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NEW STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT FOR ANALYSIS OF ZIPRASIDONE IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, inexpensive, rapid and novel stability indicating isocratic HPLC method has been developed and validated for quantitative analysis of ziprasidone in the bulk drug and in a pharmaceutical dosage form. An isocratic separation of ziprasidone was achieved on Thermo C_{18} column (4.6 x 250mm, 5µ particle size) as the stationary phase with a flow rate of 1.0 ml/min and using a UV detector to monitor the eluate at 254 nm. The mobile phase consisted of Methanol: Acetonitrile (60:40v/v) enabled separation of the drug from its degradation products. The method was validated for linearity, accuracy (recovery), precision and specificity. The linearity of the method was satisfactory over the range 5-25µg/ml (correlation coefficient 0.999). Recovery of ziprasidone from the pharmaceutical dosage form ranged from 99.69 to 99.78%. ziprasidone was subjected to stress conditions (hydrolysis (acid, base), oxidation, photolysis and thermal degradation) and the samples were analyzed by this method. The forced-degradation study with ziprasidone was shown that it was degraded under acidic condition. The drug was stable under the other stress conditions investigated. The degradation products were well resolved from main peak. The forced degradation study prove the stability indicating power of the method and therefore, the validated method may be useful for routine analysis of ziprasidone as bulk drug, in respective dosage forms, for dissolution studies and as stability indicating assay method in pharmaceutical laboratories and industries.

Keywords: RP-HPLC, ziprasidone, Forced degradation, Method validated.

INTRODUCTION

Ziprasidone hydrochloride (ZPR) chemically is 5-[2-[4-(1, 2-benz Isothiazol-3-yl)-1- Piperazinyl] ethyl]-6-chloro-1, 3-dihydro-2H-Indol-2-one hydrochloride [Fig. 1]. It has an empirical formula of C21H22Cl2N4OS and molecular weight of 449. 40 g/mol [1-2]. Ziprasidone is a new second generation or atypical antipsychotic drug that chemically differs from butyrophenone. It is used for the treatment of schizophrenia, mixed states associated with bipolar disorder and the acute mania. ZPR has a selective antagonist action for the serotonin type 2 (5-HT2), dopamine D2 and H1 histamine receptors [3-4].

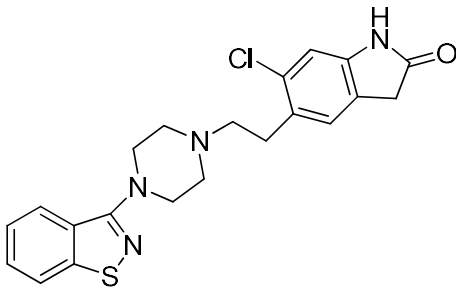


Fig. 1 Chemical Structure of Ziprasidone

Literature survey for ziprasidone revealed several analytical methods based on different techniques, viz, LC-MS[5-7] assay for their quantification in plasma and brain, high performance liquid chromatography (HPLC)[8-10] method for simultaneous determination of ziprasidone in capsule formulation, and HPLC-UV[11] methods for determination ziprasidone in human plasma and urine, LC[12] with fluorescence for determination of plasma ziprasidone, and capillary zone electrophoresis[13] for determination of ziprasidone in pharmaceutical formulations. None of the reported procedures enables analysis of the ZPR alone in pharmaceutical dosage forms in the presence of their degradation products. This manuscript describes the development and validation, in accordance with ICH guidelines [14], of a rapid, economical, precise and accurate stability-indicating isocratic reversed phase HPLC method for analysis of ZPR in the presence of its degradation products. This paper mainly deals with the forced degradation of ZPR under the stress conditions such as acidic and basic hydrolysis, oxidation, heat, light and validation of the method for accurate quantification of ZPR in the bulk drug and pharmaceutical dosage form.

EXPERIMENTAL

Chemicals and reagents

Ziprasidone was gift sample from the Dr.Reddy's laboratories, Hyderabad and Capsules Zipwell (20mg) was purchased from the local market. Acetonitrile (HPLC grade), Methanol (HPLC grade) were obtained from Merck Fine Chemicals Mumbai, India. Double HPLC grade water was used throughout the experiment. Other chemicals used were of analytical or HPLC grade. Standard stock solution (200 µg/ml) of ZPR was prepared by Accurately weighed 10 mg of ziprasidone was transferred into 50 ml volumetric flasks and dissolved in 10 ml of Methanol, then volume was made up to 50 ml with acetonitrile and vortex it to get complete dissolution of drug. Stand it aside for few minute.

Chromatography

A high performance liquid chromatographic system from Waters comprising of manual injector, waters 715 pump for constant flow and constant pressure delivery and U.V. vis. Detector connected to software data Ace for controlling the instrumentation as well as processing the data generated was used. The chromatographic analysis was performed by using a mobile phase of Methanol: Acetonitrile (60:40v/v). These were filtered through 0.45µ membrane filter and degassed by sonication before use. The mobile phase was pumped isocratically at a flow rate of 1.0ml/min during analysis at ambient temperature. The run time was set at 10 min and the volume of injection was 20µl and eluent was detected at 254 nm on a Thermo C_{18} column (4.6 x 250mm, 5µ particle size).

Analysis of dosage form

Twenty capsules were taken and their average weight was determined. They are crushed to fine powder; amount equal to 20 mg of Ziprasidone was taken in 100-ml volumetric flask. The volume is made up to the mark by mobile phase and filtered by whatmann filter paper (no.41) and the filtrate was used to prepare samples of different concentration.

Forced degradation study

To study the effect of acid, 10 mg of pure drug sample was taken into a 50 ml round bottom flask, 10 ml of 0.1 M HCl solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve.

To study the effect of alkali, 10 mg of pure drug sample was taken into a 50 ml round bottom flask, 10 ml of 0.1 M NaOH solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve.

To study the effect of oxidizing conditions, 10 mg of pure drugs sample was taken into a 50 ml round bottom flask, 10 ml of 3% hydrogen peroxide solution was added, and contents were mixed well and kept for constant stirring for 24 hr at room temperature. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve.

METHOD VALIDATION

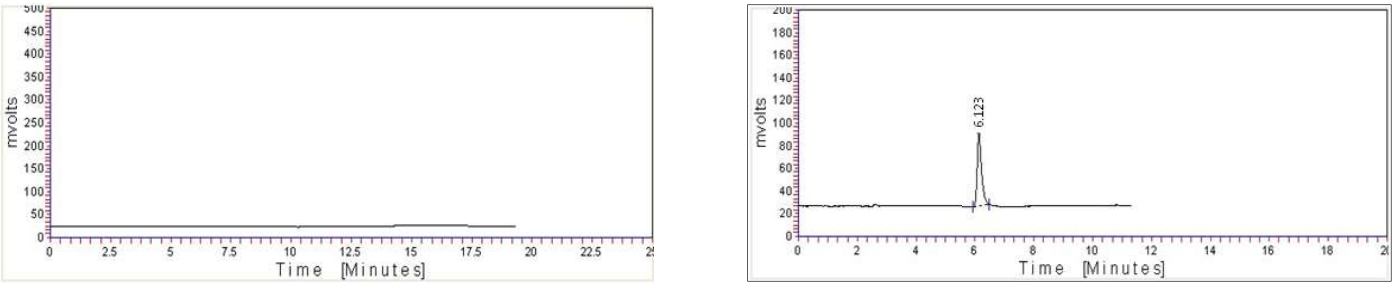
The method was validated for linearity, specificity, limits of detection (LOD) and quantification (LOQ), system in accordance with ICH guidelines. To assess specificity, peak purity was determined by use of

U.V. vis detector. To test linearity, test solutions of ZPR were prepared at six concentrations 5-25µg/ml. Each solution was injected in triplicate and calibration graphs were obtained by plotting peak area against concentration. Linearity was checked over the same concentration range on three consecutive days. RSD (%) of the slope and Y-intercept of the calibration plot were also calculated. The limits of detection (LOD) and quantification (LOQ) for ZPR were determined, as the amounts for which signal-to-noise ratios were 3:1 and 10:1, respectively, by injecting a series of dilute solutions of known concentration. Precision, as RSD (%) was determined by measuring the concentration of drug in the injection six times. Intermediate (inter-day) precision was evaluated by two analysts on different days in the same laboratory. The accuracy of the method was studied by measurement of recovery after adding known amounts of the drug (80, 100 and 120% of the label claim of 20mg ZPR per capsule) to the placebo. Three samples were prepared for each recovery level and results were calculated by use of the calibration plot. The stability of ZPR and sample solutions (at ambient temperature) were tested by analysis after 24, 48, and 72 h, comparison of the results with those obtained from freshly prepared standard solutions and calculation of RSD.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The primary objective in developing this stability indicating HPLC method was to achieve resolution between ZPR and its degradation products. To achieve this, Waters with U.V. vis. detector and C18 column was employed for envisaged work. Combination of Methanol: Acetonitrile (60:40v/v) as mobile phase was attempted for quantitation of ZPR with acceptable system suitability parameters (RT, tailing factor, number of theoretical plates and HETP) at 254nm as detection wavelength. Linearity was found 5-25 µg/ml with correlation coefficient r2 = 0.999 having equation as: AUC = 80.42Conc. +30.28. The column temperature was 25°C. The tailing factor for ZPR was <2 and retention times were approximately 6.12 ± 0.5 min for main peak and less than 10 min for the degradation products (Fig.2). This low total runs time resulted in high productivity and low cost of analysis as per sample.



(A) **(B)**

Figure 2: Chromatogram of (A) blank diluents (B) pure drug

Forced degradation study

Singh and Bakshi [15] suggested target degradation of 20– 80% when establishing the stability-indicating properties of analytical methods, because even intermediate degradation products should not interfere with any stage of drug analysis. Although conditions used for forced degradation were adjusted to achieve degradation in this range, this could not be achieved for conditions other than exposure to acid, base and oxidising agent, even after long exposure. Peak purity test results confirmed the ZPR peak was homogeneous under all the stress conditions tested. The mass balance of ZPR in stress samples was close to 100% and, moreover, assay of unaffected ZPR in the injection confirmed the stability-indicating nature of the method. The results from forced degradation studies are summarized in Table 1. Chromatographic peak-purity data were obtained from the spectral analysis report; peak purity greater than 99 is indicative of a homogeneous peak. The peak purity for ZPR from degradation studies was in the range 99.9-100.0, indicating homogeneous peaks and thus establishing the specificity of the method. No peaks co-eluted with the ZPR peak, suggesting the method enabled specific analysis of ZPR in the presence of its degradation products.

Table 1. Results of Forced degradation studies

Stress conditions	Drug recovered (%)	Drug decomposed (%)
Standard drug	99.50	0
Acidic hydrolysis	85.45	14.55
Alkaline hydrolysis	83.21	16.79
Oxidative degradation	80.12	19.88

Method validation

Peak purity was >99.9% for drug substance and drug degradation products at 254 nm, which shows the analyte peaks were pure and that formulation excipients and degradation products were not interfering with analyte peaks. LOD and LOQ for ZPR were 0.095and 0.500 µg/ml, respectively, for 20 µl injection volume. Results from regression analysis are listed in Table 2, with system suitability data. When precision was determined by six-fold analysis of drug injection, the RSD of ZPR peak area was less than 2%, indicating that the method is reliable. Results from assessment of precision are listed in Table 3. Results obtained from determination of recovery are listed in Table 4.

Table 2: Results from regression analysis and system suitability data

Parameters	Ziprasidone
Retention Time*	6.123 ± 0.5 min
Tailing Factor*	1.24
Theoretical Plate*	3544
Linear range (µg/ml)	5-25
Limits of detection (µg/ml)	0.095
Limits of quantification (µg/ml)	0.500
Linear Equation	80.42x +30.28
Slope	80.42
Intercept	30.28
Correlation coefficient	0.999

**Mean of six readings*

Table 3. Result of precision of test method of Ziprasidone

Std. Conc. (µg/ml)	Repeatability	Intermediate precision	
		Day To Day	Analyst To Analyst
5	100.2	99.80	99.26
10	102.7	103.9	100.19
15	99.60	102.3	99.80
20	101.00	100.5	99.02
25	100.19	100.9	100.12
Mean	100.738	101.48	99.678
S.D.	1.205	1.632	0.519
% R.S.D.	**1.196**	**1.608**	**0.520**

* Mean of fifteen determinations (3 replicates at 5 concentration level)

Table 4. Recovery of Ziprasidone

Level of addition	Std. Drug sol. Added (µg/ml)	% Mean* recovered
80	6	99.56
100	12	99.83
120	18	99.96

**Average of five determination*

CONCLUSION

The method developed for quantitative analysis of Ziprasidone is rapid, precise, accurate and selective. Peak purity studies under all the stress conditions showed the drug peak to be pure and hence the method is stability indicating. In other words it can be entioned that the method developed can be utilized for the successful quantification of the drug in presence of its degradation product and excipients. The method was completely validated and satisfactory results were obtained for all the characteristics tested. The method is stability-indicating and can be used to assess the stability of ziprasidone in the bulk drug. The method can be conveniently used for routine analysis of ziprasidone as bulk drug, in respective dosage forms, for dissolution studies and as stability indicating assay method in pharmaceutical laboratories and industries.

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