Journal of Chromatography B, 951–952 (2014) 38–43

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Relative determination of the alkaloid metabolites of Er Miao San in rat urine by LC–MS/MS and its application to pharmacokinetics

Fei Yan [b], Huiwen He [b], Rui Yan [a,*]

[a] College of Pharmacy, Liaoning University, 66 Chongshan Road (M), Shenyang 110036, China
[b] China Electric Power Research Institute, Wuhan 430074, China

ARTICLE INFO

Article history:
Received 25 July 2013
Accepted 9 January 2014
Available online 20 January 2014

Keywords:
Metabolite
Determination
LC–MS/MS
Cortex Phellodendri Chinensis
Er Miao San preparation

ABSTRACT

In the present study, five metabolites of *Cortex Phellodendri Chinensis*, an important herbal drug, were identified using liquid chromatography multi-stage tandem mass spectrometric techniques (LC–MS^n). A sensitive and rapid high-performance liquid chromatographic tandem mass spectrometry (LC–MS/MS) method was developed for the quantitation of the five metabolites, utilizing chlorobenzylidine as the internal standard in rat urine. Urine samples were precipitated with acetonitrile. Chromatographic separation was achieved on a Waters C18 analytical column. Detection was performed by a multiple reaction monitoring (MRM) mode via an electrospray ionization (ESI) source operating in the positive ionization mode. The method was linear over the concentration range of 0.05–1.00c for all components. The intra- and inter-day precision values were less than 14.6% and the deviations ranged from −4.4 to 13.8%. The recoveries at three levels were more than 73.7%. The fully validated method was used to determine the metabolites amount in rat urine to investigate the changes caused by coupling with *Atractylodes lancea* in Er Miao San preparation on metabolism.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Traditional Chinese medicine (TCM) mainly uses combinations to produce new pharmacological activities through a synergistic effect or antagonistic action. Studies on the interaction between herbs are useful for probing the mechanism of TCM [1,2]. There were investigations on several pharmaceutical properties after combination, like chemical, pharmacokinetic and pharmacologic [3,4], while few reports of the changes of metabolic properties after combination, especially quantitative analysis of metabolites. Metabolite analysis is important for drug development. At present, research on metabolites is limited to qualitative analysis [5]. The complex composition of biological fluids and low contents make the isolation and purification of metabolites quite difficult. Hence, the quantification of metabolites is often impracticable. Fundamentally, kinetic research usually pays more attention to the variation in the trends of drugs, other than the absolute quantity. This paper proposes a relative quantitation method for the elimination kinetics of metabolites in urine samples without the separation of pure compounds.

Er Miao San is an ancient TCM prescription, composed of *Cortex Phellodendri Chinensis* and *Atractylodes lancea* powder (1:1, g/g). In the clinical practice of TCM, the prescription has been widely used to treat gastrointestinal disorders, inflammation, liver disorder for a long history. The protoberberine alkaloids in *Cortex Phellodendri Chinensis* like berberine and jatrorrhizine (structures shown in Fig. 1) are the main active components. Despite the extensive literature on the pharmacology of *Cortex Phellodendri Chinensis*, *A. lancea*, berberine and jatrorrhizine [6–9], there was no report about the compatibility mechanism of Er Miao San based upon the metabolite studies. In this paper, a sensitive and selective liquid chromatography-tandem mass spectrometry (LC–MS/MS) method for the determination of the metabolites of Er Miao San in rat urine was developed and applied to a comparative pharmacokinetics study of the metabolites in rat urine after oral administration of Er Miao San and *Cortex Phellodendri Chinensis*.

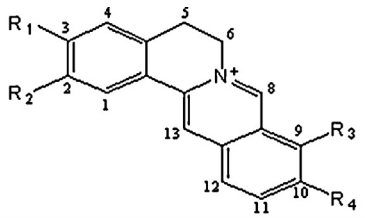
2. Experimental

2.1. Chemicals and reagents

Cortex Phellodendri Chinensis and *A. lancea* were purchased from the Sifang Pharmacy (Shenyang, China). The reagents used for LC–MS/MS measurement were of HPLC grade and were obtained from Sigma–Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade (Yuwang Reagent Company, Shandong, China). Double-distilled water was used throughout the study.

* Corresponding author. Tel.: +86 15671040801.
E-mail address: yrchn@163.com (R. Yan).

1570-0232/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jchromb.2014.01.015



Compound	$[M]^+$	R_1	R_2	R_3	R_4	N
Berberine	336	O-CH_2-O		-OCH_3	-OCH_3	=
Jatrorrhizine	338	-OH	-OCH_3	-OCH_3	-OCH_3	=
M1	402	O-CH_2-O		-OCH_3	-OSO_3H	=
M2	418	-OSO_3H	-OCH_3	-OCH_3	-OCH_3	=
M3	498	O-CH_2-O		-OCH_3	-OGlu	=
M4	514	-OGlu	-OCH_3	-OCH_3	-OCH_3	=
M5	676	-OGlu	-OGlu	-OCH_3	-OCH_3	=
IS (chlorobenzylidine)	464	O-CH_2-O		-OCH_3	-OCH_3	-CH_2-PhCl

Fig. 1. The chemical structures of metabolites and IS (chlorobenzylidine).

2.2. LC–MS/MS conditions

Qualitative analysis was performed on a ThermoFinnigan LCQ linear ion-trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA) fitted with an electrospray ionization source over the mass range from *m*/*z* 50–2000 in the positive ionization mode. Xcalibur1.2 data analysis system was used. The spray voltage was set to 4.2 kV. The capillary voltage was fixed at 13 V. The heated capillary temperature was fixed at 200 °C. Nitrogen used as the sheath and the auxiliary gas was set to 70 and 20 arbitrary units, respectively. The isolation width for MS^n was 1.0 Da. The HPLC system consists of an Agilent 1100 series equipped with an Agilent 1100 series photodiode-array detector (PDA) and autosampler Data analysis (Agilent, Palo Alto, CA). Chromatographic separation was carried out on a Waters C18 (150 mm × 4.6 mm I.D., 5 μm) with a SecurityGuard Guard Cartridge Kit (4.0 mm × 3.0 mm I.D., Phenomenex). The mobile phases consisted of 0.3% formic acid (A) and acetonitrile (B) using a gradient elution of 20% B at 0 min, 45% B at 25 min, 45% B at 40 min, at a flow rate of 0.5 mL/min. The column temperature was 30 °C, detection wavelength was at 245 nm and the injection volume was 10 μL.

For quantitative determination of the analytes, the LC–MS/MS instrument consisted of an AcQuity™ ultra-performance liquid chromatograph and a QuattroMicro API mass spectrometer (Waters, Milford, MA). The mobile phase consisted of water (containing 0.3% formic acid)/acetonitrile (30:70, v/v). The flow rate was 0.45 mL/min with an injection volume of 10 μL. Source conditions were optimized for quantitative analysis: electrospray capillary voltage 3.2 kV, source temperature 105 °C and desolvation temperature 300 °C. The cone voltage was set at 60 V. Nitrogen and argon were used as cone and collision gases, respectively. The cone and desolvation gas flows were 50 and 500 L/h, respectively. The collision gas was set at 2.40×10^{-3} mbar and the multiplier voltage was set at 650 V. The MRM transition, cone voltage and collision energy used for each of the analytes are summarized in Table 1. Total data acquisition was controlled using MassLynx™ V4.0 software with QuanLynx™ program (Waters, Milford, MA).

2.3. Preparation of calibration standards and quality control (QC) samples

An aliquot of 1 mL mixed urine 24 h after the administration of Er Miao San was added with 4 mL acetonitrile. The mixture was vortex-mixed for 2 min and centrifuged at 10,000 × *g* for 5 min.

Table 1
The constituents and metabolites in rat urine.

Analyte	MRM	Collision energy (eV)	t_R (min)
M1	402 → 322	20	3.53
M2	418 → 338	21	3.42
M3	498 → 322	20	2.61
M4	514 → 338	21	3.03
M5	676 → 500	20	2.43
IS (chlorobenzylidine)	464 → 340	22	4.07

The supernatant was separated out and blown to dryness under a gentle stream of nitrogen at 40 °C. The residue was reconstituted in 250 μl of the mobile phase as the standard stock solution of metabolites, presuming a concentration of 2c (the c means a certain but unknown concentration, denoting the quantity of the metabolites). The stock solution was then serially diluted with methanol to obtain working solutions at concentrations of 0.10c, 0.20c, 0.40c, 0.80c, 1.20c, 1.60c and 2.0c. A 100 ng/mL IS working solution was prepared by diluting a stock standard solution of chlorobenzylidine with methanol.

100 μl of the working solution was added to 200 μl of blank rat urine, vortex-mixed for 30 s. All of the spiked urine samples were then treated according to the "2.4 Sample preparation" procedure. The calibration samples ranged from 0.05c to 1.0c (equal to urine concentration) for each metabolite. QC samples were prepared in a similar manner at low, medium and high levels (0.05c, 0.20c, 0.80c). The standards and QC samples were stored at −20 °C until analysis.

2.4. Sample preparation

To 200 μL rat urine in a 1.0 mL Eppendorf tube, 50 μL of the internal standard solution (100 ng/mL), 800 μL of acetonitrile were added. This mixture was vortex-mixed 2 min and centrifuged at 4000 × *g* for 5 min. The supernatant was separated out and blown to dryness with nitrogen at 40 °C. Then the residue was reconstituted in 100 μL mobile phase and mixed to make final testing samples. A 10 μL aliquot of the final testing samples was injected onto the LC–MS/MS system for analysis.

2.5. Method validation

The method was validated according to the currently accepted USA Food and Drug Administration (FDA) bioanalytical method validation guidance.

Download English Version:

https://daneshyari.com/en/article/7617915

Download Persian Version:

https://daneshyari.com/article/7617915

Daneshyari.com