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Simultaneous multi-component quantitation of Chinese herbal injection *Yin-zhi-huang* in rat plasma by using a single-tube extraction procedure for mass spectrometry-based pharmacokinetic measurement

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ABSTRACT

Ying-zhi-huang injection (YZH-I) is an injectable multi-herbal prescription derived from the ancient Chinese remedy "*Yin-chen-hao-tang*", which is widely used in the clinic for the treatment of jaundice and chronic liver diseases. To date, little information is available on the pharmacokinetic properties of this poly-herbal formulation. Herein, we reported a simple, rapid and sensitive liquid chromatography–tandem mass spectrometry (LC–MS/MS) method for quantitative multiple reaction monitoring (MRM) of eight major ingredients of YZH-I (including baicalin, baicalein, wogonoside, geniposide, geniposidic acid, chlorogenic acid, neochlorogenic acid, and caffeic acid) in rat plasma. A fast single-tube multi-impurity precipitation extraction ("SMIPE") procedure was introduced for straightforward plasma preparation, based on one-pot deproteinization precipitation with acidified methanol extraction and in-situ multifunction impurity removal by a solid sorbent mixture (anh. magnesium sulfate plus octadecylsilane). Particularly, the addition of ascorbic acid in methanol (10 mg/mL) was found to exhibit a pronounced protective effect and significantly increase extraction effectiveness of the herbal phenolic components. Some pretreatment variables (protein precipitating solvent, acidifying agent and sorbent) were optimized with acceptable matrix effect (−18 to 7.7%), extraction recovery (65–88%) and process efficiency (62–91%) for the SMIPE-based LC-MRM multi-analyte quantitation using matrix-matched calibration (5–1000 ng/mL) without using internal standard. Mean accuracies were obtained in the range of 83–114% at three different fortification levels, with intra- and inter-day variations within 13%. This validated method was successfully applied to the simultaneous measurement and pharmacokinetic investigation of the chemical constituents in rats following an intravenous administration of YZH-I.

1. Introduction

Herbal remedy (or "herbalism") has a long history of the use of medicinal plants for cure of various ailments throughout the world. Today, traditional phytotherapy is still the primary form of healthcare in developing countries and also increasingly used as complementary/alternative medicine (CAM) in modern Western medicine [1]. Moreover, herbal medicines have drawn renewed interest and global popularity in the last decade due to high incidence of adverse effects of synthetic drugs and failure of modern allopathic medicine for a number of diseases [2,3]. Inspired by traditional medicine, herbal medicinal products also have served as an enormous repository of new drug candidates, and new drug discovery strategy based on synergistic polypill concept is re-emerging as an attractive option for multi-target therapeutic and prophylactic applications of polygenic syndromes [4].

Yin-zhi-huang (YZH) is a classical multi-herbal prescription derived from the famous traditional Chinese medicine (TCM)

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formula of "*Yin-chen-hao-tang*" ("*Inchin-ko-to*" in Japanese [5]), which was originally recorded in ancient Chinese medical text *Shanghanlun* by Zhongjing Zhang (about 150–219 A.D.) in Han dynasty and has been widely used in many oriental countries for more than 1000 years to treat jaundice and chronic liver diseases [6–8]. According to the Chinese Pharmacopoeia (2010) [9], the oral polyherbal formulation of YZH is a decoction (concentrated aqueous extract) comprised of four Chinese herbal medicines, i.e., *Yinchen* (young shoot of *Autemisia capillaries* [Compositae]), *Zhizi* (fruit of *Gardenia jasminoides* Ellis [Rubiaceae]), *Huangqin* (root of *Scutellaria baicalensis* [Labiatae]) and *Jinyinhua* (flower bud of *Lonicera* Japonica [Caprifoliaceae]) [10]. Afterwards, an injectable multicomponent herbal medicine of YZH [10] was also developed to make up for the deficiencies of the traditional oral administrations, in that many TCM injections have been recognized by their more rapid and potent therapeutic effects for tracking some acute or complicated diseases [11]. As an innovative dosage formulation concocted from the quadri-herb extract combinations, *Yin-zhi-huang injection* (YZH-I) has been used clinically in China for more than 40 years to relieve infantile jaundice and treat acute and chronic hepatitis [10–13]. As specified in the Drug standards of Chinese traditional patent formulation promulgated by the Department of Health (WS3-B-2736-97) [14], the typical Chinese herbal intravenous injection of YZH-I is prepared by combining 20 g of baicalin from *Radix Scutellariae* (*Huangqin*) and 6 g *Herba Artemisiae* Scopariae (extract of *Yinchen* by percolation in 90% ethanol), 3.2 g *Fructus* Gardeniae (extract of *Zhizi* by reflux in 70% ethanol) and 4 g *Flos Lonicerae* (extract of *Jinyinhua* by water decoction), mixing well with an aqueous solution containing glucose (20 g) plus *N*-methyl-D-glucamine (5 g), and diluting the mixture into water for injection to yield 1000 mL of the herbal injection solution (pH 6.5–8.0) for sealing, filtrating and terminal sterilization. In YZH formulations, the main constituents of YZH-I are coumarins and flavonoids from *Yinchen*, iridoids (geniposides) from *Zhizi*, chlorogenic acids from *Jinyinhua* and baicalin from *Huangqin*, and they are indicated for bilirubin clearance by activating the nuclear receptor CAR [15,16], and potential inhibition of T-cell activation [10] in the management of liver diseases [7,13].

Towards a more critical interpretation of the link between herb consumption and medicinal effects, pharmacokinetic (PK) measurements are needed to demonstrate systemic exposure to an herbal medicinal product [17–19]. Particularly, as stated in the FDA's guidance on botanical drug products [20], it is strongly encouraged to monitor the blood levels of known active ingredients, representative markers or major chemical components from a botanical drug that often consists of more than one chemical constituent and the active constituents are unknown. As to YZH-I that contains a mixture of bioactive ingredients, however, only the content of baicalin (20.0–22.5 mg/mL) [14] has been officially adopted for the quality control (QC) of the multi-ingredient formulation. Although some current methods by using biological fingerprints as a useful complement to chemical fingerprinting were reported for quality estimation of the YZH-I products [13,21], to date there is still no published data on the PK properties of this poly-herbal preparation. Recently, several analytical techniques have been reported for the bioanalysis of the purified herbal components [22] and the measurement of one or several active ingredients in single herb extract [23,24] or other herbal formulas [7,8,25], including liquid chromatography coupled with ultraviolet (LC-UV) [22,25] or mass spectrometry (LC-MS) [7,8,23,24]. Unfortunately, the majority of the PK studies for YZH only focused on measuring one or two major components such as baicalin [26], and little information is available regarding PK studies of YZH-I. Hence, considering the complexity of the chemical constituents of YZH-I, it is imperative to develop new analytical methods for pharmacokinetic evaluation of the herbal prescription.

Herein, we aim to establish a sensitive mass spectrometry-based method for rapid measurement of eight major bioactive constituents contributing to therapeutic effects of the YZH-I formula in rat plasma. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) in multiple reaction monitoring (MRM) mode has served as the gold standard for accurate simultaneous multi-analyte quantitation across large sample sets to provide high-quality data from complex systems, owing to its impressive sensitivity and inherent selectivity [19,27]. In our previous work [28–30], a semi-targeted approach based on MRM ion intensity ratios and fast one-pot multifunction extraction/cleanup procedures has been proposed for the purpose of enlarging applicability of the classical LC–MRM–MS platform. To the best of our knowledge, no LC–MRM-based assays has been reported for the simultaneous pharmacokinetic investigation of multiple constituents in YZH-I following intravenous administration of YZH herbal injection in rats. In this study, a new sample preparation method based on shortcut multi-impurity precipitation is introduced and adapted as an alternative to traditional protein precipitation procedure for rapid and facile extraction of different phyto-constituents of YZH-I in plasma samples.

2. Materials and methods

2.1. Chemicals and materials

Standards of geniposidic acid (GSA, 96%), geniposide (GPS, 99%), baicalin (BCL, 98%), baicalein (BCE, 98.5%), chlorogenic acid (CGA, 99%) and caffeic acid (CFA, 99%) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), Wogonoside (WGS, 98%) and neochlorogenic acid (NCA, 98%) were of analytical grade provided by Tauto Biotech Co., Ltd (Shanghai, China) and Sigma-Aldrich (Shanghai, China), respectively. Their chemical structures and product ion mass spectra are shown in Fig. 1. The stock solutions of each standard (1 mg mL^{-1}) was individually prepared in methanol and was stored refrigerated at −20 °C in the dark. Working standard solutions of mixed standards were prepared by diluting a stock-standard mixture (100 μg/mL) with methanol containing 1% formic acid and 50 mg/mL ascorbic acid. All of the standard working solutions were stored at 4 °C when not in use.

HPLC-grade acetonitrile (ACN), methanol (MeOH) and *n*-hexane were obtained from Merck (Darmstadt, Germany). Analytical-grade sorbents of primary secondary amine (PSA, 40 μm) and octadecylsilane (ODS, C_{18}, 40 μm) were purchased from Varian, Inc. (Palo Alto, CA, USA). Formic acid (HCOOH), acetic acid (CH_3COOH), anhydrous magnesium sulfate ($MgSO_4$) and ascorbic acid (AA) were obtained from Dima Technology, Inc. (Richmond Hill, USA). Unless otherwise specified, all other reagents were of analytical grade. Ultrapure water prepared by a Milli-Q-Plus system (Millipore, Bedford, MA, USA) was used throughout the study. The intravenous herbal solutions of *Yin-zhi-huang injection* (YZH-I) used in the animal study (lot no. 12061543; 10 mL/ampoule; pH 6.6; expiration: 05/2015) were supplied by Shenwei Pharmaceutical Co., Ltd (Shijiazhuang, Hebei, China).

2.2. Sample preparation procedures

Plasma samples were pretreated by using a new procedure similar to the previously described SEP/MAC method based on straightforward one-pot extraction/solid-phase adsorption cleanup [29,30] with some modifications. In brief, an aliquot of thawed plasma sample (50 μL) was blended with 100 μL of methanol (containing 10 mg/mL AA), and the mixture was vigorously vortexed for approximately 3 min. Then the sample tube