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DEVELOPMENT AND VALIDATION OF A ROBUST RP-HPLC ANALYTICAL METHOD FOR QUANTIFICATION OF ZIPRASIDONE

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ABSTRACT

This study presents the development and validation of a reversed-phase high-performance liquid chromatography (RP-HPLC) method for the quantification of ziprasidone (ZP) in bulk drug and formulation samples. The method utilized a Lichrospher® 100 RP-18 column (250 × 4.6 mm, 5 µm) and a mobile phase comprising buffer and acetonitrile (65:35, %v/v) at pH 3.0. Detection was performed at 318 nm. Method validation was conducted per ICH guidelines, confirming linearity (R^2 = 0.9999), precision, accuracy (recovery: 99.81–101.34%), and sensitivity (LOD: 40.88 ng/mL, LOQ: 123.91 ng/mL). Robustness and ruggedness assessments demonstrated method reliability under variable conditions. This RP-HPLC method exhibits excellent potential for routine analysis of ZP in quality control settings.

Keywords *– RP-HPLC, Ziprasidone, Method validation, Linearity, Robustness, Quantification.*

1. INTRODUCTION

High-performance liquid chromatography (HPLC) has emerged as a cornerstone of modern analytical chemistry, renowned for its sensitivity, precision, and versatility. It is widely used in pharmaceutical analysis, where accurate quantification of active pharmaceutical ingredients (APIs) is critical for ensuring product quality, therapeutic efficacy, and patient safety. The technique is particularly suitable for complex mixtures, offering high resolution and reproducibility under controlled chromatographic conditions. In recent years, the adoption of reversed-phase HPLC (RP-HPLC) has further streamlined the quantification process, enabling the efficient analysis of both hydrophilic and lipophilic compounds.

Ziprasidone (ZP), an atypical antipsychotic drug, is extensively prescribed for the management of schizophrenia and bipolar disorder. It acts as a serotonin (5-HT) and dopamine (D2) receptor antagonist, contributing to its efficacy in controlling psychotic symptoms and mood disturbances [1,2]. Given its therapeutic significance, the accurate quantification of ZP in pharmaceutical formulations is essential. Ensuring the correct dosage and uniformity across batches is not only a regulatory requirement but also a critical factor in minimizing adverse drug reactions and maximizing clinical outcomes [3].

While several chromatographic methods for ZP quantification have been reported in the literature, many face challenges such as the use of complex mobile phases, lengthy analysis times, or insufficient validation data. These limitations can hinder their applicability in routine quality control settings, where efficiency and reliability are paramount [4,5]. Additionally, variability in

methods underscores the need for a standardized analytical approach that is robust, reproducible, and compliant with global quality standards.

This study addresses these gaps by focusing on the development of a simple and efficient RP-HPLC method for ZP quantification. The method was designed with a deliberate emphasis on minimizing complexity while achieving high sensitivity and specificity. Method optimization included careful selection of column parameters, mobile phase composition, and detection wavelength to ensure compatibility with routine laboratory workflows.

The developed method was validated following the International Council for Harmonisation (ICH) Q2(R1) guidelines, covering key analytical performance characteristics such as linearity, accuracy, precision, sensitivity, robustness, and ruggedness [6]. These validations not only demonstrate the method's reliability but also highlight its potential for widespread application in the pharmaceutical industry, from quality control to regulatory compliance. This work aims to contribute a validated and practical analytical tool for the quantification of ZP, ensuring its therapeutic integrity in pharmaceutical formulations.

2.MATERIALS AND METHODS

Instrumentation and Chromatographic Conditions

The analysis was conducted using a Shimadzu HPLC system equipped with a quaternary LC-10 AT VP pump, SCL 10A VP system controller, SPD-10 AVP column oven, and a Rheodyne injector with a 20 µL loop. Chromatographic separation was achieved on a Lichrospher® 100 RP-18 column (250 × 4.6 mm, 5 µm) using buffer-acetonitrile (65:35, %v/v) as the mobile phase at pH 3.0. The flow rate was 1 mL/min, and detection was performed at 318 nm (8–9).

Preparation of Mobile Phase: The buffer was prepared with o-phosphoric acid and mixed with acetonitrile in a 65:35 ratio (%v/v). The pH was adjusted to 3.0, and the mixture was degassed using sonication and filtered through a 0.45 µm membrane filter [7].

Preparation of Solutions:

- **Stock Solution:** Prepared by dissolving 10 mg of ZP in 40 mL of mobile phase and diluting to 100 mL.
- **Working Solutions:** Freshly prepared by diluting stock solution to desired concentrations (0.5–100 µg/mL) [8].

Validation Parameters:

The analytical method was validated with respect to the ICH guidelines (ICH, 2005) parameters such as linearity, accuracy, precision, and specificity, limit of detection (LOD), limit of quantification (LOQ), robustness and ruggedness [9]:

Linearity: Calibration curves were constructed using concentrations from 0.5 to 100 µg/mL. A stock solution of ZP (1000 µg/mL) was prepared by dissolving accurately weighed amount of ZP (100 mg) in 40 mL of the mobile phase in 100 mL of volumetric flask. Then make up the volume to 100 mL with the mobile phase. Solutions of different concentrations 0.5, 1, 2, 4, 8, 10, 16, 20, 30, 40, 50 and 100 µg/mL were prepared by diluting with the mobile phase. Calibration curve was constructed between the peak area and concentration. The linearity was evaluated by linear regression analysis, that was calculated by least square regression method using Microsoft excel 2007 software

Accuracy: Recovery studies were conducted by spiking known ZP amounts into pre-analyzed samples at 50%, 100%, and 150% levels. The recovery of the method was determined by spiking a previously analyzed test solution with additional drug standard solution. Preparation of stock solution, blank solution and different concentrations were done in the following way: Stock solution = drug 10 mg dissolved in 100 mL Blank solution = 10 µg/mL (only from the stock solution) 15 µg/mL = 1.0 mL of stock + 5 mL blank diluted up to 10 mL 20 µg/mL = 1.5 mL of stock + 5 mL blank diluted up to 10 mL 25 µg/mL = 2.0 mL of stock + 5 mL blank diluted up to 10 mL The value of recovery (%), RSD (%) and SE were calculated.

Precision: Intra-day and inter-day precision were evaluated.

Sensitivity: LOD and LOQ were determined using standard deviation and slope of the calibration curve [10].

Robustness and Ruggedness: Small deliberate changes in mobile phase composition and flow rate were assessed [11-15].

4.RESULTS AND DISCUSSION

Optimization of mobile phase

In the development of an HPLC method, for analysis of ziprasidone in the bulk drug, a variety of mobile phases were investigated. These included acetonitrile-water 75:25 (% v/v), methanol- water 75:25 (% v/v), acetonitrile-methanol-water 60:25:15 (% v/v/v), methanol-buffer 70:30 (% v/v), acetonitrile-buffer 60:40 (% v/v), acetonitrile- methanol-buffer 60:25:15 (% v/v/v), buffer-acetonitrile 65:35 (% v/v) and buffer- methanol 65:35 (% v/v) . The same solvent mixture was used for extraction of the drug from the formulation containing excipients.

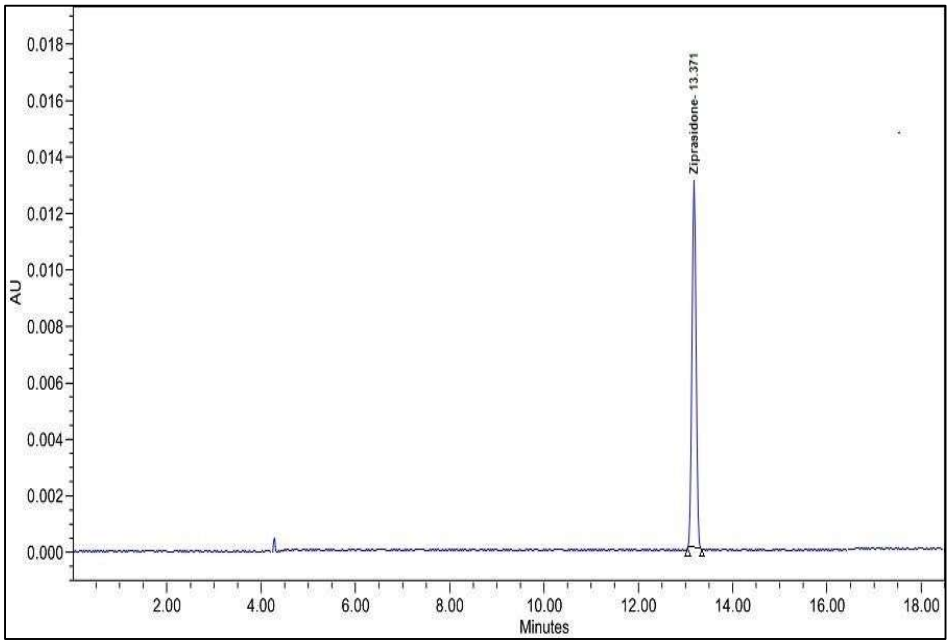

Fig. 1: Chromatogram of ziprasidone in the mobile phase buffer: acetonitrile (65:35, % v/v)

The selection of the mobile phase was based on sensitivity, ease of preparation, availability and suitability for drug content estimation and cost of the solvent systems. A mobile phase consisting of buffer-acetonitrile 65:35 (% v/v) at pH 3.0 adjusted with o-phosphoric acid, was optimized at a flow rate of 1 mL/min for further studies after several preliminary investigatory chromatographic runs. Under the described experimental conditions, the peak was well defined and free from tailing (Fig. 1).

Validation of Analytical Method

Linearity

The linearity was calculated by least square linear regression analysis of calibration curve (Miller and. Miller., 1984). The constructed calibration curve was linear over the concentration range of 0.5-100 µg/mL (n=6). The linear regression equation was Y = 12106X + 5056 with regression coefficient (R^2) of 0.9999. The mean value of slope and intercept were (12106 ± 54.47) and 5056 ± 20.73) respectively (Table 2 & 3).

Accuracy as recovery and Precision

The proposed method afforded recovery of 99.81-101.34% after spiking the additional standard drug concentration to the previously analyzed test solution, the values of % recovery, % RSD and SE are shown in the Table 4, in each case all the values for % RSD were found to be less than 1% which indicates the accuracy for the proposed method. The intra- and inter-day variability or precision data is summarized in Table 6. Low values of % RSD (<2%) indicate the repeatability of the proposed method.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Limit of detection and limit of quantification where calculated by the method based on standard deviation (S y/x) of the response for the blank injection in triplicate and the slope (S) of the calibration curve. The peak area of the blank was calculated. The LOD and LOQ were determined using slope of the calibration curve and standard deviation of the blank sample by the following formulae:

Limit of Detection = *Std. deviation × 3.3/slope*

Limit of Detection = 150 × 3.3 **/ 1**2106 = **0.040888 µg/mL = 40.88 ng/mL Limit of Quantification** = *Std. deviation × 10/slope*

Limit of Quantification = 150 × 10**/ 1**2106 = **0.123905 µg/mL = 123.905 ng/mL**

LOD and LOQ of the method were determined by standard deviation method as described above and were found to be 40.88 ng/mL and 123.905 ng/mL respectively, which indicate that the proposed method can be used for detection and quantification of ZP in a very wide concentration range.

Robustness and Ruggedness

This was done by making small deliberate changes in the chromatographic conditions at 3 different levels and retention time of ZP was noted. For the present study the chromatographic conditions selected were flow rate (0.75 mL/min, 1.0 mL/min and 1.25 mL/min) and mobile phase ratio buffer: acetonitrile (63:37, 65:35 and 67:33).

The ruggedness of the method was assessed by comparison of the intra- and inter-day assay result of ZP that has been performed by two analysts in the same laboratory.

There was no significant change in the retention time of ZP by changing the composition of the mobile phase and flow rate of the mobile phase. The low values of the % RSD indicate the robustness and ruggedness of the method (Table 5 and Table 6).

Table 1: Optimized process parameters for HPLC method development

S. No.	Process parameter	Optimized value
1	Instrument	Shimadzu (LC-10 AT VP)
2	Column	5µm RP 18 (C18), Lichrospher® 100,(250×4.6mm)
3	Mobile phase	Buffer + Acetonitrile
4	Ratio (B: A)	65:35
5	pH of mobile phase	3.0
6	Flow rate	1 mL/min
7	Run time	20 min
8	Retention time	13.371 min
9	Detector	UV-spectrophotometer
10	Detection wavelength	318 nm
11	Temperature	25 ± 0.5° C
12	Pressure	270 ± 4 kgf / cm²

Table 2: Calibration Curve of Ziprasidone by RP-HPLC Method

Concentration (µg/mL)	Mean Area ± SD (n=6)	% RSD
0.5	6185.4 ± 60.61	0.979
1	12531.7 ± 112.78	0.899
2	26741.6 ± 200.56	0.749
4	51076.4 ± 306.45	0.599
8	104262.0 ± 705.83	0.676
10	119373.8 ± 586.37	0.491
16	206189.5 ± 814.75	0.395
20	252852.5 ± 341.81	0.135
30	389290.0 ± 1002.08	0.257
40	495309.5 ± 3640.00	0.730
50	588359.3 ± 4164.18	0.707
100	1216274.1 ± 5351.60	0.439

***Note: Data represents mean values of six replicates (n=6) along with standard deviation (SD) and percentage relative standard deviation (%RSD*).**

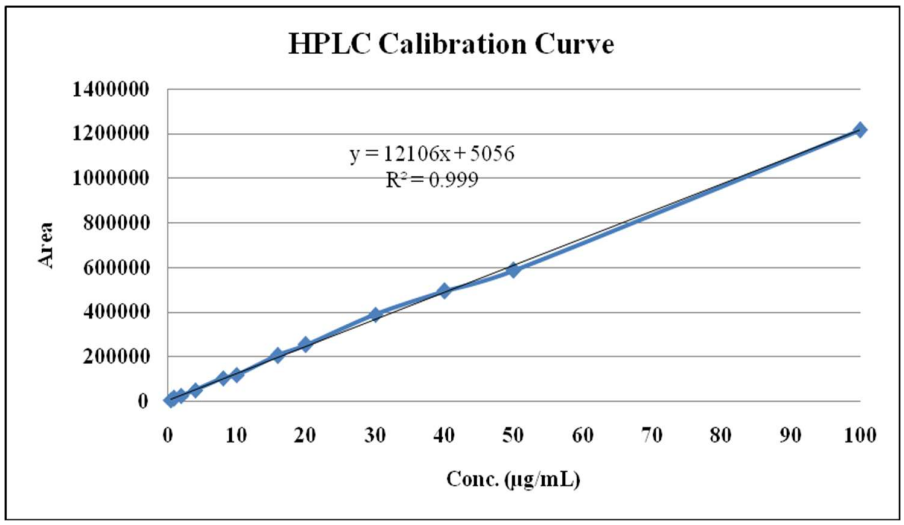

Fig. 2: Calibration curve of ziprasidone in buffer: acetonitrile (65:35, pH-3.0) by RP-HPLC method

Table 3: Linear regression data for the calibration plot (n=6)

Parameters	Values
Linearity range (µg/mL)	0.5-100
Regressed equation	Y = 12106X + 5056
Correlation coefficient (R^2)	0.9999
Slope ± SD	12106 ± 54.47
Slope without intercept ± SD	12196 ± 57.32
Intercept ± SD	**5056 ± 20.73**

Table 4: Accuracy as recovery of the proposed RP-HPLC method (n=6)

Excess drug added to analyte %	Theoretical content (µg/mL)	Conc. found (µg/mL) ± SD	% Recovery	% RSD	SE
0	10	9.981 ± 0.074	99.810	0.741	0.030
50	15	15.038 ± 0.121	100.253	0.804	0.049
100	20	20.269 ± 0.051	101.345	0.251	0.021
150	25	25.146 ± 0.173	100.584	0.687	0.069

Table 5: Robustness of the RP-HPLC method (n=3, Concentration = 18 µg/mL)

Parameters	Study conditions			Mean Area ± SD	SE	Mean Rt (min) ± SD	% RSD
	Original	Used	Level				
Mobile Phase	65:35	63:37	-2	204683.1 ± 2353.85	1359.03	13.264 ± 0.07	1.149
		65:35	0	216852.8 ± 1973.36	1139.35	13.371 ± 0.03	0.910
		67:33	+2	203179.3 ± 3047.68	1759.63	13.428 ± 0.02	1.499
Flow Rate	1.0	0.75	-0.25	194516.6 ± 2149.40	1240.99	13.451 ± 0.15	1.105
		1.0	0	216852.8 ± 1973.36	1139.35	13.371 ± 0.03	0.910
		1.25	+0.25	235132.5 ± 2748.69	1587.00	13.147 ± 0.19	1.169

Rt = Retention time

Table 6 Intra and inter day ruggedness effect on the developed RP-HPLC method

Ruggedness conditions	Mean area ± SD (At zero hrs)	SE	% RSD
Intra day	221379.4 ± 2235.93	1290.95	1.001
Inter day	240083.1 ± 2729.74	1576.06	1.137

5.CONCLUSION

A simple, precise, and robust RP-HPLC method was developed and validated for the quantification of ZP in bulk and formulation samples. The method complies with ICH guidelines and demonstrates excellent linearity, accuracy, precision, and robustness. It is suitable for routine quality control applications. This study successfully developed and validated a robust RP-HPLC analytical method for the quantification of ziprasidone in bulk drugs and pharmaceutical formulations. The method employed a Lichrospher® 100 RP-18 column with a mobile phase of buffer and acetonitrile (65:35, %v/v) at pH 3.0, achieving chromatographic separation with high precision and accuracy. The method's detection wavelength at 318 nm ensured the reliable quantification of ziprasidone, even at low concentrations, as evidenced by its LOD (40.88 ng/mL) and LOQ (123.91 ng/mL).

Validation studies, conducted in accordance with ICH Q2(R1) guidelines, demonstrated excellent linearity over the range of 0.5–100 µg/mL (R^2 = 0.9999). Accuracy was confirmed through recovery rates of 99.81%–101.34%, with %RSD values below 1%,

indicating the method's reliability for routine analysis. The robustness and ruggedness assessments highlighted the method's stability under minor variations in analytical conditions and across different analysts, emphasizing its versatility for quality control applications.

Compared to existing methods, the developed RP-HPLC method is simpler, cost-effective, and time-efficient, making it highly suitable for industrial and regulatory environments. Its adaptability to routine quality control ensures the consistent quantification of ziprasidone in bulk and formulations, essential for ensuring therapeutic efficacy and product safety.

The findings provide a reliable analytical tool for pharmaceutical industries, particularly for quality control and regulatory compliance in the production of ziprasidone. Future work may explore the method's application in stability studies and other related pharmaceutical formulations. This validated method aligns with global standards for pharmaceutical analysis, contributing to the enhancement of analytical methodologies in the field.

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