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LC-MS/MS analysis and pharmacokinetic study on five bioactive constituents of Tanreqing injection in rats

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[ABSTRACT] Tanreqing injection (TRQ), a well-known traditional Chinese medicine formula, is commonly used to treat respiratory diseases. In the present study, a rapid, selective, and sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed and validated to simultaneously determinate the plasma contents of 5 major constituents of TRQ, including chlorogenic acid (CHA), caffeic acid (CFA), baicalin (BA), ursodeoxycholic acid (UDCA) and chenodeoxycholic acid (CDCA) in rats after intravenous administration of TRQ. Chromatographic separation was performed on an Agilent Zorbax SB-C_{18} column (3.5 μm, 100 mm × 2.1 mm), with acetonitrile and 0.1% aqueous formic acid as mobile phase at a flow rate of 0.3 mL·min^{-1}. The calibration curves were linear over the ranges of 27.0–13 333.0 ng·mL^{-1} for CFA, 30.0–14 933.0 ng·mL^{-1} for CHA, 50.0–50 333.0 ng·mL^{-1} for BA, 550.0–55 000.0 ng·mL^{-1} for UDCA, and 480.0–48 000.0 ng·mL^{-1} for CDCA, respectively. Intra- and inter-day precisions (relative standard deviations, RSDs) were from 3.11% to 14.08%. The extraction recoveries were greater than 71% and accuracy (relative recovery) was from 89% to 137% for all analytes, except endogenous bile acids. This validated method was successfully applied to the first pharmacokinetic study of CFA, CHA, BA, UDCA and CDCA in rat plasma after intravenous administration of TRQ.

[KEY WORDS] Tanreqing Injection; Flavones; Phenolic acids; Bile acids; Pharmacokinetics

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Introduction

Traditional Chinese medicine (TCM), which originated in ancient China, has been widely used for prevention and treatment of various diseases in China and other countries [1]. Tanreqing injection (TRQ) is a well-known TCM formula and won a big total sale in 2010. Produced from five herb raw materials, including Scutellariae Radix, Fel selenarcti, Cornu naemorhedi, Lonicerae japonicae Flos, and Forsythiae fructus, TRQ has markedly curative effects on the infections of upper respiratory tract and some serious influenza in China [2-4]. Our previous chemical profile analyses have found that TRQ contains flavones from Scutellariae Radix and Forsythiae fructus, cholic acids from Fel selenarcti, amino acids from Cornu naemorhedi, phenolic acids from Lonicerae japonicae Flos as major constituents. Beside, its fingerprint and qualitative analyses are performed by using a high performance liquid chromatography coupled with photodiode array detection and evaporative light scattering detection (HPLC-DAD-ELSD) method, which is useful for its quality control [5]. Establishing the pharmacokinetic basis for TCM efficacy favors the clinical application [6], but it is challenging to simultaneously determine major chemical markers representing all kinds of compounds in TRQ, in biological fluids within a single run.

Recently, several studies have developed HPLC, LC-MS/MS and/or UPLC-MS/MS methods for major chemical markers in different biological samples, such as baicalin, baicalein, wogonin, wogonoside, oroxylin A, chrysin, forsythiaside A, chlorogenic acid, neochlorogenic acid, cryptochlorogenic acid, 3, 4-dicaffeoylquinicacid, and 3, 5-dicaffeoylquinic acid (all belong to flavones and phenolic

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acids), following oral administration of Scutellariae Radix, Forsythiae fructus, Lonicerae japonicae Flos, and their other TCM formulae [7-13]. Pharmacokinetics study about TRQ has been found only in a few reports, which have focused on three compounds including baicalin, ursodeoxycholic acid and chenodeoxycholic acid [14-15], ignoring other bioactive compounds in TRQ.

FDA has strongly encouraged monitoring the active ingredients, representative markers and/or major chemical components of TCM and TCM formulas [16]. An *in vitro* analytical method for TRQ has been established in our previous work, using chlorogenic acid (CHA), caffeic acid (CFA), baicalin (BA), ursodeoxycholic acid (UDCA) and chenodeoxycholic acid (CDCA) as main bioactive compounds [5] (Fig. 1). The present study was designed to develop a simple, rapid, and reliable LC-MS/MS method for *in vivo* assay of aforementioned five bioactive compounds in rat plasma which was successfully applied in its pharmacokinetic study after intravenous administration of TRQ.

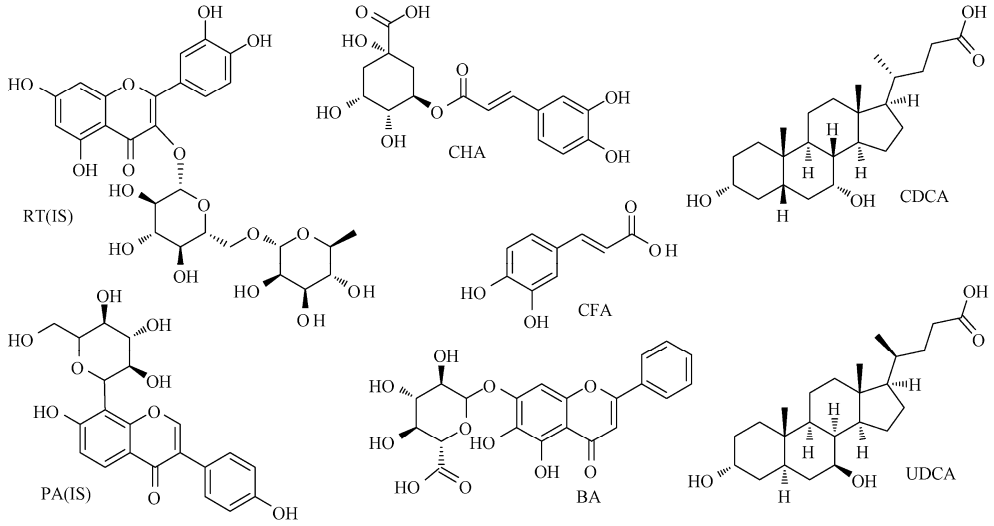

Fig. 1 Chemical structures of five analytes and two internal standards

Materials and Methods

Chemicals and materials

Methanol and acetonitrile (MS-grade) were purchased from Merck (Darmstadt, Germany). Deionized water was prepared by a Milli-Q50 Reagent Water System (Bedford, MA, USA). Other reagents were of analytical grade.

Reference standards of CHA (batch number 110753-200413), CFA (110885-200102), UDCA (110755-9003), CDCA (110806-200704), puerarin (PA, internal standard-1, 110648-200954), and rutin (RT, internal standard-2, 110903-200825) were purchased from the Chinese Institute for the Control of Pharmaceutical and Biological Products (Beijing, China); BA (26695) was purchased from Aladdin Reagents (Shanghai, China) (all purities > 98%). TRQ injections (batch number 110616) were obtained from Shanghai Kaibao Pharmaceutical Co., Ltd. (Shanghai, China). Concentrations of CHA, CFA, BA, UDCA, and CDCA used in the present study were 43.9, 192.6, 8 121.0, 7 794.0 and 1 564.0 μg·mL^{-1}, respectively, using the previous HPLC- DAD-ELSD method [5].

Instrumentation and chromatographic conditions

LC-MS system consisted of an Agilent 1200 series HPLC and an Agilent 6410 triple-quadrupole mass spectrometer equipped with an electrospray ionization source, and Agilent 6410 Quantitative Analysis version B.01.02 analyst data processing software was applied for data acquisition and processing (Santa Clara, CA, USA). An Agilent Zorbax SB-C_{18} column (3.5 μm, 2.1 mm × 100 mm) was kept at 35 °C. Gradient elution program using acetonitrile with 0.1% formic acid (A) and 0.1% aqueous formic acid solution (B) as mobile phases was as follows: 0–1 min, 10%–55% A; and 1–10 min, 55% A. The flow rate was set at 0.3 mL·min^{-1} and the injection volume was 10 μL.

MS detection was conducted in multiple reaction monitoring (MRM) in the negative mode with the optimized parameters: capillary voltage, 4.0 kV; nebulizer pressure, 40 psi; gas temperature, 350 °C; drying gas flow, 10 L·min^{-1}; and high-purity nitrogen for collision-induced dissociation, 0.1 MPa. The MRM conditions were *m/z* 353.1→191.1, 179.0→135.1, 445.1→269.1, 391.3→391.3, 391.3→391.3, 609.1→300.1, and 415.1→267.0 for CHA, CFA, BA, UDCA, CDCA, RT, and PA, respectively (Fig. 1). The values of fragmentation/collision energy were set at 100 V/20 eV, 70 V/13 eV, 120 V/12 eV, 120 V/18 eV and 175 V/20 eV, for CHA, CFA, BA, RT, and PA, respectively.

Preparation of standard and quality control samples

Stock solutions of CHA, CFA, BA, UDCA, and CDCA were prepared separately in methanol at 1.0 mg·mL^{-1}. Working standards were prepared by independent dilution of above stock solutions for each compound with 50% methanol