Journal of Chromatography B, 983–984 (2015) 10–17

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Simultaneous determination of six alkaloid components in rat plasma and its application to pharmacokinetic study of Danmu preparations by an ultra fast liquid chromatography–electrospray ionization-tandem mass spectrometry

Rong Yin[a,1], Jiaquan Chen[b,1], Yonggang Zhao[c], Xiaobin Jia[a], Zhiyuan Zhang[d], Liang Feng[a], Hui Wang[a,b], Jingjing Wang[a], Fenxia Zhu[a,*]

[a] Key Laboratory of New Drug Delivery System of Chinese Meteria Medica, Jiangsu Provincial Academy of Chinese Medicine, Nanjing 210028, China
[b] Department of Analytical Chemistry, China Pharmaceutical University, Nanjing 210009, China
[c] Jiangsu Environmental Monitoring Center, Nanjing 210036, China
[d] College of Pharmacy, Hainan Medical University, Haikou 570102, China

ARTICLE INFO

Article history:
Received 8 July 2014
Accepted 22 December 2014
Available online 3 January 2015

Keywords:
UFLC–MS/MS
Danmu injection
Danmu tablet
Alkaloid components
Pharmacokinetic study

ABSTRACT

Danmu injection and Danmu tablet are two widely used traditional Chinese medicine made of *Nauclea officinalis* (commonly known as Danmu), in which the alkaloids are the major active substances. In this paper, an ultra fast liquid chromatography–tandem mass spectrometry (UFLC–MS/MS) method was developed for simultaneous determination and the pharmacokinetic characteristics study of six main active alkaloids (naucleamide A-10-O-β-D-glucopyranosid, naucleamide G, pumiloside, 3-epi-pumiloside, strictosamide and vincosamide) of the two above-mentioned Danmu preparations in rat plasma. In the course of the experiment, following sample preparation by protein precipitation with methanol–ethyl acetate (2:1, v/v), the nitrogen-dried extraction was reconstituted in methanol and assayed on a C18 column using a gradient elution program with mobile phase consisting of acetonitrile and water containing 0.1% formic acid. The MS detection was performed in positive ionization mode with selected ion transitions. The established method was fully validated and proved to be sensitive and specific with lower limits of quantification (LLOQs) all less than 0.32 ng/mL in rat plasma and matrix effects ranged from 88.87 to 108.27%. Good linearities of six alkaloids were obtained in respective concentration ranges ($r^2 > 0.995$). The average extract recoveries for each compound at three quality control concentration levels were no less than 79.70%, and the precision and accuracy were within the acceptable limits. The validated method was successfully applied to the pharmacokinetic study of six alkaloid components of Danmu injection and tablet in rat plasma. The obtained results may be helpful to reveal the action mechanism and guide the clinical application of Danmu preparations.

1. Introduction

Nauclea officinalis, also known as Danmu in Chinese, is the dried stem bark of *Nauclea oftcinalis Pierrc ex Pitard* and has been used effectively to treat cold, fever, sore throat, pink eyes and so on for many years [1]. Modern pharmacological studies have shown that it has antibacterial, antimalarial and anti-inflammatory effects thanks to the possible enhancement of lysozyme production and inhibition of prostaglandin E_2 release [2–5]. Up to now, two kinds of drug dosage forms made of *Nauclea officinalis*, namely Danmu injection and Danmu tablet, have been approved for clinical application in China. They are applied to defense against diseases of acute tonsillitis, acute pharyngitis and acute conjunctivitis as well as upper respiratory tract infection [6,7].

Phytochemical investigations have found that *Nauclea officinalis* and its preparations contain many constituents such as alkaloids, triterpenoids, phenol acids, iridoids and flavonoids [6,8–14]. Among them, alkaloids, the most abundant components, have been proved to be the major active substances [6,12–14,11]. There are

* Corresponding author at: Key Laboratory of New Drug Delivery System of Chinese Meteria Medica, Jiangsu Provincial Academy of Chinese Medicine, Nanjing 210028, Jiangsu Province, China. Tel.: +86 25 85608672; fax: +86 25 85637809.
E-mail address: sunshinezhu@yeah.net (F. Zhu).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jchromb.2014.12.026
1570-0232/

some papers published concerning determination of chemical constituents in *Nauclea officinalis* and its preparations. Most of these studies are performed by determination of the single compound of strictosamide by high performance liquid chromatography (HPLC) [7,15]; only one paper reporting simultaneously determined five alkaloids in *Nauclea officinalis* leaves by HPLC [16]. Recently, in our laboratory, twelve constituents including four phenol acid, one iridoid and seven alkaloids were simultaneously determined in a rapid single-run process by ultra performance liquid chromatography with photodiode array (UPLC-PDA) method for the quality control of Danmu injection and Danmu tablet [8], and the results showed that the contents of six alkaloids (naucleamide A-10-O-β-D-glucopyranosid, naucleamide G, pumiloside, 3-epi-pumiloside, strictosamide and vincosamide) are relatively higher.

According to the literatures [6,12–14,11], all the six alkaloids mentioned-above are responsible for the various activities and there is a real need to evaluate the pharmacokinetic parameters for each component. However, there are just two papers concerning the pharmacokinetic study of single component (strictosamide) individually so far [17,18]. This is not enough to describe the complicated synergistic interactions and the kinetic behaviors of Danmu preparations, thus, to reveal the action mechanism and guide the clinical application, it is necessary to study their pharmacokinetic properties in experimental animals first and evaluate the results of relative bioavailability.

At present, an ultra fast liquid chromatography (UFLC) technology is developed and widely used because of its better separation capacity and column efficiency than classical liquid chromatography; while tandem mass spectrometry is the current technology for the quantification of drugs in biological fluids for its high sensitivity and selectivity. Therefore, in this paper, a rapid and sensitive UFLC–MS/MS method for simultaneous determination of naucleamide A-10-O-β-D-glucopyranosid, naucleamide G, pumiloside, 3-epi-pumiloside, strictosamide and vincosamide in rat plasma was established. The established method was fully validated and proved to be sensitive and specific with lower limits of quantification, and successfully applied to the pharmacokinetic study of six alkaloids after intramuscular and oral administration of Danmu injection and Danmu tablet respectively.

2. Experimental

2.1. Chemicals and reagents

Six alkaloid standards, naucleamide A-10-O-β-D-glucopyranosid, naucleamide G, pumiloside, 3-epi-pumiloside, strictosamide and vincosamide, were isolated from *Nauclea officinalis* in our laboratory, and their structures were confirmed by analysis data of MS, ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) [10]. The purities of six alkaloid standards were over 98%, and their chemical structures are shown in Fig. 1. Diazepam (17122-200302) used as the internal standard (IS) in this study was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Chromatographic-grade methanol, acetonitrile and formic acid were purchased from Merck (Merck KGaA, Darmstadt, Germany). All the other reagents were of analytical grade. Demonized water prepared by Milli-Q Water Purification System (Millipore, Billerica, USA) was used throughout the experiment. Danmu injections (110616) and Danmu tablets (110702) were provided by Hainan Pharmaceutical Factory, Co., Ltd. (Wuzhishan, China) [8].

2.2. Liquid chromatography

The liquid chromatographic separation was carried out by a SHIMADZU ultra fast liquid chromatography (UFLC) XR system (Shimadzu, Kyoto, Japan) consisting of an LC-20ADXR binary pump, a SIL-20AXR auto-sampler, a CTO-20AC column oven and a DGU-20A3 degasser. Six alkaloids and IS were separated on an Agilent Eclipse Plus C_{18} column (2.1 mm × 10 mm, 3.5 μm, Santa Clara, USA), and eluted with a gradient mobile phase composed of water with 0.1% formic acid (A) and acetonitrile (B) at a constant flow rate of 0.4 mL/min. The gradient elution program was developed as follows: in 0–3 min, solvent B linearly increased from 15 to 40%, subsequently it linearly increased to 100% by 1 min and hold on 100% for another 1.5 min before returning to the initial ratio. The temperature of auto-sampler was 10 °C and temperature of the column was 30 °C, the injection volume was 5 μL.

2.3. Mass spectrometer

UFLC system was equipped an AB Sciex QTRAP 5500 mass spectrometer (AB SCIEX, Fleming Han, USA) as a detector. The mass spectrometer was performed using electrospray ionization source in positive mode. The curtain gas, nebulizer gas (Gas 1) and auxiliary gas (Gas 2) was set at 40 psi, 55 psi and 55 psi, respectively. Spray voltage was 5.5 kV and ion source temperature was 550 °C. Collision gas was at medium flow. Compounds parameters were optimized as follows: declustering potential (DP), collision energy (CE), and collision exit potential (CXP) were 151, 23 and 28 V for naucleamide A-10-O-β-D-glucopyranoside, naucleamide G, strictosamide and vincosamide, 161, 27 and 24 V for pumiloside and 3-epi-pumiloside, and 71, 35 and 16 V for IS, respectively. Entrance potential (EP) was set at 10 V for all the chemicals. All the samples were detected in the multiple reaction monitoring (MRM) modes with a dwell time of 100 ms. The precursor ions and product ions were monitored at m/z 519.0 → 357.0 for naucleamide A-10-O-β-D-glucopyranoside, 489.0 → 327.0 for naucleamide G, 512.8 → 351.1 for pumiloside and 3-epi-pumiloside, 499.1 → 337.2 for strictosamide and vincosamide, and 285.1 → 154.1 for IS, respectively. Nitrogen with high purity was used to assist the detections. Data acquisition and processing were accomplished by Analyst software (AB SCIEX Version 1.5.2, Fleming Han, USA).

2.4. Preparation of standard and quality control (QC) samples

The appropriate amounts of above six standards and IS were separately accurately weighted and dissolved by methanol in amber colored volumetric flasks to obtain stock solutions. The six stock solutions were mixed and diluted with methanol to prepare a primary mixed stock solution containing 1140 ng/mL for naucleamide A-10-O-β-D-glucopyranoside, 255 ng/mL for naucleamide G, 5200 ng/mL for pumiloside, 1020 ng/mL for 3-epi-pumiloside, 10250 ng/mL for strictosamide and 4100 ng/mL for vincosamide, respectively. A series of mixed standard working solutions were freshly prepared by diluting the primary mixed stock solution with methanol at appropriate ratios, achieving concentrations of 2.80–1140 ng/mL for naucleamide A-10-O-β-D-glucopyranoside, 3.20–255 ng/mL for naucleamide G, 2.60–5200 ng/mL for pumiloside, 2.60–1020 ng/mL for 3-epi-pumiloside, 2.10–10,250 ng/mL for strictosamide and 2.10–4100 ng/mL for vincosamide, respectively. The stock solution of IS with concentration of 208,000 ng/mL was diluted 400-fold to obtain the working solution. All the solutions were kept away from light at 4 °C.

The plasma samples of standard calibration curve were prepared by spiking 20 μL of each mixed standard working solution into 180 μL blank rat plasma, achieving a series of concentrations of standard samples. Quality control samples were prepared by independently diluting the stock solutions at 3 concentrations (low, middle and high) for each analyte, and adding to blank plasma in the same way as plasma samples of standard calibration curve. The final concentrations were 0.57, 22.80, 91.20 ng/mL for naucleamide