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Research Article

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Liquid-liquid extraction method for Ziprasidone (ZRS) bioanalysis by using ZRS-D_8 (stable isotope) as internal standard

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ABSTRACT

A selective, sensitive, liquid chromatography mass spectrometry/ mass spectrometry (LC-MS/MS) method was developed and validated for the quantification of Ziprasidone in human plasma by using Ziprasidone D_8 as internal standard. Sample preparation process was accomplished by liquid-liquid extraction technique. The processed sample was chromatographed and analyzed on Zorbax Eclipse XDB-C18,100×4.6 mm (3.5µm) column using mobile phase [Acetonitrile: Buffer-1 (85:15%, v/v)] and 50% Acetonitrile in water as diluent. Ziprasidone was chromatographed and analyzed by MS Detector. This method shows good linearity over a range of 0.2980 ng/ml to 201.4820 ng/ml. Signals from the detector were captured in a computer and processed using Mass Lynx version 4.1SCN 843 software.

Key words: Ziprasidone, ZiprasidoneD_8, internal standard, LC/MS/MS and validation.

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 (Fig. 1) with the IUPAC name 5-{2-[4-(1, 2-benzisothiazol-3-yl)-1-piperazinyl] ethyl}-6-chloro-1, 3-dihydro-2H-indol-2-one [1,2]. ZRS functions as an antagonist at the D_2, $5HT_{2A}$ and $5HT_{1D}$ receptors and as an agonist at the $5HT_{1A}$ receptor. The $5HT_{2A}$ affinity of ZRS is approximately eight times that of D_2, which creates a balance that promotes antipsychotic effects and limits extra pyramidal 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 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 clinical studies. Only limited methods have been reported for determination of Ziprasidone in human plasma. The objective of the work is to develop and validate more sensitive LC-MS/MS method than pre-existing methods by HPLC and GC for quantification of Ziprasidone in Human Plasma.

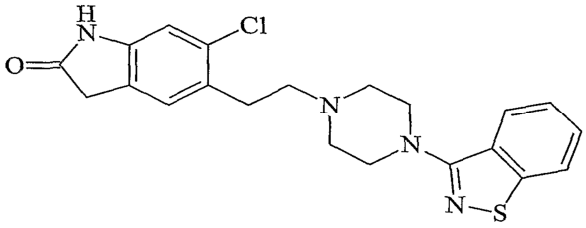

Fig.1: Structure of Ziprasidone

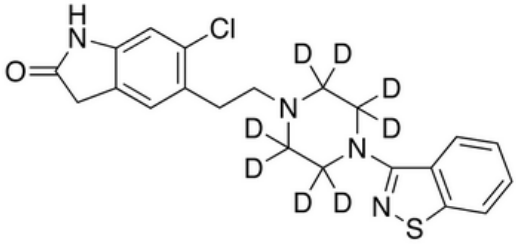

Fig.2: Structure of Ziprasidone D_8

EXPERIMENTAL SECTION

Chemicals and reagents

Ziprasidone (Fig.1) and Ziprasidone D_8 (Fig. 2) as internal standard (IS) were purchased from Clearsynth Research Center, Hyderabad. The purity of these compounds was higher than 98%. Acetonitrile (HPLC grade), Formic acid (AR grade), Human Plasma (K_3EDTA), Plastic containers (Tarson), Ammonium acetate (AR Grade), Tert Butyl Methyl Ether (HPLC grade) and Water (HPLC grade) were used throughout the analysis.

Preparation of Solutions

Buffer-1- (5 mM Ammonium Acetate in water), Buffer-2(0.1% Formic acid in 5mM Ammonium Acetate), Buffer-3(0.1% Formic acid in water),Mobile Phase- Acetonitrile: Buffer-1 (85:15, v/v), Diluent- Acetonitrile: Water (50:50, v/v), Reconstitution solution (Acetonitrle: Buffer-2,90:10,V/V), Strong Wash Solution-(1% formic acid in Acetonitrile: water,90:10%,V/V), Weak Wash Solution-(1% formic acid in Acetonitrile: water, 50:50%,V/V) were used throughout the analysis. All the solutions were used for four days from date of preparation.

Preparation of Standard Stock Solutions (w/v)

5.00 mg of Ziprosidone working standard was weighed and transferred into 5ml volumetric flask and 2.5ml methanol was added to dissolve working standard. The volume was made up to mark with methanol with final concentration of 1000.00µg/ml for Ziprosidone. 5.00 mg of Ziprosidone D_8 working standard was weighed and transferred into 5 ml volumetric flask and 2.5ml methanol was added to dissolve working standard. The volume was made up to mark with methanol to a final concentration of 1000.00µg/ml for Ziprosidone$D_{8.}$ Then final concentration of 100ng/ml was made for internal standard. All the calculations were made on the basis of molecular weight and purity. The stock solutions of analyte and internal standard were stored in a refrigerator at 2°C -8° to avoid degradation.

Instrumentation and Chromatographic Conditions

The chromatography was performed on WATERS XEVO TQMS with cooling auto sampler and column oven having temperature control. Zorbax Eclipse XDB-C18 with configuration 100×4.6 mm (3.5µm) column was employed. The column temperature was maintained at 40 °C and chromatographic separations were achieved with isocratic elution using a mobile phase composed of [Acetonitrile: Buffer-1 (85:15, v/v)] .The flow rate was set at 1000 µl/min, run time was 2.50 minutes, positive mode of ionization and retention time for Analyte and IS were 1.20±0.40 min and 1.20±0.40 min respectively. The auto sampler was conditioned at 10°C and a volume of 10µl of sample solution was programmed to be injected through auto sampler mode. Other tuning parameters and mass spectroscopic conditions like Capillary at 3.50 kV, Desolvation Gas at 900 L/Hr, Cone Gas Flow at 100 L/Hr, Desolvation Temp. at 550 ^{0}C, Source Temp. at 150 ^{0}C , cone at 45V, and D_{well} time at 0.200 sec. were employed. The samples were analyzed by mass spectrometry in the multiple reaction monitoring mode using the $[M+H]^+$ ionization i.e. *m/z* 413.00 to 193.99 for Ziprasidone and *m/z* 421.10 to 193.98 for the internal standard (Ziprasidone D_8) with collision energy of 30eV for both the ionizations.

Sample Preparation

The thawed samples were vortexed to ensure complete mixing of contents.50 µl of internal standard solution (100ng/ml) was added to all the samples except blank. 100 µl of plasma sample was pipetted out into respective vials containing internal standard solution.100 µl of Buffer-2 was added to all the samples and they were vortexed. 2.5 ml of TBME (tert butyl methyl ether) was added to all the samples and capped tightly. The samples were kept on vibramax at 2000 rpm for 10 minutes. The samples were centrifuged at 3500 rpm for 5 minutes in a refrigerated centrifuge between 2^oC to 8^oC. 2.0 ml of upper layer was collected from each labeled RIA vials and all the samples were dried under nitrogen evaporator at 40^oC and 15 psi. The dried residue was reconstituted with 250 µl of reconstitution solution. All the samples were vortexed and transferred into respective labeled auto-injector vials and loaded into LC-MS/MS.

Method Validation

The method validation parameters were performed according to the United State Food and Drug Administration (FDA) guidelines[7]. Selectivity, Linearity, Accuracy, Precision, Recovery, Stability studies and Matrix effect were performed. Each batch of spiked plasma samples were includes one complete calibration curve (consisting of one blank plasma, one blank plasma with internal standard and eight different non-zero concentration samples) and six replicates of quality control samples like LLOQ, LQC, INTQC, MQC and HQC (Fig. 3-8) were used for performing validation parameters.

Selectivity

A selectivity study was designed to investigate whether endogenous constituents and other substances existing in samples would interfere with the detection of Ziprasidone and IS. Selectivity was studied by comparing the chromatograms of six different batches of human blank plasma with the corresponding spiked plasma.

Linearity, LLOQ and LOD

The linearity of calibration curves were determined by plotting the peak area ratio of Ziprasidone to IS versus the nominal concentration of Ziprasidone with weighted ($1/x^2$) least square linear regression. The lower limits of quantification (LLOQ) and limits of detection (LOD) were calculated based on signal-to-noise ratio of 10:1 and 3:1, respectively, indicating that this method was sensitive for the quantitative evaluation of Ziprasidone.

Precision and accuracy

For the evaluation of intra-batch precision and accuracy, six replicate of QC samples were analyzed at five concentration levels. For the evaluation of inter-batch precision and accuracy, six replicates of QC samples were analyzed at five concentration levels on three consecutive days.

Extraction recovery

The extraction recoveries were evaluated by comparing peak areas obtained from the spiked samples before extraction with those after extraction at corresponding concentrations. The extraction recoveries of Ziprasidone and IS were determined by analyzing six replicates of QC samples at three concentration levels, respectively.

Matrix effect

The matrix effect was evaluated by comparing the peak areas of the post-extracted spiked QC samples with those of corresponding standard solutions. The matrix effect of Ziprasidone was determined by analyzing nine plasma samples at two concentration levels in tripilicate.

Stability

The stability experiments were performed to evaluate the stability of Ziprasidone in human plasma under the following conditions: short-term stability at room temperature for 24 h; long-term stability at −70◦C for 30 days; three freeze–thaw (room temperature) cycles on consecutive days. All stability tests in plasma were performed by analyzing three replicates of QC samples at two concentration levels. The determined concentrations were compared with the nominal values.

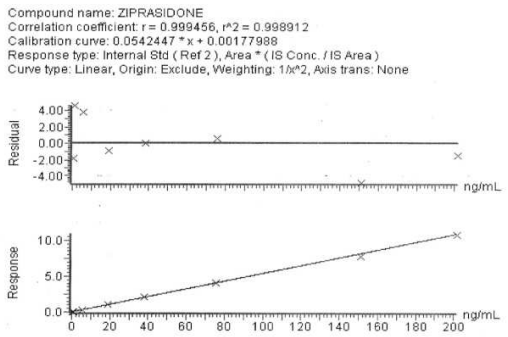

Fig.3 Std. calibration curve

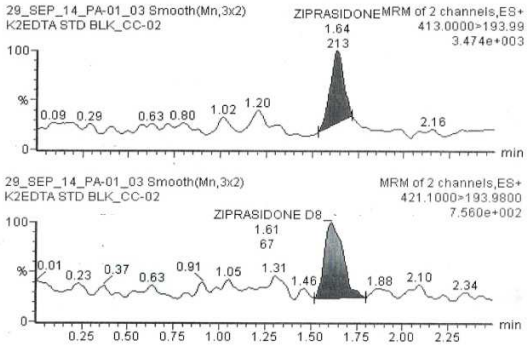

Fig.4 chromatogram of blank plasma

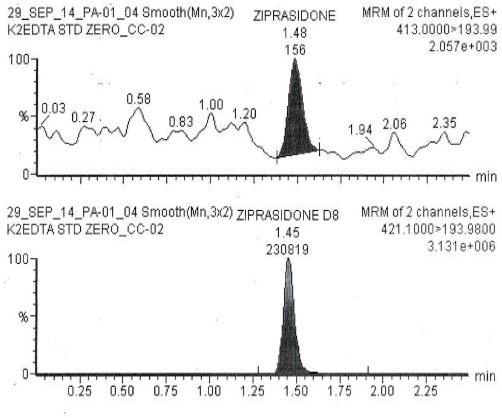

Fig.5 chromatogram of Std zero

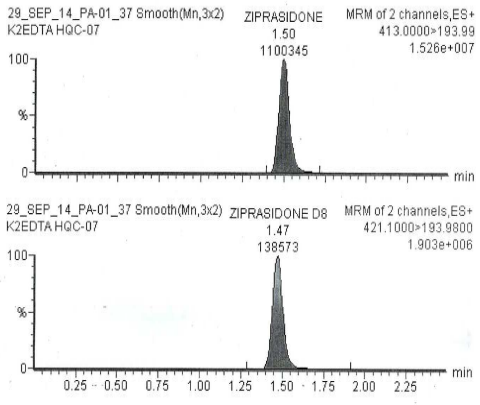

Fig.6 chromatogram of LQC

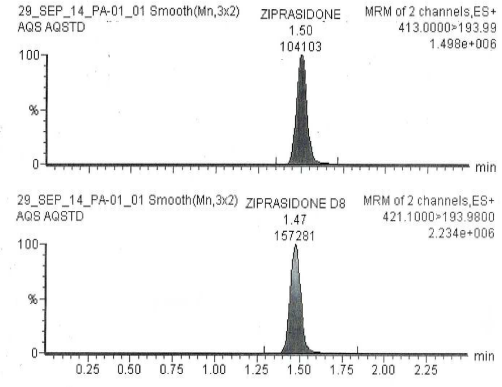

Fig.7 chromatogram of MQC

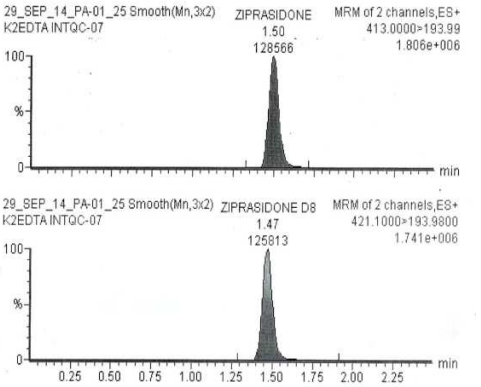

Fig.8 chromatogram of HQC

RESULTS AND DISCUSSION

Selectivity

A selectivity study was designed to investigate whether endogenous constituents and other substances existing in samples would interfere with the detection of Ziprasidone and IS. There was no interference at retention times of 1.20 min (IS) and 1.20 min Ziprasidone .The detection of IS and Ziprasidone by SRM was highly selective with no significant interference.

Linearity

The calibration curves calculated in the range 0.2980 to 201.4820 ng/ml were linear to analyze Ziprasidone from human plasma. The slopes, intercepts, and correlation coefficients of the regression equations were determined by least squares linear regression using a weighting factor of $1/x^2$. The typical equation for the standard curves was y = 0.0410x + 0.00427 (r = 0.9953) for Ziprasidone. Deviations were within ±15% for all regression equations.

Precision and accuracy

The intra-batch and inter-batch precisions and accuracies of human plasma samples were analyzed by taking six replicates each of LOQQC, LQC, INTQC, MQC, and HQC samples in each batch. The results are summarized in Table 1. At all levels, the results obtained for % CV and % Nominal were acceptable, which indicate that the method is reliable and reproducible for the determination of Ziprasidone in human plasma.

Table 1: Precision and Accuracy of Ziprasidone

QC ID	LOQQC	LQC	INTQC	MQC	HQC
Actual Conc. (ng/mL)	0.2120	0.6080	16.9160	105.7280	211.4560
Calculated Conc. (ng/mL)	0.2418	0.6023	15.0632	92.5669	182.8692
	0.2223	0.5874	14.2291	94.3191	187.4166
	0.2078	0.5691	15.5748	96.1949	186.4671
	0.2160	0.5692	14.8890	91.5529	185.6936
	0.2356	0.5729	15.1593	90.2424	185,6780
	0.2087	0.5746	14.8724	90.8370	185.5635
Mean	0.22203	0.57925	14.96463	92.61887	185.61467
SD	0.014083	0.013143	0.441622	2.264528	1.518160
%CV	6.34	2.27	2.95	2.44	0.82
%Nominal	104.73	95.27	88.46	87.60	87.78

Recovery

Extraction recoveries of Ziprasidone from the low, medium, and high QC samples ranged from 75.2 to 81.4% with a maximum RSD of 12.7%. Mean while, the extraction recoveries of IS ranged from 70.9 to 75.0% with a maximum RSD of 12.5%. The results revealed that the sample pretreatment approach employed in the present work gave reproducible recoveries for Ziprasidone and IS.

Stability

The stability of Ziprasidone in human plasma was checked and it was concluded that Ziprasidone in human plasma was stable in the reconstituted solution for 24 hrs at 20◦C. The relative error (%RE) of Ziprasidone in human plasma between the initial concentrations and the concentrations following the three freeze–thaw cycles was within ±15.0%. In addition, the processed samples were also stable at −70◦C for 30 days.

Table 2: Matrix effect of Ziprasidone

QC ID	LQC	LQC	LQC	HQC	HQC	HQC
Actual Conc.(ng/ml)	0.5900	0.5900	0.5900	210.6420	210.6420	210.6420
1	0.6444	0.6454	0.6506	209.8127	210.4185	211.0701
2	0.6247	0.6331	0.6287	210.4297	211.0784	220.5715
3	0.6221	0.6169	0.6374	211.7847	211.3595	209.9958
4	0.6099	0.6359	0.6516	213.7671	207.7483	212.9317
5	0.6528	0.6460	0.6522	214.0864	212.8923	216.4808
6	0.6290	0.6287	0.6320	217.5233	213.8969	215.7260
7(Haemolysed)	0.6350	0.6462	0.6282	213.3019	210.7508	214.3789
8(Lipemic)	0.6452	0.6344	0.6405	218.7565	209.9084	210.0211
9(Heparin)	0.6383	0.6468	0.6195	212.2880	217.3989	220.6088
Mean	0.63904	0.63704	0.63787	213.51781	211.71693	214.64274
SD	0.016634	0.010175	0.011783	2.992981	2.752067	4.092839
%CV	2.60	1.60	1.85	1.40	1.30	1.91
% Nominal	108.31	107.97	108.11	101.37	100.51	101.91

Matrix Effect

The potential for co-extracted matrix component to influence the detector response of Analyte and Internal Standard was evaluated in six independent lots of blank K_3EDTA human normal plasma, one lot of heamolyzed plasma, one lot of lipemic plasma and one lot of heparin plasma. Aqueous standard equivalent to LQC and HQC level

concentration along with intended concentration of Internal Standard were spiked to the post extracted blank matrix respectively for both LQC and HQC samples in triplicates, respectively. The percentage CV of Matrix effect for IS normalized at LQC level were found to be 2.60%, 1.60% and 1.85% and HQC level were found to be 1.40%, 1.30% and1.91% respectively. The percentage nominal of Matrix effect for IS normalized at LQC level were found to be 108.31, 107.97 and 108.11 whereas HQC level were found to be 101.37, 100.51 and 101.90 respectively. The results are summarized in Table 2

CONCLUSION

This analytical method is valid for determination of Ziprasidone (over a range of 0.2980 ng/ml to 201.4820 ng/ml) using as Ziprasidone D_8 internal standard in human plasma. Signal from the detector were captured in a computer and processed using Mass Lynx SCN 4.1 V 843 software. This method for quantification of Ziprasidone in human plasma is accurate, precise, rapid, and selective. It is a simple, practical, and economical alternative for studies of the bioavailability, bioequivalence, and pharmacokinetics of this drug in human plasma.

Acknowledgement
I avail this opportunity, with great pleasure and deep sense of gratitude, to express my thanks to my research fellows for providing enough patience, zeal and strength that enabled me to complete this work successfully.

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