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Pharmacokinetic Study of Vortioxetine in Mice

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***SUMMARY*. A sensitive and rapid ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method was developed to determine vortioxetine in mice plasma using pirfenidone as the internal standard (IS). Sample preparation was accomplished through a protein precipitation procedure with acetonitrile. The analyte and IS were separated on an Acquity UPLC BEH C18 column (2.1 × 50 mm, 1.7 μm) with the mobile phase of acetonitrile and 0.1% formic acid in water with gradient elution at a flow rate of 0.40 mL/min. The detection was performed on a triple quadrupole tandem mass spectrometer equipped with electrospray ionization (ESI) by multiple reactions monitoring (MRM) of the transitions at *m/z* 299.2→150.1 for vortioxetine and *m/z* 186.2→92.1 for IS. The linearity of this method was found to be within the concentration range of 0.1-700 ng/mL with a lower limit of quantification (LLOQ) of 0.1 ng/mL. Only 3.0 min was needed for an analytical run. The method herein described was superior to previous methods and was successfully applied to the pharmacokinetic study of vortioxetine in mice after oral administration.**

RESUMEN. Se desarrolló un método sensible y rápido de cromatografía líquida de ultra resolución en tandem con espectrometría de masas (UPLC-MS/MS) para determinar vortioxetina en plasma de ratones usando pirfenidona como estándar interno (IS). La preparación de la muestra se llevó a cabo a través de precipitación de proteínas con acetonitrilo. El analito y el IS se separaron en una columna Acquity UPLC BEH C18 (2,1 × 50 mm, 1,7 μM) con acetonitrilo y ácido fórmico 0,1% en agua como fase móvil, con gradiente de elución a un caudal de 12:40 mL/min. La detección se realizó en un espectrómetro de masas en tándem de triple cuadrupolo equipado con ionización por electrospray (ESI) con monitoreo de múltiples reacciones (MRM) de las transiciones *m/z* 299,2→150,1 para vortioxetina y *m/z* 186,2→92.1 para IS. La linealidad de este método está dentro del intervalo de concentración de 0,1 a 700 ng/mL, con un límite inferior de cuantificación (LLOQ) de 0,1 ng/mL. Sólo se necesitan 3,0 min para una serie de análisis. El método descrito fue superior a los métodos anteriores y se aplicó con éxito para el estudio farmacocinético de vortioxetina después de la administración oral a ratones.

INTRODUCTION

Vortioxetine (Fig. 1) is a novel antidepressant with multimodal action approved by the FDA for the treatment of major depressive disorder [1-3]. In addition to blocking the serotonin (5-HT) transporter, vortioxetine is an antagonist at 5-HT_{3A}, 5-HT_7 and 5-HT_{1D} receptors, a partial agonist at 5-HT1B receptors, and a full agonist at 5-HT_{1A} receptors [4,5]. Vortioxetine has a linear, dose-proportional pharmacokinetic profile following once daily administration of 2.5 to 60 mg doses [6]. Steady state plasma concentrations are typically reached within 2 weeks, and vortioxetine has an accumulation index of 5 to 6 [7]. Steady-state mean maximum plasma concentrations for vortioxetine 5, 10 and 20 mg/day were 9, 18, and 33 ng/mL, and were reached within 7-11 h of administration. Absolute bioavailability was 75% [6]. Food had no effect on the pharmacokinetics of vortioxetine [8].

Preclinical and clinical studies have demonstrated antidepressant properties of vortioxetine [9-11], yet little is known about pharmacokinetics profile of vortioxetine. Pharmacokinetic study

KEY WORDS: pharmacokinetics, plasma, UPLC-MS/MS, vortioxetine.

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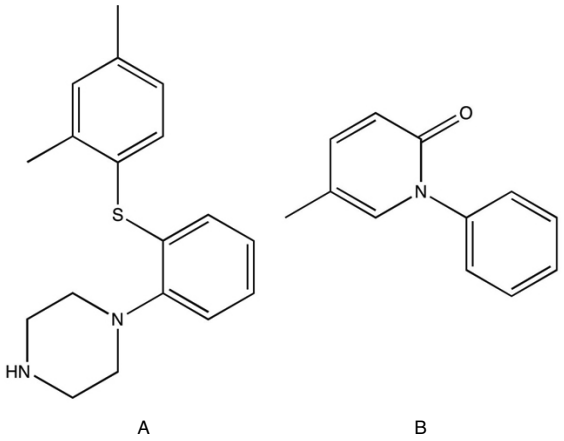

Figure 1. The chemical structures of vortioxetine and IS in the present study: (**A**) vortioxetine; (**B**) pirfenidone (IS).

constitutes an important phase in drug development. Learning the pharmacokinetic files of vortioxetine could help us get a fuller picture of the compound. Earlier publications have reported methods for the determination of vortioxetine in plasma [12,13]. However, the pharmacokinetic files of vortioxetine in mice is unknown. Thus, to monitor vortioxetine in the plasma precisely, we need a method with sufficient sensitivity and specificity.

UPLC-MS/MS has been evaluated as a faster and more efficient analytical tool compared with current chromatography [14-17]. Therefore, in this study, a specific and sensitive method for quantifying vortioxetine base on UPLC-MS/MS was developed, validated, and successfully applied to the pharmacokinetic study of vortioxetine in mice after oral administration.

MATERIALS AND METHODS

Chemicals and materials

Vortioxetine and pirfenidone (internal standard, IS) were obtained from Sigma (St. Louis, MO, USA). Acetonitrile and methanol were HPLC grade and purchased from Merck Company (Darmstadt, Germany). HPLC grade water was obtained using a Milli Q system (Millipore, Bedford, USA).

UPLC-MS/MS conditions

Liquid chromatography was performed on an Acquity ultra performance liquid chromatography (UPLC) unit (Waters Corp., Milford, MA, USA) with an Acquity BEH C18 column (2.1 × 50 mm, 1.7 µm) and inline 0.2 µm stainless steel frit filter (Waters Corp.). A gradient elution program was conducted for chromatographic separation with mobile phase A (acetonitrile), and mobile phase B (0.1% formic acid) as follows: 0-0.5 min (20-20% A), 0.5-1.0 min (20-95% A), 1.0-2.0 min (95-95% A), 2.0-2.1 min (95-20% A), 2.1-3.0 min (20-20% A). The flow rate was 0.40 mL/min. The overall run time was 3.0 min. A XEVO TQD triple quadrupole mass spectrometer equipped with an electro-spray ionization (ESI) source was used for mass spectrometric detection. The detection was operated in the multiple reaction monitoring (MRM) mode under unit mass resolution in the mass analyzers. The MRM transitions were *m/z* 299.2 → 150.1 and *m/z* 186.2 → 92.1 for vortioxetine and IS, respectively. After optimization, the source parameters were set as follows: curtain gas, 35 psig; nebulizer gas, 50 psig; turbo gas, 60 psig; ion spray voltage, 3.5 kV; and temperature, 350 °C. The Masslynx 4.1 software (Waters Corp.) was used for data acquisition and instrument control.

Standard solutions, calibration standards and quality control (QC) sample

The stock solution of vortioxetine used to make the calibration standards and quality control (QC) samples was prepared by dissolving 10 mg in 10 mL methanol to obtain a concentration of 1.00 mg/mL. The stock solution was further diluted with methanol to obtain working solutions at several concentration levels. Calibration standards and QC samples in mice plasma were prepared by diluting the corresponding working solutions with blank plasma samples. Final concentrations of the calibration standards were 0.1, 0.2, 1, 2, 10, 20, 100, 200, 500, and 700 ng/mL for vortioxetine. The concentrations of QC samples were prepared independently in the same way at three levels (0.2, 40, and 560 ng/mL). IS stock solution was made at an initial concentration of 1 mg/mL. The IS working solution (50 ng/mL) was made from the stock solution using acetonitrile for dilution. All stock solutions, working solutions, calibration standards and QCs were immediately stored at -20 °C.

Sample preparation

Before analysis, frozen plasma sample was thawed to room temperature. In a 1.5 mL centrifuge tube, an aliquot of 200 µL of the internal standard working solution (50 ng/mL) was added to 0.1 mL of plasma sample. The tubes were vortex mixed for 1.0 min. After centrifuga-

tion at 13,000 *g* for 10 min, the supernatant (2 µL) was injected into the UPLC-MS/MS system for analysis.

Method validation

Before using this method to determinate vortioxetine in plasma, the method was fully validated for specificity, linearity, precision, accuracy, recovery, matrix effect and stability according the United States Food and Drug Administration bioanalytical method validation guidances (2001).

Specificity was determined by analysis of blank plasma samples from six different mice, every blank sample was handled by the procedure described before and confirmed that endogenous substances did not have the possible interference with the analyte and the IS.

To evaluate the linearity, calibration standards of nine concentrations of vortioxetine (0.1-700 ng/mL) were separately extracted and assayed on three separate days. The linearity for vortioxetine was investigated by weighted (1/x2) least-squares linear regression of peak area ratios against concentrations. The sensitivity of the method was determined by quantifying the lower limit of quantification (LLOQ). The LLOQ was defined as the lowest acceptable point in the calibration curve, which were determined at an acceptable precision and accuracy.

To determine the matrix effect (ME), six different blank plasma samples were utilized to prepare QC samples and used for assessing the lot-to-lot matrix effect. ME was evaluated at three QC levels by comparing the peak areas of analytes obtained from plasma samples spiked with analytes after extraction to those of the pure standard solutions at the same concentrations. The ME of IS was evaluated at the working concentration (50 ng/mL) in the same manner.

The precision and accuracy of the method were assessed by determination of QC samples in plasma at three different concentrations (0.2, 40, and 560 ng/mL) on three separate days. Precision was expressed as % relative standard deviation (RSD) and accuracy was expressed as % relative error (RE) between the measured and nominal value. The precision for QC samples was within 15%, and accuracy between -15 and 15%.

Extraction recovery experiments which showed an ability to extract the analyte from the test biological samples, were evaluated by comparing the peak areas obtained from extracted QC samples with non-processed standard solutions at three concentrations at the same concentration. Recovery of IS was determined at the working concentration (50 ng/mL) similarly.

The stabilities in plasma were tested by analyzing five replicates of plasma samples at three concentration levels (0.2, 40, and 560 ng/mL) in different conditions. The short-term stability was determined after the exposure of the spiked samples at room temperature for 4 h, and the ready-to-inject samples (after extraction) in the autosampler at 4 °C for 48 h. The freeze-thaw stability was evaluated after three complete freeze-thaw cycles (-20 to 25 °C) on consecutive days. The long-term stability was assessed after storage of the standard spiked plasma samples at -20 °C for 21 days. Samples were considered to be stable if assay values were within the acceptable limits of accuracy (RE % ≤ ± 15%) and precision (RSD % ≤ 15%).

Pharmacokinetic study

Kunming mice (20-25 g) were obtained from Laboratory Animal Center of Henan University of Science and Technology (Henan, China) used to study the pharmacokinetics of vortioxetine. All experimental procedures and protocols were reviewed and approved by the Animal Care and Use Committee of Henan University of Science and Technology and were in accordance with the Guide for the Care and Use of Laboratory Animals. Diet was prohibited for 12 h before the experiment but water was freely available. Blood samples were collected from eyeball at 0.25, 0.5, 1, 2, 3, 4, and 8 h (six mice for each time point) after the administration of 5.0 mg/kg of vortioxetine dissolved in 0.5% sodium carboxymethylcellulose. After centrifugation at 13,000 g for 10 min, plasma samples were stored at -20 °C till analysis. The pharmacokinetic parameters were calculated using DAS (Drug and statistics) software (Version 2.0, Shanghai University of Traditional Chinese Medicine, China) according to the non-compartmental analysis as sparse data.

RESULTS AND DISCUSSION

Method development and optimization

An Acquity BEH C18 column (2.1 × 50 mm, 1.7 µm) was observed to provide adequate separation of analytes after a simple protein precipitation sample preparation, and formic acid was added to the mobile phase to improve peak shape. Pirfenidone was chosen as internal stan-

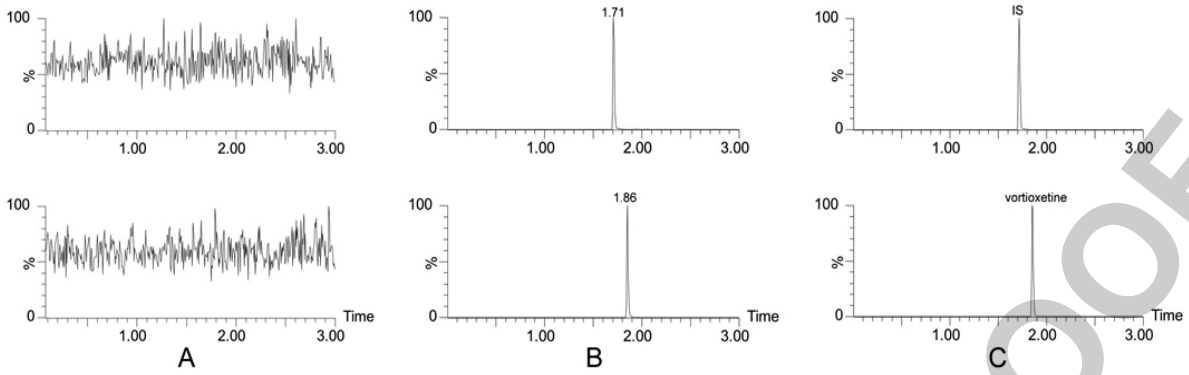

Figure 2. Representative chromatograms of vortioxetine and IS in mice plasma samples. (**A**) a blank plasma sample; (**B**) a blank plasma sample spiked with vortioxetine and IS; (**C**) a plasma sample from a mice 1.0 h after an oral administration 5.0 mg/kg.

Concentration (ng/mL)	Intra-day		Inter-day		Recovery (%)
	RSD %	RE %	RSD %	RE %	
0.2	6.7	-8.6	9.5	10.6	84.3
40	5.6	-4.9	10.2	-5.0	88.7
560	6.1	3.4	6.7	3.2	82.9

Table 1. Precision, accuracy, and recovery for vortioxetine of QC samples in mice plasma (n = 6).

dard due to its relatively high recovery, similar polarity and retention time to vortioxetine. A mobile phase gradient was developed to separate vortioxetine from other potentially interfering peaks. The gradient allowed analytes to enter the mass spectrometer after at least 1.5 column dead volumes thus enabling diversion of hydrophilic matrix components to waste prior to directing flow back to the mass spectrometer for analyte detection. Ultimately, these conditions enabled good separation, acceptable matrix effect and recovery to yield a highly sensitive and sufficiently robust assay.

Specificity and ME

UPLC-MS/MS chromatogram of the analyte in plasma samples are shown in Fig. 2.

The retention times of vortioxetine and IS were 1.86 and 1.71 min, respectively. Compared with chromatogram of blank plasma sample, no interference of endogenous peaks was observed. The ME for vortioxetine at concentrations of 0.2, 40, and 560 ng/mL was measured to be 98.4, 95.6, and 101.2% (n = 6), respectively. The ME for IS (50 ng/mL) was 103.7% (n = 6). As a result, ME from plasma was negligible in this method.

Linearity and sensitivity

The calibration curve of nine concentrations of vortioxetine were established by weighted ($1/\chi^2$) linear regression analysis, based on the peak area ratio of vortioxetine to IS *versus* the vortioxetine concentration, showing a good linear relationship over the range of 0.1-700 ng/mL with a regression coefficient (r^2 = 0.9996). The LLOQ in mice plasma was 0.1 ng/mL with the RSD and RE of 7.8 and 9.6%, respectively.

Precision, accuracy and recovery

The precision, accuracy and recovery values for six replicates of QC samples at low, medium and high concentrations are summarized in Table 1. The precision expressed as relative standard deviation (RSD%) and accuracy expressed as relative error (RE %). The method was reliable and reproducible since RSD% was below 10.2% and RE% was between -8.6 and 10.6% for all the investigated concentrations of vortioxetine in mice plasma. The recovery for vortioxetine ranged from 82.9 to 88.7% over three QC sample levels and the recovery for IS was 84.3%. It proved that the method to analyze vortioxetine was accurate, reliable and reproducible.

Analytes	Concentration added (ng/mL)	Room temperature, 4 h		4 °C 48 h		Three freeze-thaw		-20 °C 21 days	
		RSD (%)	RE (%)	RSD (%)	RE (%)	RSD (%)	RE (%)	RSD (%)	RE (%)
Vortioxetine	0.2	7.8	8.9	8.2	-9.7	10.5	9.0	8.2	5.7
	40	5.7	-6.2	6.8	-7.4	8.8	8.3	6.3	-7.1
	560	3.4	4.8	5.3	3.8	6.5	5.2	4.9	2.9

Table 2. Stability results of vortioxetine in mice plasma in different conditions (n = 5).

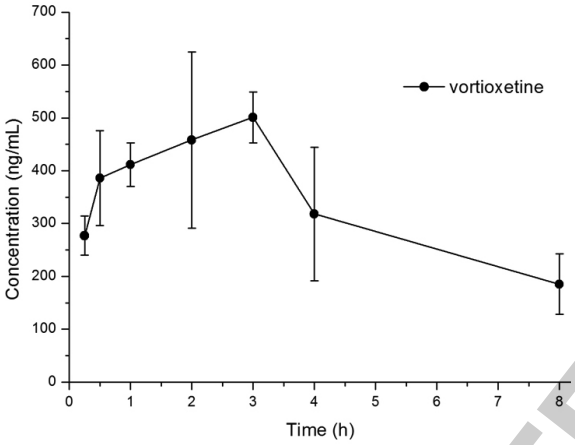

Figure 3. Concentration *versus* time curves of vortioxetine in Kunming mice after a single oral administration at 5.0 mg/kg.

Parameters	Mean ± SD
$t_{1/2}$ (h)	3.96 ± 0.74
C_{max} (ng/mL)	559.19 ± 85.25
T_{max} (h)	2.13 ± 1.18
$AUC_{0\rightarrow t}$ (ng/mL.h)	2647.90 ± 600.51
$AUC_{0\rightarrow \infty}$ (ng/mL.h)	3739.85 ± 1048.53

Table 3. The pharmacokinetic parameters of vortioxetine in mice plasma after oral administration 5.0 mg/kg.

Stability

Stability tests were performed at the low, medium and high QC samples with five determinations for each under different storage conditions. The RSDs of the mean test responses were within 15% in all stability tests.

Table 2 shows the stability data for vortioxetine in plasma samples under different storage and temperature conditions. There was no effect on the quantitation for plasma samples kept at room temperature for 4 h and at 4 °C for 48 h. No significant degradation was observed when samples of vortioxetine were taken through three freeze (-20 °C)- thaw (room temperature) cycles. As a result, vortioxetine in samples were stable at -20 °C for 21 days.

Application of the method in a pharmacokinetic study

The present method was successfully applied to determine the concentration of vortioxetine in mice plasma after oral administration. The mean plasma concentration-time curves after oral administration of vortioxetine were shown in Fig. 3. The main pharmacokinetic parameters from non-compartment model analysis are summarized in Table 3.

CONCLUSIONS

A rapid, sensitive, and specified method was developed and validated for the quantification of vortioxetine. The method showed excellent performance as follows: low LLOQ (0.1 ng/mL), wild range (0.1-700 ng/mL), short running time (3.0 min), and simple preparation process. It was successfully applied to the pharmacokinetic study of vortioxetine in mice after oral administration.

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