ASIAN JOURNAL OF PHARMACEUTICAL SCIENCES 9 (2014) 286–292

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Short communication

Development and validation of a rapid and sensitive UHPLC–MS/MS method for the determination of paliperidone in beagle dog plasma

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ARTICLE INFO

Article history:
Received 9 April 2014
Received in revised form
19 June 2014
Accepted 1 July 2014
Available online 28 August 2014

Keywords:
Paliperidone
Dog plasma
UHPLC–MS/MS
Pharmacokinetics

ABSTRACT

In order to evaluate the pharmacokinetic profile of paliperidone extended-release tablets *in vivo*, a simple and rapid ultra-high performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) method was developed and validated for the determination of paliperidone in beagle dog plasma. Paliperidone and diazepam (internal standard) were extracted from plasma samples with diethyl ether, and then separated on a C_{18} column (2.1 × 50 mm, 2.6 μm) under gradient elution with methanol–0.1% formic acid at a flow rate of 0.3 ml/min. The compounds were detected using a triple-quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source. The validated method was linear over the concentration range of 1.00–1000.00 ng/ml and the lower limit of quantitation was 1.00 ng/ml. The intra-day and inter-day precision values were not more than 15% (relative standard deviation < 20% at low levels), while the accuracy was within ±10% of nominal values. The validated UHPLC–MS/MS method was successfully applied to an oral pharmacokinetic study of paliperidone extended-release tablets in a beagle dog.

1. Introduction

Risperidone is a second-generation antipsychotic agent widely used in the treatment of schizophrenia. It is converted to the active metabolite, paliperidone, *in vivo*. In fact, the "active fraction" during risperidone treatment is considered to be the sum of risperidone and paliperidone [1–3].

Among neurological diseases, schizophrenia is a long-term recurrent and chronic disease which requires a long-term

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Peer review under responsibility of Shenyang Pharmaceutical University.
http://dx.doi.org/10.1016/j.ajps.2014.07.008

treatment with antipsychotic agents [4,5]. However, after long-term continuous medication with routine medicines, a lot of adverse reactions, poor drug tolerance and compliance may occur. In the light of these problems, an osmotic-controlled release oral delivery system (OROS) has been used to prepare paliperidone extended-release tablets. This results in relatively stable and effective plasma concentrations over 24 h, which permits once a day dosing and treatment using a potentially effective dose, to reduce any negative side effects and improve patient compliance and tolerance [6–8].

Earlier publications have described methods for simultaneous determination of paliperidone and risperidone in a biological matrix using high performance liquid chromatography (HPLC) methods with ultraviolet detection (UV) or electrochemical detection [9–13], and liquid chromatography–tandem mass spectrometry (LC-MS/MS) methods [14,15]. However, these methods are not suitable for the determination of paliperidone alone because of the long run time and/or complicated sample processing procedures. Several methods for the determination of paliperidone together with a variety of other drugs and metabolites in biological samples have been published using LC-MS/MS methods [16,17]. However, using protein precipitation (PPT), the samples are dirty and cause damage to the chromatographic column and mass spectrometry system.

To date, some publications for the determination of paliperidone alone in biological fluids have been reported. Xu et al. [18] reported a method involving liquid–liquid extraction (LLE)-HPLC with UV-detection but this required large volumes of biological samples and the sample processing was complicated. Vermeir et al. [19] developed an LC-MS/MS method for the determination of paliperidone in human plasma, which achieved a better sensitivity and the chromatographic separation time was about 6 min. However, as far as sample preparation was concerned, it used a solid-phase extraction (SPE) method which was complex and expensive.

In this paper, we attempted to develop a simple and rapid method for the determination of paliperidone in beagle plasma using a UHPLC–MS/MS method to evaluate the oral pharmacokinetics of paliperidone extended-release tablets. Compared with previous methods, the present method had the following advantages: less plasma was required, sample preparation was simpler, and the analysis time was shorter. The lower limit of quantitation (LLOQ) was 1 ng/ml with LLE using 200 μl plasma, which was sensitive enough to measure the low concentrations of paliperidone in beagle plasma. Moreover, the analysis time for a single sample was 3 min. Finally, the method was successfully applied to evaluate the pharmacokinetic characteristics after oral administration of paliperidone to a beagle dog.

2. Materials and methods

2.1. Materials

Paliperidone (99.82% purity) was provided by a laboratory in Shenyang Pharmaceutical University, and its structure was validated by comparison with chemical and spectroscopic (UV, MS and NMR) data, and its purity was measured by the area normalization method. Diazepam (purity > 99.0%) was purchased from the Zhejiang Xinhua Pharmaceutical Co. Ltd (Zhejiang, China). Methanol (HPLC grade) was purchased from Fisher Scientific (Pittsburgh, PA, USA). Formic acid (HPLC grade) was obtained from Dikma Reagent Company (Ohio, USA). Diethyl ether was purchased from Concord Chemical (Shenyang, China). Paliperidone extended-release tablets were prepared in the laboratory (3 mg).

2.2. Instrumentation

Chromatography was performed on a Waters ACQUITY™ TQD system, which consisted of an ACQUITY™ Ultra Performance Liquid Chromatography system coupled to an ACQUITY™ triple-quadrupole tandem mass spectrometer with an electrospray ionization (ESI) interface (Waters Corp., Milford, MA, USA). A phenomenex C_{18} column (2.1 × 50 mm, 2.6 μm; Phenomenex Co., USA) was used to separate the analytes.

2.3. UHPLC-MS-MS conditions

Gradient elution was conducted for chromatographic separation with methanol as mobile phase A and 0.1% formic acid as the mobile phase B. At a flow rate of 0.3 ml/min, the amount of eluent A was increased linearly from 20% to 80% over 1.5 min, then held at 80% for 1 min and returned to the initial conditions and re-equilibration took place for another 0.5 min. This resulted in a total run time of 3 min per sample. The column and auto-sampler temperature was 40 °C and 4 °C, respectively and the sample volume injected was 5 ml.

Regarding MS detection, the ESI was operated in positive mode. The capillary voltage was 3.8 kV and the cone voltage was 35 V for both paliperidone and diazepam; the collision energy of paliperidone and diazepam was 28 and 23 V, respectively; nitrogen was used as the desolvation and cone gas at 500 L/h and 50 L/h, respectively; the source temperature was 120 °C and the desolvation temperature was 350 °C. Quantification was performed in multiple-reaction monitoring (MRM) mode of the transitions *m/z* 427.31 → 207.18 for paliperidone and *m/z* 285.12 → 193.13 for diazepam. The full-scan product ion spectra of paliperidone and diazepam are shown in Fig. 1.

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

Stock solutions of paliperidone (1 mg/ml) and diazepam (0.1 mg/ml) were separately prepared in methanol. The stock solution of paliperidone was further diluted with methanol to obtain working solutions for the preparation of calibration standards. All solutions were stored at 4 °C and were brought to room temperature before use.

The diazepam solution was further diluted with methanol to 500.00 ng/ml. Calibration standard samples were prepared by spiking 200 μl samples of blank beagle plasma with 50 μl paliperidone working standard solutions. The final concentrations in standard plasma samples were 1.00, 2.00, 10.00, 50.00, 100.00, 500.00, and 1000.00 ng/ml. The QC samples were

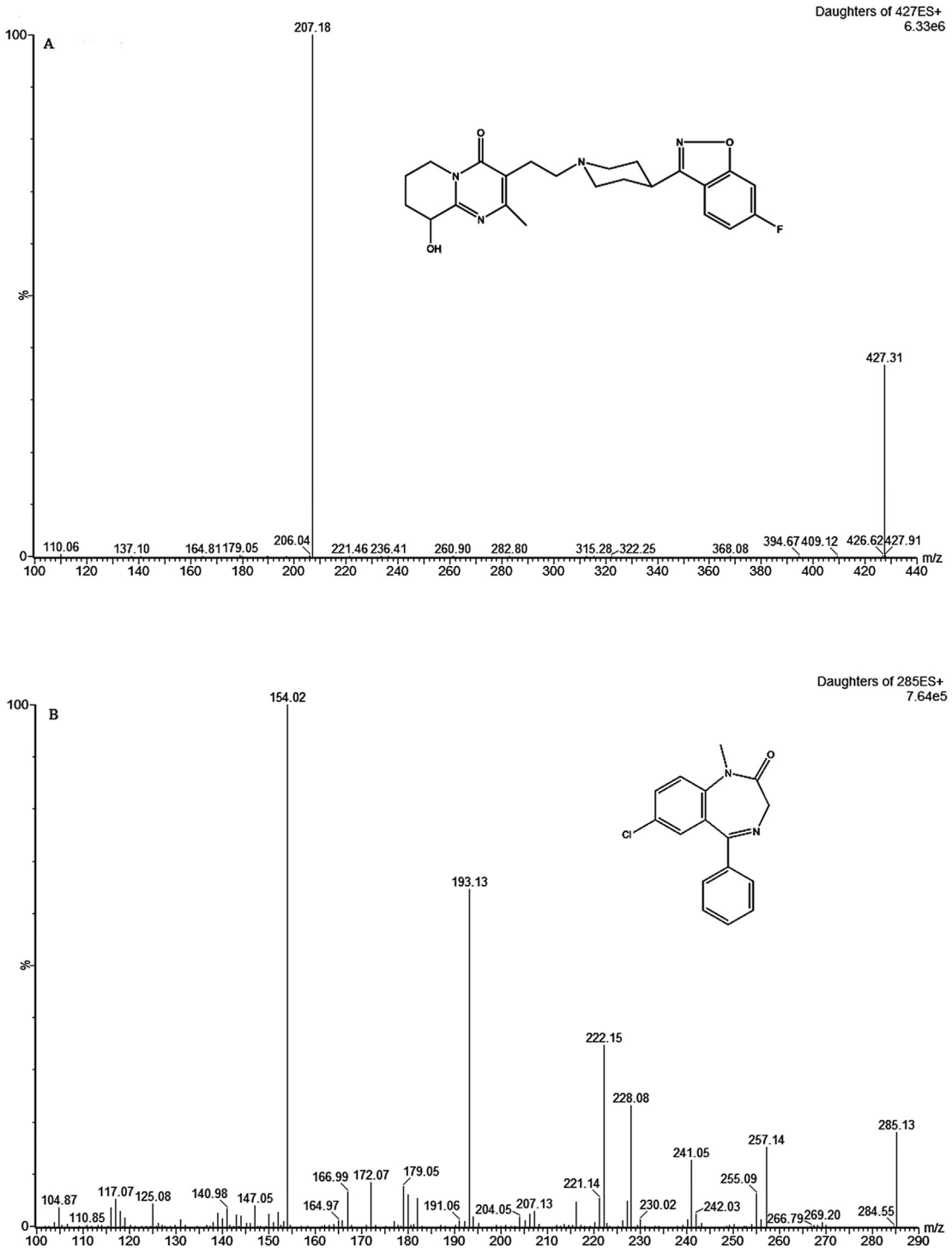

Fig. 1 – Full-scan positive mode product ion mass spectrum of (A) paliperidone and (B) diazepam.

prepared with blank plasma at low, middle and high concentrations of 2.00, 50.00 and 800 ng/ml.

2.5. *Plasma sample preparation*

Plasma (200 μl), 50 μl diazepam solution (500 ng/ml) and 50 μl methanol were added to 10.0 ml screw-capped tubes. After briefly vortexing for 1 min, 2 ml diethyl ether was added and the mixture was vortexed for another 3 min. This was followed by centrifugation at 3500 rpm for 10 min, then the upper organic layer was transferred to another 10.0 ml centrifuge tube and evaporated to dryness under a nitrogen stream at 37 °C. The residue was then reconstituted with 100 μl methanol-water (80:20, v/v). After centrifugation at 13,000 rpm for 10 min, 5 μl aliquots were injected into the UHPLC–MS/MS system for analysis.

2.6. Method validation

Selectivity was investigated by comparing the chromatograms of blank beagle plasma with the corresponding spiked beagle plasma. Linearity was assessed by weighted ($1/x^2$) linear regression analysis of the paliperidone/diazepam peak area ratio versus the nominal concentration (x) of paliperidone in plasma. LLOQ was used to define the sensitivity with the concentration of 1.00 ng/ml, with an acceptable accuracy between 80% and 120% and a precision with a deviation not exceeding 20%. The intra-and inter-day precision and accuracy were determined by analysis of six replicates at low, middle, and high QC levels on the same day and three batches on three different days. The matrix effect was investigated by comparing the peak areas of the post-extracted blank plasma with those of the corresponding standard solutions. The extraction recovery was evaluated by comparing the responses of six replicates of extracted QC samples with the mean peak area response of those samples extracted before adding paliperidone and diazepam at three QC levels. The stability of low, middle and high QC samples ($n = 3$) was measured under different conditions: freeze–thaw stability (−20 °C to ambient temperature), post-preparation stability (kept in an auto-sampler at 4 °C for about 12 h), short-term stability (ambient temperature for 12 h) and long-term stability (−20 °C for 40 days).

2.7. Application to a pharmacokinetic study

After a 24 h fast, a healthy dog was given two paliperidone extended-release tablets (6 mg). All animal experiments were performed in accordance with the institutional guidelines and approved by the Shenyang Pharmaceutical University Animal Care and Use Committee .The blood samples (5 ml) were collected in heparinized plastic tubes at 0, 2, 4, 8, 12, 16, 18, 20, 22, 24, 26, 30, 32, 36, 48, 60, and 72 h, respectively. Following centrifugation at 13,000 rpm for 10 min, the plasma samples were separated and stored at −20 °C until analysis.

3. Results and discussion

3.1. Method development

Gradient elution was applied to shorten the run time. Using methanol as eluent A, and formic acid (0.1%) as eluent B, eluent A was increased linearly from 20% to 80% over the first 1.5 min, followed by a 1 min hold at 80%. Formic acid (0.1%) is important because it improves the peak shape.

LLE, SPE and protein precipitation (PPT) are the most widely used methods for sample preparation. Although the matrix effect and recovery of SPE are adequate, the method is complicated and expensive. Although PPT is rapid and simple to perform, the method resulted in a strong matrix effect. LLE was examined to obtain cleaner samples. In this experiment, diethyl ether and methyl *tert*-butyl ether were examined as the extraction solvent. Methyl *tert*-butyl ether exhibited a strong matrix effect. Finally, diethyl ether was selected as the extraction solvent because it produced a higher recovery and lower matrix effect.

3.2. Method validation

3.2.1. Selectivity

Selectivity was assessed by comparing the chromatograms of blank plasma, standard solution (1.00 ng/ml), blank plasma spiked with paliperidone (1.00 ng/ml) and a diazepam and plasma sample about 18 h after oral administration of paliperidone. As shown in Fig. 2, there was no interference with paliperidone and diazepam in the blank plasma samples.

3.2.2. Linearity and sensitivity

The calibration curves exhibited excellent linearity with regression correlation coefficients ($r^2 > 0.99$) over the concentration range of 1.00–1000.00 ng/ml in dog plasma. A typical regression equation was: $y = 8.77x + 7.51$ with a determination coefficient (r^2) of 0.9960. The LLOQ was 1.00 ng/ml for paliperidone, the precision was 13% of the relative standard deviation (RSD) and the accuracy was 11% of the relative error (RE).

3.2.3. Precision and accuracy

The intra-day and inter-day precision and accuracy for paliperidone are summarized in Table 1.The intra-day and inter-day precisions were all below 15% (RSD < 20% at low levels) and the maximum deviation in accuracy was below 10%.

3.2.4. Extraction recovery and matrix effect

The extraction recoveries from beagle dog plasma were excellent for paliperidone: 92.10 ± 2.99%, 87.83 ± 1.60%, 92.25 ± 1.81% for the low, middle and high QC levels, respectively, and the extraction recovery of diazepam was 90.22 ± 2.10%.

In our experiment, all the ratios of the matrix effect evaluated were between 88.47% and 95.64%, which indicated that the endogenous substances had no significant effect on the two study compounds.

3.2.5. Stability

The stability of paliperidone in dog plasma was investigated under a variety of storage and process conditions, which are summarized in Table 2. The results indicated that paliperidone was stable under the conditions described.

3.3. Pharmacokinetic application

The validated method was successfully applied to the pharmacokinetic study of paliperidone following oral administration of paliperidone extended-release tablets to a beagle dog. The plasma concentration–time profile of paliperidone is illustrated in Fig. 3. The pharmacokinetic data indicated that paliperidone reached a peak concentration in plasma about 18 h after oral administration and paliperidone could still be quantified in plasma 72 h after oral administration. The application to the pharmacokinetic study of paliperidone indicated that this analytical method was suitable and

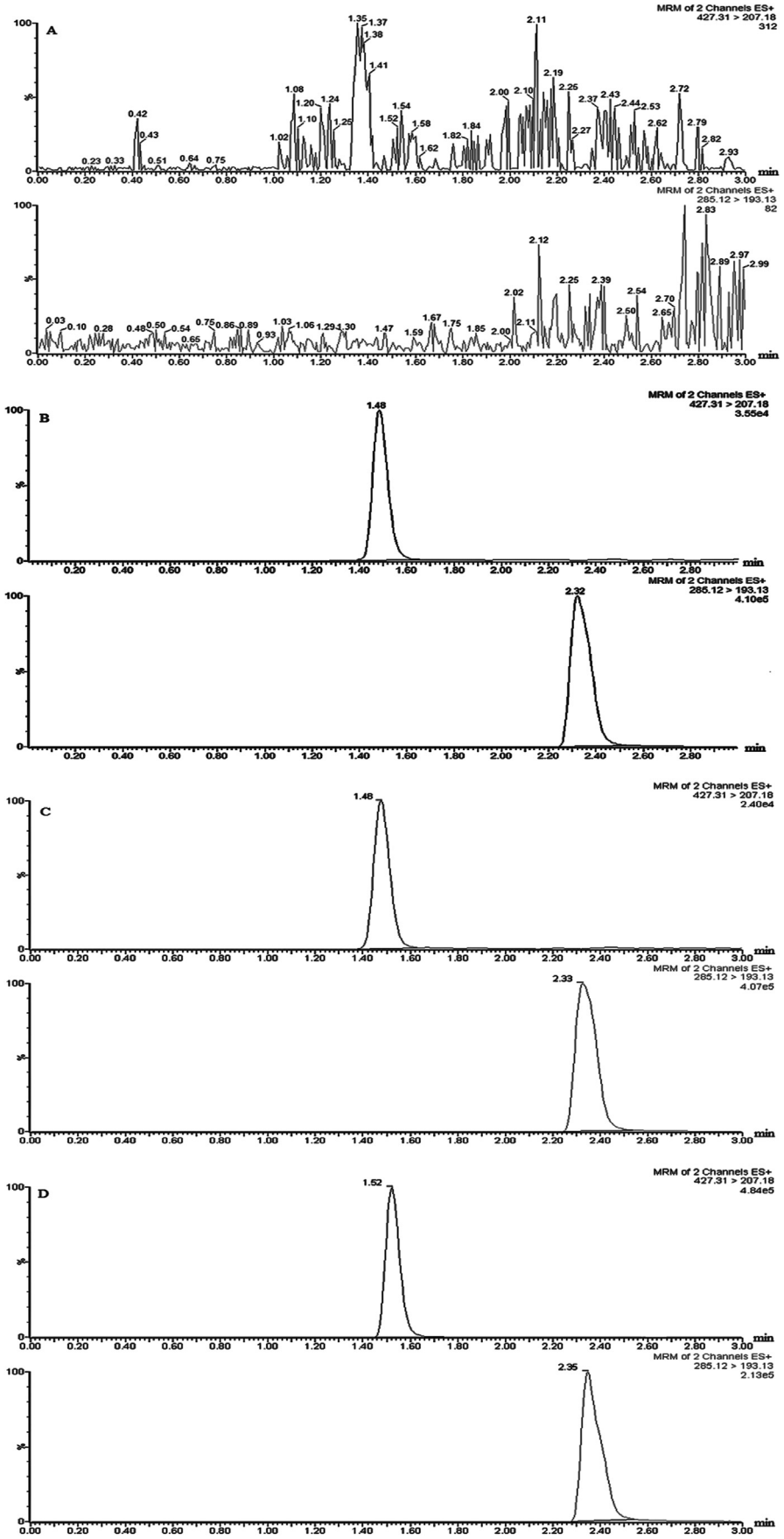

Fig. 2 – MRM chromatograms of paliperidone and diazepam from (A) a blank plasma sample; (B) standard solution sample (1.00 ng/ml); (C) a plasma sample with added paliperidone and diazepam at the LLOQ level; (D) a plasma sample from a dog following oral administration of paliperidone (6 mg).

Table 1 – Precision and accuracy data for assay of paliperidone (intra-day: $n = 6$; inter-day: $n = 6$ series per day, 3 days).

Added (ng/ml)	Found (ng/ml)	Intra-day RSD (%)	Inter-day RSD (%)	Accuracy RE (%)
2.00	1.99 ± 0.16	6.7	15	0.50
50.00	45.39 ± 1.70	3.9	2.4	9
800.00	794.94 ± 19.10	2.4	2.1	0.63

Table 2 – Stability of paliperidone in dog plasma under indicated conditions (mean ± SD, $n = 3$).

Conditions	Add (ng/ml)	Found (ng/ml)	RSD%
Post-preparation stability	2.00	2.11 ± 0.34	16
	50.00	44.52 ± 1.22	2.7
	800.00	792.90 ± 28.90	3.6
Short-term stability	2.00	2.28 ± 0.11	4.8
	50.00	52.13 ± 1.19	2.3
	800.00	725.28 ± 17.88	2.5
Freeze–thaw stability	2.00	2.10 ± 0.19	9
	50.00	43.07 ± 0.30	0.70
	800.00	815.17 ± 15.28	1.9
Long-term stability	2.00	1.78 ± 0.17	10
	50.00	43.26 ± 0.88	2.0
	800.00	808.61 ± 58.18	7.2

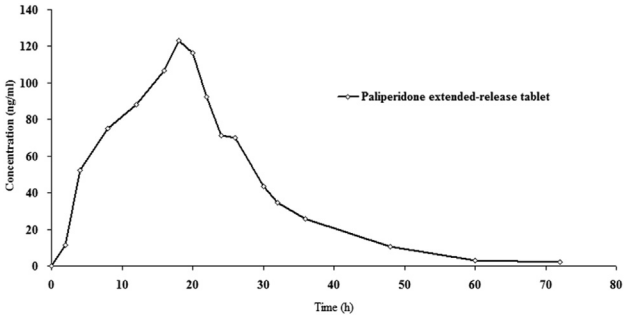

Fig. 3 – Concentration–time profile of paliperidone in beagle plasma after a single oral dose of 6 mg paliperidone extended-release tablets ($n = 1$).

sufficiently sensitive for analyzing paliperidone in beagle dog plasma.

4. Conclusion

This paper describes a rapid, sensitive and specific UHPLC–MS/MS method developed for the quantification of paliperidone and its validation for the analysis of paliperidone in beagle plasma. The UHPLC–MS/MS method has significant advantages over other techniques including the simplicity of sample preparation; sharper peaks and a higher extraction recovery and a shorter chromatographic run time. The method has been successfully applied to the pharmacokinetic study of paliperidone in dog plasma samples.

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