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## Validated stability-indicating high-performance liquid chromatographic method for determination of ziprasidone hydrochloride in bulk drug and dosage form

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### ABSTRACT

A stability-indicating high-performance liquid chromatographic assay method was developed and validated for quantitative determination of ziprasidone hydrochloride in bulk drugs and the degradation products generated from forced decomposition. An isocratic, reversed phase HPLC method was developed to separate the drug from the degradation products, using an YMC Pack ODS-A C18 (250mm×4.6mm, 5 $\mu$ ) column and the mobile phase containing the mixture of triethylamine-phosphoric acid buffer (pH-3 by orthophosphoric acid), acetonitrile and methanol (53:15:32, v/v/v). The detection was carried out at wavelength 230nm. The chromatographic resolution between its degraded products was found to be greater than three. The ziprasidone hydrochloride was subjected to stress conditions of hydrolysis acid, base, oxidation (30% H<sub>2</sub>O<sub>2</sub>), photolysis and thermal degradation. The negligible degradation was observed for ziprasidone hydrochloride in acid and oxidative degradation while in base hydrolysis, and thermal degradation, considerable degradation was observed. The mass balance was close to 100 in all the stress conditions. The degraded products were well resolved from main peak. The developed method was validated with respect to linearity, accuracy recovery, precision, system suitability, selectivity, robustness and forced degradation studies prove the stability indicating ability of the method.

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### KEYWORDS

Ziprasidone hydrochloride;  
Stability indicating;  
RP-HPLC;  
YMC pack ODS-AC18  
column;  
Forced degradation;  
Validation.

### INTRODUCTION

Ziprasidone hydrochloride is described chemically as: 5-[2-[4-(1, 2-benzisothiazol- 3-yl)-1-piperazinyl] ethyl]-6-chloro-1,3-dihydro-2H-indol-2-one mono hydrochloride salt<sup>[1-3]</sup>. Ziprasidone hydrochloride is marketed with brand names Zeldox and Geodon. It is the fifth typical antipsychotic to gain FDA approval (in

midst 2000) for the treatment of schizophrenia and schizoaffective disorder<sup>[4,5]</sup>. Ziprasidone hydrochloride has a high affinity for dopamine serotonin and alpha-adrenergic receptors and medium affinity for histaminic receptors<sup>[6]</sup>. The systemic bioavailability of ziprasidone administered intra-muscularly is 100 % and 60 %, for oral administration. Literature surveys reveal, high-performance liquid chromatographic methods were re-

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ported for the determination of ziprasidone in bulk drugs<sup>[7,8]</sup>. We are gratified to report a stability indicating HPLC method for the analysis and separation of drugs from the degradation products formed under ICH suggested conditions (hydrolysis, oxidations, photolysis and thermal stress)<sup>[9]</sup>. In present article, reversed phase HPLC method was developed for the separation ziprasidone in bulk drug and the impurities formed from its forced degradation under stress conditions like acid hydrolysis, base hydrolysis, oxidation, heat and light.

## EXPERIMENTAL

### Material and reagents

Ziprasidone hydrochloride bulk drug was made available from Cipla Ltd. India (purity 99.8). Orthophosphoric acid, triethylamine, and hydrochloric acid were obtained from Qualigens fine chemicals, India Limited. Acetonitrile, methanol, hydrogen peroxide, sodium hydroxide were obtained from Rankem laboratories, India. All chemicals and reagent were used as HPLC grades. UV cabinet was used of Kumar made, (India). Milli-Q-Water was used throughout the experiment.

### Chromatographic conditions

A chromatographic system (Shimadzu Corporation, Japan) consisting of quaternary solvent delivery pump, a degasser, an auto-injector, column oven and UV detector, 10A-VP series with Class-VP software. The chromatographic column of 250mm length and internal diameter of 4.6 mm filled with octadecyl silane YMC pack-C18 (procured from YMC Corporation, Kyoto, Japan) stationary phase with particle size 5micron and pore size 100Å was used. The instrumental settings were a flow of 1ml/min, the injection volume was 10µl.

### Mobile phase

The mobile phase consisted of a mixture of triethylamine-phosphoric acid buffer (pH-3 by orthophosphoric acid), acetonitrile and methanol (53:15:32,v/v/v). The mobile phase was premixed and filtered through a 0.45µm nylon filter and degassed.

### Preparation of standard stock solutions

Standard solution of ziprasidone was prepared by

dissolving the bulk drugs sample in the diluents and diluting it to the desired concentration. Diluents used for the standards and sample preparations, was prepared as follow: diluent was composed of buffer, a cetonitrile and methanol(53:15:32,v/v/v). A 50mg of ziprasidone was accurately weighed, transferred in a 50ml volumetric flask, and dissolved with the diluent. From this stock solution, by transferring 5ml of ziprasidone standard solutions in a 50ml volumetric flask and diluted with acetonitrile. This solution contained 100µg/ml of ziprasidone.

### Sample solution (Tablets)

Ten tablets of zeldox (80mg) were finely ground using agate mortar and pestle. The ground material, which was equivalent to 80mg of the active pharmaceutical ingredient, was extracted into buffer (pH-3) in a 100ml volumetric flask by vortex mixing followed by ultra sonication and makeup the volume by acetonitrile. The solution was filtered through 0.45-micron filter and an appropriate concentration of sample (100µg/ml-assay concentration) was prepared in diluents at the time of analysis.

### Selectivity

Selectivity is the ability of the method to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically, these might include degradants, matrix etc<sup>[10]</sup>. The selectivity of the developed LC method for ziprasidone was carried out in the presence of its degradation products.

Stress studies were performed for ziprasidone bulk drug to provide an indication of the stability indicating property and selectivity of the proposed method. Intentional degradation was attempted to stress condition exposing it with acid (0.05 N hydrochloric acid), alkali (0.025N NaOH), hydrogen peroxide (30%), heat (60°C) and UV light (254 nm and 366nm wavelength)

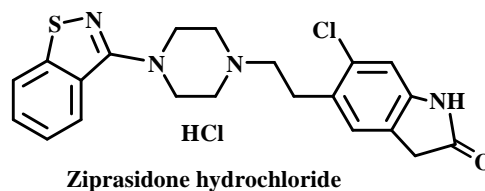


Figure 1 : Chemical structure of ziprasidone

RESULTS AND DISCUSSION

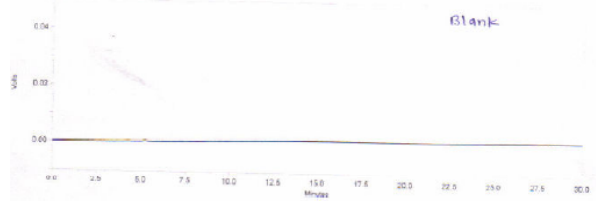


Figure 2 : A typical chromatogram of the blank for ziprasidone

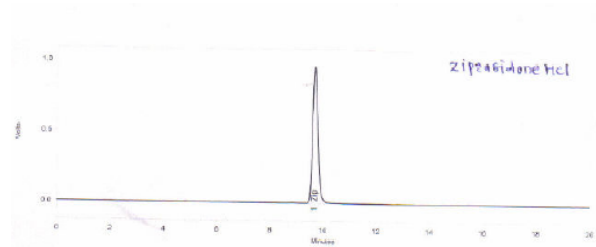


Figure 3: A typical chromatogram of the tablet: ziprasidone

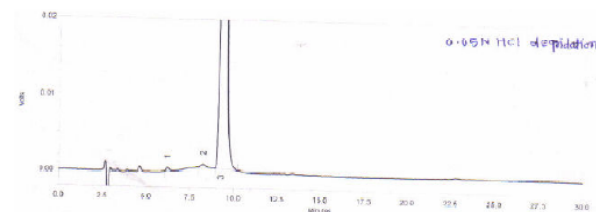


Figure 4: Chromatogram of ziprasidone in acid degradation

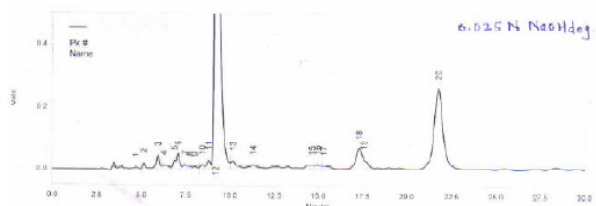


Figure 5: Chromatogram of ziprasidone in base degradation

to evaluate the ability of the proposed method to separate ziprasidone from its degraded products. For heat and light study, study period was 7 days where as for acid, oxidation 48 hr and for base 2 hour. Assay studies were carried out for stress samples against ziprasidone reference standard and the mass balance (% assay + % sum of all impurities + % sum of all degraded products) was calculated. The excipient mixture present in Zeldox tablets was injected in the optimized conditions to show the selectivity of the method in formulation of ziprasidone.

Optimization of chromatographic conditions

The main target for the development of chromatographic method was to get the reliable method for the quantification of ziprasidone from bulk drug and which will be also applicable for the degradable products. Initially, we took the effort for the development of HPLC method quantification of standard ziprasidone from bulk. For this purpose, we have used Hypersil-BDS C18, Kromasil C18, Water nova pack C18, Star ODS-II C18, Inertsil ODS C18 and YMC pack ODS-A. Out of these used HPLC column, YMC Pack ODS-A found to comparatively better and gave the graph with better gaussian shape at retention time 9.65 min. To improve the shape and width of the graph, the solvent system and buffer used were varied. The solvent systems used were 0.5% ortho phosphoric acid in water and acetonitrile(30:70, v/v), 0.25% ortho phosphoric acid in water and acetonitrile(20:80, v/v), 0.3% ortho phosphoric acid in water and acetonitrile (40:60, v/v). But, there was not satisfactory improvement in the shape and width of graph. Then, to improve it, the buffer used varied to ammonium acetate, TFA, and  $\text{KH}_2\text{PO}_4$ . Use of triethylamine-ortho phosphoric acid buffer(pH-3), acetonitrile and methanol (53:15:32, v/v/v) gave satisfactory improvement in chromatogram.

Result of forced degradation experiments

Considerable degradation was not observed in ziprasidone hydrochloride bulk samples, under stress

TABLE 1 : Summary of forced degradation results

Stress conditions	Time	Assay of Active substance (%)	Mass balance (% Assay + %impurity)	Remarks
Acid hydrolysis (0.05 NHCl)	48Hrs	99.62	99.85	No degradation
Base hydrolysis (0.025 N NaOH)	2Hrs	53.24	99.78	Degradation
Oxidation (30% $\text{H}_2\text{O}_2$ )	48Hrs	99.30	99.82	No degradation
Thermal (80°C)	7days	62.37	99.80	Degradation
UV 254nm	7days	99.13	99.88	No degradation
UV 366nm	7days	99.33	99.90	No degradation

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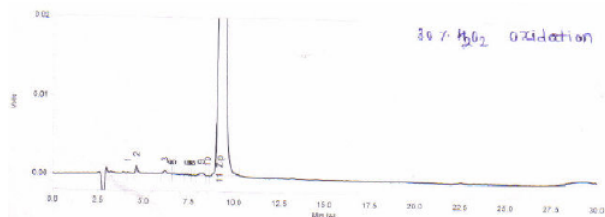


Figure 6: Chromatogram of ziprasidone in oxidative degradation

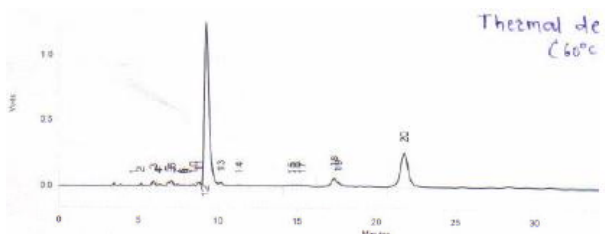


Figure 7: A chromatogram of ziprasidone in thermal degradation 80°C

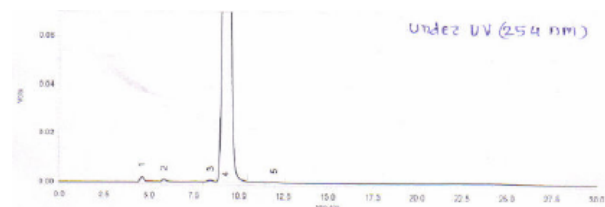


Figure 8: A chromatogram of ziprasidone in ultraviolet at 254nm

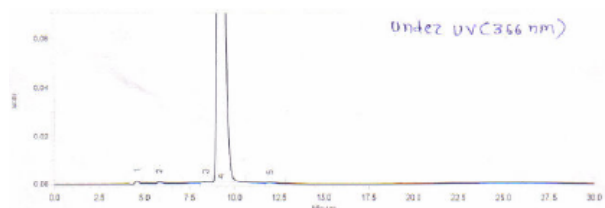


Figure 9: A chromatogram of ziprasidone in ultraviolet at 266nm

conditions such acid (figure 4) and oxidative hydrolysis (figure 6). Mild degradation of ziprasidone hydrochloride was observed under thermal stress (figure 7). Considerable degradation of the drug substance was observed under base hydrolysis (figure 5) leads to the formation of some unknown degradation peaks. The mass balance of ziprasidone hydrochloride in stress samples was close to 100% and moreover, the unaffected assay of ziprasidone hydrochloride in the tablets confirms the stability indicating power of the method. The summary of forced degradation studies is given in TABLE

TABLE 2: System suitability reports

Compound (n=3)	Retention time	% RSD	USP Resolution	USP Tailing	Theoretical plates
ziprasidone	9.658	0.18	-	1.41	11134
Acid degraded	9.267	0.26	1.27	1.44	10316
Product Base					
Degraded	9.192	0.70	3.81	1.44	9815
Product H <sub>2</sub> O <sub>2</sub>					
Degraded	9.183	0.76	1.33	1.43	10645

TABLE 3: Results of the linearity study and precision

Ingredient	Precision (% RSD)	Linearity Slopes* (µg/ml)	Coefficients of correlation (n = 3)
ziprasidone	0.18	10-150	5265.4 0.99943

\*Standard deviation shown in parentheses

TABLE 4 : Assay results of active ingredients in tablets

Set(n=3)	Label value (mg)	Found(mg)*	% assay	SD	RSD%
1	80	80.88	100.1	1.13	0.26
2	80	80.34	100.3	1.44	0.36

\*Average of six analysis

1.

## Method Validation

### 1. System suitability

For system suitability studies, five replicate injections of acid, base and oxidative degraded solutions were used and the RSD of peak area ratio, resolutions, tailing factor and number of theoretical plates of the peak were calculated. The system suitability results are shown in TABLE 2.

### 2. Precision

The precision of the method was studied by determining the concentrations of the drug ziprasidone hydrochloride in the tablet for six times. The results of the precision study (TABLE 3) indicate the reliability of the method (%RSD < 2).

### 3. Intermediate precision (reproducibility)

Intermediate precision of the method was determined by analyzing the samples for six times on different days, by different chemists, by using different analytical columns of the same make and different HPLC systems. The percentage assay was calculated using calibration curves. The assay results are shown in TABLE 4.

### 4. Accuracy (Recovery test)

**TABLE 5 : Results of the recovery tests for the ziprasidone**

Level of Addition (%)	Amount added(n=3)(mg)	% Recovery*	% Average recovery
80	70	100.8	100.77
100	80	100.67	100.69
120	90	100.92	100.90

\*RSD shown in parenthesis; ^Average recovery=the average of three levels, nine determinations

**TABLE 6: Results of robustness study**

Sr. no.	Parameters	Variations	Resolutions between ziprasidone and base degraded product
1	Temperature	(a) At 25°C	3.4
		(b) At 35°C	3.7
2	Flow rate	(a) 0.8 ml/min	4.3
		(b) 1.2ml/min	3.8
3	Mobile phase	(a) 40.5ml	4.4
		(b) 49.5ml	3.9

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found<sup>[11]</sup>. Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at three levels, 80%, 100% and 120% of the label claim of the tablet (80mg of Zeldox). Placebo equivalent to one tablet was transferred into a 200-mL volumetric flask, and the amounts of ziprasidone hydrochloride at 80%, 100% and 120% of the label claim of the tablet were added to it. The recovery samples were prepared as afore mentioned procedure, and then 5ml of ziprasidone hydrochloride solutions were transferred into a 50mL volumetric flask and diluted to volume with acetonitrile. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery values for ziprasidone hydrochloride ranged from 100.8 % to 100.92% (TABLE 5). The average recoveries of three levels nine determinations for ziprasidone hydrochloride were 100.77-100.90%.

## 5. Calibration and linearity

Linearity test solutions for the method were prepared from ziprasidone hydrochloride stock solutions at six concentrations levels from tested from 10% to 150% of the targeted level (100µg/ml), of the assay concentration ziprasidone hydrochloride. Standard so-

lutions containing 10-150µg/ml of ziprasidone hydrochloride in each linearity level were prepared. Linearity solutions were injected in triplicate. The calibration graphs were obtained by plotting peak area verses the concentration data was treated by least-squares linear regression analysis. The equations of the calibration curves for ziprasidone hydrochloride obtained were  $y=5265.4x - 4508.3$ , the calibration graphs were found to be linear in the mentioned concentrations (the slopes and correlation coefficients are shown in TABLE 3).

## 6. Robustness

To determine the robustness of the developed method experimental condition were purposely altered and the resolution between ziprasidone hydrochloride and acid degraded product were evaluated. The flow rate of the mobile phase was 1.0ml/min. To study the effect of flow rate on the resolution, it was changed by 0.2 unit from 0.8 to 1.2ml/min while the other mobile phase component were held as stated in chromatographic conditions. The effect of percent organic strength on resolution was studied by varying acetonitrile from -10 to +10 % while other mobile phase components were held constant as stated in chromatographic condition. The effect of column temperature on resolution was studied at 25 and 35°C instead of 30°C while the other mobile phase components were held constant stated in chromatographic condition. The results are shown in TABLE 6.

## 7. Stability of analytical solution

The stability of the standard solutions and the sample solutions was tested at intervals of 24, 48 and 72h. The stability of solutions was determined by comparing results of the assay of the freshly prepared standard solutions. The RSD for the assay results determined up to 72h for ziprasidone hydrochloride was 0.18%. The assay values were within  $\pm 2\%$  after 72h. The results indicate that the solutions were stable for 72h at ambient temperature.

## 8. Determination of active ingredients in tablets

The contents of drug in tablets were determined by the proposed method using the calibration curve. The results are shown in TABLE 4. The chromatogram of the tablet sample is shown in (Figure 3).

**CONCLUSION**

The method developed for quantitative determination of ziprasidone hydrochloride is rapid, precise, accurate and selective. The method was completely validated showing satisfactory data for all method-validated parameters tested. The developed method is stability indicating and can be used for assessing the stability of ziprasidone hydrochloride as bulk drugs. The developed method can be conveniently used for the assay determination of ziprasidone hydrochloride in bulk drugs and pharmaceutical dosage form. The developed method can be conveniently used for dissolution of tablets of the pharmaceutical dosage forms containing ziprasidone hydrochloride in quality control laboratory.

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