 1 mL/min, and used ultraviolet detection at 292 and 306 nm. The established method was validated in terms of linearity, precision, reproducibility, stability and recovery. The calibration curves showed good linear regression ($R^2 > 0.9994$). Limits of detection and quantification fell in the ranges of 1.47–2.48 and 2.93–4.97 µg/mL, respectively. The mean recovery of dihydromyricetin and resveratrol was 104.1% [relative standard deviation (RSD): 2.94%] and 100.8% (RSD: 2.80%), respectively. The quantitative results indicated that the HPLC–DAD method can be effectively applied to the quality control of Yeputaoteng and its preparations.

Introduction

Yeputaoteng is the dried ground part of *Ampelopsis sinica* (Miq.) W.T. Wang, which is widely used in traditional Chinese medicine in the Zhejiang and Shanghai areas for preventing and treating tumors, chronic nephritis, hepatitis, rubella, traumatic bleeding, stomach heat and vomiting (1). Yeputaoteng has been officially listed in *Shanghai Chinese Herbal Pieces Processing Standards* (2008 edition), but there has been no determination about its quantitative analysis. Due to the shortage of Yeputaoteng, the market has recently been flooded with adulterants. It is especially important to use advanced chromatographic technology to establish a reliable and accurate analytical method for the determination of the active components in Yeputaoteng.

Previous studies have shown that *Ampelopsis* contains quercetin, dihydromyricetin (2), resveratrol, gallic acid (3), stigmastanol (4) and volatile oil (5), of which dihydromyricetin (6–7) and resveratrol (8–10) are the primary active compounds researched in chemical analysis and pharmacokinetics.

Dihydromyricetin, called ampelopsin or ampeloptin, belongs to the flavonoids and has antioxidant (11), antibacterial (12), antihypertensive, anticancer (13), hepatoprotective and disinfecting (14) functions. Resveratrol, a phenolic substance, has antioxidant (15), antitumor (16), hepatoprotective and cardiovascular (17) functions. These two constituents can be extracted efficiently by ultrasonic and microwave techniques (18).

In addition, many chromatographic techniques have been described in the literature for the determination of active compounds, including liquid chromatography–mass spectrometry (LC–MS) (19), high-performance liquid chromatography (HPLC), HPLC–diode array detection (DAD) (20–21), HPLC–MS–MS (22–23), HPLC–fluorimetric detection (FLD) (24) and gas chromatography (GC)–MS (25). However, the HPLC–DAD method is the most economical and extensive for the simultaneous determination of multiple components.

Current studies have shown that the HPLC–DAD method has become one of the most frequently applied approaches for the quantitative analysis and quality control of traditional Chinese medicine.

This study aims to develop a simple, accurate and practical HPLC–DAD method for the simultaneous determination of dihydromyricetin and resveratrol in Yeputaoteng and provide the basis for its identification and quality control.

Materials and Methods

Apparatus

Equipment used for the study included an electronic analytical balance (BS110S; Beijing Sartorius Balance Co., Beijing, China), an electronic balance (Mettler XS105DU; Mettler-Toledo China, Beijing, China), an electric heated water bath (HHS; Shanghai Boxun Co., Shanghai, China), an Agilent-1100 series HPLC (G1311A quaternary pump, G1315D DAD detector, G1379A online degasser, 7725i manual injection valve and ChemStation chromatography workstation; Shanghai, China), Agilent 8453 UV-visible Spectrometer (8453, Agilent Technologies Co., Shanghai, China).

Materials, reagents and chemicals

One sample of Yeputaoteng (Lot Number: 100406, 101015, 110331-1, 110331-2) was purchased from Shanghai Kangqiao Medicinal Materials Electuary Co. (Shanghai, China). Another sample of Yeputaoteng (Lot Number: 120225-1) was purchased from Shanghai Wanshicheng Medicinal Materials Electuary Co.
(Shanghai, China) (Table I). The Yeputaoteng was identified by Jun-Song Li (Experiment Center for Teaching and Learning, Shanghai University of Traditional Chinese Medicine, Shanghai, China). Dihydromyricetin (Lot Number: 11091631, quantitative reference material) and resveratrol (Lot Number: 11082521, quantitative reference material) were obtained from Shanghai Tauto Biotech Co. (Shanghai, China) (Table I).

HPLC-grade acetonitrile and methanol (Anhui Fulltime Specialized Solvents & Reagents Co., Anhui, China) were used to prepare the mobile phase. Other solvents were of analytical grade.

**Chromatographic conditions**
The analysis of samples was performed on a Dikma C18 column (250 × 4.6 mm, 5 μm) with a gradient eluent composed of acetonitrile and 0.1% phosphoric acid (Table II). The flow rate was 1 mL/min and the detection wavelengths were 292 and 306 nm. The column temperature was 30°C and the injection volume was 20 μL.

**Preparation of standard solution**
Dihydromyricetin and resveratrol were accurately weighed and dissolved in methanol to produce a solution containing 0.414 mg/mL of dihydromyricetin and 0.489 mg/mL of resveratrol, which was used as the reference solution.

**Preparation of sample solution**
Each sample (5.0 g powder) was accurately weighed and immersed in 100 mL methanol, which was extracted by ultrasonication using 100% methanol for 30 min. After this, the sample was weighed again, methanol was added for the loss and the solution was shaken, filtered and desiccated. Finally, the sample solution was completed to a volume of 5 mL.

**Results**

**Determination of detection wavelengths**
Dihydromyricetin and resveratrol were accurately weighed and dissolved in methanol to produce solutions containing 0.01 mg/mL. The sample was scanned from 220 to 600 nm. The results showed that the maximum absorption wavelength of dihydromyricetin was 292 nm (Figure 1). The maximum absorption wavelength of resveratrol was 306 nm (Figure 2).

**Optimization of HPLC–DAD conditions**
Different HPLC–DAD parameters, including various columns, mobile phases and gradient elution conditions, were examined and compared to determine the best separation mechanism for the chromatograms (Figure 3 and Figure 4).

Two kinds of reversed-phase columns were investigated and compared, namely Elite Hypersil ODS2 C18 (250 × 4.6 mm, 5 μm) and Dikma Diamonsil C18 (2) (250 × 4.6 mm, 5 μm). The Dikma Diamonsil C18 column (2) showed good peak separation and sharp peaks.

The effect of mobile phase composition was investigated (acetonitrile–water and acetonitrile–water with phosphoric acid). Adding 0.1% phosphoric acid in the mobile phase provided better resolution and separation of the two components, resulting in high precision sensitivity and selectivity.

Based on the maximum absorption and full-scan experiments of the components in UV spectra, the detection wavelengths were set at 292 and 306 nm.

**Optimization of extraction methods**
Satisfactory extraction efficiency was obtained by comparing water-refluxing and ultrasonic methods. Ultrasonic extraction was simpler and more effective for dihydromyricetin and resveratrol. Therefore, this method was used in further experiments. In this study, different concentrations of methanol (0, 50 and 100%), sample to solvent ratios (1:5, 1:10 and 1:20, w/v), extraction times (15, 30 and 60 min) were studied for the Yeputaoteng extraction procedure. As a result, the best extraction conditions of sample consisted of extraction by ultrasonication using 100 mL of 100% methanol (1:20, w/v) as the extraction solvent with a duration of 30 min.

**Method validation of quantitative analysis**
The method was validated in terms of linearity, precision, reproducibility, stability and recovery.

**Calibration curves, limit of detection and limit of quantification**

Methanol stock solutions containing the two analytes were diluted to appropriate concentrations for construction of the calibration curves. The analyte solutions with six different concentrations were injected twice and the calibration curves were established by plotting the peak area (Y) versus the concentration (X) of each component. The limit of detection (LOD) and limit of quantification (LOQ), expressed by 3 and 10 times the signal-to-noise (S/N), were also determined. Detailed information regarding the calibration curves and linear ranges is listed in Table III.

**Precision, reproducibility, stability and recovery**
As shown in Tables IV and V, the precision was divided into intra-day (n = 6) and inter-day (n = 5). The former was based on six replicated injections with different standards in one day and the
latter was one injection with different standards per day for five days. The reproducibility \((n = 6, \text{Sample 110331-1})\) of the proposed method and the stability \((n = 7, \text{Sample 110331-1})\) of the measurements in 24 h were both acceptable. A recovery test was performed using the standard addition method. Low, medium and large amounts of the standards were added to the known sample \((110331-1)\). Extraction and analysis were performed as described previously. The mean recovery was calculated according to the following formula: 

\[
\text{recovery (\%)} = \left(\frac{\text{amount found} - \text{original amount}}{\text{amount spiked}}\right) \times 100\%;
\]

relative standard deviation (RSD) was calculated by 

\[
\text{RSD (\%)} = \left(\frac{\text{SD}}{\text{mean}}\right) \times 100\%.
\]

The mean recovery of dihydromyricetin was 104.1%, RSD was 2.94%. The mean recovery of resveratrol was 100.8%, RSD was 2.80%.

**Sample analysis**

The newly established analytical method was applied to determine the contents of different batches of Yeputaoteng. All samples were analyzed by using the optimized extraction method under HPLC–DAD conditions. Each sample was analyzed in triplicate to determine the mean content and the results are recorded in Table VI.

The results showed that the contents of two compounds in different samples, especially those from different factories and resources, were significantly different. Therefore, it is necessary to apply this efficient method to the quality control of Yeputaoteng.

**Discussion**

Yeputaoteng is widely used in clinical treatment. In order to distinguish it from adulterants, it is urgent and significant to provide an effective detection method for the quality control of Yeputaoteng.

Moreover, previous studies have shown that UV detection is often used for single wavelength detection. This is very cumbersome when used in multiple wavelengths. However, DAD detection can overcome these shortcomings, selecting multiple wavelengths to determine a variety of ingredients at the same time. HPLC–DAD is considered to be an economical, accurate and reliable technology, which can greatly reduce the time of quantitative analysis. In further studies, a combination of quantitative and chemical fingerprint analysis can be tried (26).

**Conclusion**

In conclusion, the first HPLC–DAD quantitative analytical method for Yeputaoteng was established in this study. The
method was sensitive and quick to determine the two bioactive constituents in Yeputaoteng and helpful for the reasonable development of Yeputaoteng and its preparations.

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References


