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Improved quality control method for Danshen products—Consideration of both hydrophilic and lipophilic active components

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Abstract

The current study intends to provide an improved quality control analysis for Danshen product—a representative herbal product with known active components that are both hydrophilic and lipophilic in nature. A simple HPLC method with photodiode-array (PDA) ultraviolet detection was developed for the simultaneous determination of three major lipophilic components (cryptotanshinone, tanshinone I and tanshinone IIA) and three major hydrophilic components (danshensu, protocatechuic aldehyde and salvianolic acid B) of Danshen (*Salvia miltiorrhiza*). These six components were successfully separated using Radial-pak C18 cartridge with the elution gradient consisting of 0.5% acetic acid in water and 0.5% acetic acid in acetonitrile at a flow rate of 1 ml/min. The intra-day and inter-day precisions of the analysis were within 2.32 and 2.0%, respectively. The detection limits were 0.02, 0.01, 0.01, 0.05, 0.005 and 0.02 μg/ml for cryptotanshinone, tanshinone I, tanshinone IIA, danshensu, protocatechuic aldehyde and salvianolic acid B, respectively. The developed method has been applied to the simultaneous determination of above six major components in Fufang Danshen Tablet and Dripping Pill products by extraction with methanol and water. It has been demonstrated that salvianolic acid B and danshensu are the major components among the eight commercial Fufang Danshen products studied. The current developed method with methanol as extraction solvent provides a simple and efficient method for simultaneous detection of both lipophilic and hydrophilic major components in Danshen products.

Keywords: Cryptotanshinone; Tanshinone I; Tanshinone IIA; Danshensu; Protocatechuic aldehyde; Salvianolic acid B; HPLC

1. Introduction

Danshen, the dried root of *Salvia miltiorrhiza* (Fam. Labiatae), is used as a traditional Chinese medicine for promoting circulation and improving blood stasis. It is also widely used for the treatment and prevention of coronary heart diseases, hyperlipidemia [1–3] and cerebrovascular diseases [4]. At present, numerous Danshen products are commercially available, especially in China. These consist of tablets, capsules, granules, injection preparations, oral liquids, dripping pills and sprays of either Danshen or Fufang Danshen which is the composite of *Salvia miltiorrhiza*, *Panax notoginseng* and *Cinnamomum camphora* [5–7]. Among all the available dosage forms of Danshen, Fufang Danshen Tablet and Fufang Danshen Dripping Pill, the two most commonly used ones in China, have already been officially listed in the Chinese Pharmacopoeia.

The chemical constituents of Danshen include both lipophilic and hydrophilic components. As shown in Fig. 1, the major lipophilic components are cryptotanshinone, tanshinone I and tanshinone IIA [8–10] and the major hydrophilic components include danshensu (salvianic acid A), protocatechuic aldehyde and salvianolic acid B [11]. Various in vitro and in vivo pharmacological activity studies have demonstrated that both lipophilic and hydrophilic components can improve microcirculation [12], dilate the coronary arteries [13], increase the blood flow and prevent myocardial ischemia [14–16].

For the quality control of medicinal products, chemical markers are often utilized. In Chinese Pharmacopoeia 2000, danshensu has been selected as the marker for the quality control of Fufang Danshen Dripping Pill (content of danshensu per pill should not be less than 0.08 mg), and tanshinone IIA is the marker for Fufang Danshen Tablet (content of tanshinone IIA per tablet should not be less than 0.2 mg) [17]. However, according to the Chinese Pharmacopoeia 2005, tanshinone IIA and salvianolic acid B have both been selected as the marker components for the quality control of Fufang Danshen Tablet [18].

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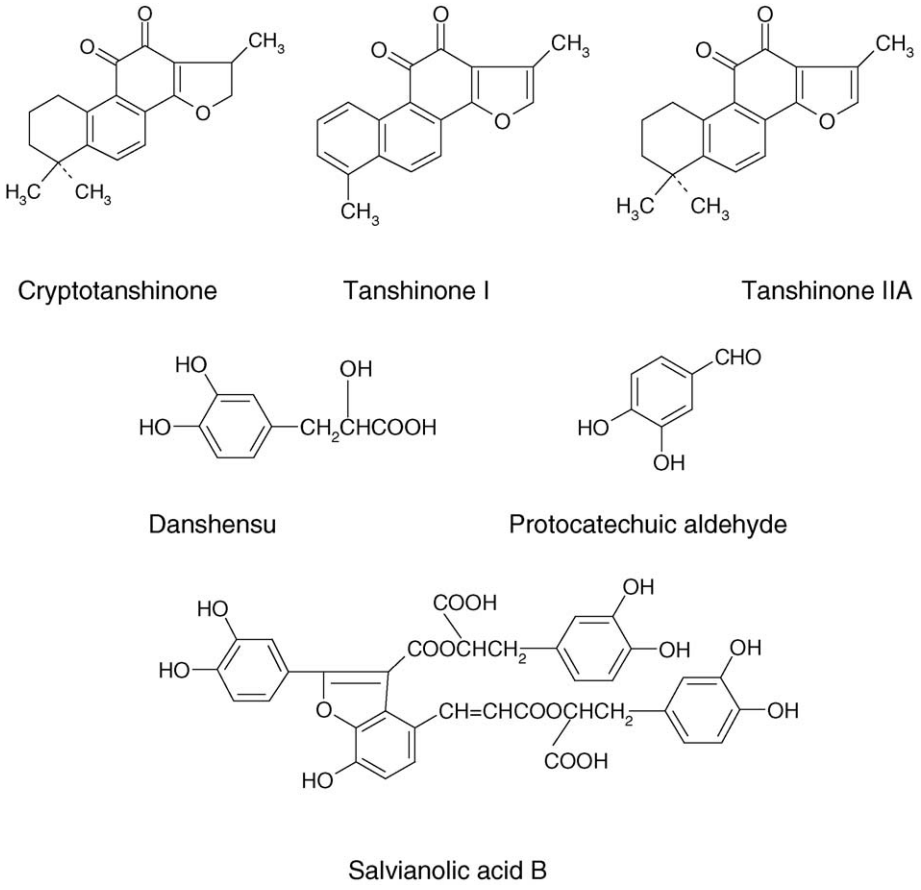

Fig. 1. Chemical structures of the six studied Danshen components.

This latest recommendation reflects the current consideration of the need to include both lipophilic and hydrophilic markers for the quality control of Danshen products.

As a result of the long history use of Danshen, a number of assays have been developed for detecting chemical markers in various Danshen products. One of the recent HPLC study was able to simultaneously determine three lipophilic components (namely cryptotanshinone, tanshinone I and tanshinone IIA) in Danshen medicinal preparations after methanol extraction [19]. Hu et al. developed a multi-component fingerprinting method using reversed phase HPLC and LC-MS-MS for detection of rosmarinic acid, salvianolic acid B and tanshinone IIA in Danshen crude drugs and processed medicinal materials using methanol extraction followed by boiled water extraction [20]. Moreover, assays have been developed to detect lipophilic components and hydrophilic components in Danshen products using separated HPLC assays with water extraction for hydrophilic components and methanol extraction for lipophilic components [21,22]. In both Chinese Pharmacopoeia 2000 and 2005, methanol was recommended as the extraction solvent for the quantifications of danshensu in Fufang Danshen Dripping Pill and tanshinone IIA in Fufang Danshen Tablet. In Chinese Pharmacopoeia 2005, water was recommended as the extraction solvent for the determination of salvianolic acid B.

In summary, previous studies focused on using separate assays or extraction solvents for hydrophilic and lipophilic markers present in Danshen products. For herbal products, such as Danshen products that contain both hydrophilic and lipophilic active markers, a rapid and simple assay method for simultaneous detection of both types of markers will be desirable. The present report presents a simple HPLC method for the simultaneous determination of essential hydrophilic and lipophilic components of Danshen including danshensu, protocatechuic aldehyde, salvianolic acid B, cryptotanshinone, tanshinone I and tanshinone IIA. This method will be applied to evaluate the contents of the studied six components in eight commercial available Danshen products including seven brands of Fufang Danshen Tablets and one brand of Danshen Dripping Pill. In addition, the effect of the extraction solvent will be investigated to find the simplest and most efficient sample preparation method for Danshen products.

2. Material and methods

2.1. Chemicals and instrument

Cryptotanshinone, tanshinone I, salvianolic acid B and protocatechuic aldehyde were purchased from National Institute for Control of Pharmaceutical and Biological Products (Beijing, PR China). Tanshinone IIA, sodium danshensu was purchased from School of Pharmacy, Fudan University (Shanghai, PR China). Griseofulvin, used as internal standard, was purchased from