August 2008 Volume 7 Issue 8

Trade Science Inc.

Analytical CHEMISTRY
An Indian Journal

Full Paper

ACAIJ, 7(8) 2008 [582-585]

New RP-HPLC method for the estimation of ziprasidone hydrochloride in pharmaceutical dosage forms

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Received: 16th July, 2008 ; Accepted: 21st July, 2008

ABSTRACT

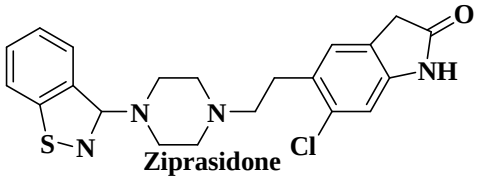
A simple reverse phase HPLC method was developed for the determination of Ziprasidone Hydrochloride present in pharmaceutical dosage forms. A Hypersil ODS C18, 4.6mm × 250 mm, 5µm column, with the mobile phase acetonitrile: ammonium acetate buffer (pH-5) (70:30 %v/v) was used. The flow rate was 1.2 ml/min and effluent was monitored at 225 nm. Abacavir sulfate is used as internal standard. The retention times were 4.88 min and 2.43 min for ziprasidone hydrochloride and abacavir sulfate respectively. The linearity range was found to be 0.5-200µg/ml for ziprasidone hydrochloride. The proposed method was also validated.

KEYWORDS

Ziprasidone hydrochloride;
RP-HPLC;
Pharmaceutical formulations;
Recovery studies.

INTRODUCTION

Ziprasidone, 5-[2-[4-(1, 2-benzisothiazol-3.2-yl)-1-piperazinyl] ethyl]-6-chloro-1,3.2-dihydro-2 H - indol-2-one is an antipsychotic agent that is chemically unrelated to phenothiazine or butyrophenone antipsychotic agents.



Ziprasidone

Ziprasidone's unique pharmacology offers advantages in the areas of antipsychotic-induced weight gain and possibly the treatment of depressive symptoms in patients with schizophrenia or schizoaffective disorder [1-2]. The efficacy of ziprasidone in schizophrenia is primarily attributable to dopamine (i.e, D_2) and serotonin (specifically $5HT_2A$) receptor antagonism, which contributes to its more favorable effect on weight gain than clozapine, olanzapine, and risperidone[3].

Literature survey reveals that a few HPLC methods are available for the determination of ziprasidone hydrochloride in biological fluids[4-9], and two methods are available for the determination of ziprasidone hydrochloride in Pharmaceutical formulations[10,11]. The present paper describes the simple, precise, accurate and new HPLC method for the determination of ziprasidone hydrochloride in pharmaceutical dosage forms.

EXPERIMENTAL

Instrumentation

Quantitative HPLC was performed on a binary gradient HPLC with Shimadzu LC10AT and LC10AT VP series HPLC pumps, with a 20µl injection of sample loop (Hemilton) (manual), and SPD 10A VP UV-Visible Detector. The output signal was monitored and in

tegrated using Shimadzu CLASS-VP Version 6.12 SP1software. Hypersil ODS C_{18}(46 mm× 25 cm, 5mm) column was used for the separation. The pH of the solution was adjusted by using digital pH meter, Model DI 707 (Digisun electronics, Hyderabad, India).

Standards and chemicals

Ziprasidone hydrochloride and Abacavir sulfate used as an internal standard (IS) were gifts obtained from Torrent pharma limited (Ahemadabad, India) and Aurobinbo pharma Limited (Hyderabad, India) respectively. Zipsydon tablets (Sun pharma. Ltd, India) containing 40 mg of Ziprasidone, were purchased from local market. Purified water was prepared using a Millipore Milli-Q (Bedford, M.A., USA) water purification system. Acetonitrile of HPLC grade was purchased from Ranbaxy Fine Chemicals Ltd (New Delhi, India), ammonium acetate and formic acid, both of A.R. grade, were purchased from Merck Ltd. (Mumbai, India).

A solution of ammonium acetate (0.01 M, pH: 5.0) was prepared by dissolving 0.77g of ammonium acetate in 800 ml water and diluting to 1000ml with water. The pH was adjusted to 5.0 with formic acid.

Preparation of standard drug solutions

Stock solution of ziprasidone hydrochloride was prepared by dissolving 25 mg of ziprasidone hydrochloride in 25 mL of volumetric flask containing 20 mL acetonitrile. The solution was sonicated for about 20 min and then made up to volume with acetonitrile. Working standard solution of ziprasidone hydrochloride was prepared by suitable dilution of the stock solution with appropriate mobile phase. Similarly stock solution of internal standard was prepared by dissolving 25mg of abacavir sulfate in 25 mL of acetonitrile.

Chromatographic conditions

The mobile phase used in this study was a mixture of ammonium acetate buffer (0.01M, P^H 5.0 adjusted with formic acid) and acetonitrile 30:70 %v/v. The contents of the mobile phase were filtered before use through a 0.45μ membrane. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1.2ml/min for 15 minutes. The column temperature was maintained at 23 ±1^{0}C. The eluate was monitored at 225nm using UV-detector. The identification of the separated ziprasidone hydrochloride and abacavir sulfate were confirmed by running the chromatograms of the individual compounds under identical conditions.

TABLE 1: Linearity range of ziprasidone

Concentration (μg/mL)	Peak area ratios	Statistical analysis
0.5	0.0163	
1.0	0.041	
2	0.071	Slope: 0.025
5	0.120	Intercept: 0.004
10	0.238	
25	0.667	Correlation
50	1.273	Coefficient:0.9997
100	2.455	
200	5.061	

Regression equation from 0.5-200 (μg/mL): Y=0.0252X+0.0046 (R^2=0.9997)

TABLE 2: Amount of ziprasidone in tablet dosage forms by proposed HPLC method

Formulation	Labeled amount (mg)	Mean ±s.d (amount mg recovered) n=6	Mean ±s.d (% of recovery)
Zipsydon	40	40.25 ± 0.35	100.63 ± 0.87

TABLE 3: Precision of proposed HPLC method

S. no	Concentration taken (μg/ml)	Intra-day Measured concentration (μg/ml) Mean ± S.D	Intra-day % C.V	Inter-day Measured concentration (μg/ml) Mean ± S.D	Inter-day %C.V
1	25	24.91 ± 0.13	0.54	25.23 ± 0.17	0.67
2	50	50.26 ± 0.03	0.05	50.97 ± 0.11	0.21
3	100	100.54 ± 0.11	0.01	99.85 ± 0.21	0.21

Calibration of standards

Calibration standards were prepared by spiking working standard solutions into mobile phase contained in 5 mL volumetric flasks to yield concentrations of 0.5, 1, 2, 5, 10, 25, 50, 100, and 200μg /mL. To the above solutions 20μg /mL of abacavir sulfate (internal standard) was added and the final volume was made up to the mark with mobile phase. A 20μL aliquot was injected into the analytical column. The linearity data were shown in TABLE 1. Calibration curve was plotted between peak area ratios of drug vs. internal standard against concentration of the drug.

Recovery of ziprasidone in tablets

Twenty tablets were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 25 mg of ziprasidone hydrochloride was extracted with acetonitrile in a 25mL volumetric flask using ultra sonicator. This solution was filtered through Whatmann no 1 filter paper. The solution obtained was

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diluted with the mobile phase so as to obtain a concentration in the range of linearity previously determined. An aliquot (20µg/mL) of the internal standard was added to the sample solution prior to the dilution. All determinations were carried out in triplicate. The represented data were shown in TABLE 2.

RESULTS

The developed method was used based upon its ability to detect and quantify ziprasidone hydrochloride with the use of standard high-performance liquid chromatographic system equipped with UV-Visible detector.

Method validation[12,13]

Specificity and selectivity

The HPLC chromatograms recorded for the drug-matrix (mixture of the drug-excipients) showed almost no peaks within a retention time range of 10 min. as shown in figure 1. The figure shows that ziprasidone hydrochloride is clearly separated from its internal standard. The retention time, asymmetric factor and peak area ratio of the marketed formulations were not affected with excipients present in formulation. Thus, the HPLC method presented in this study is selective and specific for ziprasidone hydrochloride.

Linearity

The standard curve was obtained in the concentration range of 0.5-200µg/mL. The linearity of this method was evaluated by linear regression analysis, which was calculated by least square method. The mean ± standard deviation (SD) for the slope, intercept and correlation coefficient of standard curves (n=6) were calculated.

Limit of detection (L.O.D) and Limit of quantification (L.O.Q)

Limit of detection was found to be 0.142µg/mL (signal to noise ratio is 3) and Limit of quantification was found to be 0.473µg/mL(signal to noise ratio is 10).

Precision and accuracy

The precision of the method was demonstrated by inter day and intra day variation studies. In the intra day studies, six repeated injections of standard solutions

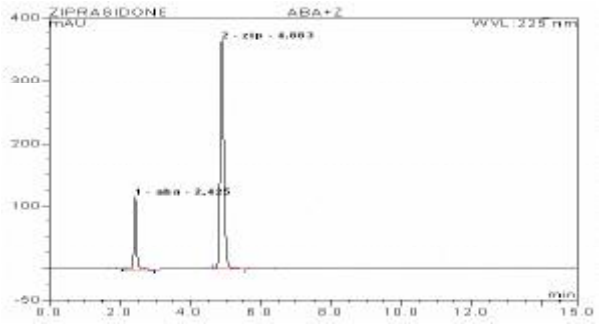

Figure 1: A typical chromatogram of ziprasidone in formulations

TABLE 3: Precision of proposed HPLC method

S. no	Concentration taken (µg/ml)	Intra-day Measured concentration (µg/ml) Mean ± S.D	Intra-day % C.V	Inter-day Measured concentration (µg/ml) Mean ± S.D	Inter-day %C.V
1	25	24.91 ± 0.13	0.54	25.23 ± 0.17	0.67
2	50	50.26 ± 0.03	0.05	50.97 ± 0.11	0.21
3	100	100.54 ± 0.11	0.01	99.85 ± 0.21	0.21

TABLE 4: Accuracy studies

S. no	Amount of drug added (µg) to 20µg of preanalysed formulation	Mean ±s.d amount (µg) recovered (n=6)	Mean ±s.d % of recovery
1	40	40.56± 0.3	101.40 ± 0.75
2	50	49.87± 0.14	99.74 ± 0.28
3	60	59.49 ± 0.44	99.15 ± 0.73

were made and the response factor of drug peaks and percentage Coefficient of variance (C.V) were calculated and presented in TABLE 3. In the inter day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks and percentage C.V were calculated and presented in TABLE 3. From the data obtained, the developed RP-HPLC method was found to be precise. The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in TABLE 4. From the data obtained, added recoveries of standard drugs were found to be accurate.

System suitability

For system suitability, six replicates of standard sample were injected and studied the parameters like plate number (N), tailing factor (k), resolution (R) and relative retention time (α), Height Equivalent Theoretical Plate (HETP), capacity factor (k^l), plates per meter

TABLE 5: System suitability parameters of ziprasidone

S.no	Parameter	Value
1	Resolution	2.51
2	Capacity factor	1.9
3	Theoretical plates	6328
4	Tailing factor	0.997
5	HETP	3.95×10^{-5}
6	Asymmetry	1.13

and peak symmetry of samples. The represented data was shown in TABLE 5.

Robustness

The percent recovery of ziprasidone hydrochloride was good under most conditions and didn't show any significant change when the critical parameters were modified. The tailing factor for ziprasidone hydrochloride was always less than 2.0 and the components were well separated under all the changes carried out. Considering the modifications in the system suitability parameters and the specificity of the method, as well as carrying the experiment at room temperature may conclude that the method conditions were robust.

DISCUSSION

The chromatographic method was optimized by changing various parameters, such as pH of the mobile phase, organic modifier and buffer used in the mobile phase. Retention time of ziprasidone hydrochloride has more dependence on pH of the mobile phase. The separation of peaks was also dependent on pH of the buffer and the percentage of acetonitrile.

Under the presently prescribed conditions, the recovery studies of ziprasidone hydrochloride were found to be from 99.15 to 101.40% respectively. This method is very useful for determination of ziprasidone hydrochloride in pharmaceutical dosage forms, clinical studies and pharmacokinetic studies. The observation of C.V less than 2.0 for both intra and inter-day measurements indicates high degree of precision. In the present method, a Hypersil ODS C18 column has been used and the buffer pH in the mobile is 5.0, which is within the limits (pH 2-8) specified by the manufacturers. In the present method, we have established a linearity range of 0.5-200µg /mL; this linearity range covers all the strengths of ziprasidone hydrochloride. Hence this method can be successfully applied for quantifying the low levels of ziprasidone hydrochloride in pharmaceutical dosage forms and other pharmacokinetic studies.

ACKNOWLEDGMENTS

The authors are thankful to the Roland Institute of Pharmaceutical Sciences, Berhampur for providing the laboratory facilities.

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