Journal of Pharmaceutical and Biomedical Analysis 49 (2009) 487–491

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Short communication

Determination of strychnine and brucine in rat plasma using liquid chromatography electrospray ionization mass spectrometry

Yanyan Xu[a,b], Duanyun Si[b], Changxiao Liu[b,*]

[a] School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China
[b] Tianjin State Key Laboratory of Pharmacokinetics and Pharmacodynamics, Tianjin Institute of Pharmaceutical Research, Tianjin 300193, China

ARTICLE INFO

Article history:
Received 10 August 2008
Received in revised form 14 October 2008
Accepted 19 October 2008
Available online 28 October 2008

Keywords:
Strychnine
Brucine
Semen Strychni
LC–ESI-MS
Rat plasma

ABSTRACT

A simple, sensitive and selective liquid chromatography–electrospray mass spectrometric (LC–ESI-MS) method was developed and validated for simultaneous determination of strychnine and brucine in rat plasma, using tacrine as the internal standard (IS). Sample preparation involved a liquid–liquid extraction of the analytes with *n*-hexane, dichloromethane and isopropanol (65:30:5, v/v/v) from 0.1 mL of plasma. Chromatographic separation was carried out on a Waters C_{18} column using a mobile phase of methanol–20 mM ammonium formate–formic acid (32:68:0.68, v/v/v). Positive selected ion monitoring mode was used for detection of strychnine, brucine and the IS at *m*/*z* 335.2, *m*/*z* 395.2 and *m*/*z* 199.2, respectively. Linearity was obtained over the concentration range of 0.5–500 ng/mL for strychnine and 0.1–100 ng/mL for brucine. The lower limit of quantification was 0.5 ng/mL and 0.1 ng/mL for strychnine and brucine, respectively. The intra- and inter-day precision for both strychnine and brucine was less than 7.74%, and accuracy ranged from −4.38% to 2.21% at all QC levels. The method has been successfully applied to a pharmacokinetic study of processed Semen Strychni after oral administration to rats.

1. Introduction

Semen Strychni, officially listed in Chinese Pharmacopoeia, is the dried mature seed of *Strychnos nux vomica* L., a tree native in India. In classic Chinese Materia Medicas, the herbal drug is listed as a toxic herb and its biological effects (pharmacological and toxic effects) are dose-dependent, needing processed to reduce the toxicity before clinical uses required by Chinese medicine prescriptions. Nowadays, processed Semen Strychni is clinically used as an important ingredient in various remedies of traditional herbal medicines to treat nervous diseases, vomiting, arthritic, traumatic pains [1] and to promote blood circulation and remove blood stasis [2]. The main bioactive components of Semen Strychni are alkaloids, and 16 of them have been isolated and identified by now [3]. The most abundant alkaloids existing in the processed Semen Strychni are strychnine and brucine (Fig. 1) [2], which have been reported to possess analgesic, anti-inflammatory and anti-tumor effects [4,5], despite their toxicity in nature [6,7].

Several analytical methods for quantitative determination of each or both of the two strychnos alkaloids have been described, including HPLC with UV detection [8,9], TLC [10], fluorescence spectrophotometric method [11], ^{1}H NMR [12], GC–MS [13], LC–MS [14] and LC–MS/MS [15], etc. However, few reports are available regarding simultaneous determination of strychnine and brucine in biological matrix for pharmacokinetic studies of Semen Strychni. The published HPLC–UV method [8] was developed for simultaneous determination of four strychnos alkaloids including strychnine and brucine in rat plasma after i.v. administration, with a LLOQ of 250 ng/mL for both of strychnine and brucine. The method was not sensitive enough for pharmacokinetic studies of strychnine and brucine after orally administered low dosage of processed Semen Strychni, due to the low levels of both alkaloids in rat plasma. Besides, the method required time-consuming sample preparation procedure, using 1 mL of plasma sample. To investigate the ADME properties of processed Semen Strychni following oral administration, a more simple and sensitive bioanalytical method is required for simultaneous determination of strychnine and brucine.

In the present study a simple, sensitive and selective liquid chromatography–electrospray mass spectrometric (LC–ESI-MS) method for simultaneous determination of strychnine and brucine in rat plasma was established. The method has been successfully applied to a pharmacokinetic study after oral administration of processed Semen Strychni to Wistar rats.

* Corresponding author. Tel.: +86 22 23006863; fax: +86 22 23006283.
E-mail address: tjpks@163.com (C. Liu).

0731-7085/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2008.10.020

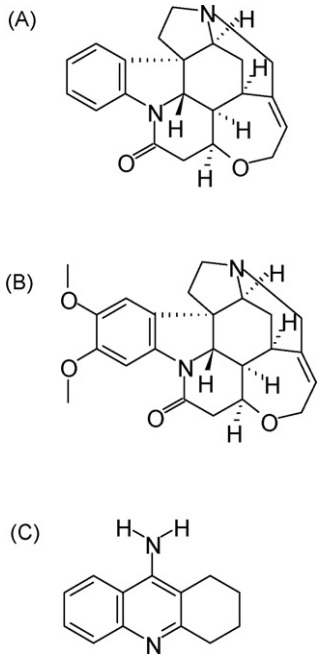

Fig. 1. Chemical structures of strychnine (A), brucine (B) and tacrine (C, internal standard).

2. Experimental

2.1. Chemicals and reagents

Reference substances of strychnine, brucine and tacrine (IS, Fig. 1) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Processed Semen Strychni, namely Semen Strychni pulveratum, was kindly provided by Tianjin Darentang Jingwanhong Pharmaceutical Co., Ltd. (Tianjin, China). HPLC grade of methanol, formic acid (88%) and isopropanol were from Tianjin Concord Tech Reagent Co., Ltd. (Tianjin, China). Ammonium formate and other reagents were of analytical grade. Distilled water, prepared from demineralized water, was used throughout the experiment.

2.2. Instrumentation and LC–MS conditions

The LC–MS system consists of a Surveyor autosampler, a Surveyor LC pump, a TSQ Quantum Discovery Max^{TM} triple–quadrupole mass spectrometer and Xcalibur 1.4 software (Thermo Finnigan, USA). A Waters Symmetry^{TM} C_{18} column (100 mm × 4.6 mm I.D., 5 μm, Waters, USA) kept at 30 °C was used for chromatographic separation. The mobile phase was composed of methanol, 20 mM ammonium formate and formic acid (32:68:0.68, v/v/v) at a flow rate of 0.4 mL/min.

Electrospray ionization (ESI) source in positive mode was used for mass spectrometric detection. Mass spectrometric conditions were optimized to achieve the maximum sensitivity. The ionspray voltage was set at 4000 V. The sheath and auxiliary gas was nitrogen, with the pressure and flow rate of 35 psi and 5 L/min, respectively. The heated capillary temperature was 300 °C. The extra energy of 10 V was added for source collision-induced dissociation (CID). Selected ion monitoring (SIM) mode was used for the quantification at m/z 335.2 for strychnine, m/z 395.2 for brucine and m/z 199.2 for tacrine with a dwell time of 0.2 s.

2.3. Calibration standard and quality control preparation

Stock solutions of strychnine and brucine were prepared in methanol at concentrations of 1 mg/mL and 100 μg/mL. Both stock solutions were diluted with methanol to get a combined standard working solution of 500 ng/mL strychnine and 100 ng/mL brucine. Then the combined standard working solution was further diluted with methanol to provide a series of standard working solutions of desired concentrations. The internal standard was prepared in methanol at a concentration of 100 μg/mL and was further diluted to 50 ng/mL as a working solution. All solutions were stored at −20 °C.

The calibration standards were prepared by spiking blank plasma (0.1 mL) with appropriate amounts of working solutions to yield final concentrations of 0.5, 1, 5, 25, 50, 250 and 500 ng/mL for strychine, and 0.1, 0.2, 1, 5, 10, 50 and 100 ng/mL for brucine, respectively. Combined quality control (QC) samples were prepared at low, medium and high concentration levels of 1, 25 and 250 ng/mL for strychine, and 0.2, 5 and 50 ng/mL for brucine, respectively. The spiked samples were then treated following the sample preparation procedure indicated in Section 2.4.

2.4. Sample preparation

To an aliquot (0.1 mL) of plasma sample, 100 μL of internal standard (50 ng/mL), 100 μL of methanol (or standard solutions for calibration curve or QC samples) and 500 μL of saturated Na_2CO_3 were added. The mixture was vortexed for 30 s and was extracted with 2 mL of *n*-hexane–dichloromethane–isopropanol (65:30:5, v/v/v) by thoroughly vortexing for 3 min. After centrifugation (3000 × *g*) for 5 min, the upper organic layer was taken and evaporated to dryness under a mild stream of N_2 at 45 °C. The residue was reconstituted in 100 μL of mobile phase. After centrifugation (12,000 × *g*) for 3 min, an aliquot of 10 μL was injected for LC–MS analysis.

2.5. Method validation

Selectivity was tested by comparison of blank plasma from six individual rats with corresponding spiked plasma samples. Calibration curves were obtained by plotting the peak–area ratio (y) of the analyte/internal standard against the spiked concentrations (x) of either strychnine or brucine, using a weighted (1/square of concentration) linear regression. Linearity of calibration was tested by analysis of three sets of calibration standards each day for three consecutive days. Deviations of the mean calculated concentrations were set at ±15% of nominal concentrations, except for the lower limit of quantification (LLOQ) where a deviation of ±20% was permitted.

Precision was expressed as the relative standard deviation (R.S.D.) and accuracy was calculated as the relative error (R.E.). QC samples at low, medium and high concentrations in six replicates were analyzed during the same day using the same calibration curve to determine the intra-day precision. Three batches of QC samples were analyzed on three consecutive days to evaluate the inter-day precision and accuracy.

Both of recovery and matrix effect were tested using triplicate of spiked samples at three QC levels. The absolute recoveries were determined by comparing the peak areas of extracted plasma samples with those of standard working solutions at equivalent concentration. The matrix effects were investigated by comparing the peak areas of post-extracted blank plasma spiked with working solutions of strychnine and brucine with those of corresponding standard solutions. The same procedure was performed for IS.