



## Original Research Article

# Development of a UPLC–MS/MS method for determination of pimavanserin tartrate in rat plasma: Application to a pharmacokinetic study



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## ABSTRACT

A simple, rapid and sensitive method based on an ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) has been developed and validated for the determination of pimavanserin in rat plasma. The analyte was extracted by protein precipitation with methanol and separated on an ACQUITY BEH C<sub>18</sub> column (100 mm × 2.1 mm, 1.7 μm; Waters, USA), with an isocratic elution of acetonitrile–water containing 10 mM ammonium acetate (70:30, v/v), at a flow rate of 0.2 mL/min for 2.5 min. The analyte and clarithromycin (the internal standard) were detected and quantified in positive ion mode using multiple reaction monitoring transitions at  $m/z$  428.2 → 223.0 for pimavanserin and  $m/z$  748.5 → 589.5 for clarithromycin. Relative coefficient ( $r$ ) for the calibration curve was more than 0.9980. The intra-day and inter-day precisions (relative standard deviation, RSD%) were less than 13.3% and 10.5%, respectively, and the accuracy (relative error, RE%) was within ± 11.5%. The analytical method was successfully applied to a routine pharmacokinetic study of pimavanserin in rats after oral administration at the dose of 10 mg/kg.

## 1. Introduction

There are many kinds of antipsychotic drugs used for the treatment of Parkinson's disease psychosis (PDP), but they almost block the dopamine (DA) D2 receptors which are the target of the DA replacement therapy in Parkinson's disease [1]. Pimavanserin, a serotonin 5-HT<sub>2A</sub> inverse agonist, is a novel drug with exciting potential for treating various neuropsychiatric disorders [2–5]. It represents a novel way to treat PDP symptoms by targeting 5-HT<sub>2A</sub> receptors, a non-dopamine receptor [6–9]. Unlike atypical antipsychotic drugs, pimavanserin does not induce catalepsy even at high doses, or cause motor effects [10]. In addition, pimavanserin increases slow-wave sleep maintenance which will improve sleep quality in people, especially PDP patients with sleep disorders [11–13].

Nuplazid (pimavanserin tartrate) has been approved by the US FDA on April 29, 2016 and marketed at a prescription status in the USA. Abundant pre-clinical research data attach importance to pharmacological and pharmacodynamic studies on animal models. However, as a new drug, there was limited pharmacokinetic information available.

Earlier publication rarely reported systematic and mature methods for determination of pimavanserin in rat plasma. Vanover et al. [6] had investigated an 8 h intravenous and oral administration pharmacokinetics of a hydrochloride salt form of pimavanserin in male rats. In their study, the validated content and pharmacokinetic parameters were not completed. Hence, it is necessary to investigate pharmacokinetic profile in rats for longer time.

Synthesized process of pimavanserin the according to previous report had low yield with the risk of polluting the environment [14]. In our previous study, we adopted a brand-new, environment-friendly, and high-yield process to produce pimavanserin tartrate (N-(4-fluorophenylmethyl)-N-(1-methylpiperidin-4-yl)-N'-(4-(2-methylpropoxy)ph-enylmethyl) carbamide – dihydroxybutanedioate (2:1)) and applied for a patent (application number: 201510259570.7) for this synthesized process in China (<http://www.pss-system.gov.cn/sipopublicsearch/portal/uiIndex.shtml>). Compared with reported HPLC and LC methods, UPLC method coupled with tandem mass (UPLC–MS/MS) is more rapid and has higher throughput. In the present study, a UPLC–MS/MS method has been developed to investigate its pharma-

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cokinetic features in male and female rats with our self-made pimavanserin tartrate. It was demonstrated that this analytical method was simple, rapid, sensitive and robust for pimavanserin plasma pharmacokinetic study in rats and according to the result, its pharmacokinetic profile was similar to that of previous publication.

## 2. Experimental

### 2.1. Chemicals and reagents

Pimavanserin tartrate (purity 99.0%) was synthesized in School of Pharmaceutical Engineering, Shenyang Pharmaceutical University (Shenyang, China). Clarithromycin (Internal standard, IS; purity 98.0%) was obtained from China Institute for Drug Control (Shenyang, China), and acetonitrile and methanol of HPLC grade were purchased from Sigma Company (Saint Louis, Missouri, USA). Other chemicals were of analytical grade.

### 2.2. Animals

Sprague-Dawley (SD) rats (male and female in half) weighing 260–300 g were purchased from Changsheng Biological Technology Company in Liaoning, China. Before this assay, the rats were bred in SPF grade room for one week. The animal study was carried out in accordance with the Guideline for Animal Experimentation of Shenyang Pharmaceutical University, and the protocol was approved by the Animal Ethics Committee of the institution.

### 2.3. UPLC–MS/MS system and operating conditions

Chromatographic separation was performed on an ACQUITY BEH C<sub>18</sub> column (100 mm × 2.1 mm, 1.7 μm; Waters, USA) using a Waters UPLC system (Waters, USA). The column and auto-sampler tray temperatures were kept constant at 35 °C and 10 °C, respectively. The analyte and IS were separated with an isocratic elution with acetonitrile (solution A) and 10 mM ammonium acetate in water (solution B) (70:30, v/v), at a flow rate of 0.2 mL/min.

The mass spectrometric data were collected on an Xevo TQS mass spectrometer (Waters Corp., Milford, MA, USA) with a triple quadrupole mass analyzer and an electrospray ionization (ESI) interface in a positive mode. The optimal parameters were set as follows: the capillary voltage, 3.0 kV; the cone voltage both for pimavanserin and IS, 27 V; the desolvation gas flow, 700 L/h; the cone gas flow, 150 L/h; the desolvation gas temperature, 350 °C; source temperature, 150 °C. Argon was used as the collision gas, and the collision energies were 10 eV for pimavanserin and 16 eV for IS. Pimavanserin was quantitated in multiple reaction monitoring (MRM) mode with the presence of the IS. The transition is  $m/z$  428.2 → 223.0 for pimavanserin and 748.5 → 589.5 for IS.

### 2.4. Preparation of calibration standards and quality control (QC) samples

For preparation of the standard samples used for construction of calibration curve, 20 μL of working standard solution of pimavanserin and 20 μL of IS working solution were added to 100 μL of blank plasma, followed by a 30 s vortex and were processed by a protein precipitation procedure (PPT). The pimavanserin concentrations of seven calibration standards were equal to 1, 2, 5, 10, 20, 40, and 80 ng/mL of plasma concentration. The QC samples were prepared in the same fashion. The final concentration of the QC samples were 2 ng/mL for low QC, 16 ng/mL for medium QC and 64 ng/mL for high QC.

### 2.5. Sample preparation

An aliquot of 100 μL plasma sample was transferred to an

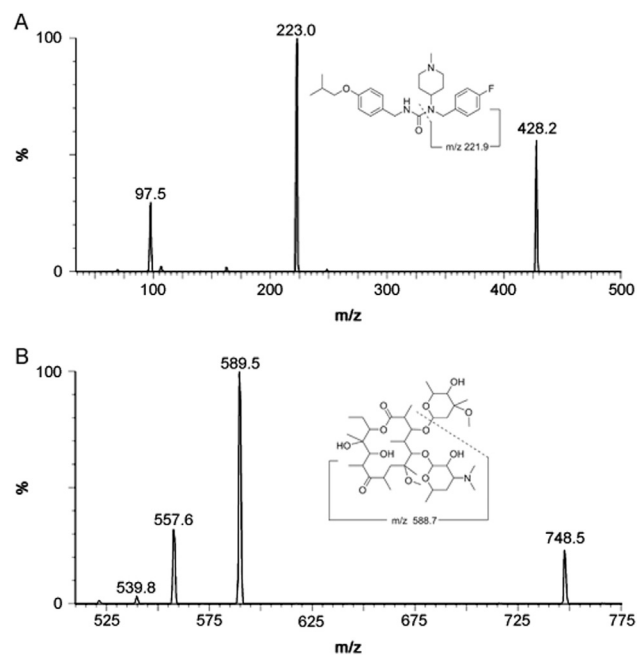


Fig. 1. Full-scan product ion spectra of pimavanserin (A) and clarithromycin (B).

Eppendorf micro tube, and vortex-mixed with 20 μL IS and 20 μL methanol for 30 s, and then 200 μL methanol was added and vortexed for 3 min. After centrifugation at 15,000 rpm for 10 min, 5 μL of supernatant was injected into the UPLC–MS/MS system for analysis.

### 2.6. Validation procedures

The assay was validated using healthy SD rat plasma following the guidelines for bioanalytical method validation issued by the US FDA [15].

#### 2.6.1. Selectivity

Selectivity was assessed by comparing chromatograms of blank plasma from six SD rats, plasma samples spiked with pimavanserin and IS, and a plasma sample after oral administration of pimavanserin.

#### 2.6.2. Linearity and sensitivity

The linearity was evaluated by weighted ( $1/x^2$ ) linear regression analysis of pimavanserin/IS peak area ratio versus the spiked concentration, and two independent calibration curves were obtained on each day for the three-day method validation [16]. Especially, one independent calibration curve was used for detection of plasma sample after oral administration of pimavanserin. The lower limit of quantification (LLOQ) was defined as the lowest concentration measurable with precision and accuracy less than 20%.

#### 2.6.3. Precision and accuracy

Precision was defined as the relative standard deviation (RSD, %) and accuracy was defined as relative error (RE, %). The intra-day and inter-day precision and accuracy were assessed at three QC concentration levels with six replicates on the same day for three consecutive days.

#### 2.6.4. Extraction recovery and matrix effect

The recoveries of pimavanserin were determined at three QC levels with six replicates by comparing the peak areas from extracted samples with those from post-extracted blank plasma samples spiked with the analyte at the same concentration. Recovery of the IS was determined in the same way at the concentration of 20 ng/mL. The matrix effect was measured at three QC levels by comparing the peak response of

blank plasma extracts spiked with pimavanserin (A) with that of pure standard solution containing equivalent amounts of the compound (B). The ratio  $(A/B \times 100)$  was used to evaluate the matrix effect.

### 2.6.5. Stability

Stability studies in plasma samples were also conducted at three QC levels under different storage conditions: at room temperature for 12 h, at  $-80^\circ\text{C}$  for 30 days, after three freeze–thaw cycles, and for 8 h at  $10^\circ\text{C}$  in an auto-sampler tray.

### 2.6.6. Dilution integrity and cross talk

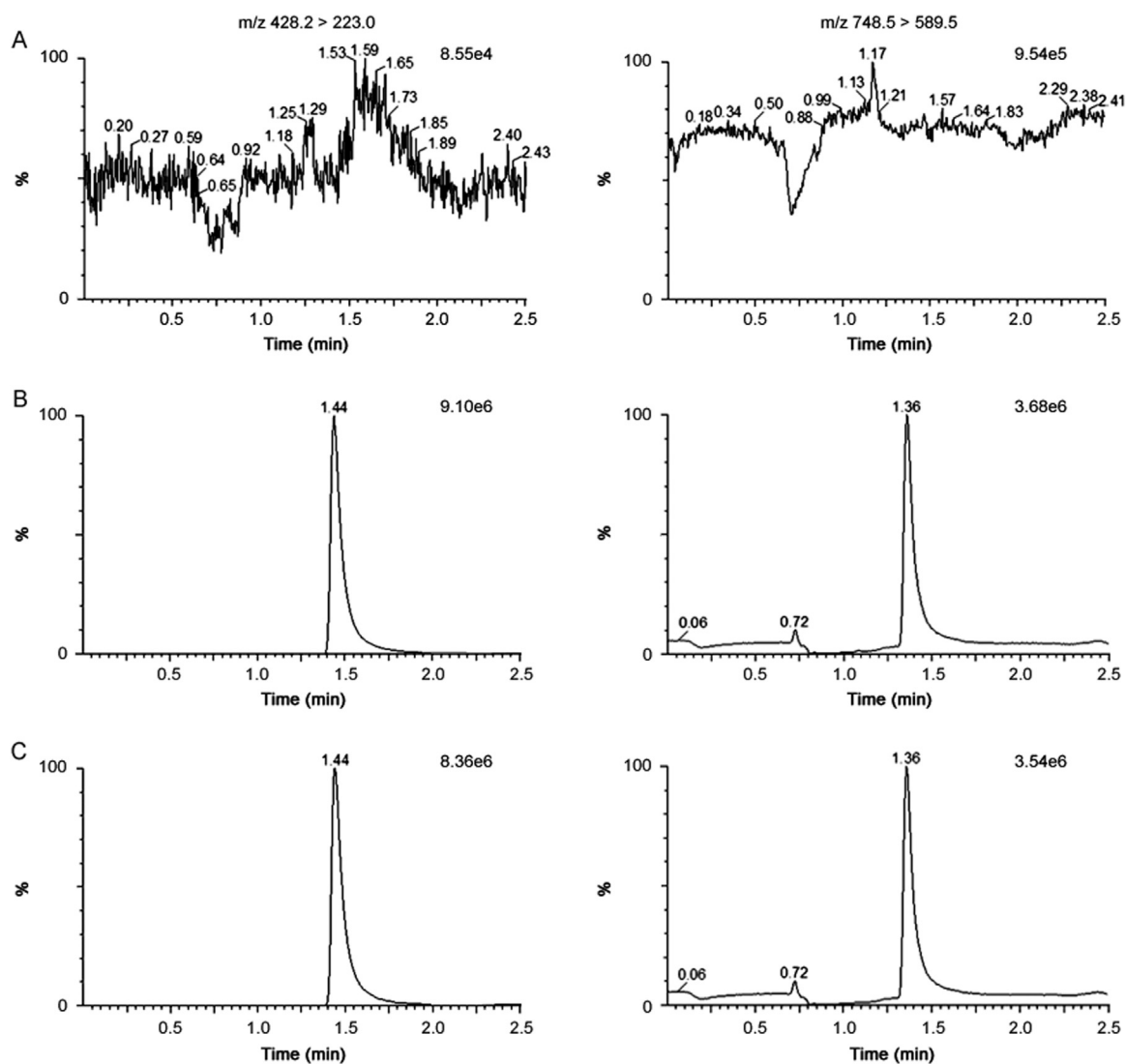
Dilution integrity was carried out by diluting the plasma sample spiked with pimavanserin at a concentration above the upper limit of quantitation (ULOQ) with blank rat plasma at a ratio of 1:2, 1:4, and 1:8. Each concentration was analyzed with five replicates. Cross talk was investigated by comparing the peak area of pimavanserin of plasma samples (no pimavanserin) with the peak area of pimavanserin of LLOQ plasma samples (no IS), requiring peak area of pimavanserin does not exceed 20% of LLOQ.

### 2.7. Pharmacokinetic study in rats

The pharmacokinetic study in rats was based on a single-dose, randomized, and sexual design. After fasting for 12 h, blood samples of about 0.3 mL were collected from orbital venous plexus into heparinized centrifuge tubes at 0.083, 0.167, 0.333, 0.667, 1, 2, 4, 6, 8, 12, and 24 h after oral administration of 1.0 mg/mL pimavanserin tartrate solution at a dose of 10 mg/kg. Plasma samples were obtained following centrifugation at 12,000 rpm for 5 min and then stored at  $-80^\circ\text{C}$  until determination (SD).

### 2.8. Statistical analysis

The pharmacokinetic parameters of pimavanserin in SD rats were calculated by DAS 2.1 software supplied by the Pharmacological Society of China (Beijing, China). The maximum plasma concentration ( $C_{\text{max}}$ ) and the time to reach  $C_{\text{max}}$  ( $T_{\text{max}}$ ) were directly obtained from the experimental data. In addition, independent T-test was made by SPSS 19 on gender difference of  $C_{\text{max}}$ ,  $T_{\text{max}}$ ,  $t_{1/2}$ ,  $AUC_{0-\infty}$ , and  $AUC_{0-24\text{ h}}$ . All data were expressed as means  $\pm$  standard deviation (SD).



**Fig. 2.** Representative MRM chromatograms of pimavanserin (1.44 min, MRM 1) and IS (1.36 min, MRM 2) in rat plasma prepared by protein precipitation. (A) Chromatogram of blank plasma. (B) Chromatogram of pimavanserin (1 ng/mL) and IS (20 ng/mL) spiked in blank plasma. (C) Representative chromatograms of rat plasma sample at 2 h after oral administration of pimavanserin at a dose of 10 mg/kg.

**Table 1**Inter- and intra-day precision, accuracy, recovery and matrix effect data for assays of pimavanserin ( $n = 6$ ).

Compound	Spiked conc. (ng/mL)	Intra-day precision (%)	Inter-day precision (%)	Accuracy (%)	Recovery (%)	Matrix effect (%)
Pimavanserin	2	8.3	14.2	7.1	77.2 ± 5.0	86.0 ± 2.4
	16	13.3	10.5	-3.0	76.6 ± 2.7	91.4 ± 1.9
	64	11.5	9.7	4.9	81.7 ± 5.9	99.4 ± 4.7
IS	20	–	–	–	77.0 ± 7.2	87.0 ± 1.5

### 3. Results and discussions

#### 3.1. Optimization of mass and chromatographic conditions

During initial infusion experiments with pimavanserin, the presence of proton adduct ions  $[M+H]^+$  was observed. According to the result of automatic compound optimization, the product ion  $m/z$  97.5 had the highest intensity, second to that of product ion  $m/z$  223.0. In this assay, two transitions were monitored; as a consequence, the transition  $m/z$  428.2 → 223.0 was robust and sensitive enough to be chosen as the final quantitative transition. The MS/MS parameters were also optimized to maximize the response for the IS of 748.5 → 589.5 in the positive ion mode. The full-scan product ion spectra of pimavanserin and IS are shown in Fig. 1.

In order to rapidly analyze the sample, an ACQUITY BEH  $C_{18}$  column was used for chromatographic separation using a Waters UPLC system (Waters, USA). The runtime was 2.5 min for one injection. To enhance the reproducible formation of  $[M+H]^+$  and sensitivity, investigations into a variety of mobile phase additives were conducted, including formic acid and ammonium acetate in different concentrations and ratios. The use of additives proved to be a necessity to control the peak type, which was achieved by the addition of 10 mM ammonium acetate in aqueous phase. Clarithromycin, a compound with a macrolide structure, was chosen as the IS because of its stable extraction recovery, appropriate chromatographic retention time (1.36 min), and ionization response similar to that of pimavanserin.

#### 3.2. Optimization of sample pre-treatment

To develop a better quantitative method, in the preliminary experiment, we investigated a PPT method, in which methanol and acetonitrile and the amount of reagent were investigated, respectively. The results revealed that twice amount ratio of plasma/methanol (v/v) could perform a higher recovery (75.2%) than that (68.6%) of acetonitrile, and had more qualified matrix effects (88.3%) than that (84.4%) of acetonitrile. As methanol protein precipitation method gained stable response, and high speed analysis, methanol was selected as the precipitating agent.

#### 3.3. Method validation

##### 3.3.1. Selectivity and matrix effect

According to the guides of the US FDA, the matrix effects should be investigated to ensure precision, selectivity, and sensitivity [15]. For this method, no endogenous interference was observed at the retention time of pimavanserin (1.44 min) or IS (1.36 min). Typical chromatograms obtained from blank plasma, blank plasma spiked with pimavanserin and IS, and an SD rat plasma sample obtained after oral administration of pimavanserin are shown in Fig. 2. The matrix effect was within the range of 86.0%–99.4%, indicating that no significant matrix effect was observed for pimavanserin and the mean matrix effect for the IS was 87.0% at a concentration of 20 ng/mL (Table 1).

##### 3.3.2. Linearity and LLOQ

The linearity was evaluated on three days with two sets of

**Table 2**Summary of stability of pimavanserin under various storage conditions ( $n = 3$ ).

Storage condition	Concentration spiked (ng/mL)	RE (%)	RSD (%)
Bench-top stability (12 h, 25 °C)	2	-0.5	4.0
	16	1.2	4.1
	64	1.5	1.1
Long-term stability (30 days, -80 °C)	2	3.2	1.8
	16	3.7	6.8
	64	–	7.9
Freeze-thaw stability (Three cycles, -80 °C)	2	-0.5	3.1
	16	1.4	5.2
	64	-2.7	2.0
Auto-sampler stability (8 h, 10 °C)	2	3.2	3.5
	16	0.5	1.4
	64	1.2	1.4

calibration curves each day. The calibration curves showed that the linearity was good over the concentration range of 1–80 ng/mL. Typical linear regression equation for the calibration curve was  $Y = 0.219X + 0.244$  ( $r = 0.9980$ ). All standards met the criteria of ≤ 15% deviation from nominal concentration. The LLOQ of pimavanserin was 1 ng/mL, stable and sufficient for this assay (the intra-, inter-day precisions and accuracy were all within 12.8%).

##### 3.3.3. Accuracy, precision, and recovery

Intra-day precision, inter-day precision, and accuracy for pimavanserin are summarized in Table 1. All results for the samples tested were within the acceptable criteria of ± 15%. The extraction efficiencies ranged from 76.6% to 81.7% for pimavanserin (Table 1), which indicated that recoveries were consistent, precise, and reproducible at different concentrations. The mean recovery of the IS was 77.0% at the concentration of 20 ng/mL.

##### 3.3.4. Stability

The stability of pimavanserin in SD rat plasma under different conditions is summarized in Table 2; the results indicated that pimavanserin was stable in plasma at room temperature for 12 h, at -80 °C for at least 30 day, after three freeze–thaw cycles, and at 10 °C in auto-sampler for 8 h after processed.

##### 3.3.5. Sample dilution and cross talk

To demonstrate the ability of dilution and analyze samples containing the analyte at concentrations above the ULOQ, a set of plasma samples were prepared containing pimavanserin at the concentration of 160 ng/mL. The samples were 2, 4, 8-fold diluted by blank matrix and analyzed in five replicates per dilution factor. The results of this experiment indicated that the dilution integrity of all the plasma samples (6.3%, 4.1% and -3.7% for 2, 4 and 8-fold diluted sample, respectively) was found to be less than 15% of their respective nominal concentrations.

Cross talk was investigated because the peaks between pimavanserin and IS were crossed. Comparing peak area of pimavanserin of plasma samples spiked only IS with that of LLOQ plasma samples spiked only pimavanserin in three replicates, respectively, indicated that the MRM transition of pimavanserin was without interference

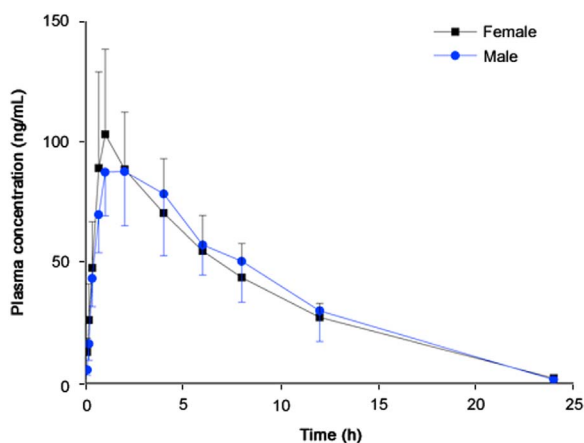


Fig. 3. Plasma concentration versus time curves for pimavanserin after oral administration of a dose of 10 mg/kg in SD rats ( $n = 4$ ).

Table 3

The main pharmacokinetic parameters of pimavanserin in SD rats after oral administration of pimavanserin at 10 mg/kg dose (mean  $\pm$  SD,  $n = 4$ ).

Parameters (unit)	Male	Female
$AUC_{0-24\text{ h}}$ ( $\mu\text{g/L}\cdot\text{h}$ )	901.22 $\pm$ 254.32	863.09 $\pm$ 219.22
$AUC_{0-\infty}$ ( $\mu\text{g/L}\cdot\text{h}$ )	938.03 $\pm$ 244.74	942.05 $\pm$ 225.21
$T_{\text{max}}$ (h)	1.75 $\pm$ 0.50	1.25 $\pm$ 0.50
$C_{\text{max}}$ (ng/mL)	95.73 $\pm$ 19.84	104.15 $\pm$ 34.47
$t_{1/2}$ (h)	4.58 $\pm$ 2.26	6.12 $\pm$ 3.39

$AUC_{0-24\text{ h}}$ : Area under the plasma concentration-time curve from zero hour to 24 h;  $AUC_{0-\infty}$ : Area under the plasma concentration-time curve from zero hour to infinity;  $T_{\text{max}}$ : The time to reach the  $C_{\text{max}}$ ;  $C_{\text{max}}$ : The maximum plasma concentration;  $t_{1/2}$ : Elimination half-life.

from IS.

### 3.4. Pharmacokinetic study in SD rats

The concentration–time curves (mean  $\pm$  SD) after oral administration of 10 mg/kg of pimavanserin are presented in Fig. 3 and the corresponding pharmacokinetic parameters are listed in Table 3. The result showed that the mean  $C_{\text{max}}$  of male and female rats were 95.73 ng/mL and 104.15 ng/mL, respectively, without significant difference between genders ( $p > 0.05$ ). The mean  $T_{\text{max}}$  of male and female rats were about 1.75 h and 1.25 h, respectively, which was the same as the result of Vanover et al. [10]. In addition, there were no significant differences in half-life,  $AUC_{0-24\text{ h}}$ , and  $AUC_{0-\infty}$  of gender ( $p > 0.05$ ) at this dosage level shown in Table 3. However, the  $AUC_{0-24\text{ h}}$  and  $AUC_{0-\infty}$  had differences among individuals whether in male or in female. It is noteworthy that a clinical pharmacokinetic research revealed that pimavanserin was well tolerated at a single oral doses of up to 300 mg and the pharmacokinetic profile of pimavanserin in healthy male volunteers was dose proportional, had low variability, and appeared reproducible, which suggested predictable exposure for future clinical trials [14]. Meanwhile, the  $T_{\text{max}}$  and  $t_{1/2}$  of these subjects were different from those of rats, probably indicating a different absorption mechanism to pimavanserin between humans and rats. It is necessary to further study pimavanserin in different animals and different doses in rats to reveal more pharmacokinetic features, which will demonstrate doses correlation with pharmacokinetics and pharmacodynamics. Nevertheless, the similar pharmacokinetic profile in rats demonstrates that the tartrate form of pimavanserin is effective.

## 4. Conclusion

A simple, rapid and sensitive UPLC-ESI-MS/MS method for the determination of pimavanserin in rat plasma has been developed and validated for the first time. This validated UPLC-ESI-MS/MS method with advantage of high speed can be used for high-throughput analysis and has been successfully applied to the pharmacokinetic study of pimavanserin in rats. Pharmacokinetic study indicated that pimavanserin plasma disposition in rat has a short  $T_{\text{max}}$  and a short terminal half-life, which is different from that in people, indicating the difference of pharmacokinetic course and action mechanism in rat.

## Conflicts of interest

The authors declare that there are no conflicts of interest.

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## References

- [1] P. Seeman, Atypical antipsychotics: mechanism of action, *Can. J. Psychiatry Rev. Can. De. Psychiatr.* 47 (2002) 27–38.
- [2] A. Abbas, B.L. Roth, Pimavanserin tartrate: a 5-HT<sub>2A</sub> inverse agonist with potential for treating various neuropsychiatric disorders, *Expert. Opin. Pharmacother.* 9 (2008) 3251–3259.
- [3] S.H. Fox, Pimavanserin as treatment for Parkinson's disease psychosis, *Lancet* 383 (2014) 494–496.
- [4] J.G. Goldman, S. Holden, Treatment of psychosis and dementia in Parkinson's disease, *Curr. Treat. Option Neurol.* 16 (2014) 1–18.
- [5] M. Horiguchi, K.E. Hannaway, A.E. Adekun, et al., Prevention of the phencyclidine-induced impairment in novel object recognition in female rats by co-administration of Lurasidone or Tansospirone, a 5-HT<sub>1A</sub> partial agonist, *Neuropsychopharmacology* 37 (2012) 2175–2183.
- [6] K.E. Vanover, D.M. Weiner, M. Makhay, et al., Pharmacological and behavioral profile of N-(4-fluorophenylmethyl)-N-(1-methylpiperidin-4-yl)-N'-(4-(2-methylpropyloxy) phenylmethyl) carbamide (2R, 3R)-dihydroxybutanedioate (2: 1)(ACP-103), a novel 5-hydroxytryptamine<sub>2A</sub> receptor inverse agonist, *J. Pharmacol. Exp. Ther.* 317 (2006) 910–918.
- [7] U. Hacksell, E.S. Burstein, K. McFarland, et al., On the discovery and development of pimavanserin: a novel drug candidate for Parkinson's Psychosis, *Neurochem. Res.* 39 (2014) 2008–2017.
- [8] D. Hubbard, U. Hacksell, K. McFarland, Behavioral effects of clozapine, pimavanserin, and quetiapine in rodent models of Parkinson's disease and Parkinson's disease psychosis: evaluation of therapeutic ratios, *Behav. Pharmacol.* 24 (2013) 628–632.
- [9] D.L. Price, D.W. Bonhaus, K. McFarland, Pimavanserin, a 5-HT<sub>2A</sub> receptor inverse agonist, reverses psychosis-like behaviors in a rodent model of Alzheimer's disease, *Behav. Pharmacol.* 23 (2013) 426–433.
- [10] K.E. Vanover, A.J. Betz, S.M. Weber, et al., A 5-HT<sub>2A</sub> receptor inverse agonist, ACP-103, reduces tremor in a rat model and levodopa-induced dyskinesias in a monkey model, *Pharmacol. Biochem. Behav.* 90 (2008) 540–544.
- [11] B.R. Teegarden, H. Al Shamma, Y.F. Xiong, 5-HT<sub>2A</sub> inverse-agonists for the treatment of insomnia, *Curr. Top. Med. Chem.* 8 (2008) 969–976.
- [12] S. Ancoli-Israel, K.E. Vanover, D.M. Weiner, et al., Pimavanserin tartrate, a 5-HT<sub>2A</sub> receptor inverse agonist, increases slow wave sleep as measured by polysomnography in healthy adult volunteers, *Sleep Med.* 12 (2011) 134–141.
- [13] R. Mills, D. Bahr, K. Chi-Burris, et al., Improved nighttime sleep and increased daytime wakefulness in patients with Parkinson's disease psychosis treated with pimavanserin, a selective 5-HT<sub>2A</sub> antagonist, *Mov. Disord.* 28 (2013) (S225–S225).
- [14] K.E. Vanover, D. Robbins-Weilert, D.G. Wilbraham, et al., Pharmacokinetic, tolerability, and safety of ACP-103 following single or multiple oral dose administration in healthy volunteers, *J. Clin. Pharmacol.* 47 (2007) 704–714.
- [15] USFDA, Guidance for Industry. Bioanalytical Method Validation, United States Department of Health and Human Service Center Food and Drug Administration, USA, 2001. ([https://www.researchgate.net/publication/50927547\\_USFDA\\_Guidance\\_for\\_Industry\\_Bioanalytical\\_Method\\_Validation](https://www.researchgate.net/publication/50927547_USFDA_Guidance_for_Industry_Bioanalytical_Method_Validation)).
- [16] R.B. Jain, Comparison of three weighting schemes in weighted regression analysis for use in a chemistry laboratory, *Clin. Chim. Acta* 411 (2011) 270–279.