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# Analytical strategy to reveal the *in vivo* process of multi-component herbal medicine: A pharmacokinetic study of licorice using liquid chromatography coupled with triple quadrupole mass spectrometry

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ABSTRACT

Although various techniques have been employed to analyze drug metabolites, the metabolism of multi-component herbal medicine has seldom been fully addressed. In contrast to chemical drugs, a number of compounds in herbal medicine could get into circulation and then be metabolized. Moreover, these compounds may have metabolic interactions which make their pharmacokinetics (PK) even more complicated. The present work aims to elucidate the multi-component pharmacokinetics of a herbal medicine, and to demonstrate how PK behaviors were altered by co-existing constituents. Licorice (*Glycyrrhiza uralensis* Fisch.), a most commonly used herbal medicine, was chosen as a model. A strategy was proposed to compare the PK profiles of licorice extract with those of nine single compounds. These compounds were major bioactive constituents of licorice, and represented various structural types (flavanone, chalcone, isoflavone, saponin, and coumarin). We established a segmented selected reaction monitoring LC/MS/MS method to simultaneously monitor 63 licorice metabolites in rat plasma, and obtained the PK profiles of 55 metabolites. The results indicated that interactions among licorice compounds altered their PK behaviors in 4 aspects: improvement in bioavailability for aglycones (133- and 109-fold increase for liquiritigenin and isoliquiritigenin, respectively), prolongation in system circulation for glycosides (0.3 h delay in $T_{max}$ for liquiritin apioside and isoliquiritin apioside), decrease of potential toxicity for saponins such as glycyrrhizic acid, and shift in plasma distribution for phase II metabolites. This is the first attempt to systematically reveal the *in vivo* process of licorice. Moreover, the study indicates noticeable interactions to alter pharmacokinetics among licorice compounds, which may be characteristic for herbal medicines.

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## 1. Introduction

Herbal medicines are playing an increasingly important role in the treatment of chronic diseases today, as chemical drugs (single compounds) are more and more challenged by drug resistance and side effects [1–4]. Distinct from chemical drugs, herbal medicines are multi-component and multi-target agents, and may exert a holistic treatment to multi-factorial diseases such as cancer [5]. More importantly, ingredients of an herbal medicine could act synergistically to reach maximal therapeutic effect [6]. It is generally considered that the maximal effect is accomplished by an array of phytochemicals. The co-existence of multiple compounds may lead to metabolic and pharmacokinetic (PK) interactions. However, further studies on the interactions are lacking because pharmacokinetics of an herbal medicine that simultaneously monitor multiple metabolites have seldom been reported, so far [7].

Although PK studies of herbal medicines have been extensively pursued, most reports follow the procedures for chemical drugs, and only monitor a few marker compounds [7]. Obviously, the results could not comprehensively reveal the pharmacokinetic behaviors of herbal medicines. Recently, Liu et al. reported the pharmacokinetics of seven ginsenosides in rat plasma after oral administration of *Panax notoginseng*, and shed clearer light on the metabolic fate of a complicated herbal extract [8]. However, systematic PK study of an herbal medicine is still challenged by desirable analytical methods, by which a big array of phytochemicals and their metabolites could be simultaneously detected at low concentrations. Due to complicated chemical composition of herbal medicines, tens of compounds could be absorbed into circulation and then converted into even more metabolites. Recently, we proposed a three-step strategy to systematically characterize the metabolites for licorice [9]. A total of 42 metabolites in rat plasma and 62 metabolites in urine were detected after oral administration of licorice water extract. The characterization of *in vivo* metabolites

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**Table 1**
Segmented SRM settings for licorice metabolites.

| Analyte | Parent | Center | Width | CE | TLO | Segment | Analyte | Parent | Center | Width | CE | TLO | Segment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 30[a], 43[a] | 255.0 | 118.9 | 1.00 | 29 | 120 | DEFG | 55 | 433.0 | 353.0 | 1.50 | 20 | 90 | GH |
| 49[a] | 267.0 | 223.1 | 2.00 | 46 | 110 | DEFG | 17 | 433.0 | 257.0 | 1.50 | 20 | 90 | BC |
| 49[a] | 267.0 | 252.1 | 2.00 | 33 | 110 | DEFG | 26 | 443.0 | 267.0 | 1.50 | 20 | 90 | D |
| 21 | 333.0 | 253.0 | 1.50 | 20 | 90 | CD | 40, 53, 56 | 447.0 | 367.0 | 1.50 | 20 | 90 | FGH |
| 23, 35 | 335.0 | 255.0 | 1.50 | 20 | 90 | CDF | 63[a] | 469.0 | 425.2 | 3.00 | 41 | 200 | H |
| 29, 39 | 347.0 | 267.0 | 1.50 | 20 | 90 | DEF | 62 | 485.0 | 441.0 | 3.00 | 41 | 200 | GH |
| 58[a] | 351.0 | 320.9 | 2.00 | 43 | 163 | H | 9, 19 | 497.0 | 417.0 | 1.50 | 20 | 90 | BC |
| 58[a] | 351.0 | 333.0 | 2.00 | 37 | 163 | H | 5, 12 | 511.0 | 335.0 | 1.50 | 20 | 90 | ABC |
| 47[a] | 353.0 | 284.9 | 2.00 | 37 | 178 | GH | 4, 18, 20 | 513.0 | 337.0 | 1.50 | 20 | 90 | ABCD |
| 59 | 353.0 | 296.9 | 2.00 | 40 | 178 | GH | 36, 45, 52 | 527.0 | 351.0 | 1.50 | 20 | 90 | FG |
| 57[a] | 367.0 | 308.9 | 2.00 | 33 | 131 | GH | 38, 41, 48 | 529.0 | 353.0 | 1.50 | 20 | 90 | FG |
| 60[a] | 381.0 | 322.9 | 2.00 | 36 | 154 | H | 32, 34, 46 | 543.0 | 367.0 | 1.50 | 20 | 90 | EFG |
| 60[a] | 381.0 | 351.0 | 2.00 | 34 | 154 | H | 14[a], 22[a] | 549.0 | 254.9 | 2.00 | 30 | 150 | BCD |
| 37 | 381.0 | 309.0 | 2.00 | 30 | 160 | FG | 50, 51 | 557.0 | 381.0 | 1.50 | 20 | 90 | FGH |
| 28 | 383.0 | 309.0 | 2.00 | 30 | 160 | DE | 31 | 559.0 | 383.0 | 1.50 | 20 | 90 | E |
| 13 | 413.0 | 333.0 | 1.50 | 20 | 90 | B | 1, 6, 11 | 593.0 | 417.0 | 1.50 | 20 | 90 | AB |
| 16[a], 24[a] | 417.0 | 254.9 | 2.00 | 27 | 150 | BCD | 2, 7 | 607.0 | 431.0 | 1.50 | 20 | 90 | A |
| 25[a] | 429.0 | 267.0 | 2.00 | 20 | 150 | BCD | 3, 8 | 629.0 | 549.0 | 1.50 | 20 | 90 | AB |
| 10 | 429.0 | 253.0 | 1.50 | 20 | 90 | B | 61 | 645.0 | 469.0 | 1.50 | 20 | 90 | GH |
| 54 | 431.0 | 351.0 | 1.50 | 20 | 90 | FGH | 44[a] | 821.0 | 351.0 | 3.00 | 40 | 222 | G |
| 15, 27 | 431.0 | 255.0 | 1.50 | 20 | 90 | BCD | 42[a] | 837.0 | 351.0 | 3.00 | 40 | 222 | FG |
| K7 | 433.0 | 271.0 | 2.00 | 24 | 152 | D | 33[a] | 983.0 | 351.0 | 3.00 | 40 | 222 | EF |
| K7 | 433.0 | 134.9 | 2.00 | 42 | 152 | D | | | | | | | |

CE, collision energy; TLO, tube lens offset; Segment, programmed SRM segments: A, 1.8–5 min; B, 5–6.5 min; C, 6.5–9 min; D, 9–11.5 min; E, 11.5–14 min; F, 14–17 min; G, 17–19 min; H, 19–25 min.
Source-induced dissociation energies were set at 5% for compounds 28, 30, 37, 43, 49, 62, 63 and K7 (internal standard); 10% for compounds 33, 42, 44, 47, 57, 58, 59 and 60; and 0% for the other compounds.
[a] Compounds identified by reference standards.

makes it possible to study multi-component pharmacokinetics. In the present study, LC/MS/MS methodology was established to facilitate multi-component quantitative analysis. The method was based on a multi-channel segmented selected reaction monitoring (SSRM) program (as listed in Table 1), which required shorter analysis time, and gave higher accuracy and sensitivity.

Licorice is still chosen as a model herb in this work. As the most frequently used herbal medicine worldwide, licorice appears in about 60% traditional Chinese medicine (TCM) prescriptions, numerous western herbal therapies, and confectionery products [9–11]. It is a typical multi-component herbal medicine as at least 400 flavonoids, saponins and coumarins were reported from *Glycyrrhiza* species [12]. More importantly, it is mainly used to moderate and harmonize the characteristics of other herbs in TCM, which strongly implies the possibility of drug–drug interactions [13]. Several groups have studied the metabolism and pharmacokinetics of licorice compounds, which lay a solid basis for our study [14–16].

A strategy comparing the PK behaviors of licorice extract and single compounds was followed. Firstly, the pharmacokinetics of 63 phytochemicals was monitored in rats after oral administration of licorice water extract (LWE). Then, the pharmacokinetics of nine representative licorice constituents was monitored following administration of pure compounds. Finally, PK profiles of the same compound in pure form and in LWE were compared. Interactions among phytochemicals were elucidated by the alteration of PK profiles. Core idea of this strategy is depicted in Fig. 1. The plasma samples were quantitatively analyzed by a multi-channel segmented selected reaction monitoring LC/MS/MS method. Following our previous study on systematic metabolites characterization [9], this study describes the pharmacokinetics of licorice (including original compounds and their metabolites) and metabolic interactions between licorice compounds.

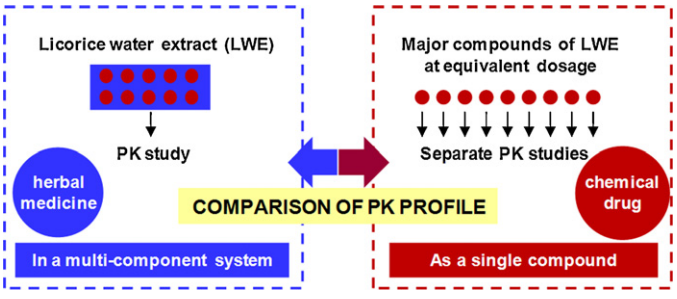


**Fig. 1.** A comparative strategy to reveal the difference in pharmacokinetics (abbreviated as PK) between single compounds (chemical drugs) and multi-component herbal medicines.

## 2. Experimental

### 2.1. Chemicals and reagents

Acetonitrile, methanol and formic acid (Mallinkrodt Baker, Phillipsburg, NJ, USA) were of HPLC grade. De-ionized water was obtained from a Milli-Q system (Millipore, MA, USA). High-purity nitrogen (99.9%) and helium (99.99%) were purchased from Gas Supplies Center of Peking University Health Science Center (Beijing, China).

Licorice (dried roots and rhizomes of *Glycyrrhiza uralensis* Fisch.) was purchased from Elion Resources Group Company (Inner Mongolia, China), and was authenticated by comparing its HPLC fingerprint with a reference sample from China National Institutes for Food and Drug Control (Beijing, China). Reference compounds were isolated from *G. uralensis* Fisch. by the authors, as shown in Fig. 2. These compounds were formononetin (FOR) [17], glycyrrhetinic acid (GA) [18], glycycoumarin (GCM) [19], glycyrrhizic acid (GLY) [20], isoliquiritin apioside (ILA) [21], isoliquiritigenin (ILG) [17], isoliquiritin (ILQ) [22], licoisoflavone A (LIFA) [23], licorice-saponin A3 (LSA3) [24], licorice-saponin G2 (LSG2) [25], licoricone (LIR) [26], liquiritin apioside (LA) [21], liquiritigenin (LG) [26], liquiritin (LQ) [22], ononin (ONO) [21], and semilicoisoflavone B (SIFB) [27]. The internal standard, butein 4-*O*-$\beta$-D-glucoside (K7) [28], was isolated from *Sophora alopecuroides* L. by the authors. For all the compounds,

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