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# One single LC–MS/MS analysis for both phenolic components and tanshinones in Radix Salviae Miltiorrihizae and its medicinal products

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**Abstract**

A LC–MS/MS method was developed for the separation and simultaneous determination of phenolic components including danshensu, protocatechuic acid, protocatechuic aldehyde and caffeic acid as well as tanshinones including cryptotanshinone, tanshinone I and tanshinone IIA in samples of Radix Salviae Miltiorrhizae and Salviae Miltiorrhizae tablet. Triple quadrupole mass spectrometry was optimized in both positive and negative ion multiple reaction monitoring modes for the simultaneous quantitative analysis of the two different types of active components by using a time-segment program. The method gave recoveries of 85.4–106.4% with relative standard deviations of 2.4–8.0% for the spiked herb samples. The limits of detection were 0.30–0.83 μg/g for the analysis of 1.0 g Radix Salviae Miltiorrhizae or tablet samples.


*Keywords:* Radix Salviae Miltiorrhizae; Danshen; Phenolic acids; Tanshinones; LC–MS/MS

## 1. Introduction

Radix Salviae Miltiorrhizae Bunge (Danshen in Chinese) is one of the most popular herbs used in many traditional Chinese medicines that have been commonly applied for promoting blood circulation to remove blood stasis, relieving vexation, nourishing the blood and cooling the blood to relive carbuncles [1–4]. Major ingredients of Danshen can be classified into water-soluble components that include danshensu, protocatechuic acid, protocatechuic aldehyde and caffeic acid, as well as lipid-soluble components including cryptotanshinone, tanshinone I and tanshinone IIA (Fig. 1).

Analysis of the active components of Danshen has become more and more important due to its increasing popularity and its intensive applications in medicinal chemistry and the pharmaceutical industry. Several reversed-phase HPLC and TLC methods have been developed for determining some of the active components of Danshen. An isocratic reversed-phase HPLC method was described to measure lithospermic acid B and rosmarinic acid in the dried tissues of the calluses, regenerated plantlets, or cultivated plants of Danshen Bunge [5]. Gradient reversed-phase HPLC methods have also been used for analyzing lithospermates and rosmarinates in an aqueous extract of *Cordia spinescens* [6] and rosmarinic acid, lithospermic acid and lithospermic acid B in root hair cultures of *Ocimum basilicum* [7]. A TLC-based method was reported for the determination of rosmarinic acid and caffeic acid in five Salvia species [8]. A method combining TLC and FAB-MS techniques was applied for identifying lithospermic acid B in the aqueous extract of Danshen Bunge [9] as well as rosmarinic acid and caffeic acid in *Sanicula europaea* L. [10]. Isocratic reversed-phase HPLC methods were also reported for the determination of cryptotanshinone, tanshinone I and tanshinone IIA in Danshen Bunge [11,12]. Recently, several studies focusing on the simultaneous determination of cryptotanshinone, tanshinone I and tanshinone IIA, as well as the water-soluble components, by using HPLC–UV and HPLC–MS were reported [13,14]. However, the methods either lacked specificity or had unbalanced sensitivity for the targeted components because the water-soluble and lipid-soluble components are significantly chemically dif-

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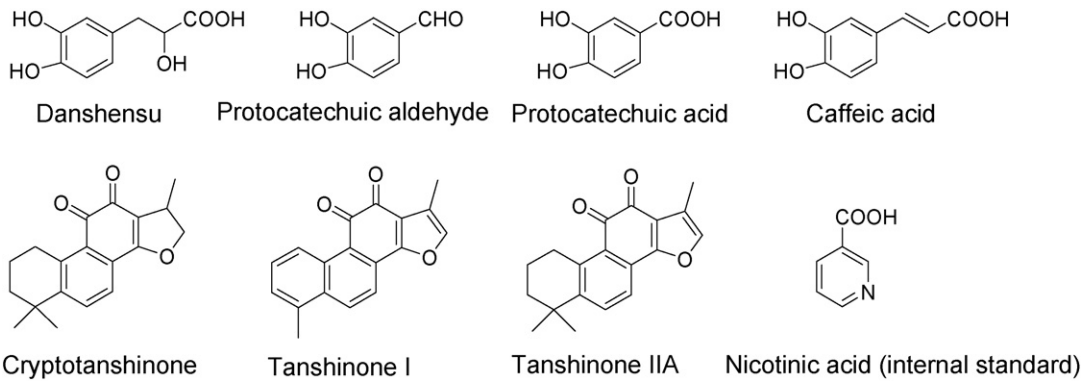


Fig. 1. Chemical structures of danshensu, protocatechuic acid, protocatechuic aldehyde, caffeic acid, cryptotanshinone, tanshinone I, tanshinone IIA and internal standard nicotinic acid.

ferent. Furthermore, most of reported methods had too high detection limits for phenolic compounds that exist in Danshen at much lower levels compared to other water-soluble acids. When electrospray LC–MS was used, positive ion mode provided good sensitivity for the tanshinones while negative ion mode suited better for the phenolic components. In this study, a time-segment program in multiple-reaction-monitoring mode was developed for the simultaneous LC–MS/MS analysis of Danshen and medicinal products. MS parameters under the positive and negative electrospray ionization conditions were optimized for achieving good sensitivity for both phenolic compounds and tanshinones in one single analytical run.

## 2. Experimental

### 2.1. Chemicals and materials

All chemicals used were of analytical grade unless specified. Formic acid, ammonium formate, HPLC-grade acetonitrile and methanol were purchased from BDH Laboratory Supplies (Poole, England). Deionized water was cleaned with a Milli-Q water purification system (Millipore, France) prior to the use as HPLC mobile phase. Nicotinic acid was purchased from Sigma (USA) and used as the internal standard. Danshensu (3-(3,4-dihydroxyphenyl) lactic acid), protocatechuic acid, protocatechuic aldehyde, caffeic acid, cryptotanshinone, tanshinone I and tanshinone IIA were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All of the Danshen standards were stored in the dark. Danshen originated from Sichuan, China was obtained from the Chinese Medicine Clinics, School of Chinese Medicine, Hong Kong Baptist University. The Danshen tablet was purchased from a local Chinese herb store.

### 2.2. Sample preparation

Individual stock standard solutions were prepared by dissolving each standard of the Danshen components in 10 ml of methanol at a concentration of 1.00 mg/ml for nicotinic acid (internal standard); 0.60 mg/ml for danshensu; 2.35 mg/ml for protocatechuic acid; 1.11 mg/ml for protocatechuic aldehyde; 1.08 mg/ml for caffeic acid; 1.00 mg/ml for cryptotanshinone; 0.19 mg/ml for tanshinone I; and 0.25 mg/ml for tanshinone IIA. A standard mixture was prepared by diluting the individual stock standard solutions in 50% ACN and 50% 10 mM ammonium formate with 0.5% formic acid to give the concentrations of the phenolic components at 100 ng/μl and the tanshinones at 50 ng/μl. Seven calibration standard mixtures were prepared by diluting 25 μl, 50 μl, 250 μl, 500 μl, 1000 μl, 2000 μl and 5000 μl of the standard mixture in 5 ml of the ammonium buffer solution.

Twenty Danshen tablets were powdered and homogenized. Six powdered tablet samples (1.0 g each) were accurately weighed. The sample was extracted with 50 ml of methanol by using an ultrasonic bath for 1 h after the internal standard addition. The extract was centrifuged at 10,000 rpm for 15 min. The supernatant was evaporated and dried by nitrogen blow-down. The dried residue was dissolved in 1 ml of the formate buffer. Finally, the extract was filtered with a 0.45-μm filter and 10 μl was analyzed by using LC–MS/MS for the Danshen components.

Three Danshen samples were pounded and homogenized by using a cyclone mill fitted with 0.45-mm seize screen (Waring Commercial, USA). The powdered samples were freeze-dried in a freeze-dryer (Heto-holten, Denmark). Three portions (1.0 g each) of the homogenized herb powder were accurately weighted. After the internal standard addition, the sample was prepared with the method described for the Danshen tablets and then analyzed by LC–MS/MS for the Danshen components.

## 3. Validation study

The linear dynamic range, limit of detection (LOD), recovery and precision were evaluated for the method developed. Due to lack of blank matrix, the linearity of calibration curve was tested with standard analysis. However, spiked matrix samples were analyzed for investigating the matrix suppression during the LC–MS/MS analysis. LOD for each analyte were measured at the signal-to-noise ration of 3:1. Recovery and precision were examined by using the spiked samples with the tablet and herb matrix. The Danshen tablet and herb samples were added with