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A sensitive and specific liquid chromatography mass spectrometry method for simultaneous determination of berberine, palmatine, coptisine, epiberberine and jatrorrhizine from *Coptidis Rhizoma* in rat plasma

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Abstract

A sensitive and specific liquid chromatography–electrospray ionization-mass spectrometry (LC–ESI-MS) method has been developed and validated for the identification and quantification of five protoberberine alkaloids, which are berberine, palmatine, coptisine, epiberberine and jatrorrhizine, in rat plasma using tetrahydroberberine as an internal standard. Following solid-phase extraction, the analytes were separated by linear gradient elution on a Shim-pack ODS (4.6 μm, 150 mm × 2.0 mm i.d.) column and analyzed in selected ion monitoring (SIM) mode with a positive electrospray ionization (ESI) interface using the respective $[M]^+$ and $[M+H]^+$ ions, $[M]^+ = 336$ for berberine; 320 for coptisine; 336 for epiberberine; 338 for jatrorrhizine; 352 for palmatine and $[M+H]^+ = 340$ for the internal standard. The method was validated over the concentration range of 0.31–20 ng mL^{-1} for all the five protoberberine alkaloids. Within-batch and between-batch precisions (R.S.D.%) were all within 15% and accuracy (%Er) ranged from −5 to 5%. The lower limits of quantification were 0.31 ng mL^{-1} for all analytes. The extraction recoveries were on average 80.8% for berberine, 67.0% for coptisine, 66.2% for epiberberine, 71.8% for jatrorrhizine and 73.2% for palmatine. The validated method was used to study the pharmacokinetic profile of the five protoberberine alkaloids in rat plasma after oral administration of *Coptidis Rhizoma* extract.

Keywords: Liquid chromatography–mass spectrometry; Protoberberine alkaloid; Rat plasma; Pharmacokinetics; *Coptidis Rhizoma*

1. Introduction

Coptidis Rhizoma (Huanglian), a widely used Traditional Chinese Medicine, has been used for centuries for the treatment of dysentery, hypertension, inflammation, and liver diseases [1,2]. It is derived from the dried rhizome of ranunculaceous plant such as *Coptis chinensis* Franch., *C. deltoidea* C.Y. Cheng et Hsiao. or *C. teeta* Wall., and is known to contain berberine, palmatine, coptisine, epiberberine and jatrorrhizine (structures shown in Fig. 1) which are all protoberberine alkaloids as its major bioactive components [3–11].

In most previous investigations, as one of the main components, berberine has been reported on the pharmacokinetics in blood, urine, bile and tissues of rats, mice, rabbits, dogs, and humans using non-specific UV spectrophotometric, fluorometric, tritium-labeled berberine, gas chromatography–chemical ionization mass spectrometry and HPLC methods [12–20]. Only a few studies regarding the pharmacokinetics of other protoberberine alkaloids such as, coptisine, dehydroapocavidine and tetradehydroscoulerine, have been carried out [21]. In our previous study, we have developed a liquid chromatography–electrospray ionization-mass spectrometry (LC–ESI-MS) method to determine berberine and palmatine in rat plasma after oral administration of Huang-Lian-Jie-Du decoction [22]. There is merit in characterizing the pharmacokinetics of five active alkaloids from *C. Rhizoma* extract in animals since there was no detailed pharmacokinetic profiles for simultaneous characterization of these alkaloids.

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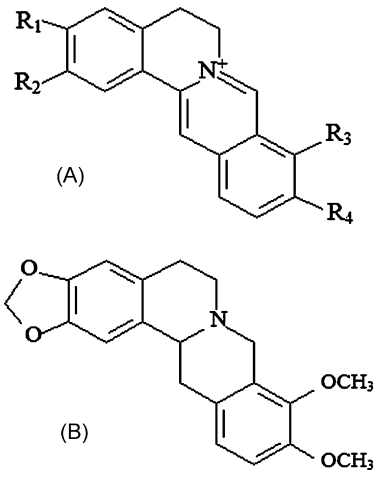

	R_1	R_2	R_3	R_4
berberine	-O-CH_2-O-		-OCH_3	-OCH_3
coptisine	-O-CH_2-O-		-O-CH_2-O-	
epiberberine	-OCH_3	-OCH_3	-O-CH_2-O-	
jatrorrhizine	-OH	-OCH_3	-OCH_3	-OCH_3
palmatine	-OCH_3	-OCH_3	-OCH_3	-OCH_3

Fig. 1. Chemical structures of berberine, coptisine, epiberberine, jatrorrhizine, palmatine (A) and internal standard tetrahydroberberine (B).

In this paper, a sensitive and selective method of liquid chromatography–electrospray ionization-mass spectrometry (LC–ESI-MS) is presented for the simultaneous determination of berberine, palmatine, coptisine, epiberberine and jatrorrhizine in rat plasma. This assay has been successfully applied to a pharmacokinetic study of the five protoberberine alkaloids after oral administration of *C. Rhizoma* extract in rats.

2. Experimental

2.1. Material and methods

2.1.1. Herbal materials

C. Rhizoma (*C. chinensis* Franch.) was purchased from Kaixin Pharmacy (Nanjing, PR China) and identified by Dr. Lina Chen (Department of Pharmacognosy, China Pharmaceutical University, Nanjing, PR China).

2.1.2. Standards and reagents

The reference standards of berberine, palmatine and jatrorrhizine were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, PR China). The reference standards of coptisine and epiberberine, were kind gifts given by Prof. Hao Zhang (West China School of Pharmacy, Sichuan University, Chengdu, PR China). The internal standard (IS), tetrahydroberberine (structure in Fig. 1), was kindly provided by Dr. Can Zhang (Department of Medicinal Chemistry, China Pharmaceutical University, Nanjing, PR China). Solid-phase extraction (SPE) columns (ODS C18, 100 mg) were obtained from Agela (Beijing, PR China). Acetonitrile was of chromatographic grade (Fisher Company Inc., USA). All other reagents were of analytical grade. Milli-Q water (Millipore, Bedford, MA) was used throughout the study.

2.1.3. Preparation of C. Rhizoma extract

C. Rhizoma (1000 g) were extracted twice by refluxing with boiling water (1:10 and then 1:5, w/v) for 1 h, and the solution obtained was concentrated to give an extract (256 g). The dried powder was stored at 4 °C before use.

2.1.4. Contents of five protoberberine alkaloids in C. Rhizoma extract

To calculate the administered dose, the contents of five protoberberine alkaloids in the extract were quantitatively determined. The HPLC analysis of these alkaloids was a modified version of a previously published method [23]. The contents of berberine, coptisine, epiberberine, jatrorrhizine and palmatine were 18.64 ± 0.61, 2.78 ± 0.24, 2.87 ± 0.26, 2.61 ± 0.20 and 3.54 ± 0.14 g $100\,g^{-1}$ extract, respectively.

2.1.5. Liquid chromatographic and mass spectrometric conditions

The LC–MS system consisted of a Shimadzu LC-10AD HPLC series liquid chromatograph and a Shimadzu LC–MS2010A single quadrupole mass spectrometer equipped with an electrospray ionization (ESI) interface and a Q-array-Octapole-Quadrupole mass analyzer. Shimadzu LCMSsolution Version 2.04 was used for data acquisition and processing.

LC separation was achieved using a Shim-pack ODS (4.6 μm, 150 mm × 2.0 mm i.d. Shimadzu) column maintained at 40 °C. The mobile phase consisted of A (0.08% formic acid and 2 mmol L^{-1} ammonium acetate) and B (acetonitrile) with linear gradient elution. The gradient cycle consisted of an initial 3 min isocratic segment (70% A and 30% B). Then, the linear gradient was started, increasing solvent B to 80% within 0.5 min and maintained from 3.5 to 5.5 min. After changing back to 30% solvent B at 6 min, the mobile phase gradient was maintained at this composition from 6 to 8 min for column equilibration. The flow rate was 0.2 mL min^{-1} during the whole gradient cycle.

The effluent from the HPLC column was directed into the ESI probe. Mass spectrometer conditions were optimized to obtain maximal sensitivity. The curve dissolution line (CDL) temperature and the block temperature were maintained at 250 and 200 °C, respectively. The probe voltage (capillary voltage), CDL voltage and detector voltage were fixed at 4.5 kV, −10 V and 1.6 kV, respectively. Vacuum was obtained by a Turbo molecular pump (Edwards 28, UK). Nitrogen (99.995%, Nanjing University, PR China) was used as the source of nebulizer gas (1.5 L min^{-1}) and drying gas (curtain gas) (4.0 L min^{-1}). Ana-