



ORIGINAL ARTICLE

# Simultaneous quantification of five major active components in capsules of the traditional Chinese medicine ‘Shu-Jin-Zhi-Tong’ by high performance liquid chromatography

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**Abstract** A simple and reliable high performance liquid chromatography (HPLC) method has been developed for the simultaneous quantification of five major bioactive components in ‘Shu-Jin-Zhi-Tong’ capsules (SJZTC), for the purposes of quality control of this commonly prescribed traditional Chinese medicine. Under the optimum conditions, excellent separation was achieved, and the assay was fully validated in terms of linearity, precision, repeatability, stability and accuracy. The validated method was applied successfully to the determination of the five compounds in SJZTC samples from different production batches. The HPLC method can be used as a valid analytical method to evaluate the intrinsic quality of SJZTC.

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## 1. Introduction

Traditional Chinese medicine (TCM) has a long history dating back several thousands of years, and is widely used for treating various chronic diseases [1] (for example, rheumatism

and arthritis), in East Asian countries such as China, Japan and Korea [2]. The increasing popularity of TCMs can be attributed to their effectiveness and relatively low toxicity. With the growing use of herbal products, quality control is required to guarantee their safety and efficacy. Hence, the development of efficient methods to evaluate and control the quality of TCMs is important.

In the past decades, a large number of analytical strategies have been designed to evaluate the quality of medicinal herbs or herbal preparations. These include quantification of a single compound or multiple components, as well as fingerprint analysis. Single marker compound quantification is simple and convenient, but it does not afford sufficient quantitative information for the other active constituents in complex TCMs [3]. Fingerprint analysis can control the quality consistency and stability of herbal products, but it cannot provide accurate quantification of analytes in TCMs [4]. Despite the

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requirement of many reference standards, multi-component determination remains widely used to evaluate and control the quality of TCMs because of the advantage of simultaneous quantification of many markers from different herbal products for control of total quality [5,6]. In the process, techniques such as HPLC [7], gas chromatography (GC) [8], high performance capillary electrophoresis (HPCE) [9], gas chromatography–mass spectrometry (GC–MS) [10] and liquid chromatography–mass spectrometry (LC–MS) [11] are often used. However, HPLC is simple, reliable and inexpensive, and has been widely used for quantitative analysis of herbal medicines.

The herbal preparation ‘Shu-Jin-Zhi-Tong’ capsules (SJZTC) has been widely used in China. It contains seven medicinal materials: Rhizoma Corydalis, Rhizoma Corydalis Decumbentis, Rhizoma Cibtii, Radix Scutellariae, Cortex Cinnamomi, Herba Lycopodii and Flos Carthami. The remedy is particularly valuable for the treatment of conditions including rheumatism, arthritis and scapulohumeral periarthritis. Various chemical and pharmacological studies have shown that tetrahydropalmatine [12,13], protopine [14,15], protocatechuic aldehyde [16], baicalin [17] and cinnamaldehyde [18] from Rhizoma Corydalis or Rhizoma Corydalis Decumbentis, Rhizoma Corydalis Decumbentis or Rhizoma Corydalis, Rhizoma Cibtii, Radix Scutellariae and Cortex Cinnamomi, respectively, possess significant pharmacological actions and are the major active components in SJZTC. Thus, the development of simple and effective methods for the simultaneous quantification of these active compounds would be of significant value for the quality control of SJZTC, but to date, none has been reported.

We present here a convenient, sensitive and reliable HPLC method, which allows the simultaneous quantification of the five major active components, protocatechuic aldehyde, protopine, tetrahydropalmatine, baicalin and cinnamaldehyde (chemical structures shown in Fig. 1), of SJZTC. The developed method was successfully applied to the quantitative analysis of the five major compounds in the samples of SJZTC from different production batches for quality evaluation and control. This is the first report of the simultaneous quantification of the five components of SJZTC.

## 2. Experimental

### 2.1. Chemicals and reagents

HPLC grade acetonitrile and methanol were purchased from Fisher Scientific (Fairlawn, NJ, USA). Deionized water was purified by a Milli-Q Water Purification System (Millipore, Billerica, MA, USA). All other reagents used were of analytical reagent grade or higher. Reference standards of protocatechuic aldehyde, protopine, tetrahydropalmatine, baicalin and cinnamaldehyde were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), in purities greater than 98%. The products of SJZTC were kindly provided by the Department of Pharmaceutical Analysis, College of Pharmacy, Shanxi Medical University (Taiyuan, China).

### 2.2. Preparation of standard solutions

Standard stock solutions of the five reference standards (protocatechuic aldehyde (173 µg/mL), protopine (138 µg/mL), tetrahydropalmatine (1060 µg/mL), baicalin (479 µg/mL) and cinnamaldehyde (14 µg/mL)) were prepared by dissolving the respective working standard substance in methanol. They were then diluted with methanol to the concentrations required. All the solutions were stored at 4 °C until use.

### 2.3. Preparation of sample solutions

Sample preparation was performed with an ultrasonic cleaning bath. In brief, the powder from SJZTC (about 0.2 g) accurately weighed was transferred into a 50-mL conical flask with stopper, and 30 mL of 60% aqueous methanol solution was added. Ultrasonication (250 W, 44 KHz) was performed at room temperature for 40 min, and then the same solvent was added to compensate for the lost weight during the extraction. The resultant mixture was centrifuged at 4000 rpm for 10 min, and then the supernatant was collected and filtered through a 0.45 µm syringe filter before injection into the HPLC system for analysis. The contents of the selected compounds in SJZTC were obtained from the corresponding calibration curves.

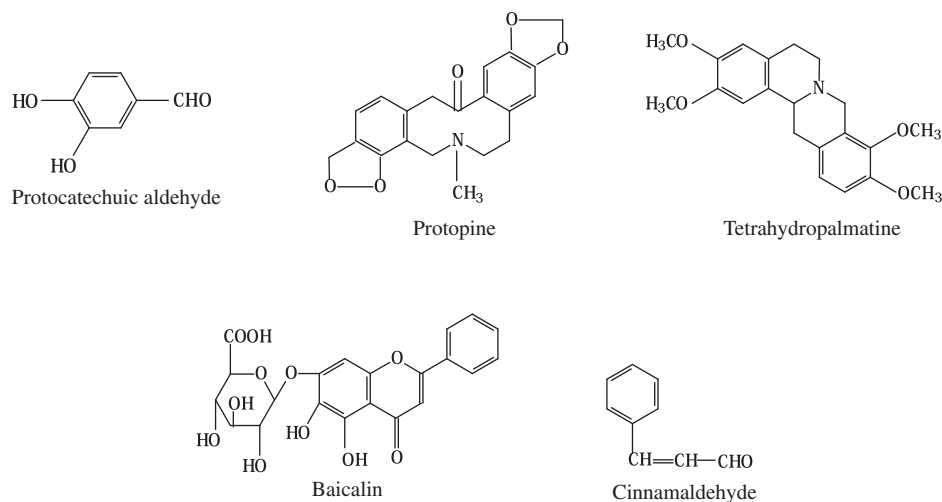


Figure 1 The chemical structures of the tested compounds.

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