Journal of Chromatographic Science 2012;50:902–908
doi:10.1093/chromsci/bms088 Advance Access publication June 7, 2012

Article

High-Performance Liquid Chromatographic Assay for Ziprasidone in Plasma Samples: Application to Pharmacokinetic Studies in Rats

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Received 1 November 2011; revised 8 March 2012

Ziprasidone (ZRS) is among the various antipsychotic drugs indicated for treating schizophrenia. The determination of the pharmacokinetic behavior of this drug is of utmost importance in evaluating its bioavailability. The objectives of the present study are: (1) to develop and validate a sensitive, specific, accurate and precise reverse-phase high performance liquid chromatographic method for quantification of ZRS in the plasma of rats; and (2) to apply the developed method to study the pharmacokinetic profile of ZRS in rats after oral administration. The method uses a C18 (250.0 × 4.6 mm, 5 μm) column and ultraviolet detector with wavelength set at 210.0 nm. The mobile phase is acetonitrile–phosphate buffer (pH 3.6) 28:72% v/v at a flow rate of 1.0 mL/min. The internal standard (IS) is escitalopram. The extraction procedure for ZRS and IS from the biological matrix (plasma) employs liquid–liquid extraction technique using a mixture of methyl *tert*-butyl ether–dichloromethane (70:30% v/v). The results show good accuracy and precision over a linearity range of 20.0–3,000.0 ng/mL with $r^2 \geq 0.9986$. The mean recoveries of ZRS and IS are 79.32 ± 1.16 and 84.10 ± 3.2%, respectively. The method has been successfully utilized to study the pharmacokinetic profile of ZRS in rats after oral administration.

Introduction

Ziprasidone (ZRS) is a typical second-generation antipsychotic drug approved by the Food and Drug Administration for use in schizophrenia, bipolar disorder and acute agitation. ZRS is a benzisoxazole derivative, more precisely a benzothiazolylpiperazine (Figure 1) with the IUPAC name 5-[2-(4–benzo[d] isothiazol-3-yl-piperazin-1-yl) ethyl]-6-chloro-1,3-dihydroindol-2-one (1, 2). ZRS functions as an antagonist at the D2, 5HT2A and 5HT1D receptors and as an agonist at the 5HT1A receptor. The 5HT2A affinity of ZRS is approximately eight times that of D2, which creates a balance that promotes antipsychotic effects and limits extrapyramidal side effects. ZRS also inhibits synaptic reuptake of serotonin and norepinephrine (3, 4). It is widely used in treating bipolar disorder, acute agitation states in schizophrenic patients and acute mania.

On oral administration, ZRS is absorbed easily, which increases two-fold in the presence of food. More than 99% ZRS is in plasma protein bound form and is metabolized extensively by phase I and II metabolic pathways (5, 6). For antipsychotic drugs, plasma level monitoring is one of the critical aspects to be considered with respect to bioavailability studies and therapeutic drug monitoring. It is important to have an efficient analytical tool to study the pharmacokinetic profiles in animal models before clinical studies.

Several published procedures describe the quantification of ZRS in preclinical and clinical plasma samples. Stability-indicating assay using chromatographic methods have been reported for the estimation of the ZRS in bulk drugs and pharmaceutical formulations (7, 8). The simultaneous estimation of five antipsychotic drugs, olanzapine, haloperidol, chlorpromazine, ziprasidone, risperidone and 9-hydroxyrisperidone (active metabolite) in rat plasma using high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection has been reported (9). This method extracted the analytes from 1.0 mL of rat plasma using liquid–liquid extraction. Determination of ZRS using HPLC with fluorescence detection and column switching with UV detection in human plasma have also been reported. These methods were highly sensitive, with a lower limit of quantification (LLOQ) 0.5 ng/mL (liquid–liquid extraction) and 10.0 ng/mL (solid-phase extraction) using 1.0 mL of human plasma (10, 11). Another published HPLC–UV method using solid-phase extraction of ZRS from human serum reported a LLOQ of 1.0 ng/mL (12). This method utilized narrow-bore columns with a weak cation exchange solid-phase extraction technique. Methods for the quantification of ZRS in human and rat plasma using ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS-MS) and LC–MS-MS have also been reported (13–18). Kul *et al.* developed an electroanalytical method for the quantification of ZRS in pharmaceutical formulation and serum samples on solid electrodes. These methods are simple and selective, but require the specific electrodes (19). A comparison of the previously reported methods is presented in Table I. Although sensitive, the previously mentioned methods lack simplicity in the sense that they use sophisticated instruments such as HPLC–MS-MS and UPLC–MS-MS, and require a high volume of plasma and lengthy extraction procedure.

The present work aims at developing a simple, selective, fully validated and sensitive HPLC method for the quantification of ZRS with very low plasma requirement. Conventional single-step liquid–liquid extraction of the analyte is the only planned extraction method. Thus, the method is designed to be useful in preclinical pharmacokinetic studies of orally administered ZRS in rats.

Experimental

Chemicals and reagents

Ziprasidone hydrochloride (99.56%) and ecitalopram hydrochloride (99.64%) (internal standard, IS) were supplied by

Lupin Pharmaceuticals (Pune, Maharashtra, India). Acetonitrile and methanol (HPLC grade) were purchased from Merck (Mumbai, Maharashtra, India). Potassium dihydrogen orthophosphate, triethylamine and orthophosphoric acid were purchased from Merck. Methyl *tert*-butyl ether (MTBE) and dichloromethane (DCM) (HPLC grade) were purchased from Spectrochem (Mumbai, Maharashtra, India). HPLC-grade water was produced in our laboratory by a Milli-Q purification system (Millipore; Vienna, Austria). Filtration of the HPLC mobile phase was performed using 0.45-μm membrane filters obtained from Pall (Bangalore, India). A TurboVap evaporator (Zymark; Hopkinton, MA) was used for evaporation of the organic solvent. A C24 centrifuge (Remi India; Bangalore, Karnataka, India) was used for centrifugation. Disodium ethylenediamine tetra-acetic acid (Na_2EDTA) vacutainers (BD; Franklin Lakes, NJ) were used for collection of blood.

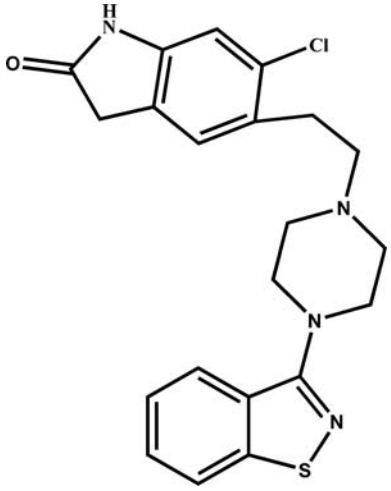

Figure 1. Structure of ziprasidone.

Chromatographic system and conditions

The HPLC system consisted of a Waters Alliance 2695 low-pressure gradient separation module (Waters, Milford, MA) with the configuration of quaternary solvent delivery pump, sampler cooler, column heater and 2487 dual wavelength UV detector. Chromatographic system operation and data recording were performed with Millenium^{32} (version 4.0) chromatography software (Waters). The analyte separation was performed on a Grace Vydac C18 monomeric (250.0 × 4.6 mm, 5 μm) column set at 25°C and the effluents were monitored using a UV detector set at 210.0 nm. The mobile phase was acetonitrile–potassium dihydrogen phosphate buffer (pH 3.6 ± 0.1; 20.0 mM containing 0.2 % v/v triethylamine) in the ratio of 28:72% v/v at a flow rate of 1.0 mL/min.

Preparation of calibration standards and quality control samples

Primary stock solutions of ZRS (1.0 mg/mL) were prepared by sonication of the drug in acetonitrile–methanol (1:1) and IS (1.0 mg/mL) in methanol and stored at 2–8°C when not in use. Working solutions of ZRS and IS (100.0 μg/mL) were prepared separately by diluting primary stock solutions with methanol. ZRS working solution was used to prepare the spiking stock solutions in methanol to concentrations in the range of 400.0 to 60,000.0 ng/mL (400.0, 600.0, 1,200.0, 2,000.0, 4,000.0, 10,000.0, 20,000.0, 40,000.0 and 60,000.0 ng/mL). The quality control (QC) standards; i.e., low quality control (LQC), middle quality control (MQC) and high quality control (HQC), spiking stock solutions were prepared by serial dilution from the stock solution to concentrations of 800.0, 8,000.0, and 50,000.0 ng/mL spiking stock solutions.

Calibration standards were prepared by bulk plasma spiking of 50.0 μL of the previously prepared working calibration solutions in 950.0 μL of blank rat plasma to achieve concentrations of calibration standards equivalent to 20.0, 30.0, 60.0, 100.0, 200.0, 500.0, 1,000.0, 2,000.0 and 3,000.0 ng/mL. Similarly, QCs were prepared from the stock solutions (i.e., 800.0, 8,000.0, and 50,000.0 ng/mL) to concentrations of 40.0, 400.0 and

Table I
Comparison of the Reported Methods

Author	Technique	Matrix (volume)	Linearity range	Extraction procedure
Zhang *et al.* (9)	HPLC–UV	Rat plasma (1.0 mL)	2.0–500.0 ng/mL	LLE and acid solution back-extraction technique
Suckowa *et al.* (10)	HPLC– fluorescence detection	Human plasma (1.0 mL)	0.5–200.0 ng/mL	LLE using MTBE
Sachse *et al.* (11)	HPLC–UV	Human serum (100.0 μL)	20.0–600.0 ng/mL	On-line sample clean up using column filled with silica CN
Janiszewski *et al.* (12)	HPLC–UV	Human serum (500.0 μL)	1.0–250.0 ng/mL	Solid-phase extraction
Al-Dirbashi *et al.* (13)	LC–MS-MS	Human plasma (1.0 mL)	0.1–200.0 ng/mL	LLE using alkalinized plasma with MTBE
Aravagiri *et al.* (14)	LC–MS-MS	Human plasma (500.0 μL)	0.25–500.0 ng/mL	LLE using 20% methylene dichloride in pentane
Zhang *et al.* (15)	LC–ESI-MS-MS	Rat plasma (250.0 μL) and brain homogenate (200.0 μL)	0.2– 200.0 ng/mL for rat plasma and 0.833–833.3 ng/g for brain tissue	LLE using isopropyl ether–methyl chloride (80:20% v/v)
Yan-Qing *et al.* (16)	UPLC–MS-MS	Human plasma (1.0 mL)	0.7–400.0 ng/ mL	Solid-phase extraction
Zhang *et. al*. (17)	LC–MS-MS	Rat brain homogenate (200.0 μL)	0.208–416.0 ng/g	LLE using isopropyl ether
Zhang *et al.* (18)	LC–MS-MS	Rat plasma (250.0 μL)	0.1–10.0 ng/mL	LLE using isopropyl ether
Kul *et al.* (19)	Electroanalytical using voltametry	Human serum (2.0 mL)	$2 \times 10^{-6} - 6 \times 10^{-5}$M	Protein precipitation using acetonitrile

2,500.0 ng/mL as LQC, MQC and HQC. All standards were stored at −80°C when not in use.

Sample extraction procedure

The procedure was developed using 150.0 μL spiked plasma aliquots. Pooled rat drug-free plasma was obtained from the central animal house facility of Manipal University, Manipal and stored frozen at −80°C. The plasma samples were thawed at room temperature and used further. To extract the drug from plasma (blank, standard or study samples), 150.0 μL of plasma was transferred to 2.0 mL propylene centrifuge tubes to which 10.0 μL of the IS (20.0 μg/mL) was added and vortexed for a few seconds. The plasma was alkalinized with 50.0 μL of 1.0 M sodium carbonate (pH 11.5) as an extraction additive for improving the extraction efficiency. Chilled acetonitrile (200.0 μL) was added and the mixture was vortexed for 20 s. A further 1.7 mL of an MTBE–DCM (70:30% v/v) mixture was added and vortexed for 1 min. The resulting solution was centrifuged at 5,000 × g for 10 min. The supernatant organic phase was transferred to nitrogen evaporation tubes and evaporated to dryness at 45°C under a gentle stream of nitrogen. The residue was reconstituted with 100.0 μL of mobile phase, vortexed, transferred to insert vials and 50.0 μL was injected to HPLC system. All procedures for sample preparation were carried out at ambient temperature.

Method Validation

Specificity and selectivity

Six blank plasma samples (from six different rats), hemolyzed plasma sample and a set of six plasma spiked samples with ZRS at LLOQ concentration were evaluated for selectivity and specificity of the method. All samples were processed using the developed extraction procedure and analyzed by proposed HPLC method.

Calibration curves and linearity

Five different calibration curves in a concentration range of 20.0–3,000.0 ng/mL were prepared. The standard calibration curve was constructed by plotting peak-area ratio of ZRS and IS versus ZRS concentration in plasma standards. The acceptance criteria for each back calculated standard was ± 15% deviation from the nominal value, except LLOQ, which was ± 20%. A linear regression analysis was performed using mathematical transformation to determine slope, intercept and coefficient of correlation.

Precision and accuracy

The accuracy and precision of the present analytical method were evaluated at LLOQ and a set of three QCs. QC samples were prepared as described previously. The different sets of solutions were injected six times into the HPLC system for quantitative determination to evaluate intra-day precision and accuracy under the same conditions and within a day. The same procedure was performed once a day for six consecutive days to determine inter-day precision and accuracy. Accuracy (expressed as percentage actual value) and precision [expressed as percentage coefficient of variation (CV)] of the QCs were calculated. The criteria for acceptability of the data for accuracy was within 85–115% deviation from the nominal values and the precision was within ± 15% CV, except for LLOQ, which could have an accuracy of 80–120% and percentage CV of ± 20% .

Recovery

The recovery of ZRS and IS through a liquid–liquid extraction (LLE) procedure was determined by comparing the peak area of the extracted plasma samples at three QC levels ($n = 6$) with control samples. As controls, equivalent amounts of the ZRS and IS were added directly into the mobile phase and injected. Recovery was determined at low, medium and high concentrations, i.e., 40.0, 400.00 and 2,500.00 ng/mL, and the recovery of the IS was determined at a single concentration of 500.0 ng/mL.

Recovery was assessed by comparing the chromatographic peak area of ZRS and IS of the extracted plasma standards to those obtained from equivalent amounts of ZRS and IS spiked directly into the mobile phase (corresponding to 100% recovery).

Stability studies

The stability of the analyte was carried out at different stability conditions. The stability studies of ZRS were carried out at LQC and HQC levels. Stability was assessed by comparing the mean concentrations of the stored QC samples with the mean concentrations of those prepared freshly. The stability of ZRS in plasma at various stability conditions such as short-term stability at room temperature for 6 h, post-preparative, freeze-thaw and long-term stability was studied. Post-preparative stability, i.e., autosampler stability, was conducted by reanalyzing QC samples kept under autosampler conditions for 24 h before injections. The freeze-thaw stability was studied at three cycles at −80°C. Long-term stability of ZRS was studied at −80°C for a period of 30 days. Samples were regarded as stable if percent accuracy was within 85–115%.

Application of method for pharmacokinetic study of ZRS in rats

The developed method was used to estimate the concentration of ZRS in animal study samples. Six young, healthy, female Wistar rats (200–230 g) were obtained from Central Animal House Facility, Manipal University (Manipal, India). Animal experiments were performed according to institutional guidelines for the care and use of laboratory animals, and approved by the Animal Ethics Committee of Manipal University.

Animals were maintained on 12 h light/dark cycle under controlled temperature (25 ± 2°C) and humidity (60 ± 5% RH) conditions in polypropylene cages filled with sterile paddy husk.

Before drug administration, the rats were fasted overnight with free access to water. The dose selected for the present study was 10.0 mg/kg in 0.5% w/v carboxy methyl cellulose as a suspension in distilled water and administered orally

(21). Animals were provided with standard diet 3 h post-dose.

The rats were anesthetized using diethyl ether, and blood samples (~0.40 mL) were collected from the retro-orbital plexus into microcentrifuge tube (containing 10 μL of saturated EDTA) at 0.25, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0 and 12.0 h after oral administration of drug, and plasma was harvested by centrifuging at 2,500 × g for 5 min. The plasma samples were transferred and stored at −80°C until analysis. The plasma concentrations of ZRS at different time points were expressed as mean ± standard error of mean (SEM).

Results and Discussion

The chromatographic conditions, analytical column, composition of mobile phase and gradient elution conditions were optimized through several trials to achieve the desired selectivity, separation, run time and symmetric peak shapes for the analyte and IS. Various columns were used, such as Hypersil C8 (250.0 × 4.6 mm, 5 μm), Kromsil C18 (250.0 × 4.6 mm, 5 μm) and Grace Vydac C18 monomeric (250.0 × 4.6 mm, 5 μm). It was found that the resolution between the analyte and IS was lower and peak tailing was higher in the presence of peak modifier using Hypersil C8 (250.0 × 4.6 mm, 5 μm) and Kromsil C18 (250.0 × 4.6mm, 5 μm) columns, respectively. An optimum resolution (Rs ≥ 2.0) was observed with good system suitability using the Grace Vydac C18 monomeric (250.0 × 4.6 mm, 5 μm), which was used for further studies. Among the various buffers tried for optimizing the chromatographic method, a maximum resolution of 3.5 between ZRS and IS was observed at pH 3.6 of phosphate buffer. Retention time was found to increase with pH. The effect of buffer strength was also evaluated at 10.0, 20.0 and 25.0 mM of potassium dihydrogen orthophosphate. Among these, 20.0 mM potassium dihydrogen orthophosphate with pH 3.6 was found to be suitable and was selected for the present study. A buffer of 72% was selected to avoid interferences with the retention times of ZRS and IS. In addition, 0.2% of triethylamine was found to play an essential role in obtaining good symmetric peak shapes.

Different mobile phases comprising several combinations of buffers (ammonium buffer and phosphate buffer) and organic solvents (methanol and acetonitrile) were tested to obtain sufficient resolution between the analyte and IS. An effective separation was achieved without any interference using a mobile phase composition of 28% of acetonitrile and 72% of phosphate buffer. Among the several columns evaluated, the column selected for the present work was the Grace Vydac C18 monomeric (250.0 × 4.6 mm, 5 μm), which gave better resolution, good peak shape and acceptable retention time.

The extraction of the drug was performed with the objectives of avoiding plasma interferences and achieving good and consistent recovery. Conventional methodologies like LLE and protein precipitation were tried. Different solvents like diethyl ether, chloroform, ethyl acetate, dichloromethane, petroleum ether and MTBE were tried individually and in combination. Protein precipitating agents like acids, alkalies, methanol and chilled acetonitrile were tried for efficiently extracting the drug. Finally, protein precipitation with chilled acetonitrile followed by LLE with MTBE–DCM (70:30% v/v) was selected for extraction. When basified, the plasma sample increased the recovery and suppressed the interference at IS. When the conditions were optimized as described, a clean chromatogram with good and consistent recovery was obtained.

Selectivity and specificity

No interference was observed at the retention times of ZRS and IS from blank plasma samples and hemolyzed plasma samples. The analytes ZRS and IS were acceptably separated from endogenous components under the described chromatographic conditions. The retention times of ZRS and IS were 13.40 ± 0.4 min and 16.66 ± 0.2 min, respectively.

The six different lots of blank plasma spiked with the LLOQ level of the drug (20.0 ng/mL) were analyzed by proposed method. The %CV was found to be 12.89% with an accuracy of 98.68 ± 12.84%, which is ± 20% of the nominal value, indicating that the method is highly specific and selective for the analysis.

Representative chromatograms of blank rat plasma, blank plasma spiked with IS, standard ZRS spiked at LLOQ with IS and animal study samples are shown in Figures 2 and 3, respectively.

Linearity

The plasma calibration curve was constructed using nine point calibration standards over a range of 20.0–3,000.0 ng/mL. The calibration curves were prepared by determining the best fit line for peak-area ratios of ZRS to IS versus ZRS concentrations, and fitted to the equation $y = mx + c$ with a $1/x$ weighing factor. The average regression ($n = 5$) was found to be 0.9984 (± 0.0002082). The regression equation was found to be $y =$ 0.00168 (± 0.000044)x − 0.0421(± 0.03281). The mean percentage accuracy observed for backcalculated concentration for five linearities was within 92.57–111.65%, while the precision (%CV) values ranged from 0.17 to 6.00%.

Precision and accuracy

The intra-day and inter-day %CV of ZRS were found to be 11.41, 4.19, 2.01 and 4.31%, and 9.76, 3.88, 2.41 and 3.67%, respectively, for LLOQ, LQC, MQC and HQC. Accuracy data were all within the acceptance interval of 85–115%. Intra-day and inter-day precision and accuracy data are shown in Table II. Thus, the intra-assay and inter-assay accuracy and precision were found to be acceptable for the ZRS analysis, in support of further pharmacokinetic studies. The data demonstrated good accuracy and reproducibility, which gave confidence for the applicability of the method for pharmacokinetic studies.

Recovery

The mean relative recovery (± standard deviation, SD) of ZRS at LQC, MQC and HQC was 80.32 ± 3.92%, 79.96 ± 1.46% and 77.68 ± 3.55%, respectively. The overall mean recovery of ZRS was found to be 79.32 ± 1.16%. The recovery of IS was found to be 84.11 ± 3.2%.

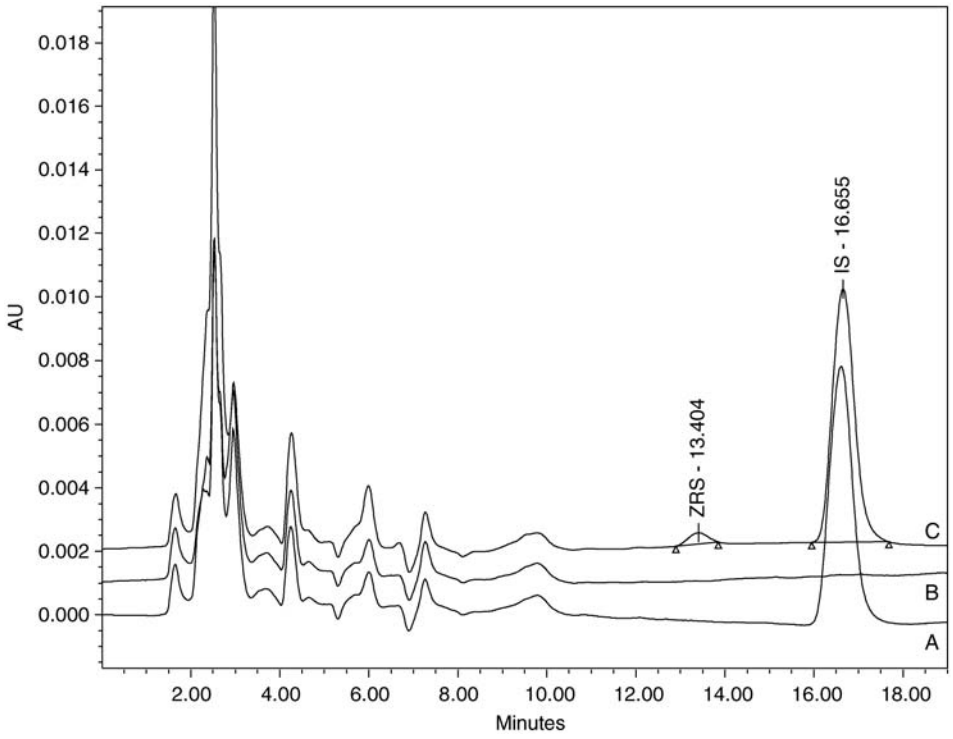

Figure 2. Representative chromatograms: blank plasma spiked with IS (A); blank rat plasma (B); blank plasma spiked with IS and LLOQ level of ZRS (C).

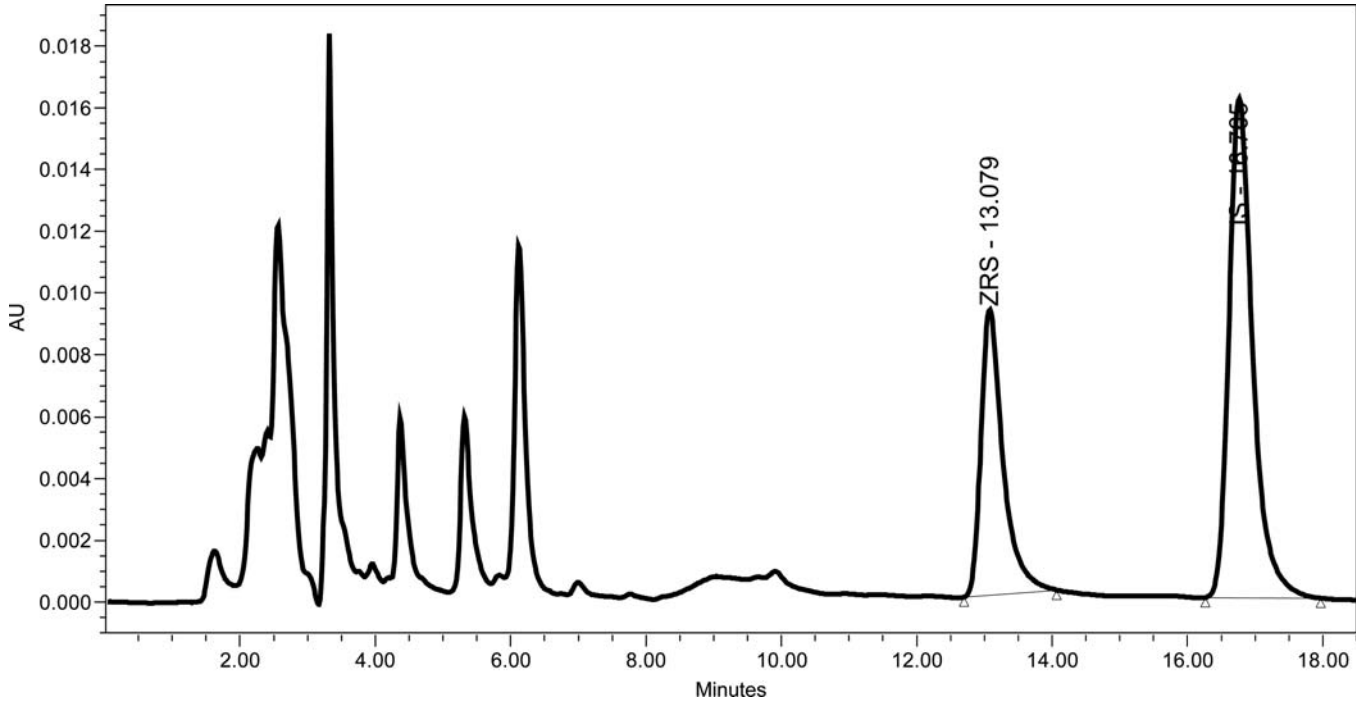

Figure 3. Representative chromatogram of animal study sample of ZRS at 4.0 h.

Table II
Intra-Day and Inter-Day Precision and Accuracy of the Assay for ZRS in Rat Plasma ($n = 6$)

Nominal concentration (ng/mL)	QC concentration (ng/mL)	Estimated concentration (ng/mL); mean ± SD	Precision (%CV)	Accuracy (%)
Intra-day				
LLOQ	20.0	19.73 ± 2.25	11.41	98.68
LQC	40.0	37.68 ± 1.57	4.19	94.20
MQC	400.0	398.27 ± 7.00	2.01	99.56
HQC	2,500.0	2,572.95 ± 111.05	4.31	102.98
Inter-day				
LLOQ	20.0	18.72 ± 1.83	9.76	93.64
LQC	40.0	37.27 ± 1.45	3.88	93.04
MQC	400.0	395.80 ± 9.54	2.41	98.95
HQC	2,500.0	2,627.45 ± 96.54	3.67	105.09

Table III
Stability Study Results for ZRS ($n = 6$)

Stability	QC	Spiked concentration (ng/mL)	Average calculated comparison sample concentration (ng/mL)	Average calculated stability sample concentration (ng/mL)	Average percentage (%)
Bench top*	LQC	40.0	42.05 ± 0.65	41.01 ± 1.66	97.52
	HQC	2,500.0	2,521.71 ± 10.31	2,512.23 ± 11.24	99.62
Autosampler†	LQC	40.0	41.71 ± 0.57	41.32 ± 0.86	99.06
	HQC	2,500.0	2,523.37 ± 29.44	2,501.60 ± 15.07	99.35
Freeze and thaw‡	LQC	40.0	41.29 ± 2.81	39.94 ± 0.58	96.74
	HQC	2,500.0	2,514.02 ± 54.75	2,451.18 ± 12.64	97.50
Long-term§	LQC	40.0	42.71 ± 0.57	41.15 ± 0.58	96.35
	HQC	2,500.0	2,551.04 ± 17.28	2,468.60 ± 12.32	96.77

*After 6 h at room temperature.
†After 24 h at 4°C.
‡After three freeze and thaw cycles at −80°C.
§At −80°C for 30 days.

Table IV
Pharmacokinetic Parameters of ZRS in Female Rats

Pharmacokinetic parameters	Average ± SEM
C_{max} (ng/mL)	724.91 ± 94.63
t_{max} (h)	2.00 ± 0.00
$AUC_{(0-t)}$ (ng-h/mL)	3,462.84 ± 554.81
$AUC_{(0-inf)}$ (ng-h/mL)	3,663.30 ± 596.03
λ_z (1/h)	0.23 ± 0.05
$t_{1/2}$	0.33 ± 0.07

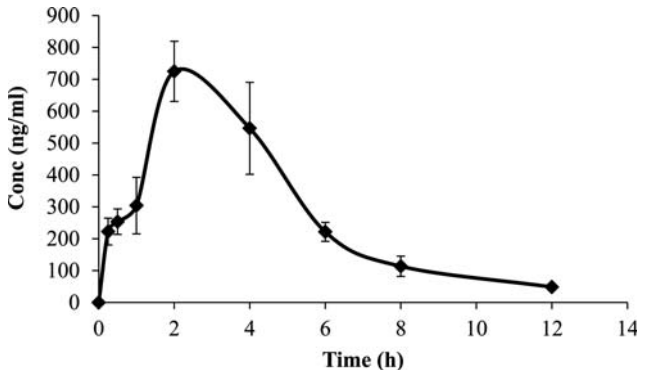

Figure 4. Plasma concentration−time curves of ZRS after oral administration of ZRS (10.0 mg/kg) to female Wistar rats ($n = 6$).

Stability

The results of stability studies (Table III) showed that ZRS was stable at all stability conditions.

Application of the method for pharmacokinetic study of ZRS in rats

The described method was successfully applied to study the pharmacokinetic parameters in female rats. Plasma concentrations of ZRS were determined up to 12 h after oral administration of ZRS at a dose of 10.0 mg/kg as a suspension in 0.5% w/v carboxy methyl cellulose. The C_{max} and t_{max} values were obtained from plasma concentration versus time curve, with interpolation. The area under the plasma concentration−time curve after oral administration to the last measured (AUC_{0-t}), extrapolated to infinity ($AUC_{0-\infty}$), elimination rate constant (λ_z) and half−life ($t_{1/2}$) were estimated by using PK Solutions 2.0™ noncompartmental pharmacokinetics data analysis software.

The pharmacokinetic parameters are listed in Table IV and the pharmacokinetic profiles of ZRS are shown in Figure 4. The results showed that the C_{max} of ZRS was 724.91 ± 94.63 ng/mL and systemic exposure (AUC_{0-t}) was found to be 3462.84 ± 554.81 ng-h/mL. These results are in agreement with the reported literature values (21).

Conclusion

A simple, sensitive, specific, accurate and reproducible reversed-phase HPLC method was developed using the commonly available UV-visible detector for the determination of ziprasidone in rat plasma. The sample extraction technique developed was a single step LLE, which is convenient and requires a smaller volume of plasma (150.0 μL). The present method has been successfully applied to study the pharmacokinetic profile of ziprasidone in female rats. This method may find useful applications in pharmacokinetic profiling of ziprasidone in preclinical or clinical studies.

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