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Metabolic profile of danshen in rats by HPLC-LTQ-Orbitrap mass spectrometry*

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Abstract: Danshen, the dried root of *Salvia miltiorrhiza* Bunge (Lamiaceae), is one of the traditional Chinese medicines (TCMs) most commonly used for the treatment of cardiovascular and cerebrovascular diseases. However, little is known about the chemical and metabolic profiles of danshen in vitro or in vivo. In particular, more information is needed in relation to the 50% ethanol extracts usually used in danshen formulations such as Fufang Xueshuantong Capsules and Fufang Danshen tablets. High-performance liquid chromatography coupled with a linear ion trap-Orbitrap mass spectrometer (HPLC-LTQ-Orbitrap) provides a sensitive and accurate method for analyzing the composition of samples. This method was used to determine the in vitro and in vivo chemical and metabolic profiles of danshen. Sixty-nine components of danshen extract and 118 components of danshen in rat plasma, urine, feces, and bile were unambiguously or tentatively identified. These results not only revealed the material composition of danshen, but also provided a comprehensive research approach for the identification of multi-constituents in TCMs.

Key words: Danshen; Chemical profile; Metabolic profile; HPLC-LTQ-Orbitrap
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1 Introduction

Recently, high-performance liquid chromatography-mass spectrometry (HPLC-MS), especially for high-resolution mass spectrometry (HRMS), has become a powerful tool for detecting and identifying known and unknown metabolites of drugs owing to its high mass accuracy and high sensitivity (Liu et al., 2011; Wang et al., 2011; Liang et al., 2013). MS/MS data provide abundant information for elucidating the structure of compounds. Thus, this method provides an effective and powerful tool for the identification of compounds in complex matrices, such as traditional Chinese medicines (TCMs) and bio-samples. The linear ion trap-Orbitrap mass spectrometer (LTQ-Orbitrap), an electrostatic Fourier-transform mass spectrometer, combines a high trapping capacity and MS^n scanning function of the linear ion trap with accurate mass measurements to within 5 ppm (parts per million) and a resolving power of up to 100000 (Cai et al., 2015; Zhang et al., 2015). Data-dependent MS/MS scanning can obtain more fragmentation information, improving the efficiency and accuracy of identification (Wang et al., 2016).

Danshen, the dried root of Chinese sage, *Salvia miltiorrhiza* Bunge (Lamiaceae), is one of the TCMs most commonly used in China and elsewhere, and is used either alone or in formulations. It has been widely used in the treatment of cardiovascular and cerebrovascular diseases, such as coronary artery disease (Ji et al., 2003), myocardial infarction (Sun et al., 2005), and stroke (Lam et al., 2003). It has also been used to treat other conditions, such as renal

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diseases (Kang et al., 2004) and diabetes (Belin et al., 2009). Many formulations containing danshen, for instance the Fufang Danshen Dripping Pill and Fufang Xueshuantong Capsule, are now frequently used in the clinical treatment of cardiovascular diseases and eye diseases (Duan et al., 2013; Yang et al., 2014). There are two principal bioactive components in danshen: water-soluble phenolic acids and liposoluble tanshinones. The phenolic acids include danshensu, rosmarinic acid, lithospermic acid, salvianolic acid A, salvianolic acid B, and other salvianolic acids. The tanshinones include tanshinone I, tanshinone IIA, tanshinone IIB, cryptotanshinone, 15,16-dihydrotanshinone I, and other tanshinones (Zhang et al., 2005; Wu et al., 2006).

Previous in vivo studies have focused mainly on the water decoction of danshen (Zhao et al., 2015) or its effective parts and components (Li et al., 2007; Sun et al., 2007). Danshen has often been used only as a component of ethanol extracts, especially in formulations, because of its complex composition and compatibility with other herbs. Also, there has been limited research on the excretion of danshen in feces and bile (Sun et al., 2007). Therefore, comprehensive and systematic studies are needed of the chemical and metabolic profiles of danshen in vitro and in vivo. In the present study, we analyzed the chemical profile of a 50% ethanol extract of danshen, as such extracts are often used in its formulation. The metabolic profile of danshen was determined in bio-samples from rats. An HPLC-LTQ-Orbitrap method coupled with an extracted ion chromatogram (EIC) data-processing technique was applied to elucidate the chemical and metabolic profiles. A total of 69 components of danshen extract and 118 components of danshen in rat plasma, urine, feces, and bile were unambiguously or tentatively identified. The present study provides a basis for research on the quality control and pharmacology of danshen, and establishes a comprehensive and reliable method for identification of multi-components of TCMs both in vitro and in vivo.

2 Materials and methods

2.1 Materials and reagents

Danshen crude drug was provided by the Guangdong Zhongsheng Pharmaceutical Co., Ltd. (Guangzhou, China) and was authenticated by Professor Jian-mei HUANG. Voucher specimens were deposited in the School of Chinese Materia Medica, Beijing University of Chinese Medicine, China.

Eleven reference standards, including caffeic acid, protocatechuic aldehyde, protocatechuic acid, danshensu, ferulic acid, isoferulic acid, rosmarinic acid, tanshinone I, dihydrotanshinone I, tanshinone IIA, and cryptotanshinone, were purchased from the Chengdu Must Bio-Technology Co., Ltd. (Chengdu, China). Three reference standards of tanshinol B, danshenxinkun B, and tanshinone IIB were purchased from the Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Three authentic standards, namely salvianolic acids A, B, and C, were obtained from the School of Chinese Materia Medica, Beijing University of Chinese Medicine, China.

HPLC-grade methanol and acetonitrile, and LC/MS-grade formic acid were purchased from Fisher Scientific (Fisher, Fair Lawn, NJ, USA).

2.2 Instrumentation and analytical conditions

Chromatographic analysis was performed using a Thermo Accela 600 HPLC system (Thermo Scientific, Bremen, Germany) equipped with a binary pump and an autosampler. Samples were separated on a Waters XBridge-C18 column (5 μm, 150 mm× 4.6 mm) at room temperature. A gradient elution of solvent acetonitrile (A) and water containing 0.1% formic acid (B) was applied according to the following program: 0–10 min, 5%–20% A; 10–25 min, 20%–30% A; 25–35 min, 30%–70% A; 35–60 min, 70% A. The flow rate was set at 1.0 ml/min. Sample solution (10 μl) was injected into the HPLC-MS/MS system.

MS analysis was performed using an LTQ-Orbitrap mass spectrometer (Thermo Scientific, Bremen, Germany). The mass spectrometer was connected to the Accela HPLC system by an electrospray ionization (ESI) source and operated in both positive and negative ion modes. Compounds were detected by full scan mass analysis from m/z 100 to 1000 at a resolving power of 30000 with data-dependent MS^n (n=3) analysis. The optimized source parameters in positive (and negative) mode were as follows: capillary temperature, 350 °C; sheath gas flow, 30 arbitrary unit (arb); auxiliary gas flow, 10 arb; source voltage, 4.0 kV; capillary voltage, 35 V; tube lens voltage,

110 V. The isolation width was 2 Da, and the normalized collision energy (CE) was 35%.

2.3 Preparation of drugs

2.3.1 Preparation of danshen freeze-dried powder

Danshen freeze-dried powder was prepared by refluxing the extract twice with 50% (v/v) ethanol (100 g/700 ml for 3 h the first time, and 100 g/500 ml for 2 h the second time) after soaking in 50% ethanol for 30 min. Each decoction was mixed, filtered, vacuum-evaporated, and freeze-dried. The yield of powdered extract was about 42.3% (w/w).

2.3.2 Preparation of danshen extract

A total of 1.05 g danshen freeze-dried powder was accurately weighed and ultrasonicated with 30 ml of 50% ethanol for 30 min. The supernatants were filtered through a 0.22-μm membrane filter. The filtrates were collected and stored at 4 °C until HPLC-MS/MS analysis.

2.3.3 Preparation of danshen suspension

Danshen freeze-dried powder was accurately weighed and suspended in deionized water to obtain a final concentration of 1.5 g/ml (crude drug) for intragastric administration.

2.3.4 Preparation of standard solutions

Individual standard stock solutions of the seventeen standards were prepared by accurately weighing and then dissolving each standard in methanol, with concentrations ranging from 0.09 to 1.20 mg/ml. A working solution of each of the seventeen standards was obtained by diluting each stock solution with methanol to the desired concentration. Working solutions were stored at 4 °C before analysis.

2.4 Animals and drug administration

Twelve male Sprague-Dawley rats, weighing (250±20) g, were purchased from the Si Bei Fu Experimental Animal Science and Technology Co., Ltd. (Beijing, China). The rats were divided into two groups: a control group (n=3, one each for blank plasma, urine and feces, and bile) and a drug group (n=9, 3 for dosed plasma, 3 for dosed urine and feces, and 3 for dosed bile). The rats were housed in a controlled environment (12-h light/12-h dark cycle, at consistent temperature and humidity) for three days before the experiment. Danshen was administered orally to the drug group once a day at a dose of 1 ml/100 g body weight for three days. An equal dose of deionized water was administered by oral gavage to the rats of the control group.

Animal experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals, and all experimental protocols were reviewed and approved by the Institutional animal Experimentation Committee of Beijing University of Chinese Medicine.

2.5 Biological sample collection

Before the last administration, the rats were deprived of food for 12 h. Blood samples (0.4 ml) were collected from the orbital vein and gathered into heparinized tubes at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, and 12 h, respectively. All blood samples were then centrifuged at 3000g for 10 min to obtain plasma samples. Plasma samples from different rats and different time points in each group were then mixed in the same proportions to produce pooled plasma samples, which were stored at −80 °C until additional extraction and analysis.

The urine and feces of rats in each group were collected over a 24-h period starting immediately after the last administration. The urine and feces samples in each group were combined separately and stored at −80 °C until additional extraction and analysis.

Rats were fixed on a wooden plate and anesthetized with ethylurethanm following the last administration. An abdominal incision was made and the common bile duct was cannulated with PE10 tubing (inside diameter (ID)=0.28 mm, San Diego, CA USA) for collection of the bile samples. Bile samples from each group were collected for 24 h and combined and stored at −80 °C until additional extraction and analysis.

2.6 Biological sample pretreatment

An aliquot of 2 ml plasma for positive ion detection was suspended in 8 ml methanol. Another aliquot of 2 ml plasma for negative ion detection was suspended in 200 μl 10% (v/v) hydrochloric acid and 8 ml methanol, and then mixed by vortex for 3 min to precipitate protein, followed by centrifugation at 10000g for 10 min. The supernatants were evaporated to dryness under nitrogen gas at room temperature,

and the residues were dissolved in 200 μl 70% (v/v) methanol. After centrifugation at 12000*g* for 10 min, 10 μl of the supernatant was injected into the HPLC-MS/MS system for analysis.

Urine sample (3 ml) was dissolved in 12 ml methanol, and then mixed by vortex for 3 min to precipitate protein, followed by centrifugation at 10000*g* for 10 min. The supernatant was evaporated to dryness under nitrogen gas at room temperature, and the residue was dissolved in 600 μl 70% methanol. After centrifugation at 12000*g* for 10 min, 10 μl of the supernatant was injected into the HPLC-MS/MS system for analysis.

Bile sample (3 ml) was dissolved in 12 ml methanol, and then mixed by vortex for 3 min to precipitate protein, followed by centrifugation at 10000*g* for 10 min. The supernatant was evaporated to dryness under nitrogen gas at room temperature, and the residue was dissolved in 1.5 ml 70% methanol. After centrifugation at 12000*g* for 10 min, 10 μl of the supernatant was injected into the HPLC-MS/MS system for analysis.

Feces were dried at 37 °C and grinded into powder. Feces sample (1.5 g) was extracted with 30 ml 70% methanol in an ultrasonic bath for 30 min, followed by filtration. Filtrate (2 ml) was evaporated to dryness under nitrogen gas at room temperature, and the residue was dissolved in 400 μl 70% methanol. After centrifugation at 12000*g* for 10 min, a 10-μl aliquot of the supernatant was injected into the HPLC-MS/MS system for analysis.

2.7 Data processing

Thermo Xcalibur 2.1 workstation (Thermo Fisher Scientific, Bremen, Germany) was used for data acquisition and processing. Metworks (Thermo Scientific, Bremen, Germany) was used for data-filtering and identification of possible metabolites. The maximum mass error between the measured and calculated values was 5 ppm.

3 Results

3.1 Analysis of the chemical profile of danshen in vitro

The results from total ion chromatography (TIC) of the danshen extract and the reference standards in positive mode and negative mode are shown in Fig. 1. Based on accurate mass measurements, MS/MS fragmentations, retention time, and reference data (Liu AH et al., 2007; Liu M et al., 2007; Su et al., 2015), a total of 69 components of danshen, including 23 phenolic acids, 33 tanshinones, and 13 unknown compounds, were identified. Their accurate mass measurements, retention time, and HPLC-MS/MS data are shown in Table 1. Among them, 16 compounds were unambiguously confirmed by comparison with reference standards. Their structures are shown in Fig. 2.

3.2 Analysis of the metabolic profile of danshen in vivo

For the identification of original components in bio-samples, the extract ion chromatograms (EICs) combined with the accurate mass measurements, MS/MS fragmentations, and retention time were compared with those of blank samples. For the identification of possible metabolites in bio-samples, firstly, all of the possible metabolic pathways of one component were input in Metworks; secondly, all of the possible metabolites proposed by the software were summarized in an Excel table; thirdly, the EICs, mass measurements, and MS/MS fragmentations of each metabolite were compared with those of blank samples.

As a result, 118 components were unambiguously or tentatively identified, including 38 original components and 80 transformative components (Table 2). Among these components, 7 phenolic acids and 28 tanshinones were identified in rat plasma; 17 phenolic acids and 46 tanshinones were tentatively identified in rat urine; 25 phenolic acids and 37 tanshinones were identified in rat feces; and 1 phenolic acid and 17 tanshinones were identified in rat bile.

4 Discussion

To better identify the metabolites of danshen in vivo after oral administration, the original components of danshen were identified by HPLC-MS/MS in both negative and positive modes. According to the literature (Wei et al., 2007; Lv et al., 2010), the responses of phenolic acids are more sensitive to negative

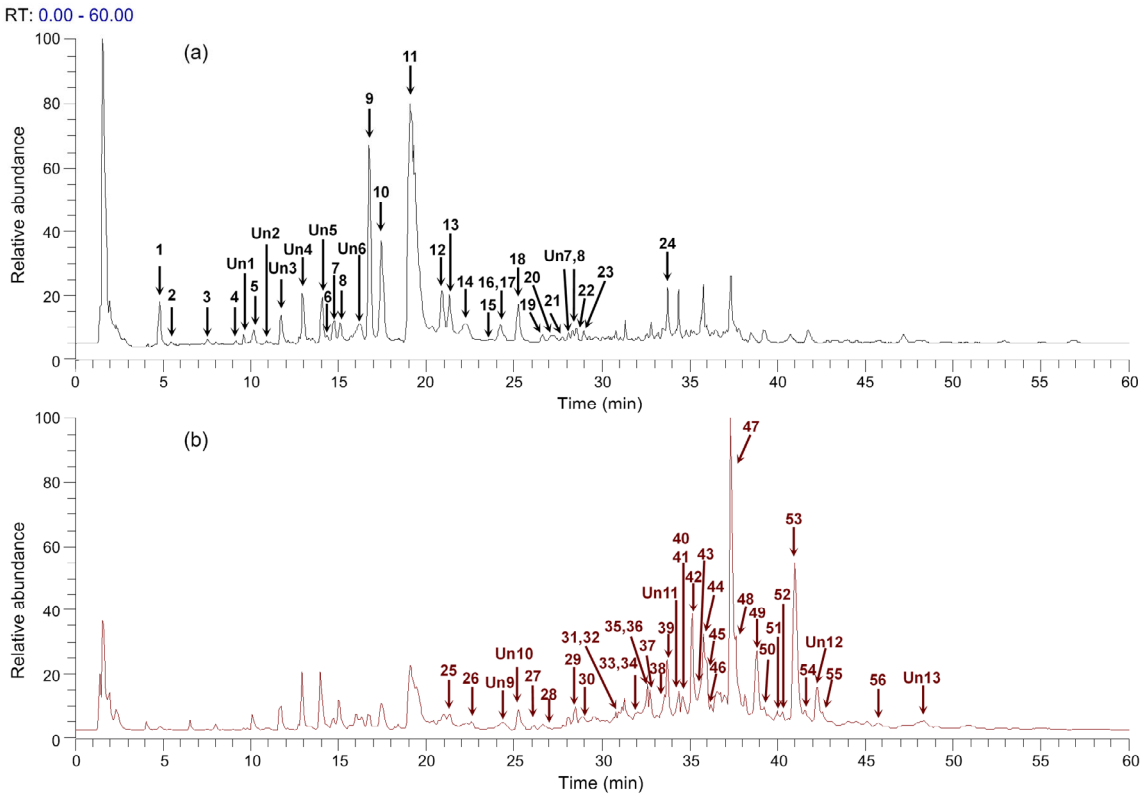

Fig. 1 Total ion chromatography of danshen extract in negative (a) and positive (b) ion modes

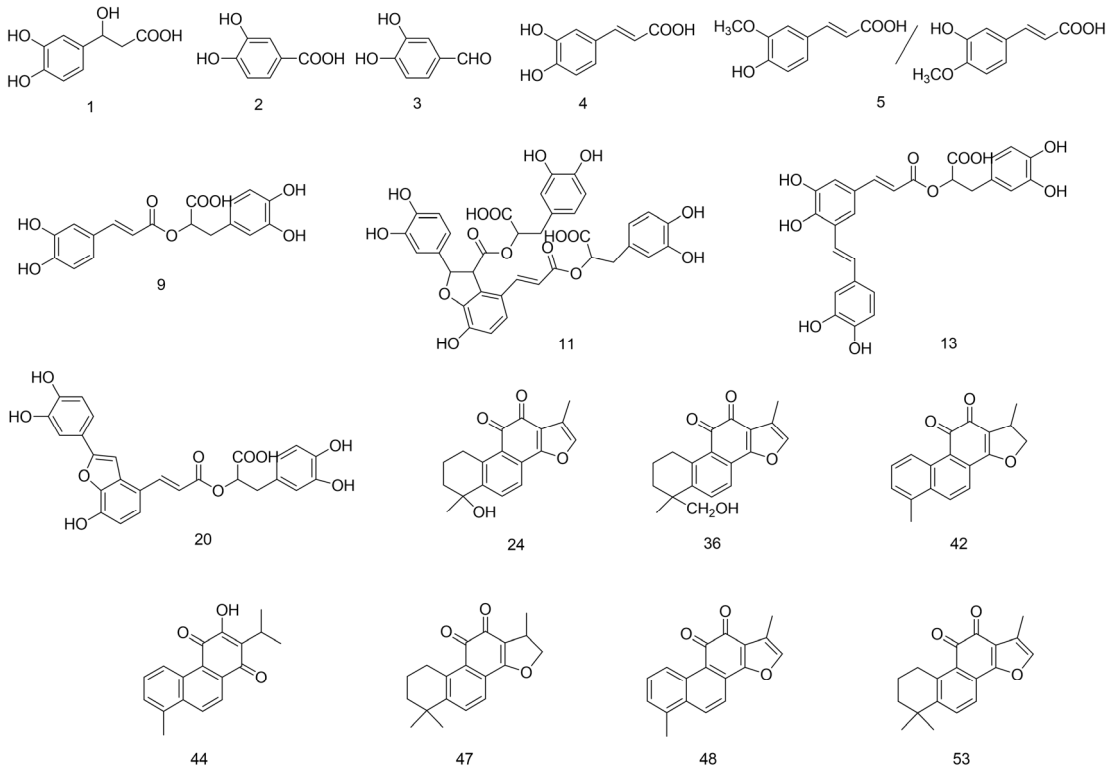

Fig. 2 Chemical structures of confirmed compounds in danshen extract

The numbering of compounds is consistent with that in Table 1

Table 1 HPLC-MS/MS data and identification of components of danshen

No.	t_R (min)	Ion	Theoretical mass (*m/z*)	Experimental mass (*m/z*)	Error (ppm)	Formula $[M-H]^-/[M+H]^+$	MS/MS fragment	Identification
Phenolic acids								
1	4.83	$[M-H]^-$	197.0444	197.0441	−1.6	$C_9H_9O_5$	MS^2[197]: 179(100) MS^3[179]: 135(100)	Danshensu[a]
2	5.70	$[M-H]^-$	153.0182	153.0184	0.9	$C_7H_5O_4$		Protocatechuic acid[a]
3	7.58	$[M-H]^-$	137.0233	137.0236	1.7	$C_7H_5O_3$	MS^2[137]: 137(100)	Protocatechuic aldehyde[a]
4	9.16	$[M-H]^-$	179.0339	179.0336	−1.3	$C_9H_7O_4$	MS^2[179]: 135(100)	Caffeic acid[a]
5	10.08	$[M-H]^-$	193.0495	193.0493	−1.2	$C_{10}H_9O_4$	MS^2[193]: 178(100), 149(38), 134(81) MS^3[178]: 134(100)	Ferulic acid/isoferulic acid[a]
6	14.42	$[M-H]^-$	735.1556	735.1552	−0.5	$C_{36}H_{31}O_{17}$	MS^2[735]: 537(100), 519(20) MS^3[519]: 519(51), 357(73), 321(65), 297(100)	Hydrated salvianolic acid B
7	14.76	$[M-H]^-$	537.1028	537.1028	0.1	$C_{27}H_{21}O_{12}$	MS^2[537]: 339(100), 295(37) MS^3[339]: 321(33), 295(100)	Salvianolic acid H/I
8	15.02	$[M-H]^-$	735.1556	735.1553	−0.4	$C_{36}H_{31}O_{17}$	MS^2[735]: 537(100), 519(49) MS^3[537]: 519(51), 339(20), 321(70), 297(100)	Hydrated salvianolic acid B
9	16.74	$[M-H]^-$	359.0761	359.0758	−1.0	$C_{18}H_{15}O_8$	MS^2[359]: 197(24), 179(23), 161(100) MS^3[161]: 161(38), 133(100)	Rosmarinic acid[a]
10	17.45	$[M-H]^-$	493.1129	493.1129	0.0	$C_{26}H_{21}O_{10}$	MS^2[493]: 295(100) MS^3[295]: 280(13), 277(65), 159(100)	Salvianolic acid A isomer
11	19.07	$[M-H]^-$	717.1450	717.1434	−2.2	$C_{36}H_{29}O_{16}$	MS^2[717]: 519(100), 321(15) MS^3[519]: 339(21), 321(100)	Salvianolic acid B[a]
12	20.89	$[M-H]^-$	717.1450	717.1450	0.0	$C_{36}H_{29}O_{16}$	MS^2[717]: 519(100), 321(18) MS^3[519]: 339(21), 321(100)	Salvianolic acid E
13	21.32	$[M-H]^-$	493.1129	493.1135	1.1	$C_{26}H_{21}O_{10}$	MS^2[493]: 295(100) MS^3[295]: 280(14), 277(66), 159(100)	Salvianolic acid A[a]
14	22.26	$[M-H]^-$	731.1607	731.1598	−1.2	$C_{37}H_{31}O_{16}$	MS^2[731]: 533(100) MS^3[533]: 353(51), 335(100)	Methyl salvianolic acid B
15	23.68	$[M-H]^-$	565.1341	565.1341	0.1	$C_{29}H_{25}O_{12}$	MS^2[565]: 519(87), 367(87), 339(15), 321(100)	Dimethyl lithospermate
16	24.23	$[M-H]^-$	491.0973	491.0982	1.9	$C_{26}H_{19}O_{10}$	MS^2[491]: 311(60), 293(100) MS^3[293]: 276(32), 275(11), 265(100), 249(89), 247(13)	Isosalvianolic acid C
17	24.44	$[M-H]^-$	565.1341	565.1341	0.1	$C_{29}H_{25}O_{12}$	MS^2[565]: 367(76), 339(17), 321(100)	Dimethyl lithospermate
18	25.23	$[M-H]^-$	565.1341	565.1345	0.7	$C_{29}H_{25}O_{12}$	MS^2[565]: 519(88), 339(15), 321(100) MS^3[321]: 293(31), 277(100), 249(76)	Dimethyl lithospermate

To be continued

Table 1

No.	t_R (min)	Ion	Theoretical mass (*m/z*)	Experimental mass (*m/z*)	Error (ppm)	Formula $[M-H]^-/[M+H]^+$	MS/MS fragment	Identification
19	26.65	$[M-H]^-$	565.1341	565.1345	0.7	$C_{29}H_{25}O_{12}$	MS^2[565]: 367(100)	Dimethyl lithospermate
20	27.27	$[M-H]^-$	491.0973	491.0977	0.9	$C_{26}H_{19}O_{10}$	MS^2[491]: 311(22), 293(100) MS^3[293]: 276(24), 275(18), 265(100), 249(21), 247(36)	Salvianolic acid C[a]
21	27.65	$[M-H]^-$	745.1763	745.1760	−0.5	$C_{38}H_{33}O_{16}$	MS^2[745]: 547(87), 519(100), 321(73)	Dimethyl salvianolic acid B
22	28.62	$[M-H]^-$	745.1763	745.1762	−0.1	$C_{38}H_{33}O_{16}$	MS^2[745]: 547(87), 519(100), 321(74)	Dimethyl salvianolic acid B
23	28.97	$[M-H]^-$	313.0707	313.0709	0.8	$C_{17}H_{13}O_6$	MS^2[313]: 269(36), 161(100) MS^3[161]: 161(29), 133(100)	Salvianolic acid F
Tanshinones								
24	33.73	$[M-H]^-$	295.0965	295.0970	1.7	$C_{18}H_{15}O_4$	MS^2[295]: 277(16), 267(27), 265(100) MS^3[265]: 265(100)	Tanshinol B[a]
25	21.24	$[M+H]^+$	313.1071	313.1056	−4.8	$C_{18}H_{17}O_5$	MS^2[313]: 295(100), 267(22), 265(87) MS^3[295]: 277(100), 267(40), 249(98)	Tanshindiol A/B/C
26	22.57	$[M+H]^+$	313.1071	313.1056	−4.6	$C_{18}H_{17}O_5$	MS^2[313]: 295(100), 267(7) MS^3[295]: 267(100)	Tanshindiol A/B/C
27	26.14	$[M+H]^+$	313.1071	313.1056	−4.7	$C_{18}H_{17}O_5$	MS^2[313]: 295(100), 267(9) MS^3[295]: 267(100)	Tanshindiol A/B/C
28	27.01	$[M+H]^+$	293.0808	293.0797	−3.9	$C_{18}H_{13}O_4$	MS^2[293]: 249(100) MS^3[249]: 234(18), 221(24), 193(100), 178(39)	Hydroxyl tanshinone I
29	28.49	$[M+H]^+$	313.1434	313.1422	−3.9	$C_{19}H_{21}O_4$	MS^2[313]: 269(100) MS^3[269]: 254(25), 251(16), 223(22), 199(100)	Hydroxyl cryptotanshinone
30	29.28	$[M+H]^+$	283.0965	283.0954	−3.7	$C_{17}H_{15}O_4$	MS^2[283]: 265(51), 255(27), 241(15), 237(100) MS^3[237]: 222(11), 219(100), 209(87), 191(35), 181(25)	Dihydronortanshinone
31	30.79	$[M+H]^+$	293.0808	293.0799	−3.3	$C_{18}H_{13}O_4$	MS^2[293]: 249(100) MS^3[249]: 234(18), 221(23), 193(100), 178(38)	Przewaquinone B
32	30.97	$[M+H]^+$	295.0965	295.0953	−4.1	$C_{18}H_{15}O_4$	MS^2[295]: 277(100), 267(44), 24(51) MS^3[277]: 259(82), 249(100), 231(27)	Hydrated tanshinone I/ trijuganone A
33	32.08	$[M+H]^+$	295.0965	295.0951	−4.8	$C_{18}H_{15}O_4$	MS^2[295]: 277(100), 249(11) MS^3[277]: 249(100), 221(17)	Hydrated tanshinone I/ trijuganone A
34	32.26	$[M+H]^+$	313.1423	313.1423	−3.5	$C_{19}H_{21}O_4$	MS^2[313]: 295(100), 267(73) MS^3[295]: 277(100), 267(55), 249(52)	Hydroxyl cryptotanshinone
35	32.44	$[M+H]^+$	301.1434	301.1424	−3.6	$C_{18}H_{21}O_4$	MS^2[301]: 283(100) MS^3[283]: 265(100), 255(26)	Salvianonol

To be continued

Table 1

No.	t_R (min)	Ion	Theoretical mass (*m*/*z*)	Experimental mass (*m*/*z*)	Error (ppm)	Formula $[M-H]^-/[M+H]^+$	MS/MS fragment	Identification
36	32.61	$[M+H]^+$	311.1278	311.1264	−4.6	$C_{19}H_{19}O_4$	MS^2[311]: 293(100), 283(22), 267(80), 225(12) MS^3[293]: 278(16), 275(100), 265(19), 251(80)	Tanshinone IIB[a]
37	32.77	$[M+H]^+$	311.1278	311.1263	−4.6	$C_{19}H_{19}O_4$	MS^2[311]: 293(14), 275(11), 267(100) MS^3[267]: 252(100), 239(11), 225(63), 185(47)	Hydroxyl tanshinone IIA
38	33.46	$[M+H]^+$	341.1384	341.1371	−3.8	$C_{20}H_{21}O_5$	MS^2[341]: 281(100), 263(43) MS^3[281]: 263(100), 235(19)	Methyl dihydrotanshinonate
39	33.69	$[M+H]^+$	327.1214	327.1248	−3.9	$C_{19}H_{19}O_5$	MS^2[327]: 309(100) MS^3[309]: 265(100)	Hydroxyl tanshinone IIB
40	34.56	$[M+H]^+$	281.1536	281.1524	−4.1	$C_{19}H_{21}O_2$	MS^2[281]: 266(38), 263(50), 253(28), 239(100) MS^3[239]: 224(22), 221(100), 193(54)	Dehydromiltirone
41	34.64	$[M+H]^+$	293.1172	293.1159	−4.4	$C_{19}H_{17}O_3$	MS^2[293]: 275(100), 265(11), 247(39) MS^3[275]: 260(13), 247(100)	Dehydrotanshinone IIA
42	35.19	$[M+H]^+$	279.1016	279.1004	−4.3	$C_{18}H_{15}O_3$	MS^2[279]: 261(100), 233(5) MS^3[261]: 233(100)	Dihydrotanshinone I[a]
43	35.71	$[M+H]^+$	315.1591	315.1576	−4.7	$C_{19}H_{23}O_4$	MS^2[315]: 297(100) MS^3[297]: 279(100), 251(57)	Neocryptotanshinone
44	35.84	$[M+H]^+$	281.1172	281.1160	−4.2	$C_{18}H_{17}O_3$	MS^2[281]: 263(100), 253(7), 235(71) MS^3[263]: 248(16), 245(10), 235(100)	Danshenxinkun B[a]
45	35.98	$[M+H]^+$	339.1227	339.1219	−2.3	$C_{20}H_{19}O_5$	MS^2[339]: 279(100) MS^3[279]: 261(100)	Methyl tanshinonate
46	36.24	$[M+H]^+$	295.1329	295.1319	−3.3	$C_{19}H_{19}O_3$	MS^2[295]: 277(100), 267(15), 249(47) MS^3[277]: 249(100)	Dehydrocryptotanshinone
47	37.34	$[M+H]^+$	297.1485	297.1474	−3.7	$C_{19}H_{21}O_3$	MS^2[297]: 279(100), 251(81) MS^3[279]: 251(100)	Cryptotanshinone[a]
48	37.62	$[M+H]^+$	277.0859	277.0850	−3.5	$C_{18}H_{13}O_3$	MS^2[277]: 249(100), 231(13)	Tanshinone I[a]
49	38.84	$[M+H]^+$	279.1016	279.1003	−4.7	$C_{18}H_{15}O_3$	MS^2[279]: 261(100), 233(6) MS^3[261]: 233(100), 205(3)	Dihydrotanshinone I
50	38.98	$[M+H]^+$	293.1172	293.1166	−2.2	$C_{19}H_{17}O_3$	MS^2[293]: 275(100), 265(11), 247(38) MS3[275]: 260(13), 247(100)	Dehydrotanshinone IIA
51	40.05	$[M+H]^+$	281.1536	281.1524	−4.1	$C_{19}H_{21}O_2$	MS^2[281]: 266(17), 263(43), 253(82), 221(100) MS^3[221]: 206(17), 193(100)	Dehydromiltirone
52	40.28	$[M+H]^+$	293.1172	293.1159	−4.5	$C_{19}H_{17}O_3$	MS^2[293]: 275(100), 265(11), 247(37) MS^3[275]: 260(13), 247(100)	Dehydrotanshinone IIA

To be continued

Table 1

No.	t_R (min)	Ion	Theoretical mass (*m*/*z*)	Experimental mass (*m*/*z*)	Error (ppm)	Formula $[M-H]^-/[M+H]^+$	MS/MS fragment	Identification
53	41.17	$[M+H]^+$	295.1329	295.1317	−4.0	$C_{19}H_{19}O_3$	MS^2[295]: 277(100), 249(14) MS^3[277]: 249(100)	Tanshinone IIA[a]
54	41.54	$[M+H]^+$	281.1536	281.1526	−3.7	$C_{19}H_{21}O_2$	MS^2[281]: 266(18), 263(43), 253(93), 221(100) MS^3[221]: 206(14), 193(100)	Dehydromiltirone
55	42.34	$[M+H]^+$	283.1693	283.1682	−3.6	$C_{19}H_{23}O_2$	MS^2[283]: 265(100), 241(47), 223(63) MS^3[265]: 237(64), 223(100)	Miltirone
56	45.75	$[M+H]^+$	557.1959	557.1942	−3.1	$C_{36}H_{29}O_6$	MS^2[557]: 539(28), 529(100), 511(48) MS^3[529]: 511(100), 501(81), 483(46)	Neoprzewaquinone A
Others								
Un1	9.62	$[M-H]^-$	509.2229	509.2230	0.2	$C_{22}H_{37}O_{13}$	MS^2[509]: 463(100) MS^3[463]: 331(100), 161(27)	Unknown
Un2	10.91	$[M-H]^-$	571.1082	571.1080	−0.5	$C_{27}H_{23}O_{14}$	MS^2[571]: 527(21), 483(100), 439(73)	Unknown
Un3	11.73	$[M-H]^-$	627.4044	627.4059	2.4	$C_{41}H_{55}O_5$	MS^2[627]: 610(14), 581(70), 564(100)	Unknown
Un4	12.96	$[M-H]^-$	723.5042	723.5013	−3.9	$C_{41}H_{71}O_{10}$	MS^2[723]: 678(100) MS^3[678]: 659(100), 451(25), 338(25)	Unknown
Un5	14.07	$[M-H]^-$	836.5856	836.5854	−0.2	$C_{44}H_{84}O_{14}$	MS^2[836]: 791(100) MS^3[791]: 773(100), 565(28)	Unknown
Un6	16.21	$[M-H]^-$	717.1450	717.1452	0.3	$C_{36}H_{29}O_{16}$	MS^2[717]: 519(100), 321(16) MS^3[519]: 339(22), 321(100)	Unknown
Un7	28.14	$[M-H]^-$	341.1020	341.1023	0.9	$C_{19}H_{17}O_6$	MS^2[341]: 297(100), 253(14) MS^3[297]: 253(100)	Unknown
Un8	28.33	$[M-H]^-$	671.1395	671.1402	1.0	$C_{35}H_{27}O_{14}$	MS^2[671]: 473(100) MS^3[473]: 429(100), 321(100)	Unknown
Un9	24.40	$[M+H]^+$	327.1227	327.1212	−4.6	$C_{19}H_{19}O_5$	MS^2[327]: 283(100), 265(8) MS^3[283]: 265(29), 254(100)	Unknown
Un10	25.27	$[M+H]^+$	369.0969	369.0955	−3.7	$C_{20}H_{17}O_7$	MS^2[369]: 323(100), 295(77) MS^3[323]: 295(100)	Unknown
Un11	34.38	$[M+H]^+$	297.1485	297.1471	−4.7	$C_{19}H_{21}O_3$	MS^2[297]: 253(100) MS^3[253]: 238(33), 211(100)	Unknown
Un12	42.22	$[M+H]^+$	283.1693	283.1677	−5.4	$C_{19}H_{23}O_2$	MS^2[283]: 265(100), 241(47), 223(63) MS^3[265]: 237(20), 223(100)	Unknown
Un13	50.83	$[M+H]^+$	587.2064	587.2059	−0.9	$C_{37}H_{31}O_7$	MS^2[587]: 569(100), 541(24) MS^3[569]: 551(100), 541(71)	Unknown

[a] Confirmed by comparison with reference standards

Table 2 Metabolites identified in bio-samples from rats after oral administration of danshen extract

No.	t_R (min)	Ion	Theoretical mass (*m/z*)	Experimental mass (*m/z*)	Formula $[M-H]^-/[M+H]^+$	Error (ppm)	MS/MS fragment	Identification	Plasma	Urine	Feces	Bile
Phenolic acids												
1	2.11	$[M-H]^-$	277.0013	277.0020	$C_9H_9O_8S$	2.8	MS^2[277]: 259(57), 215(35), 197(100)	Sulfate danshensu	×	×	√	×
2	4.26	$[M-H]^-$	277.0013	277.0010	$C_9H_9O_8S$	−0.8	MS^2[277]: 215(40), 197(100) MS^3[196]: 179(100)	Sulfate danshensu	×	√	×	×
3	4.77	$[M-H]^-$	197.0444	197.0446	$C_9H_9O_5$	0.6	MS^2[197]: 179(100) MS^3[179]: 135(100)	Danshensu[a,b]	√	√	√	×
4	7.35	$[M-H]^-$	211.0601	211.0603	$C_{10}H_{11}O_5$	0.9	MS^2[211]: 193(100), 165(23) MS^3[193]: 149(29), 134(100)	Methyl danshensu	√	√	√	√
5	7.47	$[M-H]^-$	179.0339	179.0344	$C_9H_7O_4$	3.1	MS^2[179]: 135(100) MS^3[135]: 135(100)	Demethyl ferulic acid	×	√	×	×
6	7.52	$[M-H]^-$	137.0233	137.0236	$C_7H_5O_3$	1.7	MS^2[137]: 137(100)	Protocatechuic aldehyde[a,b]	×	×	√	×
7	7.62	$[M-H]^-$	181.0495	181.0502	$C_9H_9O_4$	3.7	MS^2[181]: 163(100) MS^3[162]: 119(100)	Dihydro caffeic acid	×	√	√	×
8	8.29	$[M-H]^-$	179.0339	179.0341	$C_9H_7O_4$	1.2	MS^2[179]: 135(100) MS^3[135]: 135(100)	Acetylated protocatechuic aldehyde	×	√	×	×
9	8.59	$[M-H]^-$	165.0546	165.0550	$C_9H_9O_3$	2.4	MS^2[165]: 121(100) MS^3[121]: 121(100)	Decarbonyl ferulic acid	×	√	×	×
10	9.38	$[M-H]^-$	361.0918	361.0910	$C_{18}H_{17}O_8$	−2.2	MS^2[361]: 317(39), 273(41), 239(100), 221(68) MS^3[239]: 195(100), 151(19)	Dihydro rosmarinic acid	×	×	√	×
11	9.86	$[M-H]^-$	193.0495	193.0502	$C_{10}H_9O_4$	3.5		Ferulic acid/isoferulic acid[a,b]	×	×	√	×
12	12.03	$[M-H]^-$	343.0812	343.0806	$C_{18}H_{15}O_7$	−1.8	MS^2[343]: 299(100) MS^3[299]: 255(100)	Hydroxyl and methyl salvianolic acid F	×	×	√	×
13	13.62	$[M-H]^-$	535.1082	535.1072	$C_{24}H_{23}O_{14}$	−1.9	MS^2[535]: 359(100) MS^3[359]: 197(23), 179(20), 161(100)	Rosmarinic acid glucuronide conjugate	×	√	×	×
14	13.99	$[M-H]^-$	539.1184	539.1169	$C_{27}H_{23}O_{12}$	−2.7	MS^2[539]: 399(100), 297(67) MS^3[297]: 219(100), 201(70)	Dihydro salvianolic acid H/I	×	√	√	×
15	14.67	$[M-H]^-$	537.1028	537.1025	$C_{27}H_{21}O_{12}$	−0.4	MS^2[537]: 339(100), 295(38)	Salvianolic acid H/I[b]	×	×	√	×
16	15.03	$[M-H]^-$	735.1556	735.1539	$C_{36}H_{31}O_{17}$	−2.3		Hydrated salvianolic acid B[b]	×	×	√	×
17	15.17	$[M-H]^-$	343.0812	343.0806	$C_{18}H_{15}O_7$	−1.7	MS^2[343]: 255(100) MS^3[255]: 237(91), 148(100)	Hydroxyl and methyl salvianolic acid F	×	×	√	×
18	16.55	$[M-H]^-$	555.1133	555.1125	$C_{27}H_{23}O_{13}$	−1.5	MS^2[555]: 375(100), 357(39) MS^3[375]: 331(100), 269(60)	Hydrated salvianolic acid H/I	×	×	√	×
19	16.71	$[M-H]^-$	359.0761	359.0761	$C_{18}H_{15}O_8$	−0.2	MS^2[359]: 271(100) MS^3[271]: 149(100), 135(46), 121(74)	Rosmarinic acid[a,b]	√	×	×	×
20	17.01	$[M-H]^-$	523.1235	523.1225	$C_{27}H_{23}O_{11}$	−1.9	MS^2[523]: 505(53), 281(100) MS^3[281]: 263(26), 174(100)	Demethyl and hydroxyl salvianolic acid A	×	×	√	×

To be continued

Table 2

No.	t_R (min)	Ion	Theoretical mass (*m*/*z*)	Experimental mass (*m*/*z*)	Formula $[M-H]^-/[M+H]^+$	Error (ppm)	MS/MS fragment	Identification	Plasma	Urine	Feces	Bile
21	17.14	$[M-H]^-$	315.0863	315.0863	$C_{17}H_{15}O_6$	0.0	MS^2[315]: 297(18), 285(100), 267(12)	Dihydro salvianolic acid F	×	√	×	×
22	17.46	$[M-H]^-$	493.1129	493.1130	$C_{26}H_{21}O_{10}$	0.1	MS^2[493]: 295(100) MS^3[295]: 277(56), 159(100)	Iso salvianolic acid A[b]	√	√	√	×
23	19.02	$[M-H]^-$	717.1450	717.1439	$C_{36}H_{29}O_{16}$	−1.5	MS^2[717]: 519(100), 321(17) MS^3[519]: 339(22), 321(100)	Salvianolic acid B[a,b]	×	√	√	×
24	20.40	$[M-H]^-$	763.1869	763.1854	$C_{38}H_{35}O_{17}$	−1.9	MS^2[763]: 565(100), 520(57), 321(15)	Hydrated dimethyl salvianolic acid B	×	×	√	×
25	20.80	$[M-H]^-$	717.1450	717.1441	$C_{36}H_{29}O_{16}$	−1.3	MS^2[717]: 519(100), 321(19) MS^3[519]: 339(23), 321(100)	Salvianolic acid E[b]	×	√	√	×
26	21.26	$[M-H]^-$	493.1129	493.1129	$C_{26}H_{21}O_{10}$	−0.1	MS^2[493]: 295(100) MS^3[295]: 277(59), 159(100)	Salvianolic acid A[a,b]	√	√	√	×
27	21.70	$[M-H]^-$	551.1184	551.1177	$C_{28}H_{23}O_{12}$	−1.3	MS^2[551]: 519(43), 371(100), 353(51), 339(73)	Methyl salvianolic acid H/I	×	×	√	×
28	22.20	$[M-H]^-$	731.1607	731.1597	$C_{37}H_{31}O_{16}$	−1.4	MS^2[731]: 533(100) MS^3[533]: 353(49), 335(100)	Methyl salvianolic acid B[b]	√	√	√	×
29	24.15	$[M-H]^-$	491.0973	491.0971	$C_{26}H_{19}O_{10}$	−0.3	MS^2[491]: 293(100) MS^3[293]: 276(33), 264(100), 249(91)	Iso salvianolic acid C[b]	×	×	√	×
30	24.35	$[M-H]^-$	565.1341	565.1334	$C_{29}H_{25}O_{12}$	−1.1	MS^2[565]: 519(84), 367(78), 321(100)	Dimethyl lithospermate[b]	×	×	√	×
31	25.16	$[M-H]^-$	565.1341	565.1331	$C_{29}H_{25}O_{12}$	−1.7	MS^2[565]: 519(87), 367(79), 321(100)	Dimethyl lithospermate[b]	√	√	√	×
32	26.60	$[M-H]^-$	565.1341	565.1331	$C_{29}H_{25}O_{12}$	−1.8		Dimethyl lithospermate[b]	×	√	×	×
33	27.21	$[M-H]^-$	491.0973	491.0972	$C_{26}H_{19}O_{10}$	−0.1	MS^2[491]: 293(100)	Salvianolic acid C[a,b]	×	×	√	×
Tanshinones												
34	9.08	$[M+H]^+$	345.0969	345.0976	$C_{18}H_{17}O_7$	2.0	MS^2[345]: 327(100), 281(53) MS^3[327]: 309(14), 281(100)	Demethyl and trihydroxyl tanshinone IIB	×	×	√	×
35	12.06	$[M+H]^+$	517.1704	517.1683	$C_{26}H_{29}O_{11}$	−2.1		Methyl dihydrotanshinonate glucuronide conjugate	×	√	×	×
36	12.77	$[M+H]^+$	343.0812	343.0814	$C_{18}H_{15}O_7$	0.5	MS^2[343]: 325(92), 297(100) MS^3[297]: 279(100), 255(56)	Demethyl and carboxylated tanshindiol A/B/C	×	×	√	×
37	13.06	$[M+H]^+$	329.1020	329.1023	$C_{18}H_{17}O_6$	1.1	MS^2[329]: 311(100), 265(58) MS^3[311]: 283(25), 265(100)	Demethyl and two hydroxyl tanshinone IIB	×	×	√	×
38	13.37	$[M+H]^+$	620.1909	620.1917	$C_{28}H_{34}O_{11}N_3S$	1.3	MS^2[620]: 602(55), 584(32), 455(100)	Tanshindiol A/B/C glutathione conjugate	×	×	×	√
39	13.66	$[M+H]^+$	343.0812	343.0814	$C_{18}H_{15}O_7$	0.5	MS^2[343]: 325(95), 297(100) MS^3[297]: 279(100), 255(60)	Demethyl and carboxylated tanshindiol A/B/C	×	×	√	×

To be continued

Table 2

No.	t_R (min)	Ion	Theoretical mass (*m/z*)	Experimental mass (*m/z*)	Formula $[M-H]^-/[M+H]^+$	Error (ppm)	MS/MS fragment	Identification	Plasma	Urine	Feces	Bile
40	14.04	$[M+H]^+$	471.1286	471.1286	$C_{24}H_{23}O_{10}$	0.1	MS^2[471]: 295(57), 277(23), 267(100) MS^3[267]: 249(50), 237(100)	Hydroxyl and glucuronidated dihydrotanshinone I	×	√	×	×
41	14.25	$[M-H]^-$	487.1235	487.1232	$C_{24}H_{23}O_{11}$	−0.6	MS^2[487]: 311(100) MS^3[311]: 283(30), 281(100)	Hydroxyl and glucuronidated tanshinol B	×	√	×	×
42	14.64	$[M+H]^+$	473.1442	473.1440	$C_{24}H_{25}O_{10}$	−0.5	MS^2[473]: 297(80), 279(100), 251(39) MS^3[279]: 261(100), 251(100)	Hydroxyl and glucuronidated danshenxinkun B	×	√	×	×
43	16.60	$[M+H]^+$	327.0863	327.0866	$C_{18}H_{15}O_6$	0.7	MS^2[327]: 309(100), 281(67), 265(12) MS^3[309]: 291(13), 281(100)	Hydroxyl and dehydro tanshindiol A/B/C	×	×	√	×
44	18.89	$[M+H]^+$	345.1333	345.1338	$C_{19}H_{21}O_6$	1.5	MS^2[345]: 327(100), 309(23) MS^3[327]: 309(100), 281(19)	Hydrated and hydroxyl tanshinone IIB	×	√	×	×
45	19.32	$[M+H]^+$	301.1434	301.1433	$C_{18}H_{21}O_4$	−0.4	MS^2[301]: 283(100)	Demethyl neocryptotanshinone	√	×	×	×
46	19.43	$[M+H]^+$	301.1434	301.1439	$C_{18}H_{21}O_4$	1.6	MS^2[301]: 283(100) MS^3[283]: 265(100), 255(26)	Demethyl and two hydroxyl miltirone	×	√	×	×
47	19.81	$[M-H]^-$	313.0707	313.0708	$C_{17}H_{13}O_6$	0.4	MS^2[313]: 269(27), 252(100)	Demethyl and two hydroxyl tanshinol B	×	×	√	×
48	20.40	$[M+H]^+$	355.1176	355.1157	$C_{20}H_{19}O_6$	−2.0	MS^2[355]: 337(100), 309(45)	Hydroxyl methyltanshinonate	×	√	×	×
49	20.47	$[M+H]^+$	489.1391	489.1397	$C_{24}H_{25}O_{11}$	1.1	MS^2[489]: 313(100), 295(57)	Tanshindiol A/B/C glucuronide conjugate	√	×	×	×
50	21.17	$[M+H]^+$	313.1071	313.1077	$C_{18}H_{17}O_5$	2.0	MS^2[313]: 295(100), 265(78)	Tanshindiol A/B/C[b]	√	×	√	×
51	21.54	$[M+H]^+$	339.1227	339.1209	$C_{20}H_{19}O_5$	−1.8	MS^2[339]: 321(100)	Methyl tanshinonate	×	√	×	×
52	22.00	$[M+H]^+$	297.1121	297.1129	$C_{18}H_{17}O_4$	2.4	MS^2[297]: 279(100), 251(49), 237(36)	Hydrated dihydrotanshinone I	×	√	×	×
53	22.49	$[M+H]^+$	313.1071	313.1077	$C_{18}H_{17}O_5$	2.0	MS^2[313]: 295(100), 267(7) MS^3[295]: 267(100)	Tanshindiol A/B/C[b]	√	√	√	×
54	22.98	$[M+H]^+$	309.0758	309.0763	$C_{18}H_{13}O_5$	1.8	MS^2[309]: 291(18), 265(20), 235(100) MS^3[235]: 207(18), 179(100)	Dihydroxyl tanshinone I	×	√	×	×
55	23.60	$[M+H]^+$	295.0601	295.0603	$C_{17}H_{11}O_5$	0.6	MS^2[295]: 267(100) MS^3[267]: 239(100)	Demethyl and two hydroxyl tanshinone I	×	×	√	×
56	23.87	$[M+H]^+$	299.1278	299.1281	$C_{18}H_{19}O_4$	0.5	MS^2[299]: 281(42), 271(49), 253(100)	Demethyl and hydroxyl cryptotanshinone	×	√	×	×
57	23.89	$[M-H]^-$	311.0914	311.0915	$C_{18}H_{15}O_5$	0.3	MS^2[311]: 283(32), 281(100) MS^3[281]: 253(100)	Hydroxyl tanshinol B	×	√	×	×
58	24.85	$[M+H]^+$	299.1278	299.1284	$C_{18}H_{19}O_4$	1.1	MS^2[299]: 281(19), 271(44), 253(100)	Demethyl and hydroxyl cryptotanshinone	√	√	×	×
59	25.78	$[M-H]^-$	325.0707	325.0706	$C_{18}H_{13}O_6$	−0.3	MS^2[325]: 297(31), 295(100), 268(30) MS^3[294]: 267(100)	Demethyl and carboxylated tanshinol B	×	√	×	×
60	26.06	$[M+H]^+$	313.1071	313.1074	$C_{18}H_{17}O_5$	1.3	MS^2[313]: 295(100)	Tanshindiol A/B/C[b]	√	√	√	×

To be continued

Table 2

No.	t_R (min)	Ion	Theoretical mass (*m/z*)	Experimental mass (*m/z*)	Formula $[M-H]^-/[M+H]^+$	Error (ppm)	MS/MS fragment	Identification	Plasma	Urine	Feces	Bile
61	26.21	$[M+H]^+$	345.1333	345.1341	$C_{19}H_{21}O_6$	0.5	MS^2[345]: 327(100) MS^3[327]: 299(100), 281(13)	Demethyl and carboxylated neocryptotanshinone	×	√	×	×
62	28.01	$[M-H]^-$	297.1121	297.1124	$C_{18}H_{17}O_4$	0.9	MS^2[297]: 253(100), 239(74), 221(26) MS^3[253]: 238(100)	Dihydro tanshinol B	×	√	×	×
63	28.04	$[M+H]^+$	343.1176	343.1184	$C_{19}H_{19}O_6$	0.1	MS^2[343]: 325(100)	Dihydroxyl tanshinone IIB	×	√	√	×
64	28.27	$[M+H]^+$	372.1806	372.1811	$C_{21}H_{26}O_5N$	1.7	MS^2[372]: 354(100), 311(55), 283(60) MS^3[354]: 326(100), 311(59)	Neocryptotanshinone glycine conjugate	×	√	√	×
65	28.44	$[M+H]^+$	313.1434	313.1437	$C_{19}H_{21}O_4$	−0.5	MS^2[313]: 269(100) MS^3[269]: 254(25), 223(21), 199(100), 171(74)	Hydroxyl cryptotanshinone[b]	√	√	√	×
66	28.70	$[M-H]^-$	471.1286	471.1286	$C_{24}H_{23}O_{10}$	0.0	MS^2[471]: 295(100)	Tanshinol B glucuronide conjugate	×	√	×	×
67	28.73	$[M+H]^+$	331.1540	331.1545	$C_{19}H_{23}O_5$	0.7	MS^2[331]: 313(100), 295(38)	Hydroxyl and methyl salvianonol	√	×	×	×
68	28.80	$[M+H]^+$	459.1286	459.1287	$C_{23}H_{23}O_{10}$	1.5	MS^2[459]: 283(100) MS^3[283]: 265(25), 237(100)	Dihydronortanshinone glucuronide conjugate	×	√	×	×
69	28.99	$[M-H]^-$	471.1286	471.1286	$C_{24}H_{23}O_{10}$	0.1	MS^2[471]: 295(100)	Tanshinol B glucuronide conjugate	×	√	×	×
70	29.00	$[M+H]^+$	618.2116	618.2119	$C_{29}H_{36}O_{10}N_3S$	−0.4	MS^2[618]: 471(44), 309(100)	Tanshinone IIB glutathione conjugate	×	×	×	√
71	29.01	$[M+H]^+$	327.1227	327.1231	$C_{19}H_{19}O_5$	1.6	MS^2[327]: 309(24), 299(22), 281(100), 263(57) MS^3[281]: 263(100), 235(36)	Hydroxyl tanshinone IIB	×	√	×	×
72	29.41	$[M+H]^+$	309.1121	309.1121	$C_{19}H_{17}O_4$	−2.0	MS^2[309]: 265(100) MS^3[265]: 247(51), 223(100), 195(18)	Hydroxyl and dehydro tanshinone IIA	√	×	×	×
73	29.54	$[M+H]^+$	343.1176	343.1185	$C_{19}H_{19}O_6$	1.1	MS^2[343]: 325(100)	Dihydroxyl tanshinone IIB	×	√	×	×
74	30.06	$[M+H]^+$	313.1434	313.1438	$C_{19}H_{21}O_4$	1.1	MS^2[313]: 269(35), 251(100) MS^3[251]: 223(100)	Hydroxyl cryptotanshinone	×	√	×	×
75	30.44	$[M+H]^+$	445.1857	445.1864	$C_{24}H_{29}O_8$	−1.8	MS^2[445]: 269(100) MS^3[269]: 254(100), 239(14)	Decarbonylated and glucuronidated cryptotanshinone	×	×	×	√
76	30.63	$[M+H]^+$	293.0808	293.0813	$C_{18}H_{13}O_4$	2.4	MS^2[293]: 275(22), 263(100), 249(23) MS^3[263]: 235(100)	Hydroxyl tanshinone I	√	√	√	×
77	31.05	$[M+H]^+$	295.0965	295.0966	$C_{18}H_{15}O_4$	1.8	MS^2[295]: 251(100) MS^3[251]: 223(97), 195(95), 169(100)	Hydrated tanshinone I[b]	√	√	×	×
78	31.08	$[M+H]^+$	297.1121	297.1124	$C_{18}H_{17}O_4$	0.6	MS^2[297]: 279(100), 261(36) MS^3[279]: 261(100)	Demethyl and hydroxyl tanshinone IIA	√	√	√	×
79	31.30	$[M+H]^+$	285.1485	285.1489	$C_{18}H_{21}O_3$	1.0	MS^2[285]: 243(100), 229(6) MS^3[243]: 228(71), 225(92), 1815(100)	Demethyl and hydroxyl miltirone	√	√	×	×

To be continued

Table 2

No.	t_R (min)	Ion	Theoretical mass (*m/z*)	Experimental mass (*m/z*)	Formula $[M-H]^-/[M+H]^+$	Error (ppm)	MS/MS fragment	Identification	Plasma	Urine	Feces	Bile
80	31.32	$[M-H]^-$	311.0914	311.0912	$C_{18}H_{15}O_5$	−0.7	MS^2[311]: 267(100), 223(29)	Hydroxyl tanshinol B	×	√	×	×
81	31.36	$[M+H]^+$	473.1806	473.1806	$C_{25}H_{29}O_9$	2.3	MS^2[473]: 297(36), 269(100) MS^3[269]: 241(100), 213(92), 199(87)	Cryptotanshinone glucuronide conjugate	×	×	√	×
82	31.61	$[M+H]^+$	339.1227	339.1210	$C_{20}H_{19}O_5$	1.2	MS^2[339]: 321(100), 295(21)	Methyl tanshinonate	×	√	×	×
83	32.13	$[M+H]^+$	459.2014	459.2012	$C_{25}H_{31}O_8$	2.4	MS^2[459]: 283(100)	Miltirone glucuronide conjugate	×	×	×	√
84	32.36	$[M+H]^+$	381.1333	381.1318	$C_{22}H_{21}O_6$	2.2	MS^2[381]: 363(60), 337(100)	Acetylated methyltanshinone	√	×	×	×
85	32.65	$[M+H]^+$	311.1278	311.1282	$C_{19}H_{19}O_4$	1.4	MS^2[311]: 293(40), 275(37), 267(100) MS^3[267]: 252(100)	Tanshinone IIB[a,b]	√	√	√	√
86	32.84	$[M+H]^+$	287.1642	287.1645	$C_{18}H_{23}O_3$	0.8	MS^2[287]: 269(100) MS^3[269]: 251(58), 241(100), 213(86)	Decarbonylated neocryptotanshinone	√	×	×	×
87	33.16	$[M+H]^+$	299.1642	299.1646	$C_{19}H_{23}O_3$	1.4	MS^2[299]: 281(100), 255(68), 253(62)	Hydroxyl miltirone	×	√	×	×
88	33.24	$[M+H]^+$	267.1016	267.1022	$C_{17}H_{15}O_3$	0.3	MS^2[267]: 249(100), 221(22)	Decarbonylated hydrated tanshinone I	√	×	×	×
89	33.31	$[M+H]^+$	457.1493	457.1496	$C_{24}H_{25}O_9$	0.5	MS^2[457]: 281(100), 263(73), 261(41) MS^3[281]: 263(100)	Danshenxinkun B glucuronide conjugate	×	×	×	√
90	33.59	$[M+H]^+$	341.1384	341.1388	$C_{20}H_{21}O_5$	1.2	MS^2[341]: 281(100), 263(42) MS^3[281]: 263(100), 235(18)	Methyl dihydrotanshinonate[b]	×	×	√	×
91	33.67	$[M+H]^+$	279.1016	279.1021	$C_{18}H_{15}O_3$	−0.2	MS^2[279]: 261(100) MS^3[261]: 233(100), 205(13)	Dihydro tanshinone I	√	×	×	√
92	33.69	$[M-H]^-$	295.0965	295.0968	$C_{18}H_{15}O_4$	1.2	MS^2[295]: 277(17), 267(28), 265(100), 238(27) MS^3[265]: 237(100)	Tanshinol B[a,b]	√	√	√	√
93	33.73	$[M+H]^+$	309.1121	309.1120	$C_{19}H_{17}O_4$	2.5	MS^2[309]: 265(100) MS^3[265]: 247(55), 223(100)	Hydroxyl and dehydro tanshinone IIA	√	√	×	×
94	34.29	$[M+H]^+$	297.1485	297.1489	$C_{19}H_{21}O_3$	0.3	MS^2[297]: 253(100) MS^3[253]: 238(33), 225(24), 211(100), 209(16)	Hydroxyl dehydromiltirone	√	×	×	×
95	34.61	$[M+H]^+$	293.1172	293.1174	$C_{19}H_{17}O_3$	1.5	MS^2[293]: 275(100), 247(39) MS^3[275]: 247(100)	Dehydrotanshinone IIA[b]	×	×	√	×
96	34.63	$[M+H]^+$	297.1485	297.1490	$C_{19}H_{21}O_3$	1.6	MS^2[297]: 279(100), 251(81) MS^3[279]: 251(100), 237(67)	Dihydro tanshinone IIA	×	×	×	√
97	34.72	$[M+H]^+$	311.1278	311.1284	$C_{19}H_{19}O_4$	1.8	MS^2[311]: 283(100) MS^3[283]: 265(100), 237(17)	Hydroxyl tanshinone IIA	√	×	×	×
98	35.14	$[M+H]^+$	279.1016	279.1020	$C_{18}H_{15}O_3$	1.4	MS^2[279]: 261(100), 233(5) MS^3[261]: 233(100), 215(7), 205(14)	Dihydrotanshinone I[b]	×	√	√	√

To be continued

Table 2

No.	t_R (min)	Ion	Theoretical mass (*m/z*)	Experimental mass (*m/z*)	Formula $[M-H]^-/[M+H]^+$	Error (ppm)	MS/MS fragment	Identification	Plasma	Urine	Feces	Bile
99	35.36	$[M+H]^+$	293.0808	293.0815	$C_{18}H_{13}O_4$	2.2	MS^2[293]: 249(100) MS^3[249]: 221(26), 193(100), 178(52)	Hydroxyl hydrotanshinone I	×	×	×	√
100	35.69	$[M+H]^+$	315.1591	315.1594	$C_{19}H_{23}O_4$	1.0	MS^2[315]: 297(100) MS^3[297]: 279(100), 268(13), 254(18), 251(58)	Hydrated cryptotanshinone	√	×	×	√
101	35.83	$[M+H]^+$	281.1172	281.1178	$C_{18}H_{17}O_3$	2.2	MS^2[281]: 263(100), 235(72) MS^3[263]: 235(100)	Danshenxinkun B[a,b]	×	√	√	×
102	35.96	$[M+H]^+$	339.1227	339.1235	$C_{20}H_{19}O_5$	2.5	MS^2[339]: 279(100) MS^3[279]: 261(100)	Methyl tanshinonate[b]	×	×	√	√
103	36.22	$[M+H]^+$	295.1329	295.1331	$C_{19}H_{19}O_3$	0.9	MS^2[295]: 277(100), 249(40) MS^3[277]: 262(53), 249(100), 235(38)	Dehydrocryptotanshinone[b]	×	×	√	×
104	36.59	$[M+H]^+$	309.1121	309.1124	$C_{19}H_{17}O_4$	0.8	MS^2[309]: 265(100) MS^3[265]: 247(52), 223(100)	Hydroxyl and methyl dihydrotanshinone I	×	×	×	√
105	36.67	$[M+H]^+$	301.1798	301.1802	$C_{19}H_{25}O_3$	1.4	MS^2[301]: 271(100) MS^3[271]: 256(100)	Hydrated miltirone	×	×	√	×
106	36.96	$[M+H]^+$	313.1434	313.1439	$C_{19}H_{21}O_4$	1.5	MS^2[313]: 295(100), 277(31), 271(33), 267(25) MS^3[295]: 277(100), 253(27), 249(23)	Hydroxyl cryptotanshinone	√	×	×	×
107	37.31	$[M+H]^+$	297.1485	297.1488	$C_{19}H_{21}O_3$	1.0	MS^2[297]: 279(100), 251(81) MS^3[279]: 251(100), 237(73)	Cryptotanshinone[a,b]	×	√	√	×
108	37.58	$[M+H]^+$	277.0859	277.0863	$C_{18}H_{13}O_3$	1.4	MS^2[277]: 249(100), 231(13) MS^3[249]: 234(18), 221(89), 193(100), 178(30)	Tanshinone I[a,b]	√	√	√	×
109	38.82	$[M+H]^+$	279.1016	279.1017	$C_{18}H_{15}O_3$	0.6	MS^2[279]: 261(100) MS^3[261]: 233(100)	Dihydrotanshinone I[a,b]	×	√	√	√
110	38.97	$[M+H]^+$	293.1172	293.1178	$C_{19}H_{17}O_3$	2.1	MS^2[293]: 275(100), 247(40) MS^3[275]: 247(100)	Dehydrotanshinone IIA[b]	√	√	√	√
111	40.01	$[M+H]^+$	281.1536	281.1540	$C_{19}H_{21}O_2$	1.4		Dehydromiltirone[b]	×	×	√	×
112	40.23	$[M+H]^+$	293.1172	293.1175	$C_{19}H_{17}O_3$	1.0		Dehydrotanshinone IIA[b]	√	√	√	×
113	40.58	$[M-H]^-$	277.0859	277.0865	$C_{18}H_{13}O_3$	2.1	MS^2[277]: 249(100), 221(53)	Dehydrated tanshinol B	×	×	√	×
114	41.15	$[M+H]^+$	295.1329	295.1331	$C_{19}H_{19}O_3$	0.9	MS^2[295]: 277(100), 249(14) MS^3[277]: 262(29), 249(100)	Tanshinone IIA[a,b]	√	√	√	√
115	41.27	$[M-H]^-$	277.0859	277.0863	$C_{18}H_{13}O_3$	1.4	MS^2[277]: 249(100), 221(60)	Dehydrated tanshinol B	×	×	√	×
116	42.32	$[M+H]^+$	283.1693	283.1694	$C_{19}H_{23}O_2$	0.4	MS^2[283]: 265(100), 241(47), 223(63) MS^3[265]: 237(62), 223(100)	Miltirone[b]	×	√	√	×
117	42.70	$[M+H]^+$	269.1536	269.1537	$C_{18}H_{21}O_2$	0.4	MS^2[269]: 254(100) MS^3[254]: 239(100)	Demethyl miltirone	×	×	√	×
118	47.15	$[M+H]^+$	299.1642	299.1646	$C_{19}H_{23}O_3$	1.3	MS^2[299]: 281(100), 256(52), 253(49)	Dihydro cryptotanshinone	×	×	√	×
Total									35	63	62	18

[a] Confirmed by reference standards; [b] Original components in danshen extract. "√": detected; "×": undetected

mode, while those of tanshinones are more sensitive to positive mode. In this study, the phenolic acids were detected in negative mode, and exhibited their parent ions as $[M-H]^-$; the tanshinones were detected in positive mode, and exhibited their parent ions of $[M+H]^+$ and/or $[M+Na]^+$.

Although 10% hydrochloric acid was added to rat plasma to increase the recovery ratios of phenolic acids, few phenolic acids were detected. This may have been because of the low bioavailability and transformation of phenolic acids in vivo (Gao et al., 2009; Sun et al., 2013). Under our experimental conditions, very few phenolic acids and their metabolites were detected in rat bile, except methyl danshensu. Compared with plasma and bile, many more metabolites were detected and unambiguously identified in rat urine and feces. This suggests that urine and feces might be the major route for elimination of danshen after oral administration.

From the analysis of metabolites, we found that hydroxylation (36 out of 118), methylation/demethylation (35 out of 118), glucuronidation (14 out of 118), hydration/dehydration (8 out of 118), and hydrogenation/dehydrogenation (11 out of 118) might be the main metabolic pathways of danshen in vivo. The metabolic pathway for phenolic acids was mainly methylation/demethylation (11 out of 33), while tanshinones mostly showed hydroxylation (31 out of 85) and methylation/demethylation (19 out of 85). Hydrogenation, sulfation, acetylation, and glutathione conjugation were found to be the possible metabolic pathways of danshen. This research provided a comprehensive in vitro chemical profile and in vivo metabolic profile of danshen after oral administration, which could be useful in research on the quality control and pharmacology of danshen.

5 Conclusions

Using HPLC-MS/MS methods, our research provided the most comprehensive chemical and metabolic profiles of danshen. A total of 69 compounds in danshen extract and 118 metabolites were identified, including 35 in plasma, 63 in urine, 62 in feces, and 18 in bile. This analysis of chemical and metabolic components of danshen lays a foundation for further studies of the material composition of danshen, and provides a useful means for identification of multi-components of TCMs both in vitro and in vivo.

Compliance with ethics guidelines

Huan-huan PANG, Mei-fang JIANG, Qin-hui WANG, Xiao-ye WANG, Wei GAO, Zhi-hao TIAN, and Jian-mei HUANG declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

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中文概要

题　目：HPLC-LTQ-Orbitrap 方法分析丹参的代谢轮廓

目　的：对丹参 50%乙醇提取物的体内外物质基础进行系统全面的分析。

创新点：首次采用高效液相色谱-质谱联用（HPLC-LTQ-Orbitrap）方法对丹参 50%乙醇提取物的体内外化学和代谢成分进行全面分析。

方　法：建立 HPLC-LTQ-Orbitrap 方法，对丹参 50%乙醇提取物的化学成分以及给药后大鼠血浆、尿液、粪便和胆汁生物样品中的化学和代谢成分进行

分析。

结　论：在正负离子模式下，在丹参提取物中鉴定出共 69 个化合物，包括丹酚酸类化合物 23 个，丹参酮类化合物 33 个，以及未知化合物 13 个；在大鼠灌胃给予丹参提取物后的生物样本中鉴定出共 118 个化合物，包括血浆中 35 个，尿液中 63 个，粪便中 62 个，以及胆汁中 18 个。

关键词：丹参；化学轮廓；代谢轮廓；HPLC-LTQ-Orbitrap