RESEARCH PAPER

Development and Validation of a QBD-Driven RP-HPLC–UV Method for Stability-Indicating Analysis of Ziprasidone and LC–MS Characterization of Degradation Products

Asit Baran Panigrahy[1*], Sudhir Kumar Sahoo[2], Ghanshyam Panigrahi[3]

[1*]Research Scholar, Department of Pharmacy, Biju Patnaik University of Technology, Rourkela, Odisha, India. Email: asitpanigrahy@gmail.com ORCID: 0000-0003-0800-5300 (Corresponding Author)

[2]Professor, Department of Pharmaceutical Analysis, Royal College of Pharmacy and Health Sciences, Andhapasara Road, Berhampur, Odisha - 760002, India. Email: sudhirku.sahoo@gmail.com ORCID: 0000-0002-2222-7957

[3]Professor, Department of Pharmacology, Royal College of Pharmacy and Health Sciences, Berhampur, Odisha, India. Email: drgpanigrahi@gmail.com ORCID: 0000-0001-6358-5673

ABSTRACT

The goal of this investigation was to develop, validate and implement simple analytical techniques to quantitatively measure the amount of ziprasidone using UV-visible spectrophotometry and RP-HPLC. In addition, the study sought to demonstrate the stability-indicating capability of the new methods by using forced degradation studies and LC-MS to identify the major degradation products of ziprasidone after exposure to neutral, thermal, and hydrolytic conditions. The UV method was able to detect the maximum response for ziprasidone at 318 nm, and both methods produced linear responses from 10-50 µg/mL. The UV method produced results that were both accurate and precise, making this method suitable for routine use as an assay for ziprasidone. The optimized RP-HPLC method (C18; methanol:water; 80:20; 1.0 mL/min) produced a very narrow (RT ~3.83 minutes) peak with all validation criteria met. Forced degradation tests showed that the largest amount of degradation occurred when exposed to either neutral or thermal stress. The LC-MS results further confirmed the identity of ziprasidone and the degradation products produced under these conditions.

Keywords: Ziprasidone, RP-HPLC, UV–Visible spectrophotometry, Forced degradation, Stability-indicating method validation

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INTRODUCTION

Ziprasidone is non-typical antipsychotics that are prescribed for schizophrenia and bipolar disorder, and therefore, accurate and reliable quantification is critical to ensure compliance with regulations and protect the patient's health when preparing bulk drug and dosage form. The ultrviolet (UV vis) spectrophotometric assay method is the predominant method of pharmaceutical analysis due to its speed, cost-effectiveness, and availability; however, the UV visible method lacks selectivity when excipients, process impurities, or degradation products coelute at the same wavelength. As such, "Reverse Phase High Performance Liquid Chromatography (RP-HPLC)" is also utilized in quality control laboratories because of its ability to provide chromatographic separation of the analyte(s), increased assay specificity, and ability to apply to stability studies [2-3]. Drug products and drug substances can degrade as a result of processing, storage and/or administration, and as a result of degradation, they can lose potency and/or create degradation products that are potentially toxic to humans. Knowledge of the degradation process, and ensuring the analysis is sufficiently selective, are essential to the successful development of pharmaceuticals. Forced degradation (stress testing) is a standard activity to artificially generate degradation products via hydrolysis, oxidation, thermal or photolysis to understand how a compound is degraded and to demonstrate that a particular. It is generally advisable in peer-reviewed guidance to form moderate, controlled degradation (usually in the 520 range) to form significant stressed samples without over-stressing that can form secondarily non-representative degradants [4]. In the case of ziprasidone, UV spectrophotometric assays and stability-indicating liquid chromatographic techniques and studies that have coupled LC -UV with LC-MS to examine stress degradation behaviour and aid in the characterisation of degradants have been previously reported. Still more recent peer-based literature suggests the usefulness of the combination of HPLC and MS/MS to be

able to confirm parent identities and characterize degradation products during stability-indicating processes [5]. In this direction, the current research had the objective of designing and validating the simple and reproducible UV and RP-HPLC methods of quantitative estimation of ziprasidone, as well as determining forced degradation stability-indicating properties of the RP-HPLC method, completed with LC-MS analysis.

METHODOLOGY

Organoleptic Evaluation

The drug material was examined physically in terms of color and appearance, and taste was tested with care. Ziprasidone was found to be white to light pink or white to off-white in color which is in the form of fine crystalline powder with a bitter taste. The initial observations revealed the purity and physical identity of the drug sample.

UV–Visible Spectrophotometric Method

Solubility Studies

Researchers studied how well ziprasidone dissolved in a variety of solvents, such as distilled water; hydrochloric acid (0.1N); and both ethanol and methanol. When determining if ziprasidone was soluble in the different solvents, it was found to be insoluble in distilled water; freely soluble in both ethanol and methanol; and "somewhat" soluble in 0.1N hydrochloric acid. These results led to the decision by researchers to utilize methanol as the optimal solvent combination for future UV-Visible spectrophotometric analysis (80:20 combination of water and methanol).

Determination of the working wavelength

To determine the wavelength (max) of ziprasidone, standard drug Molarity of ziprasidone (10-50ug/ml) in Methanol:Water (80:20) were prepared. A UV-Visible spectrophotometer was utilized to scan the solutions for maximum absorbance from 200nm to 400nm using methanol:water as the blank solution. Ziprasidone produced (max) absorbance measurement at 318nm and that wavelength was selected to continue with the analysis.

Preparation of Standard Solutions

To prepare the adequate concentration of Ziprasidone, a 10 mg sample of Ziprasidone was weighed and dissolved in an 80% methanol/20% water mixture to make a final volume of 10 mL to produce a stock solution, with the concentration of 1,000 µg/mL. A secondary concentration of 100 µg/mL was produced by taking 1 mL of the primary stock solution and diluting it with the same solvent to 10 mL. The working solutions for the standard dilution were prepared utilizing the 10-50 µg/mL concentration range from the secondary stock solution.

Calibration Curve

Standard working solutions were scanned at 318 nm and absorbance values were recorded for each solution. A calibration curve was created comparing concentration vs. absorbance and linear regression analysis was performed to determine linearity.

RP-HPLC Method

Chromatographic Conditions

An RP-HPLC analysis was performed using a C-18 column (ODS) (250 x 4.6 mm, 5 µm). The mobile phase used 80ml of HPLC grade methanol and 20 ml of HPLC grade water. The frequency of flow was set to 1.0 ml/min and the dose was administered in 25 µl volumes. The study took place under normal laboratory conditions and utilized 318 nanometers for the analysis.

Preparation of Mobile Phase

The mobile phase was made by adding a combination of methanol and water in the necessary ratio, after which the mixture was filtered using a 0.45 µm membrane filter and sonicated to eliminate the dissolved gases.

Preparation of Standard Solutions

A standard stock solution was prepared using this method: The compound ziprasidone in the mobile phase (10mg/100 uL) has a concentration of 1000uG/mL. This solution is then diluted by a factor of 10 to produce a working stock concentration of 100ug/mL. Finally, five working standard solutions for use were prepared by diluting this stock solution with the same mobile phase (10, 20, 30, 40, 50ug/ml).

Calibration Curve

A calibration curve was made using HPLC, and peak areas were noted. Peak area (Y-axis) and concentration (X-axis) were taken to plot a calibration curve.

Method Validation

Both the UV-Visible and the RP-HPLC techniques were proven to be accurate, precise, robust, and rugged, with a “limit of detection (LOD)” and a “limit of quantification (LOQ)”. Recovery studies were done to assess accuracy at 80, 100, and 120 percent levels by using the standard addition method. The precision was measured in the intra-day and inter-day replicative studies. The robustness was studied through making small purposeful alterations to the parameters of analysis, whereas the ruggedness was studied through carrying out the analysis under varied conditions, such as changing the flow rate and the analyst. Standard deviation & slope of the calibration curve were used to compute LOD and LOQ.

Forced Degradation Studies

Forced degradation experiments were approved out to determine the stability of Ziprasidone in a variety of stress conditions, which include neutral, acidic, alkaline, oxidative, and thermal degradation. Each of the conditions

was subjected to the drug after seven days, and the degraded samples were analyzed by the developed RP-HPLC method. Percentage degradation was determined by determining areas of degraded samples and comparing it to the untreated standard area.

LC–MS analysis

LCMS analysis was done to identify the molecular identity of Ziprasidone as well as the degradation products that could be formed during the forced degradation experiments. A suitable reversed-phase column was used to carry out chromatographic separation under optimal conditions that were in line with the RP-HPLC technique. The flow rate of the mobile phase was maintained constant, and the eluted compounds were introduced to the mass spectrometer through an electrospray ionization (ESI) source that was used in positive ion mode. Mass spectra were measured within a suitable m/z range in order to identify the protonated molecular ion and fragment ions. The instrument was connected to the integrated software to acquire and analyze data that was used to interpret all peaks related to molecular and degradation.

RESULTS

UV–Visible Spectrophotometric Method

Solubility and Method Optimization

To select proper solvent for UV/Visible spectrophotometry of ziprasidone, the solubility of ziprasidone in various solvents was determined first. Ziprasidone did not dissolve in distilled water but dissolved completely in methanol and ethanol, and was partially soluble in 0.1N HCl. Of all the solvents tested, only methanol gave a clear consistent solution with very little background interference, therefore it was chosen as the solvent system (80% methanol : 20% distilled water) to use for future spectrophotometric analyses of ziprasidone.

Table 1. Solubility characteristics of Ziprasidone.

Solvent	Solubility
Distilled water	Insoluble
Methanol	Freely soluble
Ethanol	Freely soluble
0.1 N HCl	Soluble

Determination of Working Wavelength

The UV absorption spectrum of Ziprasidone was recorded by scanning standard drug solutions in the wavelength range of 200–400 nm using methanol:water (80:20) as blank. The overlay spectra exhibited a well-defined absorption maximum at 318 nm. This wavelength provided maximum sensitivity and was selected as the working wavelength for all further UV–Visible measurements.

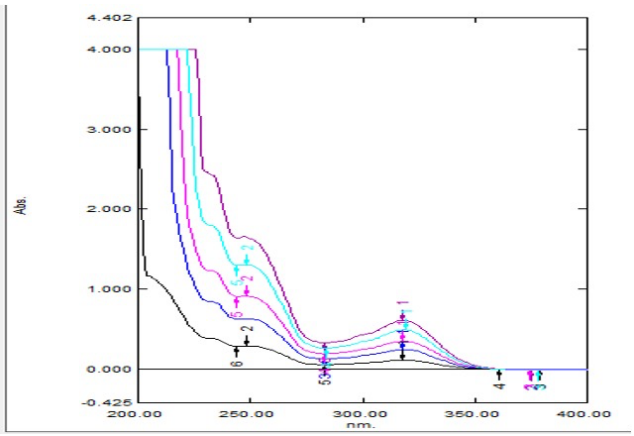

Figure 1. Overlay UV–Visible absorption spectra of Ziprasidone showing λmax at 318 nm.

Linearity and Calibration Curve

The linearity of the UV-visible spectrophotometric technique was assessed between 10 and 50 µg/mL concentrating. The absorbance values for each sample were reliable, and the calibration curve constructed from these absorbances provided a linear relationship, with $r^2 = 0.999$, indicating that the UV-visible spectrophotometric technique was linear.

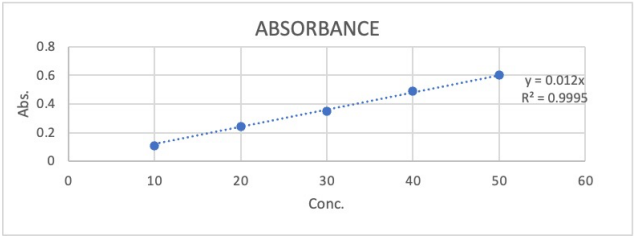

Figure 2. Calibration curve of Ziprasidone by UV–Visible spectrophotometric method.

Accuracy and Precision

Exactness of the UV–Visible method was assessed using recovery studies at three levels (80%, 100%, and 120%) by the standard addition technique. The % Recovery values were very close to 100% meaning that the method used was Accurate and Interference free. Precision was determined using repeatability, intra-day & inter-day testing. The % RSD values were all found to be acceptable confirming Precision and Reproducibility of the method. Low variance also proved that the UV-Visible method developed is reliable for daily analysis.

Table 2. Accuracy and precision results of UV–Visible spectrophotometric method.

Parameter	Result
% Recovery	99.39 – 100.65
Intra-day %RSD	≤ 0.061
Inter-day %RSD	≤ 0.045

RP-HPLC Method

Method Optimization and System Suitability

The RP-HPLC method was accustomed by assessing different mobile phase compositions. Initial trials with methanol:water (70:30) resulted in peak splitting and tailing. An optimized mobile phase consisting of methanol:methanol (80:20) produced a nice peak with a retention time of about 3.83 minutes. Performance characteristics including theoretical plates, asymmetry factor and HETP all evaluated in conjunction show that the column is functioning optimally.

Table 3. Optimized chromatographic conditions for RP-HPLC method.

Parameter	Condition
Column	ODS C18 (250 × 4.6 mm, 5 µm)
Mobile phase	Methanol:Water (80:20)
Flow rate	1.0 ml/min
Detection wavelength	318 nm
Retention time	3.83 min

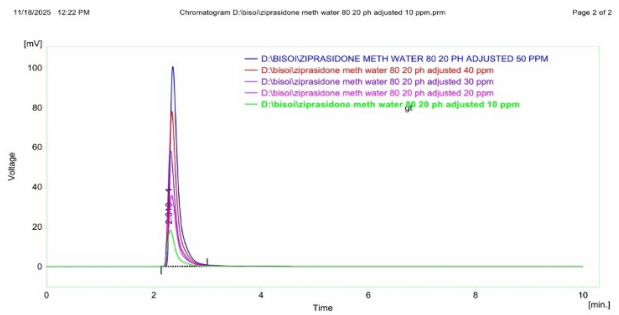

Figure 3. Representative RP-HPLC chromatogram of Ziprasidone under optimized conditions.

Linearity and Calibration Curve

There is a strong relationship between the amounts of compounds (10-50 µg/ml) and their peak areas measured by reverse phase (RP) high-performance liquid chromatography (HPLC) methods. Specifically, in this study, as the concentration of the compounds increased, so did their respective peak areas in a linear fashion; therefore, all these results are an example of a linear calibration curve; thus, one can conclude that the RP-HPLC methods used were reliable for quantifying compounds.

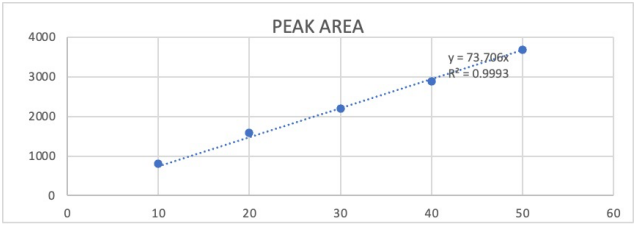

Figure 4. Calibration curve of Ziprasidone by RP-HPLC method

Accuracy, Precision, Robustness, and Sensitivity

Using methods of standard addition, studies of accuracy showed that this method results in acceptable levels of recovery, thus validating accuracy of the method's performance. Further, studies of precision verified that the method produces consistent results (i.e. low %RSD) when repeated on an intraday or inter-day basis, further confirming the consistency of the method's application for analysis purposes.

Robustness and ruggedness testing conducted for small variations in flow rate and analyst confirmed that those variations did not have a significant impact on the analytical results obtained from the analysis.

The method displays a high degree of sensitivity through an LOD value of 0.024 µg/mL and LOQ value of 0.709 µg/mL, indicating that the method is capable of detecting and quantifying very low concentrations of Ziprasidone with good reliability.

Table 4. Validation summary of RP-HPLC method.

Validation parameter	Result
% Recovery	97.24 – 99.27
Precision (%RSD)	≤ 0.59
LOD	0.024 µg/ml
LOQ	0.709 µg/ml

Forced Degradation Studies

The constancy of Ziprasidone was assessed through forced squalor studies designed to assess the drug under numerous stress conditions. The results of this study indicated significant degradation due to both neutral and thermal stressed conditions and therefore indicated that the drug is susceptible to hydrolytic and thermal stress. A moderate amount of degradation was seen under oxidative conditions, while a small amount of degradation occurred under both acidic & basic conditions. Degradation products were successfully separated from the main peak by use of the RP-HPLC method, indicating its ability to demonstrate the stability of Ziprasidone.

Table 5. Percentage degradation of Ziprasidone under stress conditions.

Condition	% Degradation
Neutral	66.64
Acidic	6.76
Basic	2.43
Oxidative	22.59
Thermal (25°C)	19.03
Thermal (50°C)	59.05

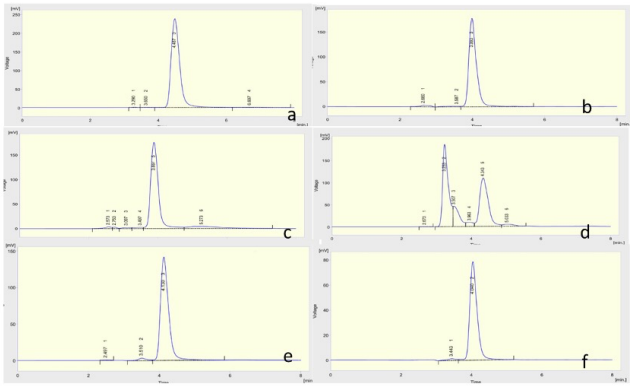

Figure 5. Representative RP-HPLC chromatograms of Ziprasidone under forced degradation conditions (a) Neutral (b) Acidic (c) Basic (d) Oxidative (e) Thermal (25°C) (f) Thermal (50°C).

LC–MS analysis

To verify the molecular mass of Ziprasidone and determine typical fragment ions and degradation-specific peaks during conditions of stress, "liquid chromatography -mass spectrometry (LC -MS)" was analyzed. The LC-MS data showed a clear molecular-level identity of the drug and consistent the stability profile of the drug in forced degradation studies by RP-HPLC. The standard of Ziprasidone had a sharp chromatographic peak of the parent compound in the LC -MS spectrum. Mass spectrometry had a distinct protonated molecular ion peak with m/z 413.9 [M+H] -1, which is equal to the theoretical molecular weight of Ziprasidone (412.9 g/mol). This ascertains the molecular identity of the drug. The peak was very intense and dominant, which showed high purity and no major impurities or pre-existing degradation products. Besides the parent ion, typical fragment ions were also seen with a lower m/z value. The large fragment peaks were identified at m/z 383.8, m/z 305.7, and m/z 194.6, which can be explained by the sequential cleavage of the piperazine ring and substitution groups of the Ziprasidone molecular structure. These fragment ions are in line with predicted fragmentation response under positive electrospray ionization conditions. Under neutral hydrolytic conditions, the LC-MS spectrum revealed a significant decrease in the intensity of the parent ion at m/z 413.9, and the emergence of several other fragment ions at m/z 369.8, 291.6, and 177.5. These ions suggest that the molecules are highly broken down because of hydrolytic cleavage, which is associated with the high percentage degradation (~66%) in the RP-HPLC analysis. The parent ion at m/z 413.9 was still preponderant in the acidic and basic degradation conditions, with small fragment ions of low intensity just being detected. This validates the comparative stability of Ziprasidone in acidic and alkaline conditions, which justifies the low degradation of the drug chromatographically. There were also additional ion peaks of m/z 429.9 and m/z 397.8 under oxidative stress that could be due to oxidized or oxygen-added Ziprasidone. These peaks justify the average degradation (~22) observed in RP-HPLC experiments. The thermal degradation LC-MS spectrum showed a severe reduction in the parent ion and a rise in the intensity of low-molecular-weight fragments such as m/z 291.6, 244.3, 177.5, and 154.4, which means that the fragmentation occurs extensively in thermal degradation. At higher temperatures, cleavage of the bonds was increased leading to more than one degradation product, supporting the high degradation (~59% at 50°C) observed chromatographically.

DISCUSSION

The current research developed and confirmed both quantitative strategies of ziprasidone-UV spectrophotometer (318 nm) and RP-HPLC (c18; methanol:water 80: 20 V: 1.0 mL/min.: 318 nm) and the RP-HPLC method was also planned as stability-indicating by forced degradation and LC-MS confirmation. The employment of a dual-method approach is in line with the modern analytical approach whereby UV methods may be used to suggest rapid routine analysis at the expense of HPLC when selectivity, separation of impurities or determination of stability is necessary. The UV procedure indicated linearity between 10-50 ug/mL with a correlation of approximately one (measured R 0.999) and a high accuracy (99-101% recovery) with extremely low intra-/inter-day RSD and therefore demonstrated to be applicable in cost-effective quantification in the event of no interference. Similar UV method validation procedures (linearity, accuracy, precision, robustness, LOD/LOQ) are common to peer-reviewed UV method development publications in pharmaceuticals, and this validation strategy is suitable to the present study. The RP -HPLC technique offered a sharp parent peak (RT 3.83 min), satisfactory recovery (97-99%), precision (RSD 0.59), and reported LOD/LOQ, which demonstrated good quantification with an enhanced analytical selectivity. Similar articles in peer-reviewed stability-indicating HPLC articles also highlight that assay procedures should be stable to degradants and impurities, and retains chromatographic resolution and Mill

on the top purity under stressed conditions [8, 9]. Forced degradation it showed that ziprasidone was significantly susceptible to neutral hydrolysis (~66.64%), high temperature (50 C; ~59.05%), moderate oxidative degradation (~22.59%), and relatively low acid/base degradation, thus showing that hydrolytic and thermal mechanisms could be prevalent under the applied stress design. The acid/base, oxidative, neutral and thermal stressors are reflective of literature-based forced degradation models which seek to produce degradation products applicable to the progress of stability-indicating methods and to test method selectivity. Since too much stress may lead to secondary degradants and make interpretation more complex, peer-reviewed forced degradation literature will also be beneficial in maximizing the stress severity to achieve controlled degradation to use in profiling and specificity of methods [10, 11]. LC–MS also supported the stability indicating assertion through the identification of the parent molecular ion (m/z = 413.9 [M+H] +) and those other stress indicative ions/fragments that were reported to exist under both the neutral, oxidative and thermal degradation conditions. This is in accord with the peer-reviewed stability-indicating method studies in which LC 3 LC -MS/MS (or LC -MS) is employed to identify/characterise degradation products and support chromatographic observations [12-14].

CONCLUSION

To sum up, the quantitative estimation of ziprasidone was successfully developed and validated using simple, specific, and reproducible UV -Visible and RP -HPLC techniques. The UV technique with a wavelength of 318 nm showed a good linearity, accuracy and precision, which calls in its favor its use as a routine assay. The RP-HPLC technique offered a fast separation with decent validation criteria and increased selectivity of the analysis. It was confirmed that ziprasidone is more vulnerable to neutral hydrolytic and thermal stress than to acidic or basic conditions by forced degradation studies. The RP-HPLC technique was suitable in separating degradation products and the parent drug peak, which is indicative of its stability. LC MS also assisted in identity and degradation characterization.

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