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Simultaneous determination of danshensu, ferulic acid, cryptotanshinone and tanshinone IIA in rabbit plasma by HPLC and their pharmacokinetic application in danxiongfang

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Abstract

A selective and sensitive reversed-phase high performance liquid chromatography method was developed and validated for the simultaneous determination of danshensu, ferulic acid, cryptotanshinone, and tanshinone IIA in rabbit plasma using *p*-hydroxybenzoic acid as internal standard. Liquid–liquid extraction was used for sample preparation. Chromatographic separation was successfully achieved on an Agilent HC-C_{18} column using a mobile phase composed of methanol–water (from 20:80 to 80:20, v/v) containing 0.5% (v/v) glacial acetic acid. The mobile phase was employing gradient elution at a flow rate of 1.0 ml/min. The method showed good linearity and no endogenous material interfered with the marked compounds and I.S. peaks. The limit of quantification of danshensu, ferulic acid, cryptotanshinone, and tanshinone IIA were 0.1, 0.03, 0.05, and 0.05 μg/ml, respectively. The average extract recoveries of the four compounds from rabbit plasma were all over 60%. The precisions determined from 5 days were all within 10%. The established method has been successfully applied in the pharmacokinetic study and drug interaction of danshensu, ferulic acid, cryptotanshinone, and tanshinone IIA in rabbits after intravenous administration of danxiongfang, a useful compound preparation of traditional Chinese medicine.

Keywords: Danshensu; Ferulic acid; Cryptotanshinone; Tanshinone IIA; Quantification; Pharmacokinetics; HPLC

1. Introduction

Traditional Chinese medicine (TCM) prescriptions are playing an indispensable role in the prevention and treatment of diseases due to their particular effectiveness in the orient public life for more than 2000 years. The remarkable efficacies of many TCM preparations are increasingly being recognized and accepted by more and more people in the world. In clinical practice, most traditional Chinese herbals are prescribed in combination to obtain the synergistic effects or diminish the adverse reactions.

Salvia miltiorrhiza Bunge, which is a well-known traditional Chinese medicine named "Danshen", has been widely adopted in TCM compound preparations and used for treating coronary heart diseases such as angina pectoris, myocardial infarction, anticoagulant and atherosclerosis [1–4], hepatitis and liver fibrosis, anti-inflammatory, antibacterial and antineoplastic action [5–8] in clinical practice. *Ligusticum chuanxiong* Hort [9–11], another famous TCM named "Chuanxiong", is similar in the therapeutic effects to *S. miltiorrhiza* Bunge. For gaining more satisfyingly therapeutic efficacy, *S. miltiorrhiza* Bunge and *L. chuanxiong* Hort were commonly combined to form the compound preparations. At present, there have already been some component mixture preparations containing the two herbs, such as guanxinning tablet [12] and danxiong tongmai pellet [13] in the Orient Market.

According to the chemical structures, the major bioactive constituents in *S. miltiorrhiza* Bunge can be classified into two groups: the phenolic compounds such as danshensu (3,4-dihydroxyphenyl lactic acid), and the tanshinone compounds (abietane-type diterpenes) such as cryptotanshinone and tanshinone IIA. From *L. chuanxiong* Hort, the high content and bioactive component is ferulic acid (3-methoxy-4-hydroxy cinnamic acid). On the basis of these investigations above, danshensu, the major water-soluble component, and tanshinones, the main lipophilic component extracted from *S. miltiorrhiza*

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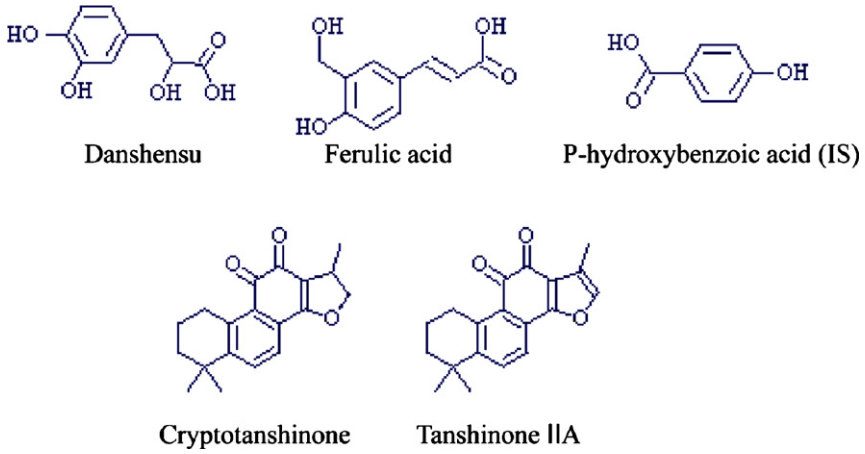

Fig. 1. Chemical structure of danshensu, ferulic acid, cryptotanshinone, tanshinone IIA, and *p*-hydroxybenzoic acid (I.S.).

Bunge, and ferulic acid, the major component from *L. chuanxiong* Hort were combined to form the danxiongfang, a new compound preparation manufactured by our laboratory. The constituents of compound preparation of the two herbs were more complex than that of the single herb preparation. So it is very necessary to develop a more efficient method for simultaneous determination of the four active components, danshensu, ferulic acid, cryptotanshinone and tanshinone IIA which were as the markers in the compound preparation for the quality control of the manufacturing process, the pharmacokinetics and drug interaction *in vivo* and the therapeutic monitoring of danxiongfang.

Each quantification method of HPLC equipped with UV [14–17] or MS, MS/MS detection [18–20] about danshensu, ferulic acid, cryptotanshinone and tanshinone IIA *in vivo* has been reported. However, those methods can only determine the water-soluble or the lipophilic component, respectively, but could not simultaneously determine the water-soluble and the lipophilic component in *S. miltiorrhiza* Bunge, especially not including ferulic acid. In our paper, a gradient HPLC–UV method has been developed for simultaneous determination of danshensu, ferulic acid, cryptotanshinone and tanshinone IIA in biological samples. This method was specific, linear, precise and accurate for the four compounds and successfully applied to the pharmacokinetic study and the drug interaction *in vivo* of danxiongfang in rabbits after an intravenous administration of the component preparations with a single dose.

2. Experimental

2.1. Chemicals and reagents

Danshensu (DS), cryptotanshinone (CT), tanshinone IIA (TS) and ferulic acid (FA) were purchased from National Institute for Control of Pharmaceuticals and Biological Products (Beijing, China). *p*-Hydroxybenzoic acid (internal standard, I.S.) was purchased from Beijing Reagent Chemical Company (Beijing, China). The chemical structures of these compounds were shown in Fig. 1. The raw materials of tanshinones, danshensu and ferulic acid were purchased from Xi'an Honson Biotechnology Co. Ltd. (Xi'an, China). Danxiongfang, which was composed of danshensu, cryptotanshinone, tanshinone IIA and ferulic acid, was prepared with distilled water containing 3% tween-80 and 0.5% 1,2-propanediol by our laboratory. Methanol used was of HPLC grade and obtained from Fisher Scientific Products (Fair Lawn, NJ, USA). Water was triply distilled. The other chemicals, reagents and solvents used were all of analytical grade.

2.2. Instrument and chromatography conditions

All analysis were performed on an Agilent high performance liquid chromatography system (Series 1100, Agilent technology, Palo Alto, CA, USA) which consisted of a G1310A quaternary pump, G1322A vacuum degasser, G1316A column thermostat, G1314A VWD and 7725I manual sample injector. The chromatography data were recorded and processed with HP chemstation software. The analytical column was an Agilent HC-C_{18} (150 mm × 4.6 mm, i.d. 5 μm) column coupled with a C_{18} guard column. All chromatography was performed at 25 °C.

The mobile phase was a mixture of methanol–water containing 0.5% (v/v) glacial acetic acid employing gradient elution (from 20:80 to 80:20, v/v) at a flow rate of 1 ml/min. The gradient elution was shown in Table 1. The solvent was filtered through a 0.45 μm filter and degassed. Danshensu and ferulic acid were determined at 281 nm. Cryptotanshinone and tanshinone IIA were detected at 254 nm. The sample injection volume was 20 μl.

Table 1
Gradient elution program using mobile phase containing A and B

Time (min)	Flow rate (ml/min)	Mobile phase A (%)	Mobile phase B (%)
0	1.0	20	80
4	1.0	45	55
9	1.0	45	55
12	1.0	80	20
25	1.0	80	20

A: methanol; B: water containing 0.5% acetic acid.