Metabolite Profiling and Pharmacokinetics of Herbal Compounds Following Oral Administration of a Cardiovascular Multi-herb Medicine (Qishen Yiqi Pills) in Rats

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Abstract: Qishen yiqi pills (QY pills) are a type of standardized cardiovascular herbal medicine, which contain four component herbs, i.e., *Astragalus membranaceus* (Huangqi), *Salvia miltiorrhiza* (Danshen), *Panax notoginseng* (Sanqi), and *Dalbergia odorifera* (Jiangxiang). After oral administration of QY pills, the *in vivo* exposure types of each component herb in rats were first uncovered and identified according to a target-directed strategy based on hyphenated chromatography techniques. The dominated metabolites in urine, blood and bile were originated from flavonoids of Huangqi and monomer phenolic acids of Danshen; no metabolites but parent drugs of Sanqi ginsenosides, namely ginsenosides Rb_1, Rd, Re and Rg_1, notoginsenoside R_1 and gypenoside XVII, were detected in rat urine and blood, and the 20(*S*)-protopanaxatriol type ginsenosides (NR_1, GRe, GRg_1) could also be excreted to bile; the high liposolubility of volatile oils from Jiangxiang restricted them to small intestine, liver and adipose tissues. The identification of metabolites in bio-samples was achieved by exact mass measurement and detailed fragmentation pathway analyses. In specific conditions, not only the types of phase II metabolism but also their conjugation positions could be determined by our established cleavage pathways, which lead to discriminate the phase II metabolites of protocatechualdehyde for the first time. Based on the metabolite study in rats, the 4 main compounds (tanshinol, astragaloside IV, GRb_1 and GRg_1) in QY pills were selected as pharmacokinetic markers. The PK results showed that their maximal concentrations in blood were obtained within one hour, much shorter than the reported values in single herbs. The rat exposure was proximately linear under the studied dosages from 1 to 6 g/kg.

Keywords: Chinese herbal medicine, metabolism, pharmacokinetics, qishen yiqi pills.

INTRODUCTION

Herbal medicine, one essential therapy of traditional Chinese medicine (TCM), is playing an important role in China's hearth care system. With the support of Chinese government, the chemical bases of some important herbs and formulas have been revealed [1-3], and their curative and preventative effects been confirmed by many research groups [4-7]. However, the chemical components in herbal drugs were not directly correlated with their *in vivo* effects since pre-systemic elimination and hepatic metabolism would alter the exposure types and levels of herbal components in body. Recently, more and more researchers have begun to study the *in vivo* components after administration of herbal drugs [8-10]. The major hindrance to detect and identify such herbal related compounds in bio-samples lies in three key points: (1) the absorbed compounds would be diluted by body fluids and masked by high abundant endogenous compounds; (2) it is hard to predict which compounds were absorbed and to what they were transformed due to limited information about the metabolism of natural products [11]; (3) no metabolic screening strategies are available for so many components in one dose and the identification of metabolites is very challenging. The current strategies for metabolite screening of synthetic drugs are heavily based on tandem mass spectrometry to selectively detect the potential metabolites deduced by biotransformation pathways. It is not realistic to apply these strategies to herbal medicines directly because there are so many parent compounds. Thus, new methodologies are desperately needed to solve the problem about *in vivo* study of herbal medicine. Not only the exposure types, but also the exposure levels in body are crucial to understand their therapeutic effects. The dynamic process and distribution of herbal components in body show the superposition effects of the whole preparation. The results from purified compounds or single herbal extracts may not infer the same results when they are included in a multi-herb preparation. To monitor all components in a multi-herb preparation is not possible and thus, the selection of representative pharmacokinetic (PK) markers is crucial. An ideal PK marker should have a significant dose-dependent exposure and an appropriate elimination half-life [12]. Collectively, the study of body systemic exposure to herbal medicines, including the chemical species, the distribution, and time-concentration profiles, would have a profound meaning for their effectiveness and safety in clinical practice.

Qishen yiqi pills (QY pills) were prepared from four component herbs, viz., *Astragalus membranaceus* (Huangqi), *Salvia miltiorrhiza* (Danshen), *Panax notoginseng* (Sanqi or sanchi ginseng), and *Dalbergia odorifera* (Jiangxiang), according to a proprietary formula in which the percentages of the four herbs were 45, 25, 5 and 25, respectively [13]. In clinical practice, this formula is used for treating chronic cardiac insufficiency [14], effort angina [15], congestive heart failure [16] and myocardial infarction [17]. In this standardized preparation, the main components [18] are astragalosides and isoflavones from Huangqi, including astragaloside IV, ononin and formononetin; phenolic acids from Danshen, including tanshinol, salvianolic acids A, B, C and D; triterpene saponins from Sanqi, including ginsenosides Rb1, Rd, Rg1, and notoginsenoside R1; and volatile oils from Jiangxiang, including Nerolidol and *E*-nerolidol. To improve the safety and patient compliance of QY pills, natural pharmaceutic adjuvants other than synthetic macrogol were utilized. QY pills had been authorized by the China State Food and Drug Administration (Drug Approval Number: Z20030139) and a randomized and multi-center clinical trial was carried out to evaluate the efficacy and safety of QY pills for secondary prevention of myocardial infarction in 2006, which was the largest randomized double blinded trial for TCM [19]. The results showed that QY pills have the same potent effect as that of enteric-coated aspirin [20]. Since last year, QY pills have been included in directory B of the National Medical and Health Insurance (NMHI) in China. With a population of more than 1.1 billion participants in NMHI, the annual sales volume of QY pills is expected to exceed 300 million Chinese Yuan (~44 million U.S. dollars) in a few years.

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Previous studies on QY pills and the component herbs have mostly focused on the identification [18, 21, 22] or quantification [23] of their main chemical compounds using HPLC or HPLC/MS techniques. The metabolism and pharmacokinetics of a single or two herbs of Danshan, Huangqi, Sanqi and their major active compounds have also been studied [24-27]. However, there is little information on *in vivo* exposure to herbal compounds after oral administration of QY pills, which remains unfavorable for disclosing the pharmacological mechanisms and could give rise to a risk of medication for QY pills.

In this study, to help better understand the *in vivo* process of QY pills, rat exposure to herbal compounds following oral administration of QY pills was originally investigated. According to the intrinsic characteristics of herbal medicine, a target-directed strategy was developed to find the buried metabolites from LC-MS datasets and then, metabolites were identified by high-resolution mass spectrometry and fragmentation pathway analyses. In addition, PK studies were also devised to reveal the exposure levels of representative PK markers of QY pills. This is the first comprehensive presentation of such a complex herbal preparation in rats.

EXPERIMENTAL

Materials and Chemicals

Qishen yiqi pills (QY pills) were provided by TASLY Pharmaceutical Co. Ltd. (Tianjin, China), in which the identified components are astragaloside IV (AIV), soyasaponin I, isomucronulatol-7-*O*-β-D-glucoside (IMG), 3-hydroxy-9,10-dimethoxy-pterocarpan (HDP), Calycosin-7-*O*-β-D-glucoside (CG), formononetin (FO) and ononin (Ono) from Huangqi; tanshinol (TSL), protocatechualdehyde (PCA), rosmarinic acid (RA), salvianolic acid B (SAA), salvianolic acid B (SAB), isosalvianolic acid C, salvianolic acids C, D (SAD), E, G (SAG), H, I, lithospermic acid and lithospermic acid B from Danshen; notoginsenside R_1 (NR_1), ginsensides Rg_1 (GRg_1), GRb_1, GRe, GRd, Ginsenoside Rh_1 (GRh_1), 20(S)GRg_3, 20(R)GRg_3, ginsenosides Rh_4 and Rk_3, notoginsenosides R_2, I and T_5, sanchinoside B_1 and gypenoside XVII (GXVII) from Sanqi; *E*-nerolidol (ENL) , (*3S*,*6R*,*7R*)-3,7,11-trimethyl-3,6-epoxy-1,10-dodecadien-7-ol (RDL) and (*3S*,*6S*,*7R*)-3,7,11-trimethyl-3,6-epoxy-1,10-dodecadien-7-ol (SDL) from Jiangxiang [18, 28]. Reference compounds, TSL, PCA, RA, SAB, NR_1, GRg_1, GRb_1, GRe and GRd, and AIV were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Ginsenoside Rh_1 (GRh_1), 20(S)GRg_3 and 20(R)GRg_3 were purchased from Jilin University (Jilin Province, China). CG, FO and Ono were purchased from Shanghai Ronghe Medicine Technology Development Co. Ltd. (Shanghai, China). *E*-nerolidol was obtained from Sigma-Aldrich (Steinheim, Germany). The purity of all of these compounds was >98% determined by peak area normalization.

HPLC grade acetonitrile (Merk KGaA, Darmstadt, Germany) were used for the HPLC analysis. Deionized water was purified by Milli-Q system (Millipore, Bedford, MA, USA). Analytical-grade formic acid was purchased from Hangzhou Reagent Company (Hangzhou, China). Chloral hydrate was a product of Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Heparin sodium (12500 IU/ml ampoule) was purchased from Shanghai First Biochemical Co. Ltd., which was diluted to 1000 IU/ml before usage. Unless specified otherwise, all other chemicals and solvents were of AR grade.

Each gram of QY pills contained the following amounts of 4 main components: 14.52 mg of TSL, 9.83 mg of GRg_1, 7.97 mg of GRb_1, and 6.22 mg of AIV, as measured by HPLC/UV. For oral administration, three concentrations (0.1, 0.3, and 0.6 g/ml) of QY pills corresponding to the low, middle and high dose levels were prepared by suspending QY pills in a quantity of distilled water and then mixing well. So the volumes given to rats were all equal to 1 ml per 100 g body weight. For intravenous administration, TSL, GRg_1 or GRb_1 was dissolved in physiological saline and AIV in ethanol/water (50:50). Then, they were centrifugated (10000 rpm, 10 min) to prepare an injectable solution of TSL (1 mg/ml), GRg_1 (2 mg/ml), GRb_1 (1 mg/ml) and AIV (1 mg/ml), respectively.

Rat Studies

Animal studies were conducted according to protocols approved by the Review Committee of Animal Care and Use at Zhejiang University (Hangzhou, China). Male Sprague–Dawley (SD) rats (220–250 g) were obtained from the Experimental Animal Center of Zhejiang University (Hangzhou, China). The rats were maintained in an air-conditioned room with controlled temperature (22-26 ºC) and humidity (40%-70%) with a 12-h light-dark cycle, and were acclimatized to the facilities for 1 week. Diet was prohibited for 12 h prior to the experiment while water was taken freely.

For screening plasma metabolites, pre-dose blood samples (1 ml per rat) were collected in heparinized tubes from the retro-orbital plexus of three rats. Then the rats were given a high dose of QY pills (6 g/kg) by oral administration. After forty-five minutes, they were anaesthetized with 5% chloral hydrate by intraperitoneal injection (0.6 ml/100 g), and blood samples were drawn from the inferior vena cava (~5 ml per rat). All the blood samples were centrifuged at 3,000 rpm for 15 min, and harvested plasma samples at the same time point were mixed and stored at -80 °C until analysis.

For screening urinary metabolites, three rats were housed in metabolic cages (1 rat per cage) and received a high dose of QY pills (6 g/kg) via gavage. Urine samples were collected at 0 to 8, 8 to 24, and 24 to 32 h. Their volumes were recorded before storage at -80 °C. For bile sampling, bile ducts of three rats anaesthetized with chloral hydrate were cannulated with polyethylene-10 tubing for collection of bile. Blank bile samples (1 ml) were collected in polypropylene tubes pre-dose. When the rats recovered from anesthesia, the bile was collected at 2-h intervals for 8 h after high dose QY pills (6 g/kg) and at 9 to 22, 22 to 24 h intervals thereafter. Bile samples were stored at -80 °C until analysis.

For screening tissue metabolites, four groups (three per group) of rats under 5% chloral hydrate anesthesia were sacrificed by bleeding the inferior vena cava at 0.21, 0.84, 3 and 7 h after an oral administration of high dose QY pills (6 g/kg), respectively. Samples of heart, liver, spleen, lung, kidney, brain, abdominal adipose tissue, testicle and small intestine were excised, trimmed of extraneous fat, residual muscle and connective tissue, rinsed in physiological saline for three times, and then blotted dry on absorbent paper. All tissue samples were weighted and frozen at -80 °C until analysis.

For pharmacokinetic studies, three randomly assigned groups (three per group) of rats received a single oral administration of QY pills at low, middle and high dose levels (1, 3, 6 g/kg body weight), respectively; four groups received a single i.v. dose of TSL, AIV, GRg_1 and GRb_1 solutions at 1 ml/100 g body weight. Serial blood samples (0.3 ml) were collected in heparinized tubes from the tail vein at 0, 0.25, 0.50, 0.75, 1, 2, 4, 8, 12, 24 and 48 h post-dose. Plasma samples were separated by centrifugation at 3000 rpm for 15 min and frozen at -80 °C until analysis.

HPLC/MS Analysis

Plasma samples for exposure study were precipitated with 3 volume of methanol followed by centrifugation (13,000 rpm, 15 min) and concentration (Speedvac® SPD121P, Thermo Savant, USA); the urine, bile and plasma samples for pharmacokinetic study were purified by solid-phase extraction; a portion of 200 mg tissue samples (100 mg for kidney) was weighted and homogenized in 1.5 ml methanol followed by centrifugation at 13,000 rpm for 15 min and concentration.

For identification of the metabolites in bio-samples (plasma, urine, bile and tissues), multistage mass spectra of all potential

compounds were recorded on a Finnigan LCQ Deca XP^{plus} ion trap mass spectrometer (ITMS) coupled with an Agilent 1100 HPLC instrument. The LC separation was achieved on an Ultimate^{TM} XB-C_{18} column (4.6×250 mm, 5 μm) with a flow rate of 0.5 ml/min. The mobile phase consisted of acetonitrile (A) and water containing 0.05% formic acid. The elution gradient was as follows: 2% A (0 min), 10% A (10 min), 26% A (20 min), 30% A (25 min), 30% A (40 min), 37% A (45 min), 45% A (65 min), 95% A (75 min) and 95% A (85 min). The acquisition parameters for ITMS were: ion spray voltage, -4.5 kV; sheath gas (N_2) 30 arbitrary units; auxiliary gas (N_2) 10 arbitrary units; capillary temperature 350 °C; capillary voltage -15V; tube lens offset voltage -30V; mass range recorded m/z 100–1500. Data-dependent scans were performed to obtain the multistage mass spectra (n=2-3). Accurate mass measurements were performed on an Agilent 6210 time-of-flight MS (TOF-MS) equipped with an electrospray ionization (ESI) interface. The ESI-TOF-MS system was set to run in negative ion mode with a capillary voltage of 3500, and an m/z range of 100-1500. The instrument calibration was performed by using manufacturer-provided calibration solution (G1969-85000).

For pharmacokinetic experiments, an Agilent 6410B tandem mass spectrometer (Agilent, Santa Clara, CA) was connected to the Agilent 1100 HPLC via an ESI interface. The chromatographic separation was achieved on a Zorbax SB-C_{18} column (100×2.1 mm, 3.5 μm). Precursor/product ion pairs used for multiple reactions monitoring in the negative ESI mode of TSL, GRg_1, GRb_1, AIV and digoxin were *m/z* 197/135, *m/z* 799/475, *m/z* 1107/945, *m/z* 783/489 and *m/z* 779/475, respectively. Matrix-matched calibration curves were constructed for the analytes (3.69-940 ng/ml for TSL, 4.16-530 ng/ml for AIV, 4.61-587.5 ng/ml for GRg_1 and 3.79-482.4 ng/ml for GRb_1) using weighted linear regressions of the analyte peak area against the corresponding nominal concentrations of the analyte, which showed good linearity (r^2>0.99).

GC/MS Analysis

Before GC/MS analysis, the plasma, urine and bile samples for exposure studies were precipitated with 3 volume of acetone followed by centrifugation (13,000 rpm, 15 min) and concentration; a portion of tissue samples (200 mg) were homogenized with 0.5 ml of acetone followed by centrifugation at 13,000 rpm for 15 min and concentration. A Hewlett Packard (hp) gas chromatograph (6890 series) with splitless injection was coupled to an Agilent 7683 series mass spectrometer (Agilent Technologies, Atlanta, GA, USA). For separation, a ZB-5MS column (30 m × 250 μm × 0.25 μm) was used with helium as the carrier gas.

Screening Metabolites of QY Pills from Bio-matrix

In this work, a target-directed strategy was introduced to find the buried metabolites from LC-MS datasets. First, the chemical components of QY pills were identified by comparing with standards or literature data using HLPC-MS technologies [18], and their metabolic pathways in rats or microorganisms were retrieved from the literature databases and summarized in Table **S1** of the supporting materials. When there was no information about the metabolism of some compounds, it was assumed that such compounds would undergo the same pathways with those compounds that have similar structures. For example, SAH and SAI were thought to be hydrolyzed and methylated as that of SAB in bio-systems. Thus, all possible metabolites of each compound in QY pills would be deduced and combined as a potential metabolite list. Then, bio-samples before and after dosing of QY pills were analyzed by HPLC-IT-MS and HPLC-TOF-MS in full scan mode (100-1500 Da). All proposed quasi-molecular ions of potential metabolites were extracted in both blank and dosed samples. Those peaks only found in dosed samples were further analyzed by multi-stage MS. Possible adduct ions and multimers were excluded by checking the full scan mass spectrum acquired at the retention time of the peak. At last, the metabolic positions were proposed by systemic fragmentation pathway analysis and/or comparing with reported data if available. The elemental composition or molecular formula of each metabolite was determined by their exact mass registered on HPLC-TOF-MS. We supposed that only five types of elements (C, H, O, N, and S) could exist in all the metabolites. To avoid the omission of some metabolites not listed in the potential metabolite list, an auxiliary procedure using the high sensitive feature detection algorithm of XCMS [29], popular R-based software for analysis of metabonomics datasets, were applied to analyze the pre- and post-dose bio-samples. Differential features were obtained by a matlab script and verified by checking the original full mass spectra and multi-stage mass spectra. The identified metabolites are listed in Table **1**.

Pharmacokinetic Data Analysis

Non-Compartmental Analysis (WinNonLin Pro 5.2.1) was utilized to obtain pharmacokinetic parameters for each rat. The Area Under the Concentration–time profile ($AUC_{0\rightarrow t}$) was calculated by the Log/Linear trapezoidal method. The first-order constant (λ_z) was determined using the last four or five sampling points. Half life ($t_{1/2\lambda z}$) was derived as $t_{1/2\lambda z} = \ln(2)/\lambda_z$. The AUC from time zero to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-last} + C_{last}/\lambda_z$. The Mean Residence Time (MRT) was equal to the Area Under the first Moment Curve from time zero to infinity, divided by $AUC_{0-\infty}$. Absolute oral bioavailability (F) was determined in each animal as $F = AUC_{oral}/AUC_{IV} \times Dose_{IV}/Dose_{oral} \times 100\%$. Total clearance was calculated as $CL = Dose/AUC_{0-\infty}$.

RESULTS

The urine, blood, bile and tissue samples after a dose of 6 g/kg QY pills to rats were analyzed by HPLC-IT-MS and HPLC-TOF-MS. As shown in the total ion chromatograms of bio-samples Fig. (**S1** to **S3**), high abundance of endogenous components was dominated and almost all metabolites were buried. So the target-directed strategy was applied to discriminate herbal metabolites from endogenous components. Due to the limited amount of tissue samples and relatively lower content corresponding to those in urine, blood and bile samples, no metabolites were detected by HPLC-IT-MS and HPLC-TOF-MS in tissues (data not shown). Since the main components of Jiangxiang belong to volatile oil that could not be ionized under atmospheric pressure ionization, all the above bio-samples (urine, blood, bile and tissue samples) were also analyzed by GC-MS. As a result, a total of 52 metabolites and 14 parent compounds were identified from bio-samples (34 in plasma, 47 in urine, 21 in bile, and 3 in tissues). Most of the metabolites of Huangqi were phase II conjugates of isoflavonoid with sulfonic acid and/or glucuronic acid, and AIV were absorbed in blood and eliminated to urine as its prototype. The metabolites of Danshen were found to be originated from monomers (TSL, PCA) and dimers (RA, SAD, SAG) of phenolic compounds in Danshen. However, the trimers (LA, SAA, SAC) and tetramers (SAB, SAE), which were rather abundant in herbal extracts of Danshen, could not be observed in all bio-samples. Low levels of Sanqi saponins (NR_1, GRe, GRg_1, GRb_1, GRd and GXVII) were detected in rat blood and urine, and only NR_1, GRe and GRg_1 in rat bile. Three volatile components (ENL, RDL, and SDL) were observed in intestine, fat and liver by GC-MS. A representative combined extracted-ion chromatogram (CEIC) of urine samples and the GC-MS chromatogram of fat tissues were given in Fig. (**1**). CEIC is constructed using all the ion traces of identified metabolites from the corresponding samples. Chromatograms of bile and blood samples and GC-MS chromatograms of other tissues were supplied as supplementary materials Fig. (**S4**). The structures of identified metabolites and their proposed metabolic pathways were shown in Fig. (**2**).

Identification of the Metabolites of Huangqi

Four types of isoflavonoid aglycones, names calycosin (MW: 284), formononetin (MW: 268), isomucronulatol (MW: 302) and

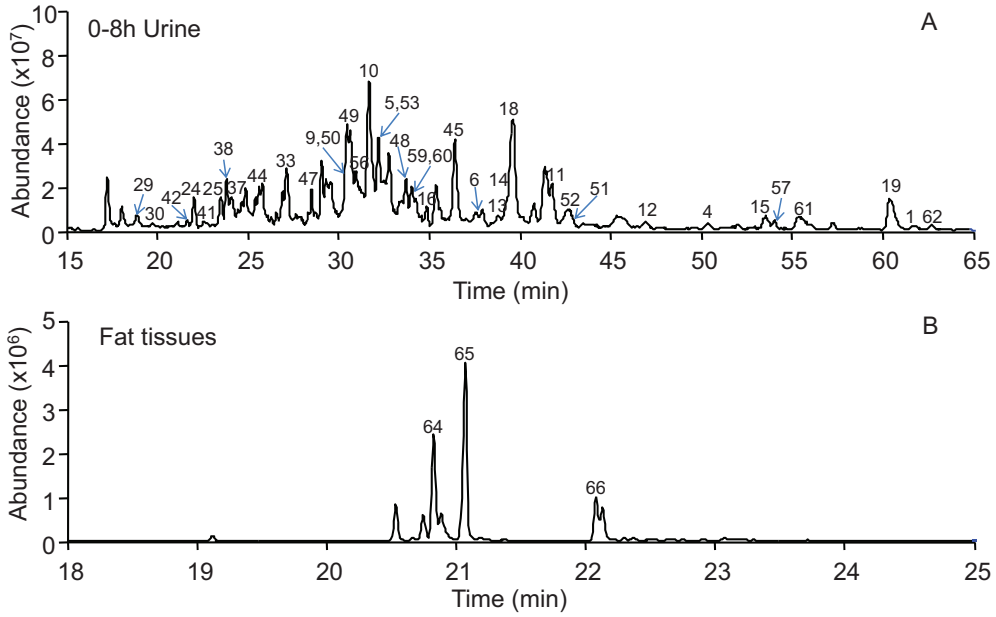

Fig. (1). Combined extracted-ion chromatogram of the 0-8h urine sample (**A**) and the GC-MS total ion chromatogram of fat tissues (**B**) after oral administration of QY pills. The peak numbers are in accord with those in Table **1**.

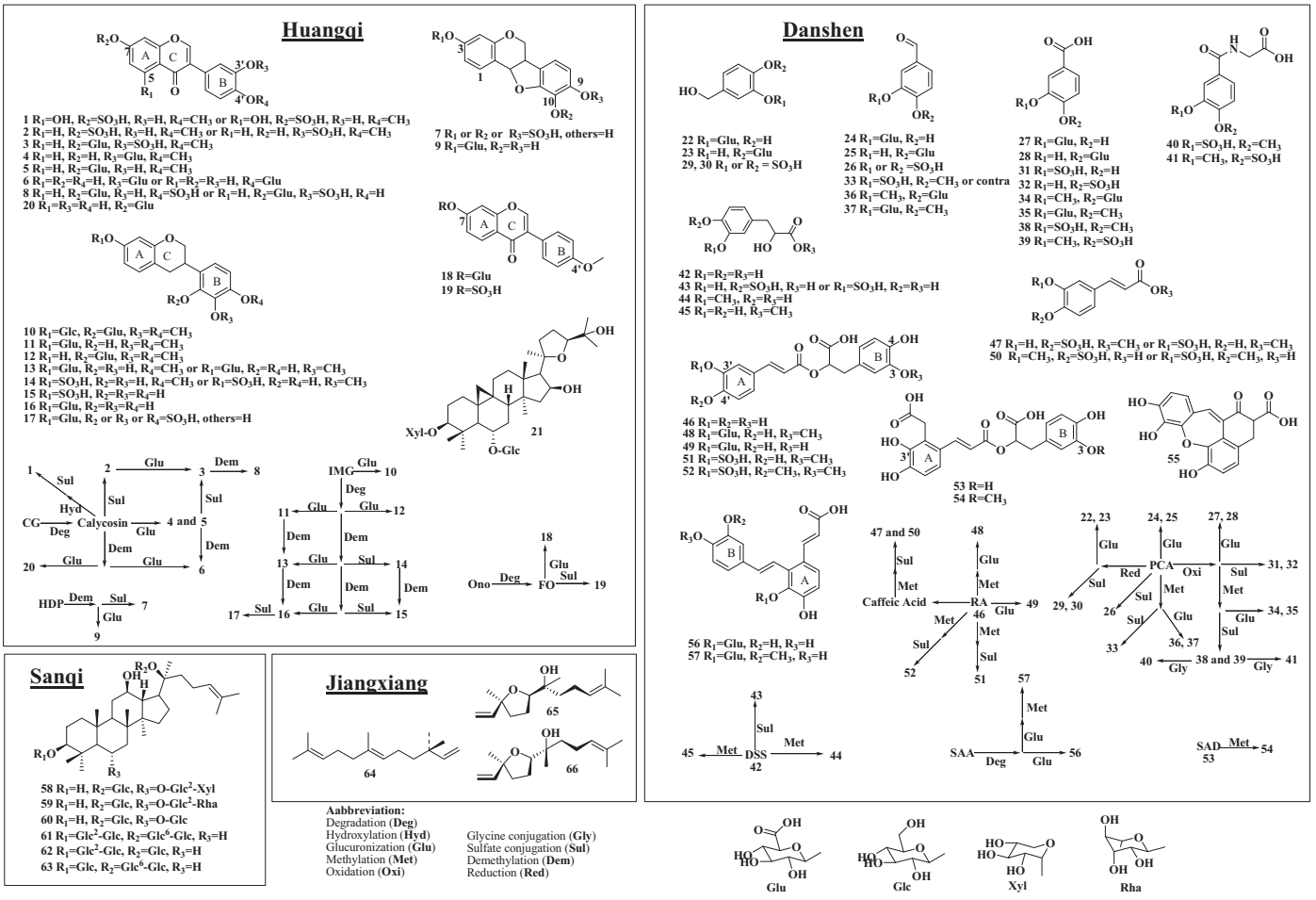

Fig. (2). Components found in rat urine, bile and blood samples following oral administration of QY pills and their proposed metabolic pathways.

Table 1. Identified Compounds in urine, Bile, Plasma and Tissues of Rats Following Oral Administration of QY Pills

No.	Identification	Observed mass (Da)	Calculated mass (Da)	Error (ppm)	Proposed formula	Measurement site[a]
Huangqi						
1	5 or 8-Hydroxyl calycosin sulfate	379.0141	379.0129	3.2	$C_{16}H_{11}O_9S^-$	U
2	Calycosin 7 or 3'-sulfate	363.0181	363.0180	0.3	$C_{16}H_{11}O_8S^-$	P/B
3	Calycosin-7-*O*-β-D-glucuronide-3′-sulfate	539.0517	539.0501	3.0	$C_{22}H_{19}O_{14}S^-$	B
4	Calycosin-3′-*O*-β-D-glucuronide	459.0950	459.0933	3.7	$C_{22}H_{19}O_{11}^-$	U
5	Calycosin-7-*O*-β-D-glucuronide	459.0944	459.0938	1.3	$C_{22}H_{19}O_{11}^-$	P/U/B
6	4'-Demethyl-calycosin-7-*O*-β-D-glucuronide	445.0790	445.0782	1.8	$C_{21}H_{17}O_{11}^-$	U
7	3,9,10-Trihydroxy-pterocarpan sulfate	351.0193	351.0180	3.7	$C_{15}H_{11}O_8S^-$	U
8	4′-Demethyl-calycosin-7-*O*-β-D-glucuronide sulfate	525.0358	525.0345	2.5	$C_{21}H_{17}O_{14}S^-$	B
9	3,9,10-Trihydroxy-pterocarpan-3-*O*-β-D-glucuronide	447.0951	447.0933	4.0	$C_{21}H_{19}O_{11}^-$	U
10	Isomucronulatol-7-*O*-β-D-glucoside-2′-*O*-β-D-glucuronide	639.1934	639.1931	0.5	$C_{29}H_{35}O_{16}^-$	P/U/B
11	Isomucronulatol-7-*O*-β-D-glucuronide	477.1434	477.1402	6.7	$C_{23}H_{25}O_{11}^-$	U/B
12	Isomucronulatol-2′-*O*-β-D-glucuronide	477.1413	477.1402	2.3	$C_{23}H_{25}O_{11}^-$	P/U
13	3' or 4'-Demethyl isomucronulatol-7-*O*-β-D-glucuronide	463.1261	463.1246	3.2	$C_{22}H_{23}O_{11}^-$	U
14	3' or 4'-Demethyl-isomucronulatol sulfate	367.0512	367.0493	5.2	$C_{16}H_{15}O_8S^-$	U
15	3′,4′-Didemethyl-isomucronulatol sulfate	353.0347	353.0337	2.8	$C_{15}H_{13}O_8S^-$	U
16	3′,4′-Didemethyl-isomucronulatol-7-*O*-β-D-glucuronide	449.1108	449.1089	4.2	$C_{21}H_{21}O_{11}^-$	U/B
17	3′,4′-Didemethyl-isomucronulatol-7-*O*-β-D-glucuronide sulfate	529.0668	529.0658	1.9	$C_{21}H_{21}O_{14}S^-$	B
18	Formononetin-7-*O*-β-D-glucuronide	443.1009	443.0984	5.6	$C_{22}H_{21}O_{10}^-$	U/B
19	Formononetin-7-sulfate	347.0244	347.0231	3.8	$C_{16}H_{11}O_7S^-$	U/B
20	4′-Demethyl-calycosin-3' or 4'-*O*-β-D-glucuronide	445.1146	445.0776	83	$C_{21}H_{17}O_{11}^-$	U
21	Astragaloside IV[b]	829.4636	829.4591	5.4	$C_{42}H_{69}O_{16}^-$	P/U
Danshen						
22	3′,4′-Dihydroxyl-benzyl alcohol-3'-O-β-D-glucuronide	315.0722	315.0722	0	$C_{13}H_{15}O_9^-$	P/B
23	3′,4′-Dihydroxyl-benzyl alcohol-4'-O-β-D-glucuronide	315.0725	315.0722	1.0	$C_{13}H_{15}O_9^-$	P/B
24	Protocatechuicaldehyde-3'-glucuronide	313.0572	313.0565	2.2	$C_{13}H_{13}O_9^-$	P/U/B
25	Protocatechuicaldehyde-4'-glucuronide	313.0571	313.0565	1.9	$C_{13}H_{13}O_9^-$	P/U/B
26	Protocatechuicaldehyde sulfate	216.9818	216.9812	2.8	$C_7H_5O_6S^-$	P/B
27	Protocatechuic acid-3'-glucuronide	329.0521	329.0514	2.1	$C_{13}H_{13}O_{10}^-$	P
28	Protocatechuic acid-4'-glucuronide	329.0520	329.0514	1.8	$C_{13}H_{13}O_{10}^-$	P

(Table 1) Contd….

No.	Identification	Observed mass (Da)	Calculated mass (Da)	Error (ppm)	Proposed formula	Measurement site[a]
29	3′,4′-Dihydroxyl-benzyl alcohol sulfate	218.9975	218.9969	2.7	$C_7H_7O_6S^-$	P/U
30	3′,4′-Dihydroxyl-benzyl alcohol sulfate	218.9972	218.9969	1.4	$C_7H_7O_6S^-$	P/U
31	Protocatechuic acid sulfate	232.9772	232.9761	4.7	$C_7H_5O_7S^-$	P
32	Protocatechuic acid sulfate	232.9789	232.9761	12	$C_7H_5O_7S^-$	P
33	Isovanillin sulfate or vanillin sulfate	230.9978	230.9969	3.9	$C_8H_7O_6S^-$	U
34	Vanillic acid-4’-glucuronide	343.0682	343.0671	3.2	$C_{14}H_{15}O_{10}^-$	P
35	Isovanillic acid-3’-glucuronide	343.0678	343.0671	2.0	$C_{14}H_{15}O_{10}^-$	P
36	Vanillin glucuronide	327.0710	327.0722	-3.7	$C_{14}H_{15}O_9^-$	B
37	Isovanillin glucuronide	327.0732	327.0722	3.1	$C_{14}H_{15}O_9^-$	U/B
38	Isovanillic acid-3’-sulfate	246.9922	246.9918	1.6	$C_8H_7O_7S^-$	P/U
39	Vanillic acid-4’-sulfate	246.9921	246.9918	1.2	$C_8H_7O_7S^-$	P
40	4-Methoxyl-3-sulfooxy-hippuric acid	304.0139	304.0133	1.8	$C_{10}H_{10}NO_8S^-$	P/U
41	3-Methoxyl-4-sulfooxy-hippuric acid	304.0138	304.0133	1.6	$C_{10}H_{10}NO_8S^-$	P/U
42	Tanshinol[b]	197.0458	197.0455	1.5	$C_9H_9O_5^-$	P/U
43	3,4-Dihydroxylphenyllactic acid sulfate	277.0029	277.0024	1.8	$C_9H_9O_8S^-$	P/U
44	4-Hydroxy-3-methyloxyphenyllactic acid	211.0619	211.0612	3.3	$C_{10}H_{11}O_5^-$	U
45	3,4-Dihydroxyphenyllactic acid methyl ester	211.0618	211.0612	2.8	$C_{10}H_{11}O_5^-$	U
46	Rosmarinic acid[b]	359.0783	359.0772	3.1	$C_{18}H_{15}O_8^-$	P
47	3-(3,4-Dihydroxy-phenyl) crylic acid methyl ester sulfate	273.0073	273.0074	-0.4	$C_{10}H_9O_7S^-$	U
48	3-Methyl rosmarinic acid-3’-O-glucuronide	549.1275	549.1250	4.6	$C_{25}H_{25}O_{14}^-$	U
49	Rosmarinic acid-4’-glucuronide	535.1111	535.1093	3.4	$C_{24}H_{23}O_{14}^-$	U
50	Isoferulic acid 4-*O*-sulfate	273.0089	273.0074	5.5	$C_{10}H_9O_7S^-$	U
51	3-Methyl rosmarinic acid 4’-sulfate	453.0512	453.0497	3.3	$C_{19}H_{17}O_{11}S^-$	U
52	3,3’-Dimethyl rosmarinic acid 4’-sulfate	467.0670	467.0654	3.4	$C_{20}H_{19}O_{11}S^-$	U
53	Salvianolic acid D[b]	417.0837	417.0827	2.4	$C_{20}H_{17}O_{10}^-$	P/U
54	3-Methyl salvianolic acid D	431.0988	431.0984	0.9	$C_{21}H_{19}O_{10}^-$	P/U
55	Salvianolic acid G[b]	339.0516	339.0510	1.8	$C_{18}H_{11}O_7^-$	P/U/B
56	Salvianolic acid F 3-O-glucuronide	489.1066	489.1039	5.5	$C_{23}H_{21}O_{12}^-$	U
57	3’-Methyl salvianolic acid 3-O-glucuronide.	503.1210	503.1195	3.0	$C_{24}H_{23}O_{12}^-$	U
Sanqi						
58	Notoginsenoside R_1[b]	977.5342	977.5327	0.2	$C_{48}H_{81}O_{20}^-$	P/U/B
59	Ginsenoside Re[b]	991.5500	991.5483	1.7	$C_{49}H_{83}O_{20}^-$	P/U/B
60	Ginsenoside Rg_1[b]	845.4906	845.4904	0.2	$C_{43}H_{73}O_{16}^-$	P/U/B
61	Ginsenoside Rb_1[b]	1153.6031	1153.6011	1.7	$C_{55}H_{93}O_{25}^-$	P/U
62	Ginsenoside Rd[b]	991.5462	991.5483	-2.1	$C_{49}H_{83}O_{20}^-$	P/U
63	Gypenoside XVII[b]	991.5503	991.5483	2.0	$C_{49}H_{83}O_{20}^-$	P/U

(Table 1) Contd….

No.	Identification	Observed mass (Da)	Calculated mass (Da)	Error (ppm)	Proposed formula	Measurement site[a]
Jiangxiang[c]						
64	*E*-nerolidol[b]	222				SI/L/A
65	(*3S*,*6R*,*7R*)-3,7,11-trimethyl-3,6-epoxy-1,10-dodecadien-7-ol[b]	238				SI/L/A
66	(*3S*,*6S*,*7R*)-3,7,11-trimethyl-3,6-epoxy-1,10-dodecadien-7-ol[b]	238				SI/L/A

[a] P, Plasma; U, Urine; B, Bile; SI, Small intestine; L, Liver; A, Adipose; [b] detected as parent drugs; [c] The three compounds of Jiangxiang were identified based on GC-MS analysis while all other compounds were analyzed by LC-IT-MS and LC-TOF-MS.

3-hydroxy-9,10-dimethoxypterocarpan (MW: 300), have been identified from Huangqi and most of them are available as 7-*O*-glucosides[22, 30]. After oral administration, such glucosides may be hydrolyzed by intestinal bacteria and then absorbed as their corresponding aglycones, which are quickly conjugated with glucuronic acid via UDP-glucuronosyltransferases (UGTs). The 7-hydroxy of isoflavonoids has priority to be glucuronided than other hydroxyl groups [31, 32]. Other minor pathways, such as hydroxylation, sulfate conjugation, methlylation and demethylation, have also been reported. Based on the above metabolic information, the possible metabolites were screened in all bio-samples and a total of 20 isoflavonoid metabolites of Huangqi have been tentatively identified Table **1**.

Eight glucuronided (neutral loss of 176 Da with or without an ion of dehydrated glucuronic acid at m/z 175) and/or sulfated conjugates (neutral loss of 80 Da) were assigned as metabolites of CG (compounds **1-6**, **8** and **20** in Table **1**). In most cases, a base peak of $[M-H-176]^-$ was observed for glucuronides, but the $[M-H-80]^-$ of sulphates was not always the base peak. It may be that some sulphates were more stable then glucuronides upon collision induced dissociation. Compounds **2**, **3**, **4** and **5** contained the same aglycone calycosin (m/z 283), which lost a $CH_3\cdot$ and yielded a characteristic ion at *m/z* 268 in their product ion spectra. Compounds **4** and **5** were a pair of isomers. It was shown that the peak area of compound **5** was greater than that of compound **4**. According to the literatures [31, 32], glucuronidation mainly occurred at the 7-hydroxyl group. Thus, compound **5** was tentatively identified as calycosin-7-*O*-β-D-glucuronide, and compound **4** inferred as calycosin-3′-*O*-β-D-glucuronide. The relative abundance of the $[M-H-80]^-$ ion of compound **2** was only 32%. It seemed that the substitute position of sulfuric acid would influence the stability of such conjugates. Due to lack of standards, compound **2** could only be tentatively identified as 7 or 3'-calycosin sulfate. Compound **3** was assigned as calycosin-7-O-β-D-glucuronide-3'-sulfate for the high concentration of calycosin-7-O-β-D-glucuronide in bio-samples. The aglycone of compound **1** was assigned as hydroxyl calycosin according to the appearance of the aglycone ion at *m/z* 299 ($C_{16}H_{11}O_6^-$), 16 Da (O) higher than the deprotonated ion of calycosin. Previous studies showed that calycosin is possible to be hydroxylated at the 2′ or 5′ position at B-ring, or 5 or 8 position at A-ring [33]. In addition, the dominant MS^3 ion at *m/z* 165 via fragmentation involving the 0 and 3 bonds further restricted the hydroxyl moiety at A-ring. Thus, compound **1** was tentatively identified 5 or 8-hydroxyl calycosin sulfate. The aglycone of compounds **6**, **8** and **20** was inferred as 4′-demethylcalycosin due to the presence of the aglycone ion at *m/z* 269 ($C_{15}H_9O_5$), 14 Da (CH_2) less than the deprotonated ion of calycosin. The base peak of compound **6** was $[M-H-176]^-$ at m/z 269, while that of compound **20** was dehydrated glucuronic acid at m/z 175. The difference of compound **20** with the above identified compounds **4** and **5** was a pair of phenolic hydroxyl groups at ortho position to each other. It was deduced that the ortho hydroxyl group would aid the loss of glucuronic acid and form the base peaks at m/z 175. Thus, compounds **6** and **20** were identified as 4′-Demethyl-calycosin-7-*O*-β-D-glucuronide and 4′-Demethyl-calycosin-3' or 4'-*O*-β-D-glucuronide, respectively. The base peaks of compound **8** was $[M-H-176]^-$ at m/z 349, which further lost another 80 Da to form the ion at m/z 269. So it was inferred as 4'-Demethyl-calycosin-7-O-β-D-glucuronide sulfate.

A total of **8** glucuronided and/or sulfated conjugates were assigned as metabolites of IMG. Among them, compound **10** was tentatively assigned as isomucronulatol-7-*O*-β-D-glucoside-2′-*O*-β-D-glucuronide, the only glucuronided conjugate of flavonoid glycoside. The possible reason is that since there is no carbonyl group (electron withdrawing group) at the 4 position of IMG, the glycosidic bond is less easy to be broken compared with CG and Non. Compounds **11** and **12** possessed the same aglycone isomucronulatol, and produced the diagnostic ion at *m/z* 286. Since the content of compound **11** was much higher than that of compound **12**, compound **11** and **12** were preliminarily characterized as isomucronulatol-7-*O*-β-D-glucuronide and isomucronulatol-2′-*O*-β-D-glucuronide, respectively. As for compound **13** and **14**, the aglycone ion at *m/z* 287 ($C_{16}H_{15}O_5$) was 14 Da (CH_2) lower than the deprotonated ion of isomucronulatol, suggesting that it could be derived from isomucronulatol via demethylation at the 3′ or 4′ position, respectively. Therefore, compound **13** and **14** were plausibly inferred as demethyl isomucronulatol-7-*O*-β-D-glucuronide and demethyl isomucronulatol sulfate, respectively. Similarly, compound **15**, **16** and **17** were assigned as 3′,4′-didemethyl-isomuc-ronulatol sulfate, 3′,4′-didemethyl-isomucronulatol-7-*O*-β-D-glucuronide and 3′,4′-didemethyl-isomucronulatol-7-*O*-β-D-glucuronide sulfate, respectively.

FO is the aglycone part of Ono. It has been well established that the absorption of isoflavonoids requires deconjugation of isoflavonoid glucosides to aglycones [34], we could therefore deduce that FO and Ono had the same metabolites. Compounds **18** and **19** were tentatively identified as formononetin-7-*O*-β-D-glucuronide and formononetin-7-*O*-sulfate, respectively, consistent with the reported data [35].

Compound **7** showed an accuracy mass $[M-H]^-$ at m/z 351.0193 in (–)ESI-TOF MS and its molecular formula was determined to be $C_{15}H_{12}O_8S$. The neutral loss of 80 Da (m/z 351→271) also confirmed that compound **7** was a sulfate. The aglycone ion at m/z 271 was prone to form a pair of complement ions (m/z 149 and 121), indicating that it was not a stable isoflavone structure. The molecular weight of compound **7** was 28 Da (C_2H_4) less than that of 3-hydroxy-9,10-dimethoxypterocarpan, suggesting the two methoxyl groups were demethylated. Thus, compound **7** were tentatively identified as 3,9,10-trihydroxy-pterocarpan sulfate. Compound **9** also showed an aglycone ion at m/z 271 after a neutral loss

of 176 Da. It was plausibly characterized as 3,9,10-trihydroxy-pterocarpan-3-O-β-D-glucuronide.

Only one astragaloside (compound **21**) was detected in rat plasma and urine samples. By comparing the retention time and multi-stage mass spectra with standards, it was identified as astragaloside IV.

Identification of the Metabolites of Danshen

Most of the metabolites of PCA in bio-samples after dosing of QY pills were conjugates. Diagnostic losses corresponding to conjugates could be observed in the mass spectra of the metabolites, such as $C_6H_8O_6$ (176 Da) for glucuronide conjugates and SO_3 (80 Da) for sulfate conjugates. Compounds **24** and **25** had identical chemical formula $C_{13}H_{14}O_9$, and the product ions were at *m/z* 175, 113 and 137, indicating they were the glucuronided conjugates of PCA, since *m/z* 175 and 113 were the product ions of glucuronide and *m/z* 137 was the precursor ion $[M-H]^-$ of PCA, consistent with the literature [36]. Accordingly, compound **26** was tentatively assigned as PCA sulfate. In a similar way, compounds **22** and **23**, **29** and **30** were preliminarily characterized as glucuronided and sulfated conjugates of 3′,4′-dihydroxyl-benzyl alcohol; while compounds **27** and **28**, **31** and **32** were plausibly inferred as protocatechuic acid glucuronided and sulfated conjugates. There are two potential phenolic hydroxyl moieties in PCA, 3′,4′-dihydroxyl-benzyl alcohol and protocatechuic acid. By comparing the MS^2 spectra of the above five pair of isomers, differential fragmentation pathways were only observed for compounds **27** and **28** -- the loss of CO_2 (44 Da) from $[M-H]^-$. When the glucuronic acid is at meta position of protocatechuic acid, the hydrogen atom of hydroxyl group at para position could be rearranged to the carboxyl group through the benzene ring. So the bond between carboxyl group and benzene showed some characters of a double bond, which is hard to be broken. However, if the glucuronic acid is condensed with the para hydroxyl group of protocatechuic acid, the meta hydrogen atom have no chance to be delocalized to the carboxyl group. After losing the dehydrated glucuronic acid, the two hydroxyl groups at meta and para position of protocatechuic acid would have opposite effects on the carboxylic acid and the loss of CO_2 could be observed (m/z 153→109). The proposed pathways of compounds **27** and **28** were demonstrated in Fig. (**S5**). Thus, compounds **27** and **28** were identified as protocatechuic acid-3'-glucuronide and protocatechuic acid-4'-glucuronide, respectively. No difference were observed in MS^2 of the other four pair of isomers: PCA glucuronides (**24** and **25**), 3′,4′-dihydroxyl-benzyl alcohol glucuronides (**22** and **23**), 3′,4′-dihydroxyl-benzyl alcohol sulfates (**29** and **30**) and protocatechuic acid sulfates (**31** and **32**). The structure of PCA glucuronides and 3′,4′-dihydroxyl-benzyl alcohol glucuronides were similar to those of protocatechuic acid glucuronide and it was deduced that such 3'- and 4'- isomers had the same elution order on reversed phase columns. Their identification was tentatively obtained based on this empirical rule. Up to now, we have no information to determine the elution order of sulfates.

The predicted molecular formula of compounds **36** and **37** was $C_{14}H_{16}O_9$, suggesting that they were methylation products of compound **24** ($C_{13}H_{14}O_9$). Besides, the presence of the product ions at *m/z* 175 and 113 also supported their assignments as glucuronided conjugates. Thus, it could be deduced that compound **36** and **37** were methylated PCA glucuronides. According to the elution order of isovanillin (3-hydroxy-4-methoxybenzaldehyde) and vanillin (4-hydroxy-3-methoxybenzaldehyde) on reversed-phase-HPLC columns [37, 38], we supposed that compound **37** might be isovanillin glucuronide and compound **36** vanillin glucuronide. Accordingly, compound **33** was tentatively assigned as isovanillin/vanillin sulfate.

Similar to the identification of compounds **33** and **37** in view of MS data, compounds **34**, **35**, **38** and **39** were preliminarily assigned as glucuronided or sulfated conjugates of methylated protocatechuic acid. Comparing with the retention behaviour of vanillic acid (4'-hydroxy-3'-methoxybenzoic acid) and isovanillic acid (3'-hydroxy-4'-methoxybenzoic acid) on reversed-phase-HPLC columns [37], we proposed that compound **34** might be vanillic acid-4'-glucuronide and compound **35** isovanillic acid-3'-glucuronide. A $[M-H-44]^-$ ion at m/z 203 was observed in MS^2 of compound **38**, suggesting the methoxy group was at para position of benzoic acid. Thus, compound **38** was tentatively identified as isovanillic acid-3'-sulfate and compound **39** vanillic acid-4'-sulfate.

Compounds **40** and **41** showed an even number $[M-H]^-$ at m/z 304, indicating one or odd number of nitrogen atoms in their structure. Their molecular formulas were determined to be $C_{10}H_{11}NO_8S$ by (−)ESI-TOF. In addition, the MS^3 spectra of the $[M-H-80]^-$ ions at *m/z* 224 of compound **40** and **41** were similar to those of the deprotonated molecular ions of glycine conjugates of methylated PCA [36], suggesting that compound **40** and **41** were glycine conjugates. This pair of isomers was discriminated in the same way as in the identification of compounds **27** and **28**. After losing the sulfuryl groups, the main fragments of compound **40** were m/z 123 and 100, a pair of complement ions by splitting the C-C bond between C-1' and amide. This suggested that the para position (C-4') was a methoxy group as shown in Fig. (**S5**). Thus, compound **40** and **41** were tentatively identified as 4-methoxyl-3-sulfooxy-hippuric acid and 3-methoxyl-4-sulfooxy-hippuric acid, respectively. The relative abundance of $[M-H-80]^-$ of compound **40** was 100% and that of compound **41** was 49%, indicating that the 4'-sulfooxy group could be stabilized by the electron-withdrawing substituents at C-1'.

Compound **42** was identified as TSL by comparing its retention time and MS^2 with those of a standard. Compound **43** was tentatively inferred as TSL sulfate based on the neutral loss of 80 Da in the MS/MS data. The predicted molecular weights of compounds **44** and **45** were 212.0685 Da ($C_{10}H_{12}O_5$), an increase of 14 Da compared to that of TSL ($C_9H_{10}O_5$), suggesting the presence of one methyl residue. By comparison with previous literature [27], compound **44** and **45** were preliminarily assigned as 4-hydroxy-3-methyloxyphenyllactic acid and 3,4-dihydroxyphenyllactic acid methyl ester, respectively. The retention time of compound **45** is 10 min longer than that of compound **44**, which is also in accordance with the assumption of a methyl ester structure in compound **45**.

Compound **46** was characterized as rosmarinic acid (RA) by comparing the mass spectra data with literature [21]. According to the neutral loss of 176 Da or 80 Da in their MS^2 spectra, compounds **47**, **50**, **51** and **52** were found to be sulfate conjugates of RA and compounds **48** and **49** were glucuronide conjugates of RA. After losing a glucuronic acid group, the main fragment ions of compound **49** in MS^3 were identical with those of RA in MS^2 spectra, indicating that compound **49** has the same parent structure as RA. After losing a glucuronic acid group, compound **48** had a fragment ion at m/z 373, which was 14 Da higher than the quasi-molecular ion $[M-H]^-$ of RA, suggesting that compound **48** was a methylated form of RA. Because of the action of catechol-3-O-methyltransferase (COMT) [2], the methylation should occur at the 3-OH of B-ring or the 3'-OH of A-ring. Since the ion at m/z 211 were observed in MS^3 of m/z 373, it suggested that the methylation occurred at the 3-OH of B-ring. Except for the $[M-H-176]^-$ in MS^2 of compound **48** and **49**, ions originated from the cleavage of a or b bond were detected at m/z 337 and m/z 355, respectively Fig. (**3**). If the glucuronic acid is connected to 3'-OH of A-ring, the fragmentation of a bond would result in a stable ketone structure at m/z 337. On the other hand, a 4'-substitent of the A-ring would hamper the formation of such a ketone structure. Thus, compound **48** were plausibly identified as 3-methyl rosmarinic acid-3'-O-glucuronide and compound **49** as rosmarinic acid-4'-glucuronide. After losing a sulfuryl group, compound **52** showed a fragment ion at m/z 387, 28 Da higher than that of RA, suggesting that the 3-OH and 3'-OH of RA were both methylated. The ester bond cleavage ion at m/z 273

is an indication of a 4'-sulfooxy group Fig. (**3**). Therefore, compound **52** were tentatively identified as 3,3'-dimethyl rosmarinic acid 4'-sulfate. In the same way, compound **51** were identified as 3-methyl rosmarinic acid 4'-sulfate Fig. (**3**). Compound **47** and **50** were methylated caffeic acid sulfates resluted from hydrolysis of RA and methylation. The $[M-H-80]^-$ ion of compound **50** produced predominant $[M-H-CO_2]^-$ ion in its MS^3 spectrum, suggesting the methyl residue was connected with one of the phenolic hydroxyl groups, not the carboxyl moiety. Compared with the literature [39], compound **50** was more likely to be isoferulic acid 4-*O*-sulfate. On the contrary, the absence of the *m/z* 149 ion in the MS^3 spectra of compound **47** indicated that the methyl residue may connect with the carboxyl group. Thus, compound **47** was plausibly inferred as 3-(3,4-dihydroxy-phenyl) crylic acid methyl ester sulfate.

Compound **53** showed a $[M-H]^-$ at m/z 417 that lost a CO_2 to form the base peak in MS^2 spectra. Its exact mass at m/z 417.0837 indicated a chemical formula of $C_{20}H_{18}O_{19}$. This was all in accordance with those of SAD [21]. Compound **54** had similar fragmentation pathways with compound **53** except for a 14 Da higher $[M-H]^-$ and $[M-H-CO_2]^-$, suggesting that it was methylated SAD. After the cleavage of a bond of compound **54**, the ion at m/z 211 suggested the methoxy group occurred at B-ring of SAD Fig. (**S6**).

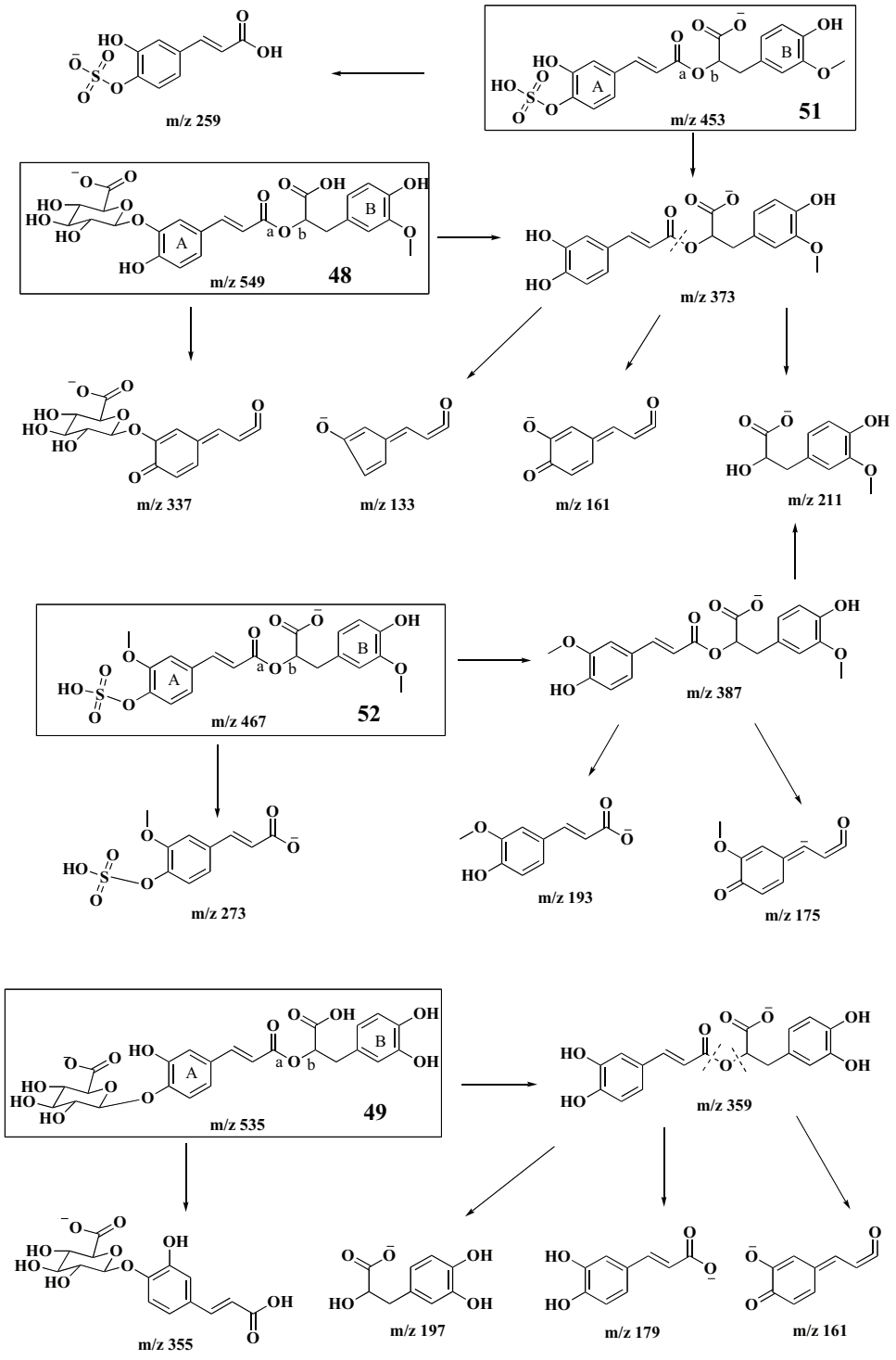

Fig. (3). Proposed fragmentation pathways of compounds **48**, **49**, **51** and **52** in Table **1**.

In view of the action of COMT, compound **54** were tentatively identified as 3-methyl salvianolic acid D. Compound **55** were characterized as salvianolic acid G by comparing with the reported data [21]. Compound **56** had an aglycone ion at m/z 313 after losing a glucuronic acid unit. The MS^3 of m/z 313 was identical with that of salvianolic acid F, suggesting that compound **56** was salvianolic acid F glucuronide. In MS^2 of compound **56**, the neutral loss of CO_2 indicated that the glucuronic acid was linked with the phenolic hydroxyl groups. The sequential loss of two molecular of H_2O (m/z 489→471→453) suggested that the glucuronic acid was condensed with the 3-OH of A-ring Fig. (**S6**). Accordingly, compound **56** were plausibly identified as salvianolic acid F 3-O-glucuronide. The chemical formula of compound **57** suggested it was a methylated product of compound **56**. No losses of CO_2 or H_2O were observed in MS^2 of compound **57**. We deduced that the 3'-OH of compound **56** was methylated by COMT, which would improve the stability of the backbone structure. Thus, compound 57 was tentatively identified as 3'-methyl salvianolic acid 3-O-glucuronide.

Identification of the Metabolites of Sanqi

The detected saponins in plasma and urine samples were presented as they were in herbal extract and no hydrolyzed metabolites were observed. Compounds **58** to **63** were identified as NR_1, GRe, GRg1, GRb1, GRd and GXVII, respectively, by comparing their retention time and multi-stage mass spectra with those of standards and literature data [40].

Identification of the Metabolites of Jiangxiang

Since no components of Jiangxiang could be detected in plasma, urine and other bio-samples by HPLC-IT-MS and HPLC-TOF-MS, GC-MS was utilized to monitor the volatile compounds of Jiangxiang in the collected bio-samples. By comparing with standards and NIST mass spectra database retrieval [28], three compounds (**64**, **65** and **66**) were detected from small intestine, liver and adipose tissues and identified as ENL, RDL, and SDL, respectively.

Pharmacokinetics of TSL, AIV, GRb_1 and GRg_1 after Oral QY Pills

As a systematic investigation of QY pills, its pharmacokinetic behaviors must be elucidated. According to the philosophy of traditional Chinese medicine, it was believed to be an integrative therapeutical effect for QY pills on the heart and blood vessel diseases. Reasonably, the markers selected in pharmacokinetic research of QY pills should be responsible for its pharmacologic actions. Previous studies reported that AIV possessed cardioprotective activity [41] and vasorelaxant effect [42], and an antioxidant effect was one of the underlying mechanisms by which AIV protects the myocardium. Lu *et al*. have summarized several literatures to show that TSL could have the potential for relief of angina [12]. In addition, the triterpenoid saponins GRg_1 and GRb_1 could significantly increase the fluidity of platelet membranes, which caused inhibition of platelet aggregation. These reports indicated that AIV, TSL, GRg_1 and GRb_1 were the main and representative bioactive ingredients, which could be responsible for therapeutical effect for QY pills. Therefore, in our study, AIV, TSL, GRg_1 and GRb_1 were selected as the pharmacokinetic maker to primarily profile pharmacokinetic behaviors of QY pills. As shown in Fig. (**4**), the representative four compounds (TSL, AIV, GRb_1, and GRg_1) were absorbed quickly after oral administration of QY pills and the maximal plasma concentrations occurred at 0.25 to 0.75 h. The concentrations of GRb_1 were detectable in rat plasma up to 48 h, whereas those of TSL, AIV, and GRg1 were measurable only within 24 h. The exposure levels of TSL, AIV, GRb_1, and GRg_1 in $AUC_{0\rightarrow t}$ and C_{max} increased directly with the dose levels of QY pills from 1 to 6 g/kg. It showed that the correlation coefficients (R^2) between $AUC_{0\rightarrow t}$ and dose were no less than 0.7951. Except for TSL (F=27%), the mean oral bioavailability (F) of AIV, GRg1 and GRb1 was quite low with F values from 0.18 to 0.49% Table **2**.

DISCUSSIONS

In QY pills, the total weight percentage of Huangqi and Danshen was 70, whereas only 5 for Sanqi. Although no quantitative determination of all the components in QY pills was reported, a rough comparison of their content could be obtained according to their peak height in LC-UV [18]. Under 254 nm, the top-ten compounds from high to low were CG, RA, SAD, calycosin-7-O-glucopyranoside-6-O-malonate, SAB, PCA, TSL, FO, SAH and SAI, all of which originated from Huangqi or Danshen. Accordingly, the metabolites of QY pills in urine, blood and bile showed the same tendency Fig. (**1** and **S4**). The most abundant metabolites were calycosin glucuronides and sulfates (**2**, **3**, **5**, **8**) in bile; PCA sulfates (**31**, **32**, **38** and **41**) and SAD (**53**) in blood; methylated TSL (**45**), PCA sulfates (**33**, **38**), RA glucuronides (**49**) and FO conjugates (**18**, **19**) in urine. As for the metabolites of Sanqi, three 20(S)-protopanaxatriol (ppt) type ginsenosides (NR1, GRe and GRg_1) were detected in bile and another three 20(S)-protopanaxadiol (ppd) type ginsenosides (GRb1, GRd and GXVII) were only found in urine and blood. This was in accordance with the reported elimination characteristics of ppt- and ppd-type ginsenosides [26].

Huangqi contained three types of flavonoid aglycones, including isoflavones, pterocarpans and isoflavans. They all underwent glucuronidation and sulfation. Calycosin, isomucronulatol and 3-hydroxy-9,10-dimethoxypterocarpan proceeded demethylation; besides, calycosin was functionalized by hydroxylation. Astragaloside IV was present as intact form in all bio-samples, and none of its hydrolysis products was detected, indicating that astragaloside IV is stable in body. Astragaloside IV could be chosen as a potential pharmacokinetic marker, considering its pharmacologic actions reported in literature.

The phenolic acids in Danshen are composed of monomers (TSL and PCA), dimers (RA, SAG and SAD), trimers (SAA, SAC and LA) and tetramers (SAB and SAE). Previous studies [12] demonstrated that monomers were highly absorbable according to *in vitro* and in silico results; however, the polymers (SAA, SAB, SAD, RA, LA) showed poor membrane permeability. Besides, Guo *et al*. has reported that intestinal bacteria played an important role on the absorption and metabolism of a tetramer SAB after oral administration, on account of no metabolites were detected in urine and bile of antibiotic treated rats [43]. In our study, only two dimers (RA and SAD) were detected in plasma and/or urine, and most metabolites of Danshen were from monomers (TSL and PCA). PCA underwent remarkably active biotransformation in rats in two distinct steps. During the first step (phase I metabolism), PCA was functionalized by oxidation and reduction. Three enzymes, aldehyde oxidase, xanthine oxidase and aldehyde dehydrogenase could contribute to the oxidation of PCA to its acid [37], however, further studies on rats should also be performed on account of species differences. In the second step (phase II metabolism), PCA and/or its primary metabolites proceeded glucuronidation catalyzed by uridine-diphosphate-glucuronosyltransferase, methylation catalyzed by cathecol-O-methyltransferase (COMT), and/or glycine conjugation depending not only upon medium chain acyl-CoA synthetases but also upon acyl-CoA: glycine N-acyltransferases [36]. In addition, PCA and its primary metabolites were also conjugated with sulfate by phenolsulfotransferase (PST) mainly in the liver. The appearance of RA in the plasma and its 7 metabolites in the urine indicated that RA was present as intact, degraded, and/or conjugated forms, consistent with literature. Recent study showed that cinnamoyl esterase played an important role in the hydrolysis of RA [44]. In addition, based on the other phenolic acid metabolites, we hypothesized that TSL may be absorbed and sulfated by PST, or methylated by COMT; SAD was functionalized by methylation;

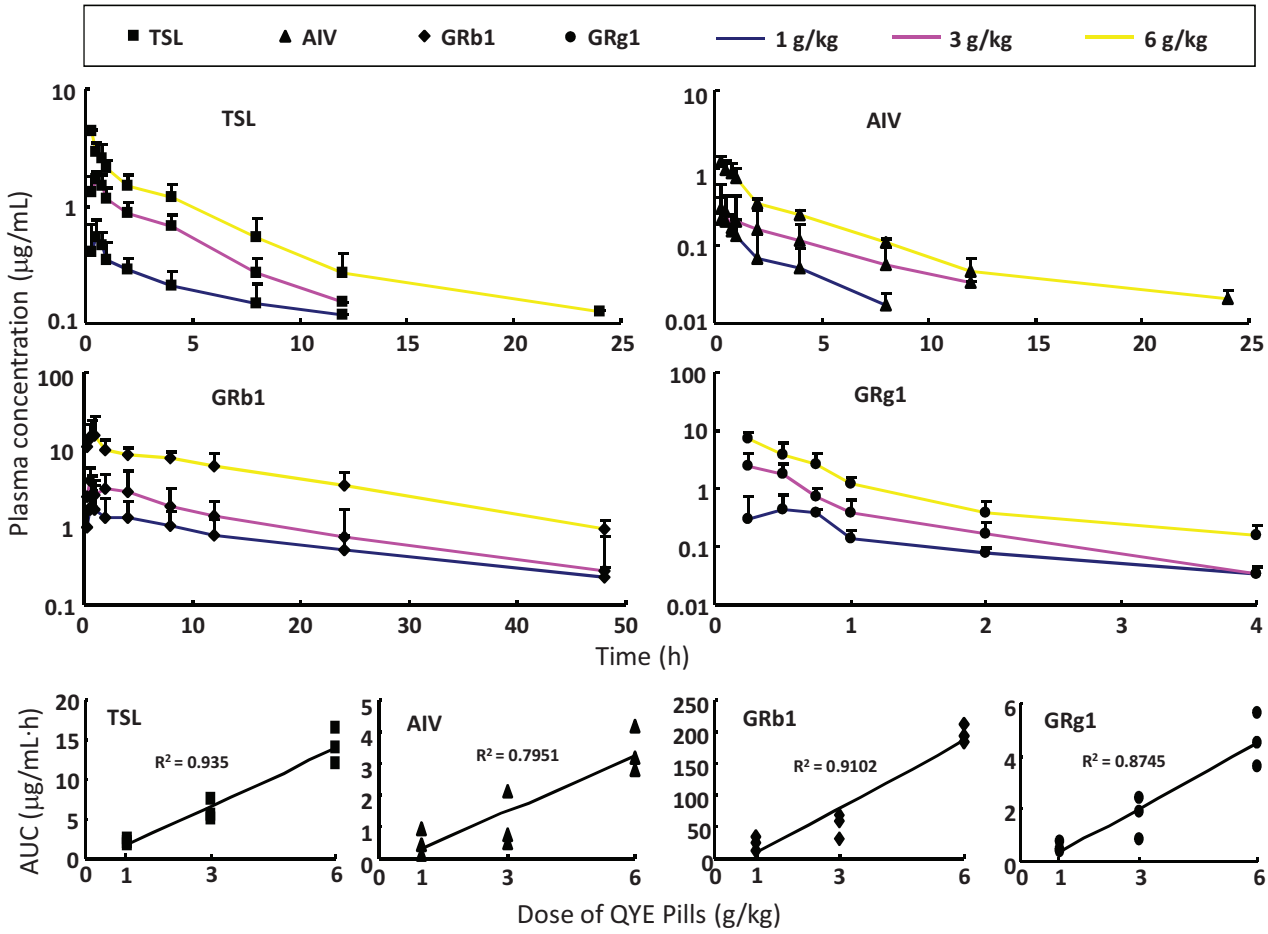

Fig. (4). Pharmacokinetic profiles and dosage responses of TSL, AIV, GRb1 and GRg1 after oral administration of QY pills at 1, 3 and 6 g/kg.

and SAA was hydrolyzed by cinnamoyl esterase before absorption and methylated and/or glucuronided conjugation.

For Sanqi saponins, their oral absorption has been investigated roundly. Previous studies have revealed that elimination in the stomach, large intestine and liver contributed to the low oral bioavailability of Rg_1 and Rb_1, but low membrane permeability might be a more important factor in determining the extent of absorption [45, 46]. In addition, some Sanqi saponins metabolites, such compound K, 20(S)-protopanaxadiol and 20(S)-protopanaxatriol, have been observed in the *in vivo* study [47]. In our study, after oral administration of QY pills, only the prototypes could be detected in rat plasma *in vivo*. However, no metabolite of Sanqi saponins was found in our study, it could be attributed to the limited percentage of Sanqi in this formula (5%), even though they had metabolites, their contents may be too low to be detected using the current method.

Three volatile oils from Jiangxiang, involving ENL, RDL, and SDL have been detected in small intestine, liver and adipose tissues and the contents of these volatile oils in small intestine were highest. Previous studies has reported that the area under curve of TSL significantly increased after administration of Danshen coupled with Jiangxiang [48]. In view of the strong liposolubility of volatile oils from Jiangxiang, it could be supposed that the volatile oils played an important role in small intestine, facilitating other compounds to be absorbed, which need further investigation in the future.

In view of the structural similarity and complexity of constituents in QY pills, the origins and structures of some metabolites could not be definitely elucidated using the current analytical method. However, our investigation of rat systemic exposure to various compounds from oral QY pills provided a global view of the fate of bioactive constituents in body, which could be very helpful in revealing the therapeutic basis of QY pills.

CONCLUSIONS

In summary, our study has investigated the rat systemic exposure to compounds from oral QY pills in detail. Although QY pills contains numerous chemical constituents, after research on the systemic exposure, we concentrated our attention on 6 phenolic acids (TSL, PCA, RA, SAA, SAD and SAG), 4 flavonoids (CG, IMG, Ono and FO), 7 triterpenoid saponins (GRb_1, GRd, GRe, GRg_1, NR_1, GXVII and AIV) and 3 volatile oils. Among them, TSL, RA, SAD, SAG and the 7 triterpenoid saponins could be detected as prototypes into plasma; 3 volatile oils have been distributed to small intestine, liver and adipose tissues; and a total of 52 metabolites were identified in the plasma, bile and/or urine, 32 of which were tentatively assigned as metabolites originating from PCA, TSL, RA, SAA and SAD, and 20 were plausibly inferred as metabolites of the 4 flavonoids. Based on these metabolites, the metabolic profiles were proposed, which made the fate of QY pills in the body more clear. In addition, we investigated the pharmacokinetics of QY pills, among which, TSL, GRb_1, GRg_1 and AIV were selected as pharmacokinetic markers and revealed the dynamic change of QY pills in the body. In brief, the present study would be remarkably helpful in deciphering the mechanism of action of QY pills. In the next step, the activity and pharmacokinetics of high abundant metabolites, such as PCA sulfates and calycosin conjugates, would be measured.

Table 2. Plasma Pharmacokinetic Parameters of TSL, AIV, GRg_1 and GRb_1 in Rats

Com-pounds	Route	Dose [a]	Cmax (μg/mL)	Tmax (h)	$AUC_{0\rightarrow t}$ (μg/mL·h)	$AUC_{0\rightarrow\infty}$ (μg/mL·h)	MRT (h)	$t_{1/2\lambda z}$ (h)	CL_F_obs[b] (L/h/kg)	F (%)
TSL	po	1	0.59±0.15	0.58±0.14	2.08±0.46	3.00±0.86	3.53±1.17	5.57±2.67	5.09±1.26	27.05±2.38
		3	1.71±0.29	0.57±0.15	6.01±1.32	6.97±1.23	3.10±0.71	3.27±0.20	6.38±1.13	
		6	4.38±0.11	0.25±0.00	13.14±3.87	14.54±3.72	4.27±1.52	5.58±3.61	6.27±1.67	
	iv	2	2.03±0.56	–	1.09±0.49	1.17±0.47	0.38±0.04	0.71±0.32	1.99±1.05	
AIV	po	1	0.24±0.21	0.67±0.14	0.51±0.42	0.55±0.45	2.02±0.65	1.90±0.73	22.43±23.11	0.26±0.05
		3	0.34±0.27	0.49±0.25	1.14±0.87	1.32±0.86	3.54±0.71	4.16±1.05	18.27±10.01	
		6	1.22±0.15	0.25±0.00	3.03±0.20	3.26±0.29	3.45±1.03	3.72±0.86	11.52±1.09	
	iv	2	38.05±5.49	–	57.85±10.68	58.37±11.29	1.52±0.70	1.37±0.57	0.04±0.01	
GRg_1	po	1	0.51±0.25	0.67±0.14	0.52±0.17	0.59±0.21	1.20±0.28	1.55±0.46	17.81±5.57	0.49±0.09
		3	2.57±1.59	0.31±0.14	1.72±0.79	1.80±0.76	0.74±0.15	0.95±0.65	19.14±10.08	
		6	7.05±2.35	0.25±0.00	4.61±1.02	4.82±0.84	0.80±0.25	0.86±0.56	12.50±2.14	
	iv	4	58.43±24.35	–	50.13±24.84	50.29±24.85	0.40±0.23	0.27±0.19	0.10±0.06	
GRb_1	po	1	2.18±1.55	0.75±0.25	22.71±13.51	36.84±21.46	10.25±0.76	18.48±0.68	0.31±0.25	0.18±0.05
		3	4.30±1.96	0.58±0.14	52.86±19.98	59.50±26.01	13.60±3.04	14.57±4.01	0.47±0.24	
		6	18.19±10.40	0.67±0.38	198.87±15.05	262.17±68.89	13.23±3.78	17.27±4.25	0.19±0.04	
	iv	2	303.98±33.32	–	3415.15±389.91	4479.99±561.69	8.58±0.20	11.87±0.43	0.45±0.05	

[a]In case of oral administration, the given drugs were QY pills (g/kg); while for iv administration, the given drugs were standard compounds of TSL, AIV, GRg_1 and GRb_1 (mg/kg).
[b]It is "CL_obs" for iv administration.

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CONFLICT OF INTEREST

None declared.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers Web site along with the published article.

REFERENCES

[1] Yang, M.; Sun, J.; Lu, Z.; Chen, G.; Guan, S.; Liu, X.; Jiang, B.; Ye, M.; Guo, D. A. Phytochemical analysis of traditional Chinese medicine using liquid chromatography coupled with mass spectrometry. *J. Chromatogr. A,* **2009**, *1216*(11), 2045-2062.

[2] Shen, Y.; Wang, X.; Xu, L.; Liu, X.; Chao, R. Characterization of metabolites in rat plasma after intravenous administration of salvianolic acid A by liquid chromatography/time-of-flight mass spectrometry and liquid chromatography/ion trap mass spectrometry. *Rapid Commun. Mass Spectrom.,* **2009**, *23*(12), 1810-1816.

[3] Zhang, Q.; Ye, M. Chemical analysis of the Chinese herbal medicine Gan-Cao (licorice). *J. Chromatogr. A,* **2009**, *1216*(11), 1954-1969.

[4] Han, S. Y.; Li, P. P. Progress of research in antitumor mechanisms with Chinese medicine. *Chin. J. Integr. Med.,* **2009**, *15*(4), 316-320.

[5] Qin, F.; Huang, X.; Ren, P. Chinese herbal medicine modified xiaoyao san for functional dyspepsia: meta-analysis of randomized controlled trials. *J. Gastroenterol. Hepatol.,* **2009**, *24*(8), 1320-1325.

[6] Tang, J.; Feng, Y.; Tsao, S.; Wang, N.; Curtain, R.; Wang, Y. Berberine and Coptidis rhizoma as novel antineoplastic agents: a review of traditional use and biomedical investigations. *J. Ethnopharmacol.,* **2009**, *126*(1), 5-17.

[7] Zhang, P.; Li, J.; Han, Y.; Yu, X. W.; Qin, L. Traditional Chinese medicine in the treatment of rheumatoid arthritis: a general review. *Rheumatol. Int.,* **2010**, *30*(6), 713-718.

[8] Liang, Y.; Kang, A.; Xie, T.; Zheng, X.; Dai, C.; Hao, H.; A, J.; Sheng, L.; Xie, L.; Wang, G. J. Influence of segmental and selected ion monitoring on quantitation of multi-component using high-pressure liquid chromatography-quadrupole mass spectrometry: Simultaneous detection of 16 saponins in rat plasma as a case. *J. Chromatogr. A,* **2010**, *1217*(26), 4501-4506.

[9] Chen, J. Z.; Chou, G. X.; Wang, C. H.; Yang, L.; Bligh, S. W.; Wang, Z. T. Characterization of new metabolites from *in vivo* biotransformation of norisoboldine by liquid chromatography/mass spectrometry and NMR spectroscopy. *J. Pharm. Biomed. Anal.,* **2010**, *52*(5), 687-693.

[10] Zhao, H.; Fan, M.; Fan, L.; Sun, J.; Guo, D. Liquid chromatography-tandem mass spectrometry analysis of metabolites in rats after administration of prenylflavonoids from Epimediums. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.,* **2010**, *878*(15-16), 1113-1124.

[11] He, S. M.; Li, C. G.; Liu, J. P.; Chan, E.; Duan, W.; Zhou, S. F. Disposition pathways and pharmacokinetics of herbal medicines in humans. *Curr. Med. Chem.,* **2010**, *17*(33), 4072-4113.

[12] Lu, T.; Yang, J.; Gao, X.; Chen, P.; Du, F.; Sun, Y.; Wang, F.; Xu, F.; Shang, H.; Huang, Y.; Wang, Y.; Wan, R.; Liu, C.; Zhang, B.; Li, C. Plasma and urinary tanshinol from Salvia miltiorrhiza (Danshen) can be used as pharmacokinetic markers for cardiotonic pills, a cardiovascular herbal medicine. *Drug Metab. Dispos.,* **2008**, *36*(8), 1578-1586.

[13] Yan, X. J. *et al*. A traditional Chinese medicine prescription treating for coronary heart disease and effort angina and its preparation method. CN patent 200310107278.0, filed December 11, 2003, and issued June 15, 2005.

[14] Long, W.; Luo, J. H.; Zhou, G. H. Analysis of treatment of chronic cardiac insufficiency with Qishen yiqi drop pills. *J. Jinggangshan Med. Coll.,* **2006**, *13*(1), 47.

[15] Lin, Y.; Liu, H. J.; Li, H. Y. Effects of QI-SHEN-YI-QI dropping pills on effort angina. *China Med. Herald.* **2006**, *3*(26), 127-128.

[16] Lai, L.; Hao, H.; Liu, Y.; Zheng, C.; Wang, Q.; Wang, G.; Chen, X. Characterization of pharmacokinetic profiles and metabolic pathways of 20(S)-ginsenoside Rh1 *in vivo* and *in vitro*. *Planta Med.,* **2009**, *75*(8), 797-802.

[17] Du, W. X.; Zhu, M. D.; Feng, L. M.; Song, Q. G.; Wei, Y.; Ma, P.; Liu, C. Y.; Liu, M. Intervention effect of Qishenyiqi Gutta Pills on early ventricular remodeling after acute myocardial infarction. *Chin. J. Evid. Based Cardiovasc. Med.,* **2008**, *1*(1), 41-43.

[18] Li, Y. F.; Qu, H. B.; Cheng, Y. Y. Identification of major constituents in the traditional Chinese medicine "QI-SHEN-YI-QI" dropping pill by high-performance liquid chromatography coupled with diode array detection-electrospray ionization tandem mass spectrometry. *J. Pharm. Biomed. Anal.,* **2008**, *47*(2), 407-412.

[19] Shang, H.; Dai, G.; Hang, J.; Xiang, Y.; Wang, Y.; Zhang, J.; Du, W.; Mao, J.; Yao, C.; Weng, W.; Wen, T.; Zhang, B. Myocardial infarction secondary prevention study (MISPS). *J. Geriatr. Cardiol.,* **2006**, *3*(2), 116-119.

[20] http://www.chinadaily.com.cn/dfpd/tianjin/2010-07-02/content_527491.html (Accessed July 28, 2010).

[21] Liu, A. H.; Guo, H.; Ye, M.; Lin, Y. H.; Sun, H. H.; Xu, M.; Guo, D. A. Detection, characterization and identification of phenolic acids in Danshen using high-performance. liquid chromatography with diode array detection and electrospray ionization mass spectrometry. *J. Chromatogr. A,* **2007**, *1161*(1-2), 170-182.

[22] Lin, L. Z.; He, X. G.; Lindenmaier, M.; Nolan, G.; Yang, J.; Cleary, M.; Qiu, S. X.; Cordell, G. A. Liquid chromatography-electrospray ionization mass spectrometry study of the flavonoids of the roots of Astragalus mongholicus and A. membranaceus. *J. Chromatogr. A.* **2000**, *876*(1-2), 87-95.

[23] Li, Y. F.; Wang, Y.; Qu, H. B.; Cheng, Y. Y. Simultaneous Determination of Seven Bioactive Compounds in Chinese Medicine "QI-SHEN-YI-QI" Dropping Pill by LC-UV and LC-ELSD. *Chromatographia,* **2008**, *67*(3-4), 293-297.

[24] Wei, Y. J.; Li, P.; Shu, B.; Li, H. J.; Peng, Y. R.; Song, Y.; Chen, J.; Yi, L. Analysis of chemical and metabolic components in traditional Chinese medicinal combined prescription containing Radix Salvia miltiorrhiza and Radix Panax notoginseng by LC-ESI-MS methods. *Biomed. Chromatogr.,* **2007**, *21*(8), 797-809.

[25] Xu, F.; Zhang, Y.; Xiao, S.; Lu, X.; Yang, D.; Yang, X.; Li, C.; Shang, M.; Tu, P.; Cai, S. Absorption and metabolism of Astragali radix decoction: in silico, *in vitro*, and a case study *in vivo*. *Drug Metab. Dispos.,* **2006**, *34*(6), 913-924.

[26] Liu, H.; Yang, J.; Du, F.; Gao, X.; Ma, X.; Huang, Y.; Xu, F.; Niu, W.; Wang, F.; Mao, Y.; Sun, Y.; Lu, T.; Liu, C.; Zhang, B.; Li, C. Absorption and disposition of ginsenosides after oral administration of Panax notoginseng extract to rats. *Drug Metab. Dispos.,* **2009**, *37*(12), 2290-2298.

[27] Zhang, Z. C.; Xu, M.; Sun, S. F.; Qiao, X.; Wang, B. R.; Han, J.; Guo, D. A. Metabolic analysis of four phenolic acids in rat by liquid chromatography-tandem mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.,* **2008**, *871*(1), 7-14.

[28] Tao, Y.; Wang, Y. Bioactive sesquiterpenes isolated from the essential oil of Dalbergia odorifera T. Chen. *Fitoterapia,* **2010**, *81*(5), 393-396.

[29] Tautenhahn, R.; Bottcher, C.; Neumann, S. Highly sensitive feature detection for high resolution LC/MS. *BMC Bioinformatics,* **2008**, *9*, 504.

[30] Zhang, X.; Xiao, H. B.; Xue, X. Y.; Sun, Y. G.; Liang, X. M. Simultaneous characterization of isoflavonoids and astragalosides in two Astragalus species by high-performance liquid chromatography coupled with atmospheric pressure chemical ionization tandem mass spectrometry. *J. Sep. Sci.,* **2007**, *30*(13), 2059-2069.

[31] Chen, J.; Lin, H.; Hu, M. Absorption and metabolism of genistein and its five isoflavone analogs in the human intestinal Caco-2 model. *Cancer Chemother. Pharmacol.,* **2005**, *55*(2), 159-169.

[32] Bursztyka, J.; Perdu, E.; Tulliez, J.; Debrauwer, L.; Delous, G.; Canlet, C.; De Sousa, G.; Rahmani, R.; Benfenati, E.; Cravedi, J. P. Comparison of genistein metabolism in rats and humans using liver microsomes and hepatocytes. *Food Chem. Toxicol.,* **2008**, *46*(3), 939-948.

[33] Wen, X. D.; Qi, L. W.; Li, B.; Li, P.; Yi, L.; Wang, Y. Q.; Liu, E. H.; Yang, X. L. Microsomal metabolism of calycosin, formononetin and drug-drug interactions by dynamic microdialysis sampling and HPLC-DAD-MS analysis. *J. Pharm. Biomed. Anal.,* **2009**, *50*(1), 100-105.

[34] Setchell, K. D.; Brown, N. M.; Zimmer-Nechemias, L.; Brashear, W. T.; Wolfe, B. E.; Kirschner, A. S.; Heubi, J. E. Evidence for lack of absorption of soy isoflavone glycosides in humans, supporting the crucial role of intestinal metabolism for bioavailability. *Am. J. Clin. Nutr.,* **2002**, *76*(2), 447-453.

[35] Li, C. Y.; Qi, L. W.; Li, P.; Wen, X. D.; Zhu, Y. F.; Liu, E. H.; Gong, Z.; Yang, X. L.; Ren, M. T.; Li, Y. J.; Ge, X. X. Identification of metabolites of Danggui Buxue Tang in rat urine by liquid chromatography coupled with electrospray ionization time-of-flight mass spectrometry. *Rapid Commun. Mass Spectrom.,* **2009**, *23*(13), 1977-1988.

[36] Xu, M.; Zhang, Z.; Fu, G.; Sun, S.; Sun, J.; Yang, M.; Liu, A.; Han, J.; Guo, D. Liquid chromatography-tandem mass spectrometry analysis of protocatechuic aldehyde and its phase I and II metabolites in rat. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.,* **2007**, *856*(1-2), 100-107.

[37] Panoutsopoulos, G. I.; Beedham, C. Enzymatic oxidation of vanillin, isovanillin and protocatechuic aldehyde with freshly prepared Guinea pig liver slices. *Cell Physiol. Biochem.,* **2005**, *15*(1-4), 89-98.

[38] Sinha, A. K.; Verma, S. C.; Sharma, U. K. Development and validation of an RP-HPLC method for quantitative determination of vanillin and related phenolic compounds in Vanilla planifolia. *J. Sep. Sci.,* **2007**, *30*(1), 15-20.

[39] Baba, S.; Osakabe, N.; Natsume, M.; Terao, J. Orally administered rosmarinic acid is present as the conjugated and/or methylated forms in plasma, and is degraded and metabolized to conjugated forms of caffeic acid, ferulic acid and m-coumaric acid. *Life Sci.,* **2004**, *75*(2), 165-178.

[40] Zhang, H.; Cheng, Y. Solid-phase extraction and liquid chromatography-electrospray mass spectrometric analysis of saponins in a Chinese patent medicine of formulated Salvia miltiorrhizae and Panax notoginseng. *J. Pharm. Biomed. Anal.,* **2006**, *40*(2), 429-432.

[41] Hu, J. Y.; Han, J.; Chu, Z. G.; Song, H. P.; Zhang, D. X.; Zhang, Q.; Huang, Y. S. Astragaloside IV attenuates hypoxia-induced cardiomyocyte damage in rats by upregulating superoxide dismutase-1 levels. *Clin. Exp. Pharmacol. Physiol.,* **2009**, *36*(4), 351-357.

[42] Zhang, C.; Wang, X. H.; Zhong, M. F.; Liu, R. H.; Li, H. L.; Zhang, W. D.; Chen, H. Mechanisms underlying vasorelaxant action of astragaloside IV in isolated rat aortic rings. *Clin. Exp. Pharmacol. Physiol.,* **2007**, *34*(5-6), 387-392.

[43] Xu, M.; Guo, H.; Han, J.; Sun, S. F.; Liu, A. H.; Wang, B. R.; Ma, X. C.; Liu, P.; Qiao, X.; Zhang, Z. C.; Guo, D. A. Structural characterization of metabolites of salvianolic acid B from Salvia miltiorrhiza in normal and antibiotic-treated rats by liquid

chromatography-mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.,* **2007**, *858*(1-2), 184-198.

[44] Bel-Rhlid, R.; Crespy, V.; Page-Zoerkler, N.; Nagy, K.; Raab, T.; Hansen, C. E. Hydrolysis of rosmarinic acid from rosemary extract with esterases and Lactobacillus johnsonii *in vitro* and in a gastrointestinal model. *J. Agric. Food Chem.,* **2009**, *57*(17), 7700-7705.

[45] Han, M.; Fang, X. L. Difference in oral absorption of ginsenoside Rg1 between *in vitro* and *in vivo* models. *Acta Pharmacol. Sin.,* **2006**, *27*(4), 499-505.

[46] Han, M.; Sha, X.; Wu, Y.; Fang, X. Oral absorption of ginsenoside Rb1 using *in vitro* and *in vivo* models. *Planta Med.,* **2006**, *72*(5), 398-404.

[47] Yang, L.; Liu, Y.; Liu, C. X. Metabolism and pharmacokinetics of ginsenosides. *Asian J. Pharmacodyn. Pharmacokinet.,* **2006**, *6*(2), 103-120.

[48] Zheng, X.; Zhao, X.; Wang, S.; Luo, K.; Wei, Y.; Zheng, J. Co-administration of Dalbergia odorifera increased bioavailability of Salvia miltiorrhizae in rabbits. *Am. J. Chin. Med.,* **2007**, *35*(5), 831-840.

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