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# Stability Indicating Method Development and Validation of Ziprasidone by using RP-HPLC Method

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## Abstract

A new simple, sensitive and stability indicating RP-HPLC method for the determination of Ziprasidone in pharmaceutical dosage form was developed. Chromatographic separation was carried on Reverse Phase C18 (Cosmosil) column (symmetry 250x4.6mm;5µm) with a mobile phase composed of acetonitrile and 0.05% (OPA) water (90:10 v/v) having pH 3.0 at an absorption maxima 318nm. The analysis of the drug was carried out on Agilent Tech. Gradient System with Auto injector DAD Detector. Equipped with Reverse Phase C18 (Cosmosil) with 250 mm x 4.6; (5µm), a SP930D pump, a 20 µl injection loop and DAD Absorbance detector and running chem station software. Linearity for detector response was observed in the concentration range of 80-120% of test concentration. The correlation coefficient of the method shows good linear relationship with 0.9996. Retention time was found to be 4.3 min. The % recovery of Ziprasidone is between 97- 101%. The %RSD for the tablet analysis is less than 2 which is indicating high degree of precision. The limit of detection and quantification are determined for Ziprasidone 0.1722 µg/ml and 0.5218 µg/ml. Drug product was exposed to acid, base, oxidation and neutral conditions and the samples were analyzed by the proposed validated method. Results of the analysis were validated statistically and by recovery studies. The developed method was found to be precise for the determination of Ziprasidone in bulk dosage form. The chromatographic method validation of Ziprasidone was simple, reliable, sensitive and less time consuming. This method may be recommended for routine and quality control analysis of the investigated drug. The developed method is specific and stability indicating. Hence this method is suitable, linear, accurate & robust for the estimation of Ziprasidone

**Keywords:** Stability indicating, RP-HPLC, Ziprasidone, Cosmosil, dosage form.

## Introduction

Ziprasidone is chemically 5-[2-[4-(1,2-benzisothiazol-3-yl)-1-piperazinyl] ethyl]-6-chloro-1,3-dihydro-2H-indol-2-one and the structural formula is as shown in Fig

1. It is an atypical antipsychotic drug.[1,2] The molecular formula is $C_{21}H_{21}ClN_4OS$ and having molecular weight 412.94 g/mol. It is atypical antipsychotic drug and it is slightly soluble in DMSO and methanol. Its melting point is about 213-215°C. The pKa value is 13.34 +- 0.20 (pe-redicted). It is brown to dark brown solid. The mechanism of action of Ziprasidone, as with other drugs having efficacy in schizophrenia, is unknown. However, it has been proposed that this drug's efficacy in schizophrenia is mediated through a combination of dopamine type 2 (D2) and serotonin type 2 (5HT2) antagonisms. As with other drugs having efficacy in bipolar disorder, the mechanism of action of Ziprasidone in bipolar disorder is unknown.[3-5]

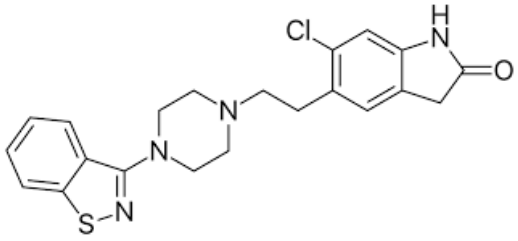


**Figure No.1: Structure of Ziprasidone**

## Materials and Method

### Materials

Ziprasidone was obtained from Swapnaroop Drug & Pharmaceutical. HPLC grade water, Acetonitrile, methanol, and OPA from Merck.ltd were used for study. A local store provided ZELDOX (20MG).

### Instrument

The analysis of the drug was carried out on Agilent Tech. Gradient System with Auto injector DAD Detector. Equipped with Reverse Phase C18 (Cosmosil) with 250mm x4.6; (5µm), a SP930Dpump, a 20µl injection loop and DAD Absorbance detector and running chemstation software.

### Mobile phase preparation

Acetonitrile and 0.05% (OPA) water were mixed in the ratio of 90:10 v/v and degassed.

### Standard stock preparation

Stock I: Standard Sample Preparation:
Std. Ziprasidone 10 mg in 10 ml Methanol 1000 µgm/ml
Stock II: formulation solution Preparation: Take 22.5 ml in 10 ml Methanol 400 µgm/ml

**Table No.1: Chromatographic conditions (HPLC) details used during method Development**

| Sr. No. | Parameters | Particulars |
|---|---|---|
| 1. | HPLC | Agilent Tech. Gradient System with Auto injector |
| 2. | Software | Chemstation |
| 3. | Column | (Agilent) C18 column (4.6mm x 250mm |
| 4. | Particle size packing | 5 µm |
| 5. | Stationary phase | C18 (Cosmosil) |
| 6. | Mobile Phase | Acetonitrile :Water (0.05% OPA) 90:10 |
| 7. | Detection Wavelength | 318 nm |
| 8. | Flow rate | 1 ml/min |
| 9. | Temperature | Ambient |
| 10. | Sample size | 20 µl |
| 11. | pH | 3.0 |
| 12. | Run Time | 15 min |
| 13. | Filter paper | 0.45 µm |

**Linearity**

The capacity of an analytical process to produce a response that is directly proportional to the concentration (quantity) of analyte in the sample is known as linearity.[6] Linearity of detector was found by injecting five standard solutions with concentration ranging from 10 to 50 µm/ml of the test concentration and a graph was plotted for concentration versus peak area. The calibration curve of Ziprasidone is depicted in Fig 2.

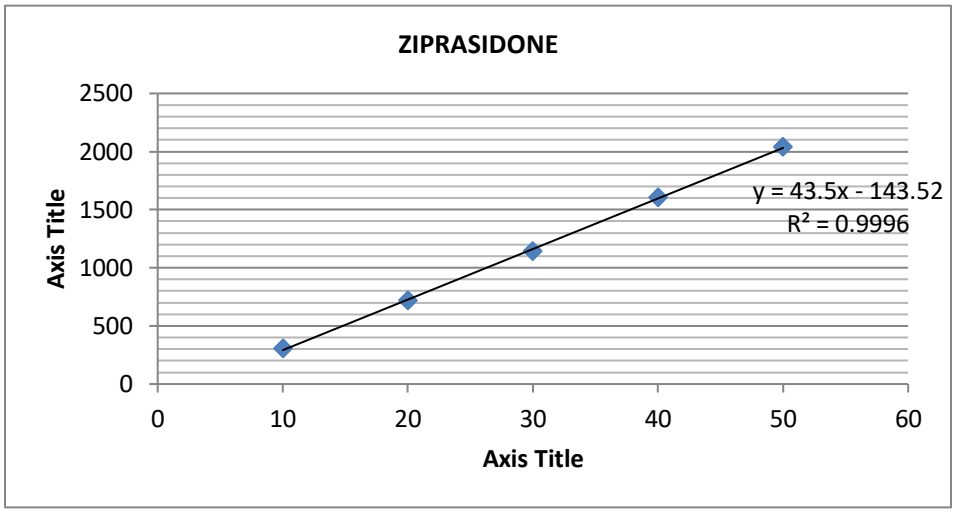


**Figure No. 2: Calibration curve of Ziprasidone**

**Accuracy**

The accuracy of a measurement is defined as the closeness of the measured value to the true value. Typically, accuracy is represented and determined by recovery studies.[7, 8] Recovery studies were performed to validate the accuracy of developed method. To pre analyzed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed (Table No.2). Statistical validation of recovery studies shown in (Table No.3)

**Table No.2: Result of Recovery data for Ziprasidone**

| Method | Drug | Level (%) | Amt. taken (µg/ml | Amt. Added (µg/ml | Absorbance/ Area Mean* ± S.D. | Amt. recovered Mean *±S.D. | %Recovery Mean *± S.D. |
|---|---|---|---|---|---|---|---|
| RP-HPLC Method | Zips | 80% | 20 | 16 | 36.28± 0.06 | 20.23± 0.13 | 101.36±0.34 |
| | | 100% | 20 | 20 | 39.64± 0.13 | 20.58±0.13 | 98.20±0.70 |
| | | 120% | 20 | 24 | 44.31 ±0.01 | 20.32±0.03 | 99.97±0.15 |

*mean of each 3 reading for RP-HPLC method and UV method

**Table No.3: Statistical Validation of Recovery Studies Ziprasidone**

| Method | Drug | Level (%) | Mean % Recovery | Standard Deviation* | % RSD |
|---|---|---|---|---|---|
| RP-HPLC Method | Zips | 80% | 101.36 | 0.34 | 0.33 |
| | | 100% | 98.20 | 0.70 | 0.71 |
| | | 120% | 99.97 | 0.15 | 0.15 |

*Denotes average of three determinations for RP-HPLC and UV method

**Precision**

Precision is described as "The degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogenous sample."[9, 10] the method was established by analyzing various replicates standards of Ziprasidone. All the solution was analyzed thrice in order to record any intra-day & inter-day variation in the result that concluded. The result obtained for intraday and interday is shown in (Table No.4) respectively.

**Table No.4: Result of Intraday and Inter day Precision studies on RP-HPLC method for Ziprasidone**

| Method | Drug | Conc (µg/ml) | Intraday Precision | | Interday Precision | |
|---|---|---|---|---|---|---|
| | | | Mean± SD | %Amt Found | Mean± SD | %Amt Found |
| RP-HPLC METHOD | Zips | 10 | 295.78 ±0.96 | 100.98 | 293.32±0.96 | 100.40 |
| | | 30 | 1141.87 ±1.10 | 98.49 | 1134.36±1.41 | 97.90 |
| | | 50 | 2043.14±0.83 | 100.53 | 2040.99±6.14 | 100.38 |

*Mean of each 3 reading for RP-HPLC and UV method

**Robustness**

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage. The robustness of a method is evaluated by varying method parameters such as percent organic solvent, pH, ionic strength, temperature and determine the effect (if any) on the results of the method.[11]

Effect of variation in flow rate, mobile phase composition and wavelength on retention time and tailing factor of drug peak was studied. The mobile phase composition was changed in (±1 ml/min$^{-1}$**)** proportion and the flow rate was varied by (±1ml/min$^{-1}$**)**.

Wavelength change (±1 ml/min$^{-1}$**)** of optimized chromatographic condition. The results of robustness studies are shown in (Table No.5). Robustness parameters were also found satisfactory; hence the analytical method would be concluded.

**Table No.5: Result of Robustness Study of Ziprasidone**

| Parameters | Conc. | Amount of detected (mean ±SD) | %RSD |
|---|---|---|---|
| Mobile phase composition-(89+11) | 50 | 2006.2±2.86 | 0.14 |
| Mobile phase composition-( 91+9) | 50 | 2017.97±3.33 | 0.17 |
| Wavelength change317nm | 50 | 2078.2±4.26 | 0.21 |
| Wavelength Change 319nm | 50 | 2082.33±2.96 | 0.14 |
| Flow rate change(0.9ml) | 50 | 2041.97±12.72 | 0.62 |
| Flow rate change(1.1ml) | 50 | 1769.69 ± 7.83 | 0.44 |

**Limit of detection and Limit of quantification**

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable Precision and accuracy.[12-14] The LOD and LOQ for this method were found to be 0.1722 and 0.5218 µg/ml respectively.

**Forced degradation studies**

To cause partial drug degradation, different forced degradation conditions were used to the zeldox tablets containing (Ziprasidone 20mg). Studies on forced deterioration were carried out to demonstrate that the technique is appropriate for the degraded products. In addition, the studies reveal the circumstances under which the medicine is unstable, enabling precautions to be made during formulation to prevent potential instabilities.[15] Forced degradation studies of the drugs namely Ziprasidone were carried out individually and in combination under different stress conditions like acid hydrolysis, alkaline hydrolysis, hydrogen peroxide oxidation and photolysis. The results are shown in (Table No.6)

**Acid degradation**

About 0.4ml standard sample solution was transferred into 10ml volumetric flask. To that 5ml 0.1N HCl was added and made up to the mark with mobile phase and sonicated for 60min. Then the solution was filtered through 0.45µm filter. After 60min sample for injection. The major degradation products for Ziprasidone were observed at 1 hr % degradation 12.79 %.

**Base degradation**

About 0.4ml standard sample solution was transferred into 10ml volumetric flask. To that 5ml 0.1N NaOH was added and made up to the mark with mobile phase and sonicated for 60min. Then the solution was filtered through 0.45µm filter. After 60min sample for injection. The major degradation products for Ziprasidone were observed at 1 hr % degradation 11.97%.

**Neutral degradation**

About 0.4ml standard sample solution was transferred into 10ml volumetric flask. To that 5ml water was added and made up to the mark with mobile phase and sonicated for 60min. Then the solution was filtered through 0.45µm filter. After 60min sample for injection. There was no major degradation observed for Ziprasidone and hence they were not sensitive to light.

**Hydrogen Peroxide degradation:**

About 0.4ml standard sample solution was transferred into 10ml volumetric flask. To that 5ml 3% $H_2O_2$ was added and made up to the mark with mobile phase and sonicated for 60min. Then the solution was filtered through 0.45µm filter. After 60min sample for injection. In the oxidation condition with 3% $H_2O_2$ for 1 hr, Ziprasidone show oxidative stress degradation peak in the chromatogram observed at 1 hr % degradation 8.55%.

**Table No.6: Degradation of different stress condition**

| Sr no | Degradation parameter | %Degradation |
|---|---|---|
| 1 | Alkali DEG. 0. 1 N NAOH - AFTER 1hr 40mcg | 11.97 |
| 2 | Acid DEG.0. 1 N HCL- AFTER 1hr (40 mcg) | 12.79 |
| 3 | 3% H202 DEG AFTER 1hr -40mcg | 8.55 |
| 4 | Neutral After 1hr -40mcg | 0.26 |

**Analysis of tablet formulation**

**Procedure**

Weigh equivalent weight of Ziprasidone 22.5mg in 10ml volumetric flask. Add about 10 ml of diluent and sonicate to dissolve it

completely and make volume up to the mark with diluent. Mix well and filter through 0.45 µm filter. Further pipette 0.5 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents (40 µg/ml). The simple chromatogram of test Ziprasidone Shown in Fig 3the amounts of Ziprasidone per tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with tablet formulation. Tablet Assay for %Lable claim for %RSD Calculated, Result was shown in (Table No.7)

Brand Name: ZELDOX (20MG)
22.5 mg Sample in 10 ml methanol
1000µgm/ml Ziprasidone stock –II
Take 0.4 ml in 10ml Mobile Phase 40µg/ml tab. solution

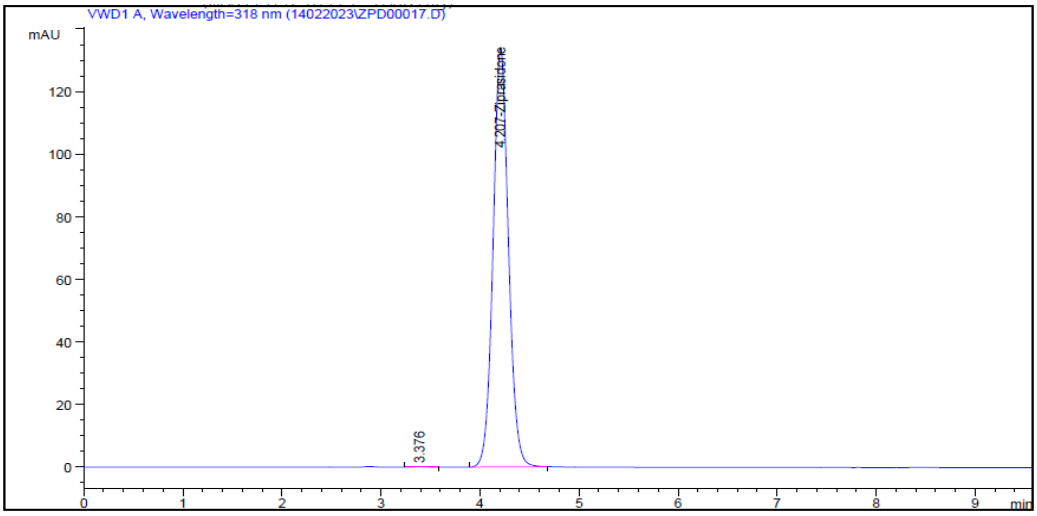


**Table No. 7: Analysis of marketed formulation**

| Assay | Drug | Label Claimed | Amt Found | %Label Claim | SD | %RSD |
|---|---|---|---|---|---|---|
| **RP-HPLC Method** | **Zips** | 40 | 40.87 | 102.18 | 0.51 | 003 |
| | | 40 | 40.89 | 101.73 | 0.51 | 0.03 |

## Results

The retention times observed as 4.3 min. The linearity for detector response was observed in the concentration range of 80 to 120% of the concentration and the correlation coefficient(r) for calibration curve was found to be 0.9996. The results of the recovery studies between 80 to 120% were in the range of 97 to 101% indicating accuracy of the method. The %RSD for the tablet analysis is less than 2 which is indicating high degree of precision. The results of the robustness study

indicates that the method is robust and is unaffected by small variations in the chromatographic conditions. The LOD and LOQ were calculated and were found to be 0.1722 and 0.5218 µg/ml respectively. Forced degradation studies were performed and degradation was found within the 20-80% range. Analysis of marketed formulation were also %Lable Claim was found to be 99-101% satisfactory are concluded.

## Discussion

The proposed method for the determination of Ziprasidone in pharmaceutical dosage form was found to be precise, selective and economical the present study describes the RP-HPLC method development and validation of Ziprasidone. The dosage form was analyzed symmetry C18 (Cosmosil) column (250mmx4.6mm) using Acetonitrile and 0.05% (OPA) water having pH 3.0 at an absorption maxima 318nm with flow rate 1.0ml/min.

## Conclusion

The chromatographic method validation of Ziprasidone was simple, reliable, sensitive and less time consuming. This method may be recommended for routine and quality control analysis of the investigated drug. The developed method is specific and stability indicating. Hence this method is suitable, linear, accurate & robust for the estimation of Ziprasidone. The present work shows, a validated, highly sensitive method for determination of Ziprasidone.

## Acknowledgement

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