Journal of Chromatography B, 903 (2012) 46–52

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

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

Simultaneous determination of blonanserin and its metabolite in human plasma and urine by liquid chromatography–tandem mass spectrometry: Application to a pharmacokinetic study

Yu-Guan Wen [a,*], Xiao-Jia Ni [a], Ming Zhang [a], Xia Liu [a], De-wei Shang [a,b]

[a] Department of Pharmacy, Guangzhou Brain Hospital, Guangzhou Medical University, 36 MingXin Road, Guangzhou 510370, China
[b] Laboratory of Clinical Psychopharmacology, Beijing Anding Hospital, Capital Medical University, 5 Ankang Lane, Dewai Avenue, Xicheng District, Beijing 100088, China

ARTICLE INFO

Article history:
Received 18 March 2012
Accepted 28 June 2012
Available online 4 July 2012

Keywords:
Blonanserin
Metabolite
HPLC–MS/MS
Human plasma
Pharmacokinetics

ABSTRACT

Blonanserin is a novel atypical antipsychotic with highly selective receptor antagonist activity to dopamine D_2 and 5-HT_{2A}. *N*-desethyl blonanserin (blonanserin C) is its major active metabolite in human plasma. Herein we report a new highly sensitive, selective, and rapid liquid chromatography–tandem mass spectrometry method to determine blonanserin and blonanserin C simultaneously in human plasma and urine, with *N*-desethyl-chlor-blonanserin (blonanserin D) as internal standard (IS). Blonanserin and blonanserin C were extracted from aliquots of plasma and urine with ethyl acetate and dichloromethane (4:1) as the solvent and chromatographic separation was performed using an Agilent Eclipse Plus C_{18} column. The mobile phase was composed of: acetonitrile and ammonium formate–formic acid buffer containing 5 mM ammonium formate and 0.1% formic acid (87:13, v/v). To quantify blonanserin, blonanserin C, and blonanserin D, respectively, multiple reaction monitoring (MRM) transition of m/z 368.2 → 297.2, m/z 340.2 → 297.1, and m/z 356.2 → 313.3 was performed in positive mode. The analysis time was about 5.5 min. The calibration curve was linear in the concentration range of 0.01–2 ng/ml. The lower limit of quantification reached 0.01 ng/ml. The intra and inter-day precision and relative errors were less than 8.0% and 6.6% for three QC levels in plasma and urine. The current LC–MS/MS method was validated as simple, sensitive, and accurate and has been successfully applied to investigate the pharmacokinetics of blonanserin and blonanserin C in humans.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Blonanserin (Fig. 1(A)), first developed by Dainippon Sumitomo Pharma Company in Japan in 2008, is a novel atypical antipsychotic that is validated for the treatment of both positive and negative symptoms of schizophrenia, without extra-pyramidal symptoms [1–6]. It has a high affinity with dopamine D_2 and 5-HT_{2A}, while being much less potent in adrenergic-α_1, histamine H_1, and muscarinic M_1 antagonist activities [4]. Its major metabolite N-deethyl blonanserin (blonanserin C) with D_3 receptors plays a role in reducing the EPS of blonanserin which contributes at least partly to the atypical nature of blonanserin with low EPS liability [5]. So blonanserin is well tolerated and such a favorable pharmacological profile makes it as potent as other atypical antipsychotics [6,7].

Pharmacokinetic data for blonanserin are limited and available from the manufacturer's prescribing information [8] or unpublished sources [9] presented by Deeks [2]. Blonanserin is rapidly absorbed orally and extensively metabolized. It is metabolized mainly by cytochrome P450 3A4, according to data from an in vitro study [8,9]. Many metabolites (deethyl form, N-oxide form, 7- and 8-hydroxylated form) were identified and estimated in vivo and vitro [8–13]. N-deethyl blonanserin (blonanserin C, Fig. 1(B)) has the highest neurotransmitter binding affinity in vitro, but it merely displays pharmaco-logical activity several-fold lower than that of the parent drug in vivo animal models [9]. Moreover, affinities of blonanserin C to the D_2 and anti-5-HT_{2A} receptors that are approximately one-tenth and one-half of blonanserin [13].

With the development of clinical use and clinical trials on blonanserin throughout the world, it is essential to establish an accurate, sensitive, and simple method for the quantification of blonanserin and its metabolite concentration in plasma and other biological matrices. A few methods have been reported for the determination of blonanserin in human plasma [10,14–16]. High-performance liquid chromatography (HPLC) with fluorescence

* Corresponding author.
E-mail address: wen.yuguan@yahoo.cn (Y.-G. Wen).

1570-0232/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jchromb.2012.06.037

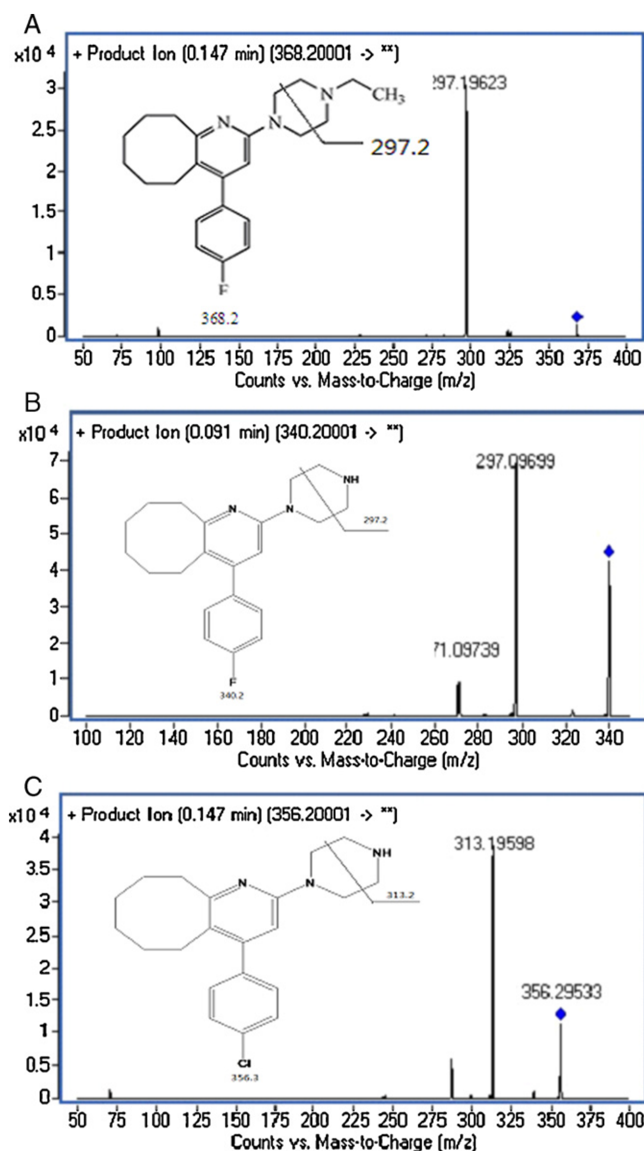

Fig. 1. Mass spectra and chemical structures for: (A) blonanserin, (B) blonanserin C, and (C) blonanserin D (IS), respectively.

detection has been reported for the determination of blonanserin and *N*-desethyl blonanserin (blonanserin C) in human plasma [10], but a complicated and expensive solid-phase extraction was used with a lower limit of quantification (LLOQ) of 0.04 ng/ml. The detection limit of the GC/MS method is only about 0.25 ng/ml [14]. A sensitive method for analysis of blonanserin in human plasma by HPLC–MS/MS using $[^2H_5]$-blonanserin as internal standard (IS) and ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) using *N*-butyl-blonanserin as IS had been developed [15,16], but involved a complicated solid-phase extraction and the metabolite was not determined.

Herein we have developed a novel, simple, and sensitive HPLC–MS/MS method for the simultaneous determination of blonanserin and its major metabolite blonanserin C in human plasmas and urine. Liquid–liquid extraction was used in sample processing and a new and similar chemical structure *N*-desethyl-chlor-blonanserin (blonanserin D, Fig. 1(C)) was synthesized as IS. It has been successfully applied to a pharmacokinetic study in healthy Chinese volunteers.

2. Materials and methods

2.1. Chemicals and reagents

Blonanserin tablets (No: 20110201, 4 mg/tablet) were manufactured by the Lizhu Pharmaceutical Limited Company (Zhuhai, China). Standard reference (purity >99.5%) of blonanserin [No: 20110105], blonanserin C [No: 20110105], and blonanserin D (IS) [No: 20110105] were identified and also supplied by the Lizhu Laboratories Limited Company. Acetonitrile was chromatographic pure grade and purchased from BurDick&Jackson. Formic acid, ethyl acetate, and dichloromethane were chromatographic pure grade and purchased from the Dikma Company (USA). Ammonium formate was mass spectrum pure grade and purchased from the Sigma Company (USA). Sodium hydrogen carbonate was analytical grade from Guangzhou Chemical Agent Factory (China). Deionized water was distilled before using. Deionized water was acquired by a Milli-Q academic reagent grade water purification, Millipore corporation (USA).

2.2. Apparatus

Chromatographic analysis was performed using an Agilent 1100 series LC system (Agilent Technologies, Inc., USA) consisting of a quaternary pump, an autosampler, a column oven, and a degasser. The analytes and IS were detected using an Agilent 6410 triple quadrupole mass spectrometer (Agilent Technologies, Inc., USA) with electrospray ionization. Ultra high pure (UHP) nitrogen was used as the drying gas. An Agilent MassHunter Chemstation (B.01.03) was used to process the raw data. A Salvis Lab vacuo drying apparatus (Vacucenter VC20, CH-6343 Rotkreuz, Switzerland) was used to evaporate the supernatant extracted from plasma or urine samples. The pharmacokinetic parameters were calculated by DAS 2.1.1 software (Mathematical Pharmacology Professional Committee of China).

2.3. LC–ESI-MS/MS

Chromatographic separation was carried out at 40 °C on an Agilent Eclipse Plus-C_{18} (4.6 mm × 150 mm, 3.5 μm). The mobile phase consisted of acetonitrile –5 mmol/l ammonium formate (0.1% formic acid) aqueous solution (87:13, v/v) with a flow rate of 0.5 ml/min. The injection volume was 4 μl.

The mass spectrometer was operated in a positive ion multiple reaction monitoring (MRM) mode. The optimized condition consisted of a nebulizer of 50 psi, a dry gas of 10 l/min at 350 °C, a HV capillary of 4000 V and a split voltage of 145 V. The impact energy of blonanserin was 27 units, with 30 units for both blonanserin C and blonanserin D. Optimized reaction monitoring of the precursor–product ion transitions were m/z 368.2 → 297.2 for blonanserin, m/z 340.2 → 297.1 for blonanserin C, and m/z 356.2 → 313.3 for blonanserin D.

2.4. Preparation of stock solutions calibration curves and quality control (QC) samples

Stock solution of blonanserin, blonanserin C, and blonanserin D were prepared separately at a concentration of 1 mg/ml by dissolving the accurately weighed reference substance in the mobile phase. The stock solution was then serially diluted with the mobile phase to give working solutions at the following concentrations: 0.1, 0.2, 1, 5, 10, 15, and 20 ng/ml. Internal standard working solutions were diluted to 10 ng/ml. We added 50 μl working solutions and 50 μl internal standard solutions into 0.5 ml black plasma or urine to obtain the final concentrations at 0.01, 0.02, 0.1, 0.5, 1, 1.5, and 2 ng/ml for blonanserin and blonanserin C with IS 1 ng/ml.

Quality control samples from plasma or urine were prepared at 0.02, 0.5, and 1.5 ng/ml for blonanserin and blonanserin C. All the solutions were kept at 2 °C and were placed at room temperature before use.

2.5. Sample preparation

All plasma or urine samples were extracted employing a liquid–liquid extraction technique. To each tube containing 0.5 ml of plasma or urine, 50 μl of internal standard, 200 μl of saturated sodium hydrogen carbonate, and 3 ml of ethyl acetate–dichloromethane (4:1, v/v) were added and then vortexed for 2 min. Samples were then centrifuged at 3000 r/min for 5 min. The upper organic phase was transferred into a 5-ml glass centrifuge tube and evaporated under vacuum drying at 42 °C. Samples were reconstituted with 200 μl of mobile phase, of which 4 μl were analyzed.

2.6. Method validation

2.6.1. Selectivity

The selectivity of the method was tested with blank human plasma or urine from six healthy volunteers. Lack of interfering peaks at the same analyte retention time was considered as acceptable selectivity.

2.6.2. Calibration curve and LLOQ

Calibration was performed by a linear weighted ($1/x^2$) least squares regression of the peak-area ratios of the drugs to the IS versus the corresponding standard concentration. Linearity was assessed by assaying calibration curves of human plasma or urine in duplicate for three separate runs. We had evaluated the residues as follows: highest concentration and blank samples were injected continuously by three repeats and the area of blank sample is no more than 20% of LLOQ; the back calculated values was calculated by S/N ≥ 5 to LLOQ. LLOQ was defined as the lowest plasma concentration in the calibration curve. The accuracy (RE) was in the range of −20 to 20% for LLOQ and −15 to 15% for other points. The precision (RSD) was less than 20% for low concentration points and 15% for other points.

2.6.3. Precision and accuracy

Precision and accuracy assays were analyzed by five sets of spiked plasma or urine QC samples of blonanserin and blonanserin C at three concentrations levels (0.02, 0.5, and 1.5 ng/ml) on the same day and over three days. The concentration of each sample was calculated using a standard curve prepared and analyzed on the same day. Precision was calculated by using the relative standard deviation (RSD (%)) and accuracy was expressed as relative error (RE (%)). RE (%) = ((measured conc. − nominal conc.)/nominal conc.) × 100.

2.6.4. Recovery

The extraction recoveries were determined at three QC concentrations levels (0.02, 0.5, 1.5 ng/ml) for blonanserin, blonanserin C, and IS (1 ng/ml), and then calculated by the peak area ratios of the analytes of extracted samples to the corresponding standard solutions at the same concentrations.

2.6.5. Matrix effects

The matrix effect on the ionization of analytes was evaluated by comparing the peak area ratios of the analytes dissolved in the extracted blank plasma or urine to that of standard solutions at three QC concentrations. Matrix effects of the IS (1 ng/ml) were evaluated in the same way.

2.6.6. Stability

The stability of the stock solutions and working solutions of blonanserin, blonanserin C, and IS, which were stored at 2 °C for 3 days and at room temperature (25 °C) for 6 h, was tested by comparing the instrument response with that of freshly prepared solutions. The analytes were considered stable when the variation ranged between 85% and 115% of the initial solutions.

The QC plasma or urine samples were subjected to short-term room temperature (24 h, 25 °C), three freeze/thaw (−20 to 25 °C) cycles and long-term (30 days for plasma, 7 day for urine, −20 °C) stability tests. Placement stability of the post-preparative (ready-to-inject samples) (24 h, 24 °C) was also investigated. Subsequently, the concentrations were measured in comparison to freshly prepared samples. The analytes were considered stable in the biological matrix when 85–115% of the initial concentrations were found.

2.7. Pharmacokinetic study

2.7.1. Volunteers

Clinical study was undertaken on 5 healthy Chinese volunteers (2 females and 3 males), aged 18–26 years, non-smokers and non-drinkers. Their height ranged from 155 to 178 cm and body weight from 52 to 62 kg. Medical history, clinical examination, and laboratory tests (hematology, blood biochemistry, and urine analysis) showed all volunteers were in a good healthy condition and qualified for the study. The volunteers were instructed to abstain from taking any medication, including over the counter (OTC) drugs, for at least 2 weeks prior to and during the study period. They were instructed to avoid any alcohol or xanthine containing food and beverages 36 h prior to or during the course of the study. There was no disturbance to the nervous system, emotion, and mood disorder. The ethics committee of the Guangzhou Brain Hospital approved the study. Tolerability was based on the recording of adverse events (AEs), physical examination, electrocardiograms, and laboratory screen controls before and after the final study.

2.7.2. Clinical study design

The subjects received a single-dose of 8 mg blonanserin (4 mg/tablet) by oral administration with 250 ml waters under fasting conditions. Blood samples were collected before administration and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, and 60 h, and put into lithium heparin tubes and immediately centrifuged at 3000 r/min for 10 min. The subjects evacuated urine before administration and drinked water freely 2 h after administration. Urine samples were collected at 0, 0–2, 2–4, 4–8, 8–12, and 12–24 h. The volumes of urine were recorded and 1.0 ml of each was reserved. All the samples obtained were frozen at −20 °C in coded polypropylene tubs until analysis.

3. Results and discussion

3.1. Selection of IS

N-butyl-blonanserin has been reported as the IS to detect blonanserin, but we found it unfit to analyze the metabolite because of the low recovery and discordant ionization efficiency. Therefore, a structural analogue *N*-desethyl-chlor-blonanserin (blonanserin D) was selected as IS and synthesized. It has a similar structure to blonanserin and blonanserin C which has a similar extraction recovery, ionization response, and retention time.

3.2. Sample preparation

Liquid–liquid extraction was developed because this technique not only extracted the analytes with sufficient efficiency and

Table 1
The precision and accuracy of determination method for blonanserin and blonanserin C in human plasma and urine.

Matrix	Measurement interval	Nominal conc. (ng/ml)	Blonanserin			Blonanserin C		
			Measured conc. (ng/ml) (mean ± S.D.)	RSD (%)	RE[a] (%)	Measured conc. (ng/ml) (mean ± S.D.)	RSD (%)	RE[a] (%)
Plasma	Intra-day (n = 5)	0.02	0.019 ± 0.002	7.9	−4.1	0.021 ± 0.001	6.4	3.7
		0.5	0.502 ± 0.018	3.6	0.4	0.527 ± 0.042	8.0	5.5
		1.5	1.478 ± 0.042	2.8	−1.5	1.598 ± 0.044	2.8	6.6
Urine		0.02	0.021 ± 0.004	2.1	3.5	0.021 ± 0.001	6.1	2.6
		0.5	0.492 ± 0.013	2.6	−1.6	0.494 ± 0.009	1.7	−1.1
		1.5	1.506 ± 0.026	1.7	0.4	1.491 ± 0.066	4.5	−0.6
Plasma	Inter-day (n = 15)	0.02	0.019 ± 0.001	5.5	−2.7	0.020 ± 0.002	7.9	0.9
		0.5	0.490 ± 0.025	5.0	−2.1	0.508 ± 0.035	6.9	1.6
		1.5	1.439 ± 0.076	5.3	−4.1	1.549 ± 0.069	4.4	3.2
Urine		0.02	0.021 ± 0.001	4.3	3.4	0.020 ± 0.001	7.2	1.9
		0.5	0.491 ± 0.010	2.0	−1.8	0.482 ± 0.024	5.0	−3.6
		1.5	1.496 ± 0.025	1.7	−0.2	1.481 ± 0.060	4.1	−1.3

[a] RE (%) = ((measured conc. − nominal conc.)/nominal conc.) × 100.

specificity, but also minimized the experimental cost. Different options were evaluated to optimize sample extraction. Initially, after basification, drugs were isolated from plasma using dichloromethane (alone or in combination), ethyl acetate, and diethyl ether as extracting solvents. However, the recovery was poor (<45%) for dichloromethane. It was found that the matrix effect was high and inconsistent when ethyl acetate and diethyl ether were selected as the extracting solvent. Ethyl acetate–dichloromethane (4:1, v/v) was adopted as extraction solvent because of its appropriate extraction efficiency with a low variability. Saturated sodium hydrogen carbonate was added to the plasma in order to accelerate the drugs' dissociation from the plasma and to reduce interference as most of the endogenous materials are of acidic nature.

3.3. HPLC–ESI-MS/MS

To produce a high mass-to-charge $[M+H]^+$ precursor ion with minimal fragmentation of the analytes and IS, we used formic acid and ammonium acetate to form a complex mobile phase. Formic acid offered an acidic condition and ammonium acetate was used to adjust the retention of analytes on the column. Under ion monitoring in the positive ion mode, the most intense product ions observed in the MS/MS spectra were m/z 368.2 → 297.2 for blonanserin, m/z 340.2 → 297.1 for blonanserin C, and m/z 356.2 → 313.3 for blonanserin D which are shown in Fig. 1.

Figs. 2 and 3 show that no interference of the analytes was observed in plasma and urine samples. The retention times of blonanserin C, blonanserin, and blonanserin D were 3.8, 4.2, and 4.4 min, respectively.

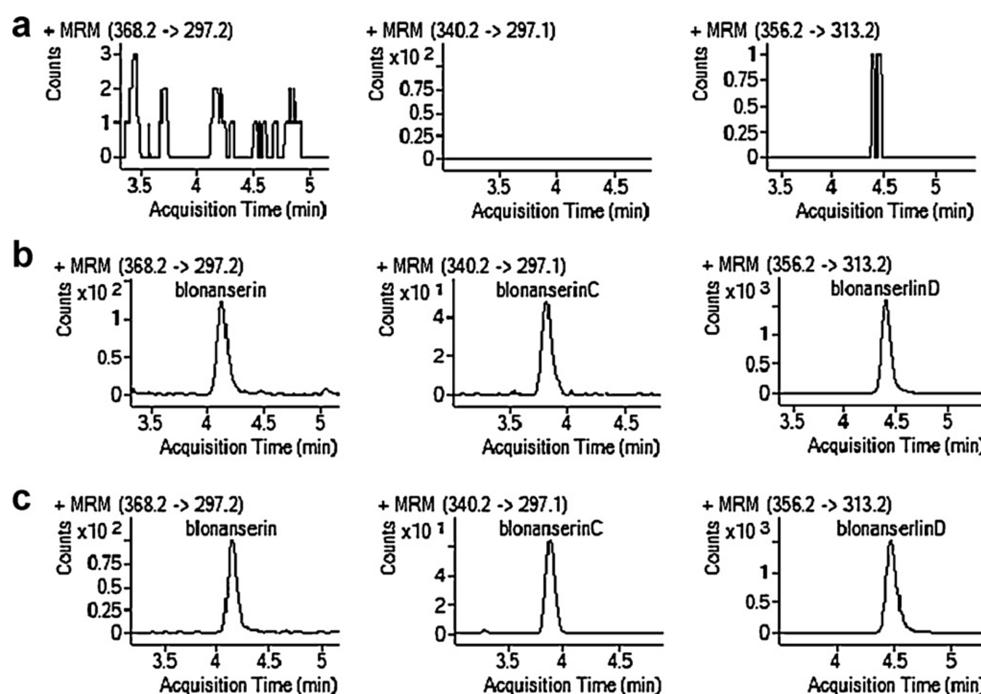

Fig. 2. MRM chromatograms of: (A) drug free plasma, (B) drug free plasma spiked with blonanserin (0.01 ng/ml), blonanserin C (0.01 ng/ml) and blonanserin D (IS, 1 ng/ml), and (C) subject's plasma sample obtained at 60 h after a single oral dose of 4 mg blonanserin with concentrations of 0.016 and 0.012 ng/ml, respectively.

3.4. Method validation

3.4.1. Selectivity

Typical MRM chromatograms from the quantification of blonanserin and blonanserin C in human plasma or urine are shown in Figs. 2 and 3. No interfering peak was observed in the free plasma or urine (Figs. 2 and 3(A)). The MRM chromatograms of blank plasma or urine spiked with blonanserin (0.01 ng/ml), blonanserin C (0.01 ng/ml), and the IS (1 ng/ml) are shown in Figs. 2 and 3(B). A plasma or urine sample from a volunteer after a single oral dose of 4 mg blonanserin is shown in Figs. 2 and 3(C). The drug, metabolite, and the IS were free from endogenous matrix interference at their respective retention times in the chromatograms.

3.4.2. Linearity and LLOQ

The method exhibited a good linear response in the range of concentrations from 0.01 to 2.0 ng/ml for blonanserin and blonanserin C. The regression equations of blonanserin ($n = 7$) in plasma and urine were $y = 1.8402{*}x + 0.0055$ ($r^2 = 0.996$) and $y = 2.0532{*}x + 0.0062$ ($r^2 = 0.996$), and of blonanserin C ($n = 7$) is $y = 1.7410{*}x + 0.0049$ ($r^2 = 0.997$) and $y = 1.4365{*}x + 0.0034$ ($r^2 = 0.999$), respectively, where y represents the ratio of blonanserin or blonanserin C peak area to that of the IS and x represents the corresponding plasma or urine concentration. The determination of the residues and the back calculated values are satisfied in analysis.

The LLOQ for blonanserin and blonanserin C in plasma was proved to be 0.01 ng/ml. The precision and accuracy were acceptable with 11.9% and 4.8% for blonanserin, and 7.0% and −3.8% for blonanserin C, respectively.

The LLOQ for blonanserin and blonanserin C in urine was proved to be also 0.01 ng/ml, with suitable precision and accuracy of 6.6% and −7.9% for blonanserin, and both 4.1% for blonanserin C, respectively.

3.4.3. Precision and accuracy

All the values are shown in Table 1. The intra- and inter-day precisions were less than 7.9% for blonanserin and 8.0% for blonanserin C in plasma, while 4.3% and 7.2% in urine at all QC levels. Relative errors ranged −4.1 to 0.4% for blonanserin and 0.9–6.6% for blonanserin C in plasma, while −1.8 to 3.5% and −3.6 to 2.6% in urine at all QC levels. The results showed good precision and accuracy and were consistent and reproducible.

3.4.4. Recovery

The extraction recoveries of blonanserin from the three concentrations of QC plasma and urine samples were 70.1, 65.8, 67.0 and 94.7, 93.5, 96.1%, respectively. And from blonanserin C were 78.1, 86.1, 82.5 and 74.5, 80.7, 74.9%, respectively. Recoveries of IS were 77.6 and 78.4% in plasma and urine, respectively.

3.4.5. Matrix effect

The matrix effects of blonanserin in plasma and urine were 97.8, 93.3, 93.3% and 109.3, 98.5, 99.6% at the three QC concentrations; for blonanserin C, the matrix effects were 109.3, 98.0, 98.3% and 102.1, 100.2, 99.3%; the matrix effects of the IS were 94.9 and 98.1%. The results indicate that no significant matrix effect for blonanserin, blonanserin C, and the IS was implied in the method.

3.4.6. Stability

The stock and working solutions of blonanserin, blonanserin C, and IS stored at 2 °C for 3 days and at room temperature (25 °C) for 6 h showed good stability with the intensities ranged between 93.8% and 106.6% of the initial solutions. All the solutions are re-prepared every 3 days.

Table 2 shows the stability data of the short-term, freeze–thaw, and long-term stability of blonanserin and blonanserin C. The short-term stability indicates reliable stability behavior under the experimental conditions of the regular runs. The results of freeze–thaw stability indicate that the analytes were stable in

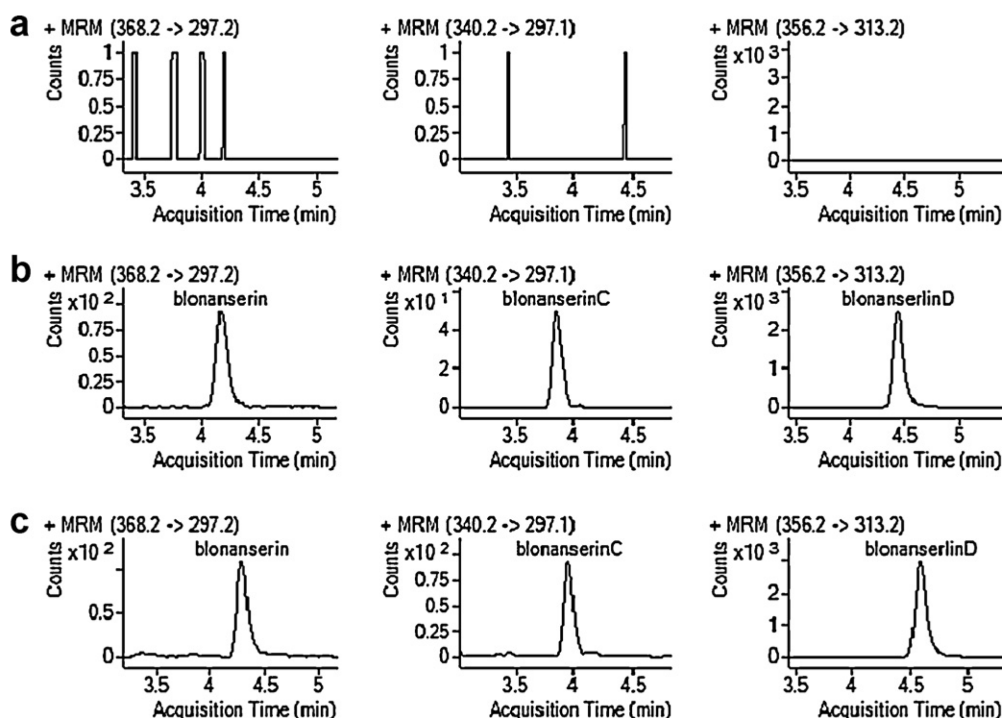

Fig. 3. MRM chromatograms of: (A) drug free urine, (B) drug free urine spiked with blonanserin (0.01 ng/ml), blonanserin C (0.01 ng/ml) and blonanserin D (IS, 1 ng/ml), and (C) subject's urine sample obtained at 2–4 h after a single oral dose of 4 mg blonanserin with concentrations of 0.011 and 0.010 ng/ml, respectively.

Table 2
The stability of blonanserin and blonanserin C in human plasma and urine under tested conditions ($n = 5$).

Matrix	Added conc. (ng/ml)	Found conc. (ng/ml) (mean ± S.D.)							
		Blonanserin				Blonanserin C			
		Short-term stability (24 h, 25 °C)	Freeze–thaw stability (3 cycles, −20 to 25 °C)	Long-term stability (−20 °C)	Post-preparative stability (24 h, 25 °C)	Short-term stability (24 h, 25 °C)	Freeze–thaw stability (3 cycles, −20 to 25 °C)	Long-term stability (−20 °C)	Post-preparative stability (24 h, 25 °C)
Plasma	0.02	0.02 ± 0.001	0.02 ± 0.000	0.02 ± 0.001	0.02 ± 0.002	0.02 ± 0.001	0.02 ± 0.003	0.02 ± 0.001	0.02 ± 0.001
	0.5	0.50 ± 0.01	0.48 ± 0.02	0.49 ± 0.02	0.48 ± 0.03	0.05 ± 0.03	0.46 ± 0.02	0.52 ± 0.01	0.50 ± 0.01
	1.5	1.48 ± 0.06	1.45 ± 0.06	1.49 ± 0.09	1.40 ± 0.05	1.49 ± 0.01	1.48 ± 0.01	1.60 ± 0.08	1.54 ± 0.03
Urine	0.02	0.02 ± 0.001	0.02 ± 0.002	0.02 ± 0.002	0.02 ± 0.001	0.02 ± 0.002	0.02 ± 0.002	0.02 ± 0.003	0.02 ± 0.002
	0.5	0.49 ± 0.01	0.48 ± 0.01	0.47 ± 0.02	0.49 ± 0.00	0.45 ± 0.02	0.50 ± 0.01	0.50 ± 0.01	0.45 ± 0.01
	1.5	1.49 ± 0.01	1.49 ± 0.02	1.46 ± 0.04	1.48 ± 0.02	1.42 ± 0.03	1.45 ± 0.06	1.47 ± 0.06	1.37 ± 0.02

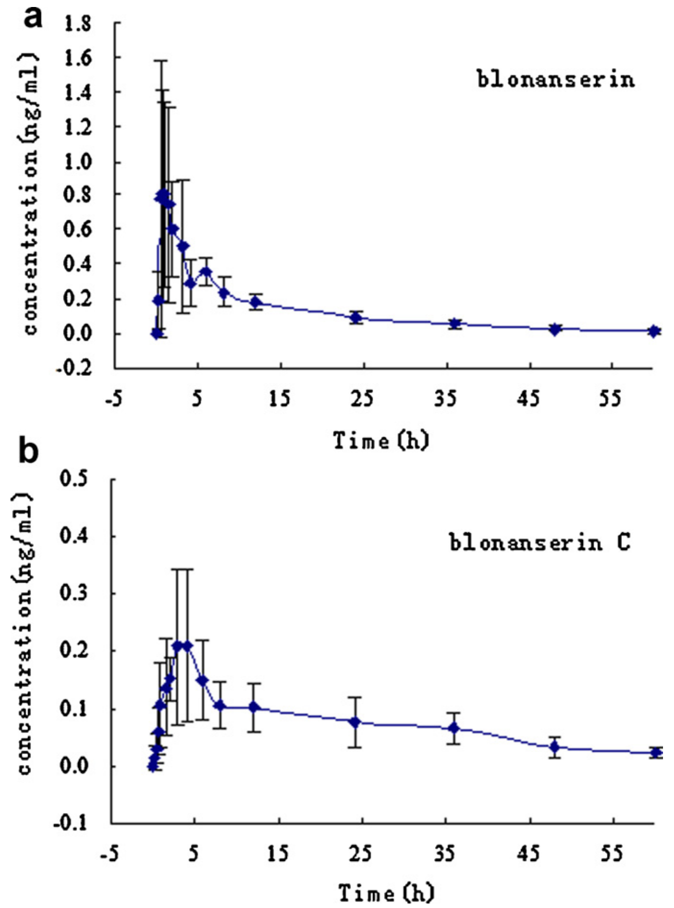

Fig. 4. Mean plasma concentration–time curves of blonanserin and blonanserin C in 5 volunteers after a single-dose oral administration of 8 mg blonanserin.

human plasma and urine for three cycles of freeze–thaw when stored at −20 °C and thawed to room temperature. The findings from long-term test indicate that storage of samples containing blonanserin and blonanserin C at −20 °C is adequate when stored for 30 days in plasma or 7 days in urine and no stability related problems existed during the samples' routine analysis for the pharmacokinetic studies.

3.5. Pharmacokinetic study

The mean plasma concentration–time curves of blonanserin and blonanserin C following single oral dose of 8 mg blonanserin in 5 healthy volunteers are shown in Fig. 4. T_{max} and C_{max} are measured values. Other pharmacokinetic parameters are calculated by DAS software and summarized in Table 3. The absorption of

Table 3
Pharmacokinetic parameters after oral administration of blonanserin 8 mg in 5 volunteers (mean ± S.D., $n = 5$).

Parameters[a]	Blonanserin	Blonanserin C
$T_{1/2}$ (h)	14.93 ± 3.66	18 .91 ± 4.91
T_{max} (h)	1.05 ± 0.67	2.90 ± 1.14
C_{max} (ng/ml)	1.13 ± 0.70	0.24 ± 0.12
AUC_{0-t} (ng h/ml)	7.18 ± 2.67	4.37 ± 1.86
$AUC_{0-\infty}$ (ng h/ml)	7.86 ± 2.97	4.96 ± 2.11
CL/F (l/h)	1.17 ± 0.53	1.96 ± 1.03
V_d/F (l)	25.28 ± 14.38	51.52 ± 25.67

[a] AUC, area under the plasma concentration–time curve; C_{max}, maximum plasma concentration; $T_{1/2}$, elimination half-life; T_{max}, time to C_{max}.

blonanserin is rapid after oral administration in healthy volunteers and a median time of T_{max} is 1 h, with a $T_{1/2}$ as long as 15 h. The data is close to the reports [8]. C_{max} and $AUC_{0-\infty}$ is higher than the reports [8]. Blonanserin is metabolized mainly by CYP 3A4 and the genetic polymorphism of CYP 3A4 may contribute to individual and racial variances among human volunteers with some other physiological factors. T_{max} of blonanserin C is about 3 h, with a $T_{1/2}$ as long as 19 h, too. The C_{max} and the AUC of blonanserin in plasma were approximately five and two times, respectively, those of blonanserin C which is also close to the reports [12].

Elimination of blonanserin was predominantly via the urine (59%) and feces (30%) after administration of a radiolabeled dose of 4 mg in six healthy Caucasian volunteers. Less than 5% of the administered dose was excreted in the feces as unchanged parent drug; unchanged drug was not detected in the urine [8]. In our study, the volumes of urine (V_{0-t}) were recorded and 1 ml of each was reserved to determine concentration (C_{0-t}). Excretion $S_{0-t} = C_{0-t} * V_{0-t}$; Urine excretory rate = $S_{0-\mathrm{t}}/S_{\mathrm{dose}}$*100%. Urine excretory rate is only 0.2% for blonanserin ($S_{0-24\,h}$ = 16.26 μg) and 0.4% for blonanserin C ($S_{0-24\,h}$ = 32.37 μg).

4. Conclusion

The highly sensitive LC–MS/MS assay with LLOQ 0.01 ng/ml for simultaneous determination of blonanserin and its metabolite in human plasma and urine was developed, validated, and successfully applied in human pharmacokinetic study for the first time. Using simple and rapid liquid–liquid extraction with short retention time makes it an attractive procedure in batch sample detection in pharmacokinetic, bioavailability, bioequivalence studies, and therapeutic drug monitoring.

Acknowledgments

We thank Lizhu Pharmaceutical Limited Company for providing us blonanserin, blonanserin C, and blonanserin D. This work was supported by a grant from Guangdong Natural Science Foundation (No. 8151037001000001) and Guangdong Province Department of Science and Technology (No. 00498500130062027).

References

[1] N. Kurotaki, H. Nobata, S. Nonaka, K. Nishihara, H. Ozawa, Psychiatry Clin. Neurosci. 65 (2011) 396.
[2] E.D. Deeks, G.M. Keating, CNS Drugs 24 (2010) 65.
[3] E. Garcia, M. Robert, F. Peris, H. Nakamura, N. Sato, Y. Terazawa, CNS Drugs 23 (2009) 615.
[4] T. Ishibashi, H. Nishikawa, T. Une, H. Nakamura, Folia Pharmacol. Jpn. 132 (2008) 351.
[5] O. Yukihiro, O. Motoki, I. Junta, T. Ayaka, O. Takahiro, S. Saki, Pharmacol. Biochem. Behav. 96 (2010) 175.
[6] J. Yang, W.M. Bahk, H.S. Cho, Clin. Neuropharmacol. 33 (2010) 169.
[7] R. Kumagai, Y. Ichimiya, Psychiatry Clin. Neurosci. 23 (2009) 593.
[8] R. Lonansen, (Blonanserin): Prescribing Information, Dainippon Sumitomo Pharma Co. Ltd., Osaka, 2009.
[9] Investigator's Brochure, Dainippon Sumitomo Pharma Co. Ltd., Osaka, 2006, February (Data on file).
[10] M. Matsuda, M. Sakashita, T. Yamaguchi, T. Fujii, J. Pharm. Biomed. Anal. 15 (1997) 1449.
[11] T. Ochi, M. Sakamoto, A. Minamida, K. Suzuki, T. Ueda, T. Une, H. Toda, K. Matsumotob, Y. Terauchi, Bioorg. Med. Chem. Lett. 15 (2005) 1055.
[12] M. Murasaki, H. Nishikawa, T. Ishibashi, Jpn. J. Clin. Psychopharmacol. 11 (2008) 845.
[13] T. Une, S. Kurumiya, Jpn. J. Clin. Psychopharmacol. 10 (2007) 1263.
[14] H. Hattori, M. Iwai, T. Ogawa, Y. Mizutani, A. Ishii, O. Suzuki, H. Seno, Forensic Toxicol. 28 (2010) 105.
[15] J. Saruwatari, N. Yasui-Furukori, Y. Inoue, S. Kaneko, Eur. J. Clin. Pharmacol. 66 (2010) 899.
[16] T. Ogawa, H. Hattori, R. Kaneko, K. Ito, M. Iwai, Y. Mizutani, T. Arinobu, A. Ishii, O. Suzuki, H. Seno, Anal. Sci. 26 (2010) 1099.