Journal of Chromatography B, 879 (2011) 2714–2719

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Ultra-performance liquid chromatography–tandem mass spectrometric method for the determination of strychnine and brucine in mice plasma

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ARTICLE INFO

Article history:
Received 4 May 2011
Accepted 25 July 2011
Available online 30 July 2011

Keywords:
Semen Strychni
Strychnine
Brucine
UPLC–MS/MS

ABSTRACT

A selective, simple and efficient method-ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) was developed for determination of two toxic alkaloids, namely strychnine and brucine in mice plasma. The UPLC separation was carried out using a 1.7 μm BEH C_{18} column (50 mm × 2.1 mm) with a mobile phase consisting of methanol:0.1% formic acid (25:75, v/v), hence providing high efficiency, high resolution and excellent peak shape for the analytes and internal standard. The method was validated over the range of 2.48–496.4 ng/ml for strychnine and 2.64–528 ng/ml for brucine, respectively. Intra- and inter-day accuracy ranged from 95.0% to 107.9% for strychnine, 93.4% to 103.3% for brucine, and the precisions were within 13.8%. The extraction recoveries of both the two alkaloids exceed 81.9%. With a simple and minor sample preparation procedure and short run-time (<3 min), the proposed method was applicable for the pharmacokinetic and toxicological analysis of strychnine and brucine *in vivo*.

1. Introduction

Semen Strychni, the seed of *Strychnos nux-vomica L.* (Loganiaceae), is an important medicinal plant in Asia. It has been effectively used in Chinese folk medicine to alleviate inflammation, joint pains, allergic symptoms and treat nervous diseases [1]. However, for extremely toxic the seeds are forbidden to use directly and must be processed. Phytochemical analysis has revealed that alkaloids are the main bioactive ingredients in this species. Strychnine (Str) and brucine (Bru) (Fig. 1), the most abundant alkaloids in Semen Strychni, have important effects on the pharmacological and toxic properties [1]. On the one side, Str and Bru possess analgesic, anti-inflammatory and anti-tumor effects [2,3]; on the other side, through inhibiting inhibitory neurotransmitter, the toxic alkaloids can result in motor disturbance, increase muscle tone, hyperactivity of sensory, while high dose can induce convulsions of the central nervous system and finally death through respiratory or spinal paralysis or by cardiac arrest [3]. Several cases of Str poisoning have been reported [4–6]. The 50% lethal dose (LD_{50}) of Str in mice is reported to be 3.27 mg/kg (i.g.) and 1.53 mg/kg (i.p.), LD_{50} of Bru for mice is 233 mg/kg (i.g.) and 69 mg/kg (i.p.) [7]. In humans, the reported LD of orally ingested Str ranges from 50 to 100 mg/kg [8]. Thus, the determination of the two alkaloids is extremely important in toxicological and forensic analysis.

In recent years, many methods have been proposed for determination of Str and Bru in biological matrix, for example, CE [9], GC–MS [10–12], HPLC-UV [5,6,13], HPLC–MS [14] and LC–MS/MS [15,16]. However, some shortcomings exist in these methods such as long analytical time, complex procedures, low selectivity and sensitivity. The published LC–MS/MS method [15] is developed for screening many kinds of toxical alkaloids but lacked of applications to practical samples. In this work, a fast and new UPLC–MS/MS method is developed for simultaneous determination of Str and Bru in mice plasma.

Recently, ultra-performance LC (UPLC) has introduced and quickly adopted in quantitative analysis of biological matrix. The van Deemter equation indicates that, as the particle size decreases to less than 2.5 μm, there is a significant improvement in efficiency that will not reduce with increased LC flow rates. Compared with conventional HPLC columns, UPLC, by utilizing 1.7 μm particle, greatly increased the separation throughput and efficiency, resulting in LC peaks as narrow as or less than 2 s [17]. By using multiple reaction monitoring (MRM) as the MS detection, UPLC–MS/MS method can offer a more sensitive and selective detection, thus it is suitable for the research of pharmacokinetics and metabolic kinetics under toxical dose for strychnine and brucine.

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doi:10.1016/j.jchromb.2011.07.033

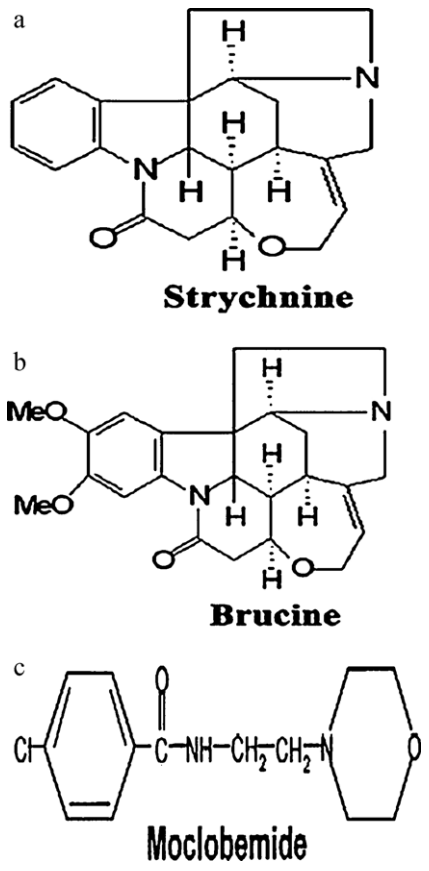

Fig. 1. Chemical structures of Str, Bru and moclobemide (IS).

2. Experimental

2.1. Chemical reagents and animals

Reference standards of Str, Bru and moclobemide (internal standard, IS) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China); HPLC grade methanol was purchased from Merck (Darmstadt, Germany); HPLC grade formic acid and tert-butyl methyl ether were purchased from Tedia (Fairfield, OH, USA); other chemicals were all of analytical grade; water was purified by redistillation and filtered through 0.22 μm membrane filter before using. Processed Semen Strychni was kindly provided by Baijia Pharmaceutical Company (Changsha, China). Male Kunming mice (Certificate No. SCXK-Xiang-2009-0004) were provided by the Slac experimental animal corporation (Shanghai, China). The study was approved by the Animal Ethics Committee of the Central South University (Approval No. 2009-0219).

2.2. Standard and working solutions

Individual standard stock solutions of Str (496.4 μg/ml), Bru (105.6 μg/ml) and IS (220.8 μg/ml) were prepared by accurately weighting the required amounts into separate volumetric flasks and dissolving in methanol. Further dilutions were made from these stocks to obtain a series of standard working solutions: Str (4.96, 0.496, 0.0496 μg/ml) and Bru (5.280, 0.528, 0.0528 μg/ml). IS stock solution was further diluted with methanol to prepare the working solution containing 44.0 ng/ml of moclobemide. The stock solutions and working solutions were all stored at 4 °C until use.

2.3. Calibration standards and quality control samples

The calibration standards were prepared by spiking blank plasma (1.0 ml) with appropriate amounts of above working solutions to yield final concentrations of 2.48–496.4 ng/ml for Str and 2.64–528 ng/ml for Bru. Quality control (QC) samples at low, middle and high concentrations of Str (4.96, 37.23 and 397.1 ng/ml), Bru (5.28, 39.6 and 422.4 ng/ml) were prepared with mice plasma for the determination of inter-day, intra-day accuracy and precision, room temperature and freeze–thaw stability.

2.4. Sample preparation

Plasma samples were kept in plastic vials at −70 °C until analysis. 100 μl of plasma sample, 20 μl of NaOH (0.1 mol/l) and 50 μl of IS solution (44.0 ng/ml) were added to a vitric tubu. The mixture was vortexed for 30 s, then extracted with 2 ml tert-butyl methyl ether by thoroughly vortexed for 2 min, followed by centrifugation at 3000 rpm for 5 min. The upper organic layer was transferred to another vitric tube and dried under a mild stream of N_2 at 38 °C. The residue was reconstituted in 100 μl mobile phase. A 2 μl aliquot of each supernatant was injected into the UPLC–MS/MS system for analysis.

2.5. Liquid chromatography

Liquid chromatography was performed on ACQUITY UPLC system (Waters, Milford, MA, USA) with autosampler and column oven. Chromatographic separations were performed on a Waters Acquity UPLC™ BEH C_{18} column (50 mm × 2.1 mm, i.d., 1.7 μm particle size). The column temperature was maintained at 40 °C. The mobile phase consisted of methanol:0.1% formic acid (25:75, v/v). The flow rate was 0.25 ml/min, the injection volume was 2 μl, and the duration of the run was 3 min.

2.6. Mass spectrometry

The MS instrument consisted of a Waters Micromass Quattro Micro™ triple-quadrupole system (Manchester, UK). Ionization was performed in the ESI positive mode. Quantification was performed using multiple reaction monitoring (MRM) mode. The transitions of m/z 335 → 184, m/z 395 → 324 and m/z 269 → 182 were selected for the quantification of Str, Bru and IS, respectively. The MS spectra and MS/MS spectra for Str, Bru and IS were shown in Fig. 2. The optimized ionization conditions were as follows: capillary voltage 4.0 kV, cone voltage 45 kV, source temperature 120 °C and desolvation temperature 400 °C. Nitrogen was used as desolvation and cone gas with the flow rate at 750 and 50 l/h, respectively. Argon was used as the collision gas at a flow rate of 0.15 ml/min. The collision energies for Str, Bru and IS were 35 eV, 32 eV and 18 eV, respectively. The dwell time was 0.05 s. Data acquisition was carried out by MassLynx 4.1 software.

2.7. Method validation

For quantitative bio-analytical procedures, the following essential parameters should be evaluated: selectivity, sensitivity, accuracy, precision, recovery and stability [18]. Matrix effects were evaluated as well.

Selectivity was assessed by comparing chromatograms of blank plasma obtained from mice without dosing with those of blank plasma spiked with Str, Bru, IS and plasma samples after administration of Semen Strychni. To evaluate linearity, calibration standards in plasma at seven concentration levels ranged from 2.48 to 496.4 ng/ml for Str and 2.64 to 528 ng/ml for Bru were prepared. Calibration curves in plasma were generated by plotting the peak