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Short communication

Determination of olprinone in human plasma utilizing liquid chromatography tandem mass spectrometry

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ABSTRACT

A sensitive and rapid method was developed for quantification of olprinone in human plasma utilizing liquid chromatography tandem mass spectrometry (LC–MS/MS). An aliquot of 1 mL plasma sample was extracted with ethyl acetate–dichloromethane. Separation of olprinone and the milrinone (internal standard, IS) from the interferences was achieved on a C_{18} column followed by MS/MS detection. The analytes were monitored in the positive ionization mode. Multiple reaction monitoring using the transition of m/z 251 → m/z 155 and m/z 212 → m/z 140 was performed to quantify olprinone and IS, respectively. The method had a total chromatographic run time of 3 min and linear calibration curves over the concentration range of 0.5–60 ng/mL. The lower limit of quantification (LLOQ) was 0.5 ng/mL. The intra- and inter-day precisions were less than 16.3% for low QC level, and 7.1% for other QC levels, respectively. The intra- and inter-day relative errors were ranged between −12.2% and 3.7% for three QC concentration levels. The validated method was successfully applied to the quantification of olprinone concentration in human plasma after intravenous (i.v.) administration of olprinone at a constant rate of infusion of 2 μg/(kg min) for 5 min in order to evaluate the pharmacokinetics.

1. Introduction

Olprinone is a specific inhibitor of phosphodiesterase III, and exerts its pharmacological effects by increasing cyclic AMP (cAMP) levels in the heart and vascular smooth muscle, which causes an increase in contractility of heart muscle and reduction in peripheral vascular resistance [1,2]. Olprinone is commonly used for the treatment of heart failure [1–4] or prevention from the occurrence of low cardiac output syndrome following open heart surgery [5,6]. Olprinone affords advantages that it does not commonly cause increase of heart rate or risk of dysrhythmias [6,7]. It has been used successfully following cardiovascular surgery in infants [8], children [9] and adults [5,6].

The desired therapeutic plasma concentration of olprinone reportedly ranges from 20 to 60 ng/mL [5,10,11]. In order to evaluate its pharmacokinetics, it is essential to establish an accurate, sensitive, and selective method for the quantification of olprinone concentration in plasma. Few methods have, however, been reported to quantify plasma concentrations of olprinone for human. A liquid chromatographic-ultraviolet spectral (LC-UV) method for the determination of olprinone in plasma has been developed successfully to support the pharmacokinetics studies in dogs [12]. However this HPLC-UV method had several problems, such as complicated and time-consuming sample preparation and low sensitivity. Recently a liquid chromatography–mass spectrometric (LC–MS) method [13] has been reported for the determination of olprinone in rat plasma. To date there is no quantification method based liquid chromatographic tandem mass spectrometry (LC–MS/MS) reported for olprinone in human plasma. Here we report the development of a LC–MS/MS method for the determination of olprinone in human plasma that allows for a sensitive, rapid, and highly selective analysis. The assay described here was fully validated and successfully used for the pharmacokinetic studies of an olprinone formulation product.

2. Experimental

2.1. Reagents

Olprinone injection (lot number: 20060801; 5 mg/5 mL) and olprinone (purity >99.5%) were kindly provided by Kangmei Phar-

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maceutical Co. Ltd. (Guangdong, China). Milrinone (purity >99.5%) which used as internal standard (IS) was kindly provided by Lunan Beite Pharmaceutical Co. Ltd. (Shandong, China). Methanol, acetonitrile, hexane, and ethyl ether were HPLC grade and were purchased from Sigma–Aldrich (Branch, China). Ethyl acetate, dichlormethane and sodium bicarbonate were AR grade and purchased from CAF (Canton, China). Blank human plasma from healthy donors was obtained from the Blood Service Center of Guangzhou (Canton, China) or from the healthy volunteers.

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) equipped with a spray interface. Ultra high pure (UHP) nitrogen was used as drying gas. Agilent MassHunter Chemstation (B.01.03) was utilized to process the raw data. A Salvis Lab vacuo drying apparatus (Vacucenter VC20, CH-6343 Rotkreuz, Switzerland) was utilized to evaporate the supernatant extracted from plasma samples.

2.3. LC–ESI-MS

Separation of the analytes from plasma was achieved at 40 °C on a Zorbax Eclipse XDB C_{18} column (150 mm × 4.6 mm i.d., 5 μm; Agilent, Wilmington, DE, USA) with a Security-Guard C_{18} guard column (4 mm × 3.0 mm i.d., 5 μm; Phenomenex, Torrance, CA, USA). The mobile phase consisting of ammonium formate–formic buffer (containing 4 mM ammonium formate and 0.4% formic) and acetonitrile (20:80, v/v) at a flow-rate of 0.8 mL/min was employed. The autosampler temperature was set at 4 °C.

The mass spectrometer was operated in a positive ion multiple reaction monitoring (MRM) mode. The instrument was operated with an ion spray voltage at +3.5 kV, heater gas temperature at 350 °C, drying gas at flow-rate of 10 L/min. Multiple reaction monitoring (MRM) was employed for data acquisition. The optimized MRM fragmentation transitions are m/z 251 → m/z 155 with collision energy (CE) of 38 eV for olprinone, and m/z 212 → m/z 140 with CE of 38 eV for milrinone (IS). The dwell time for each transition is 200 ms.

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

Stock solutions of olprinone at a concentration of 1 mg/mL were prepared by dissolving the accurately weighed reference substance in acetonitrile. The stock solution was then serially diluted with acetonitrile to give working solutions at the following concentrations: 10, 20, 50, 100, 200, 500, 1000, and 1200 ng/mL. The other stock solution was independently diluted in a similar way to achieve quality control (QC) solutions at concentrations of 1, 25 and 50 ng/mL. Internal standard working solution (500 ng/mL) was prepared by diluting the 1 mg/mL stock solution of milrinone with acetonitrile. All the solutions were kept at 4 °C and were brought to room temperature before use.

Both the calibration standard samples and the quality control samples, which were used in the pre-study validation and during the pharmacokinetic study, were prepared by spiking 1000 μL blank plasma with 50 μL working solutions correspondingly.

2.5. Extraction procedure for plasma samples

To each 1.0 mL plasma sample, 50 μL of the internal work solution (500 ng/mL acetonitrile solution), 0.5 mL of sodium bicarbonate saturated and 3 ml of ethyl acetate/dichloromethane (4:1, v/v) were added. The mixture was vortexed for 2 min and then centrifuged at 3500 × g for 5 min. Following centrifugation the supernatant was transferred to clean glass test tubes, and then was dried using vacuo drying apparatus. The residues were reconstituted with 200 μL of mobile phase and aliquots of 25 μL were injected into the LC–MS/MS system.

2.6. Method validation

2.6.1. Selectivity and lower limit of quantification (LLOQ)

To investigate the selectivity of the method, human blank plasma samples from 10 different donors were pretreated and analyzed at LLOQ.

2.6.2. Calibration curve

Linearity was assessed by assaying calibration curves in human plasma in duplicate in three separate runs. And the curves were fitted by a linear weighted ($1/x^2$) least squares regression method through the measurement of the peak-area ratio of the analyte to IS.

2.6.3. Recovery

The extraction recoveries were determined at three concentration levels (1, 25 and 50 ng/mL) for olprinone and at one concentration level for IS by comparing the analyte peak areas obtained from the quality control samples (n = 5) after extraction with those obtained from the corresponding unextracted reference standards prepared at the same concentrations.

2.6.4. Precision and accuracy

To evaluate the precision and accuracy of the method, QC samples at three concentration levels (1, 25 and 50 ng/mL) were analyzed in five replicates on three validation days. The assay precision was calculated by using the relative standard deviation (RSD) and one-way analysis of variance (one-way ANOVA). The assay accuracy was expressed as relative error (RE(%)), i.e. (measured concentration − nominal concentration)/(nominal concentration) × 100.

2.6.5. Stability

The stability of the stock solutions and working solutions of olprinone and IS, which were stored at 4 °C for 2 months and at room temperature (25 °C) for 8 h, was tested by comparing the instrument response with that of freshly prepared solutions. The analytes were considered stable when the intensities ranged between 85% and 115% of the initial solutions. The stability of olprinone in human plasma was evaluated by analyzing replicates (n = 5) of plasma samples that were exposed to different conditions (time and temperature) at three concentrations (1, 25 and 50 ng/mL). These results were compared with those obtained for freshly prepared plasma samples. The analytes were considered stable in the biological matrix when 85–115% of the initial concentrations were found. The stability was determined after exposure of the spiked samples at −20 °C for 2 months, 25 °C for 8 h and placement of the ready-to-inject samples (after extraction) on the autosampler rack (4 °C) for 24 h. The freeze/thaw stability was evaluated after three complete freeze/thaw cycles (−20 to 25 °C) on consecutive days.

2.6.6. Matrix effects

According to the method described by Matuszewski et al. [14], we assessed the matrix effects (MEs), or whether the potential ion suppression or enhancement owing to the co-eluting matrix components existed in the present experiment. The corresponding peak areas of olprinone from the spike-after-extraction samples at three concentration levels (1, 25 and 50 ng/mL) were then compared to those of the standard solution at the same concentration in mobile

phase. MEs of IS were investigated at only one concentration level (25 ng/mL) in a similar way.

2.7. Pharmacokinetic study

Pharmacokinetics of olprinone injection was assessed. The study protocol was approved by the Human Investigation Ethical Committee of Guangzhou Brain Hospital. Informed consent was obtained from all subjects after explaining the aims and risks of the study. After a thorough medical, biochemical and physical examination, ten Chinese healthy volunteers (five males and five females; aged 24–34; body weight 51–69 kg) received an intravenous administration of olprinone at a constant rate of infusion of 2 μg/(kg min) for 5 min (10 μg/kg in total). 4 mL of blood samples were collected in heparin-containing tubes before drug administration and at 5, 10, 15, 25, 35, 50, 65, 80, 95, 125, 155, 185 min after the end of the injection. Plasma samples were obtained by centrifugation at 3000 × *g* for 10 min and were frozen at −20 °C until analysis. Olprinone plasma concentration levels were determined using the present HPLC–MS/MS method.

3. Results and discussion

3.1. LC–MS/MS optimization

Olprinone is moderately polar and has a tertiary amino group in its structure, and so produces good mass spectrometric responses in positive electrospray ionization (ESI) mode. In the Q1 full scan mode $(M+H)^+$ ion with a high MS response was generated and no other additive ions were observed. Furthermore, the product spectrum of $(M+H)^+$ ion of olprinone was dependent on the collision energy (CE). Increasing the CE value to 38 eV, the most abundant fragment ion at *m/z* 155 was generated. In our case, milrinone, a structural analogue of olprinone was used as the internal standard. The base peak $(M+H)^+$ ion at *m/z* 212 in the Q1 full scan mode was selected as the precursor ion. Its fragment ion at *m/z* 140 proved to be steady and abundant which was chosen for the MRM acquisition. The MS^2 mass spectra of olprinone and the IS are shown in Fig. 1.

The effect of pH, buffer and composition of mobile phase on retention and resolution were investigated. As far as the mobile phase was concerned, methanol resulted in higher background noise than acetonitrile. After optimization, the mobile phase consisting of ammonium formate–formic buffer (containing 4 mM ammonium formate and 0.4% formic) and acetonitrile (20:80, v/v) was chosen as it gave a shortest retention time and better shapes of peaks. The retention times for olprinone and IS were at approximately 1.74 and 1.77 min, respectively (Fig. 2).

In the experiment, no significant carry-over was observed by injecting an extract of blank matrix immediately following the highest calibration standard (upper limit of quantitation).

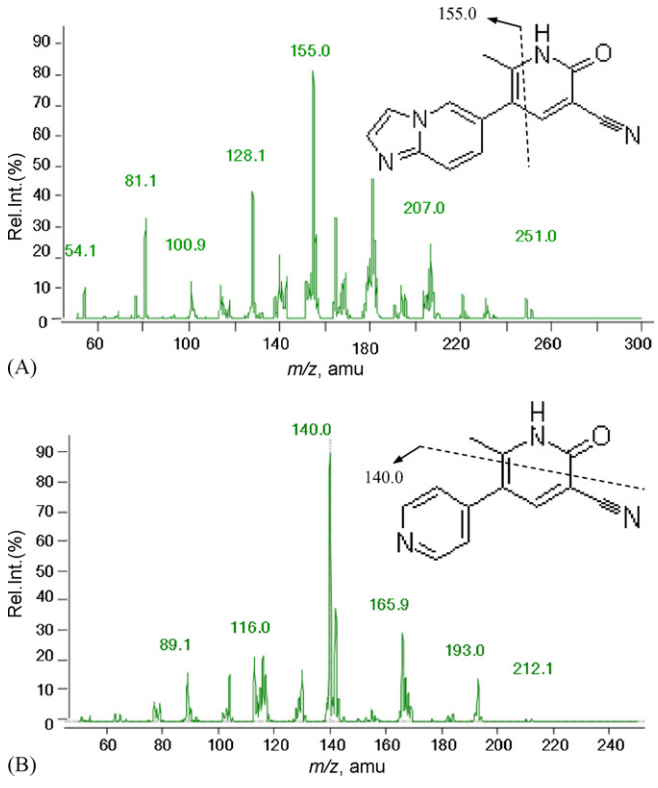

Fig. 1. Product ion mass spectra of $(M+H)^+$ with CE value 38 eV of (A) olprinone and (B) milrinone (internal standard).

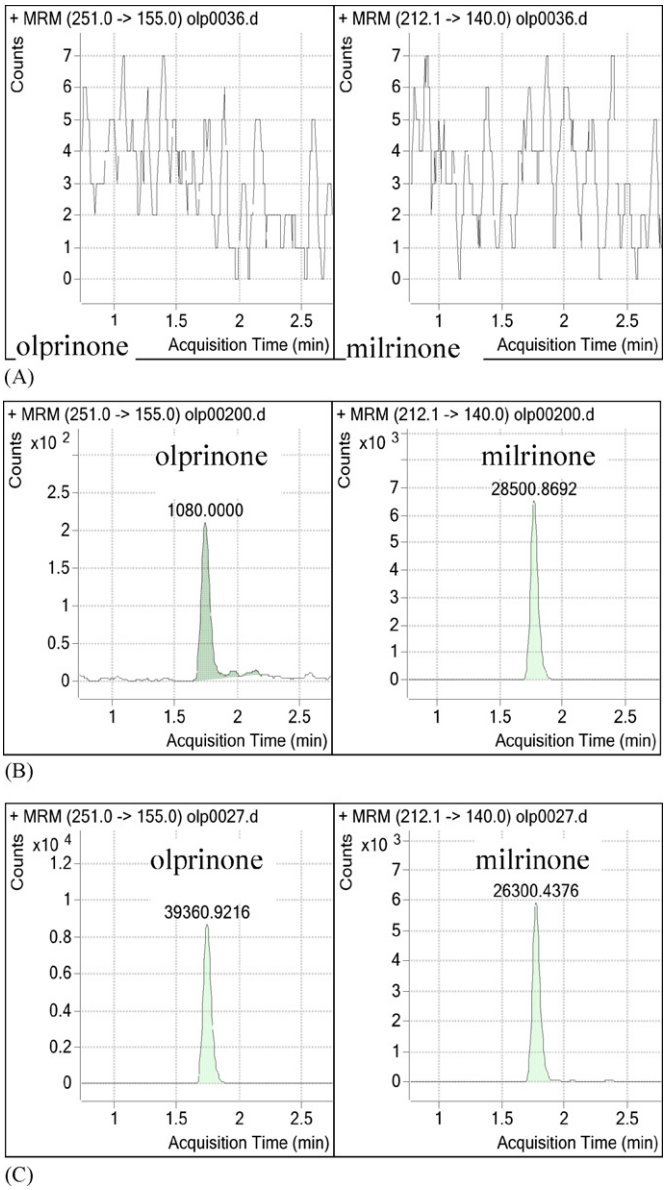

Fig. 2. Representative MRM chromatograms for olprinone and IS (milrinone) in human plasma samples: (A) a blank plasma sample; (B) a blank plasma sample spiked with olprinone (0.5 ng/mL) and milrinone (25 ng/mL); and (C) a plasma sample (olprinone concentration: 23.79 ng/mL) from a volunteer 15 min after an intravenous injection of 10 μg/kg oplrinone hydrochloride.

During method development different options were evaluated to optimize sample extraction. Initially, after basification drugs were isolated from plasma using methyl tertiary butyl ether, diethyl ether, and dichloromethane (alone and in combination) as extracting solvents. However, the recovery was poor (<45%) or inconsistent. It was found that the recovery was higher but not consistent when ethyl acetate was selected as extracting solvent. Finally ethyl acetate/dichloromethane (4:1, v/v) and saturated sodium bicarbonate buffers were found to provide the highest and consistent recoveries and there were no any interfering peaks (Fig. 2) compared with other tested extraction solvents.

3.2. Method validation

3.2.1. Assay selectivity and LLOQ

Three typical MRM chromatograms from the quantification of olprinone in human plasma are shown in Fig. 2. No interfering peak was observed in the blank plasma (Fig. 2(A)). The MRM chromatograms of blank plasma spiked with olprinone (0.5 ng/mL) and the IS (25.0 ng/mL) are shown in Fig. 2(B). A sample from a volunteer taken 15 min after an infusion of 10 μg/kg of olprinone is shown in Fig. 2(C). The chromatograms for the drugs and the IS were free from endogenous matrix interference at their respective retention times.

The LLOQ was established at 0.5 ng/mL, which was sensitive enough to investigate the pharmacokinetics of low dose (10 μg/kg) of oplrinone in human. The precision and accuracy at this concentration level were acceptable, with 7.1% of the RSD and 3.3% of the RE during within-run, and with 14.2% of the RSD and −4.3% of the RE during between-run.

3.2.2. Linearity

Typical equation of the calibration curves was as follows: $y = 1.6645x + 2.5 \times 10^{-5}$, $r = 0.997$ ($n = 8$).

3.2.3. Recovery

The recoveries of olprinone were ranged between 74.9% and 76.8% at three concentration levels. While the recovery of the IS was 77.4%. The recovery of olprinone and the IS was consistent and was not concentration-dependent.

3.2.4. Precision and accuracy

All the values are summarized in Table 1. The intra- and inter-day precisions were less than 16.3% for low QC level, and 7.1% for other QC levels, respectively. Relative errors ranged between −12.2% and 3.7%. Hence, the method was proved to be accurate and precise.

Table 1
Accuracy and precision for the analysis of olprinone in human plasma.

Nominal conc. (ng/mL)	Intra-day			Inter-day
	Day 1	Day 2	Day 3	
1				
Mean (ng/mL)	0.88	0.91	1.01	0.93
S.D.	0.05	0.10	0.09	0.1
RE[a] (%)	−12.2	−9.0	0.8	−6.8
R.S.D. (%)	5.7	11.2	8.9	16.3
25				
Mean (ng/mL)	25.01	25.91	25.47	25.46
S.D.	1.40	1.12	0.59	1.08
RE[a] (%)	0.1	3.7	1.9	1.9
R.S.D. (%)	5.6	4.3	2.3	4.0
50				
Mean (ng/mL)	51.62	48.75	51.46	50.61
S.D.	2.87	2.05	2.86	2.78
RE[a] (%)	3.3	−2.5	2.9	1.0
R.S.D. (%)	5.6	4.2	5.6	7.1

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

3.2.5. Stability

The stock solutions and working solutions of olprinone and IS stored at 4 °C for 2 months and at room temperature (25 °C) for 8 h showed good stability with the intensities ranged between 94.6% and 107.1% of the initial solutions. The stability data of olprinone in human plasma under four conditions are summarized in Table 2. As shown in Table 2, no significant degradation of olprinone was observed under any of those conditions. Take all the points into

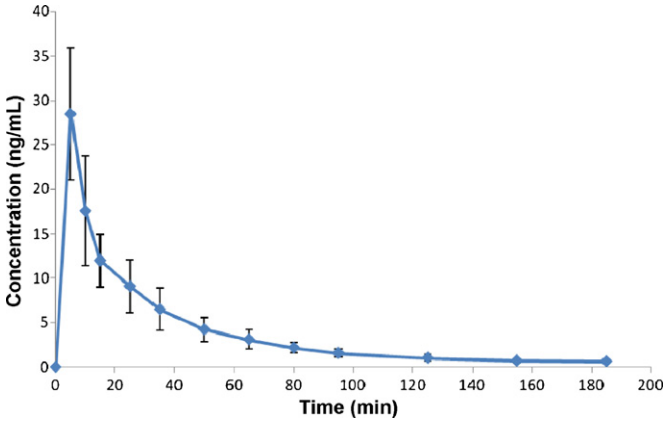

Fig. 3. Plasma concentration vs. time profiles of olprinone after intravenous (i.v.) administration of olprinone at a constant rate of infusion of 2 μg/(kg min) for 5 min in healthy volunteers ($n = 10$, mean ± S.D.).

Table 2
Stability of olprinone in human plasma[a].

Parameters	QC concentration levels ($n = 5$)		
	Low	Medium	High
Freshly prepared plasma concentration (mean ± S.D., ng/mL)	0.96 ± 0.10	25.01 ± 0.16	55.52 ± 2.49
Concentration (1) (mean ± S.D., ng/mL)	1.07 ± 0.19	25.87 ± 1.05	54.12 ± 1.50
RE[b] (%)	11	3	−3
Concentration (2) (mean ± S.D., ng/mL)	0.99 ± 0.12	26.25 ± 0.88	52.37 ± 1.75
RE[b] (%)	3	5	−6
Concentration (3) (mean ± S.D., ng/mL)	0.91 ± 0.10	23.32 ± 0.54	54.36 ± 2.01
RE[b] (%)	−5	−7	−2
Concentration (4) (mean ± S.D., ng/mL)	1.01 ± 0.12	25.25 ± 3.06	54.28 ± 1.60
RE[b] (%)	5	1	−2

[a] Experimental conditions: Concentration (1): after three freeze–thaw cycles. Concentration (2): at room temperature (25 °C) for 8 h. Concentration (3): at 4 °C for 24 h in autosampler rack. Concentration (4): at −20 °C for 2 months.
[b] RE(%)=((conc. [x] − freshly prepared plasma conc.)/freshly prepared plasma conc.) × 100.
$x = 1$–4.

consideration, olprinone can be stored and extracted under routine laboratory conditions without special attention.

3.2.6. Matrix effect

The matrix effects of olprinone, determined at three concentrations (1, 25 and 50 ng/mL), were 88.3%, 90.9% and 86.3%, respectively. The matrix effect of IS was 89.1%.

3.3. Pharmacokinetic study

Mean plasma concentration vs. time profile was presented in Fig. 3. There was no statistical significance ($P > 0.05$) in pharmacokinetic parameters between male and female.

4. Conclusion

A new LC–MS/MS method for the measurement of olprinone in human plasma has been established. The method is specific, highly sensitive with an LLOQ of 0.5 ng/mL, accurate over a concentration range of 0.5–60 ng/mL with a simple sample preparation.

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