LC-UV and LC-MS study of stress degradation behavior of ziprasidone HCl and development of rapid UHPLC stability-indicating related substances & assay method

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ABSTRACT

An attempt has been made to study the forced degradation behavior of ziprasidone hydrochloride by LC-MS/MS ,and hence to develop a new, sensitive, stability indicating gradient UHPLC Related substances & assay method for the quantitative determination of ziprasidone in the Bulk drugs. The chromatographic separation of ziprasidone & impurities was achieved on Waters Acquity BEH C18, 100×2.1mm, 1.7μm column within a short run time of 8 min, and using the simple mobile phase combination of buffer and Acetonitrile. Buffer consists of 0.01M Ammonium acetate pH 5.0, delivered in a gradient mode and quantitation was carried out using ultraviolet detection at 254nm with a flow rate of 0.2mL min^{-1}. The same method was also extended to LC-MS/MS studies which were carried out to identify the degradation product. In the developed UHPLC method the resolution (R_s) between ziprasidone and its four potential process impurities were found to be greater than 2.0. Regression analysis shows an r^2 value (correlation coefficient) of greater than 0.99 for ziprasidone and it's all the four potential impurities. This method was capable to detect the four impurities of ziprasidone at a level of 0.002 % (0.01μg mL^{-1}) with respect to test concentration of 0.5mg mL^{-1} for a 1μL injection volume & also the all possible degradants formed during the stress studies. The drug substances were subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. Considerable degradation was found to occur in base stress and oxidative conditions. The stressed test solutions were assayed against the qualified working standard of ziprasidone and the mass balance in each case was close to 99.8% indicating that the developed method was stability-indicating. The developed RP-UHPLC method was validated with respect to linearity, accuracy, precision and robustness.

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INTRODUCTION

Ziprasidone (5-(2-(4-(1, 2-benzisothiazol-3-yl-1-piperazinyl)-ethyl) - -6-chloro-1, 3-dihydro-2-(1H)-indol-2-one) (Figure 1), is a potent antipsychotic agent and is useful for treating various disorders including schizophrenia, anxiety and migraine pain. Ziprasidone is a psychotropic agent belonging to the chemical class of benzisoxazole derivatives & it also displays some inhibition of synaptic reuptake of serotonin and nor epinephrine. The mechanism of action of ziprasidone is unknown. However it has been theorized that its antipsychotic activity is mediated primarily by antagonism at dopamine receptors, specifically D_2. Serotonin agonism may also play a role in the effectiveness of ziprasidone, but the significance of 5-HT_{2A} antagonism is debated among researchers. Ziprasidone has perhaps the most selective affinity for 5-HT_{2A} receptors relative to D_2 and 5-HT_{2C} receptors of any neuroleptic[1-3]. Antagonism at histaminic and alpha adrenergic receptors likely explains some of the side effects of ziprasidone, such as sedation and orthostasis.

Extensive literature survey did not reveal any rapid, simple, sensitive and stability indicating UHPLC method for the quantification of impurities and quantitative assay of ziprasidone in bulk drugs. Few LC methods were reported in literature describing the determination of ziprasidone in biological fluids[4-8]. Also there exists some stability indicating methods for the separations of some process impurities in bulk drugs[9,10]. United States Pharmacopeia Forum PF 35 (3) published a LC method for the determination of IMP-1 to IMP-4 by two different LC methods. So here an attempt was made to determine all the above mentioned four process impurities along with the degradation impurities in a single method with shorter run time. An ideal stability indicating chromatographic method should estimate the drug and is able to resolve from its potential impurities and degradation products. The present drug stability test guideline Q1A (R2)[11] issued by International Conference on Harmonization (ICH) suggests that stress studies should be carried out on a drug to establish its inherent stability characteristics, leading to separation of degradation products and hence supporting the suitability of the proposed analytical procedures. According to ICH, stress testing of the drug substance can help the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedure used. It also requires that analytical test procedures for stability samples should be stability indicating and they should be fully validated as per ICH Q2 (R1)[12] and USP/NF[13].

Hence, an attempt has been made to develop an accurate, rapid, specific and reproducible method for the determination of ziprasidone in the presence of its degradation products and the four process impurities, IMP-1, IMP-2, IMP-3 & IMP-4, (Figure 1), along with method validation as per ICH norms.

EXPERIMENTAL

Chemicals

Samples of ziprasidone and its related impurities were procured from USP-India (P) limited, Hyderabad, India (Figure 1). HPLC grade Acetonitrile, Analytical reagent grade orthophosphoric acid purchased from Merck, Darmstadt, Germany. High purity water was prepared by using Millipore Milli-Q plus water purification system. All samples and impurities used in this study were of greater than 99.0% purity.

Equipment

The LC system used for method development, forced degradation studies and method validation was Waters Quattro Micro LC-MS/MS (Mass Lynx 4.1), Waters Alliance 2695 and Waters Acquity UPLC (Waters Millford, USA) system with binary solvent Manager plus auto sampler and a Aquity photo diode array detector. The output signal was monitored and processed using Empower software on Pentium computer (Digital equipment Co). Photo stability studies were carried out in a photo stability chamber (Mack Pharmatech, Hyderabad, India). Thermal stability studies were performed in a dry air oven (Mack Pharmatech, Hyderabad, India).

Chromatographic conditions

The chromatographic separation was performed on a Waters Acquity UPLC BEH C18 Column (100 × 2.1)mm with 1.7μm particles. The mobile phase A con-

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Compound	Structure	Chemical name	Mol. For.	Mol. weight
Ziprasidone		5-[2-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]ethyl]-6-chloro-1,3-dihydro-2H-indol-2-one Hydrochloride Monohydrate	$C_{21}H_{24}Cl_2N_4O_2S$	467.4
IMP-1		3-(1-Piperazinyl)-1, 2-benzisothiazole	$C_{11}H_{13}N_3S$	219.2
IMP-2		5-(2-(4-(1,2-benzisothiozole-3-yl) 1-piperazinyl) –1 ethyl)-3-oxo-6- chloro-1, 3-dihydro-2H-indole-2-one	$C_{21}H_{19}ClN_4O_2S$	426.9
IMP-3		5-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl)ethyl)-3-(5-(2-(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl)ethyl)-6-chloro-3-hydroxy-2-oxoindolin-3-yl)-6-chloroindolin-2-one	$C_{42}H_{40}Cl_2N_8O_3S_2$	839.8
IMP-4		5-(2-(4-(1,2-benzisothiozole-3-yl) 1-piperazinyl) –1 ethyl)-2-benzothiazole-6- chloro-1, 3-dihydro-2H-indole-2-one	$C_{28}H_{24}ClN = OS_2$	546.1

Figure 1 : Chemical structures and labels of ziprasidone and its impurities

sists of 0.01M Ammonium acetate buffer pH.5.0 & mobile phase B is acetonitrile with gradient mode (time (min) / % solution B: 0/40, 1.0/40, 2.0/80, 5.5/80, 6.0/40, 8.0/40. The flow rate was 0.2mL min^{-1}. The column temperature was maintained at 35°C and the detection was monitored at a wavelength of 254 nm. The injection volume was 1 µL. Initial mobile phase ratio of A: B (60: 40 v/v) was used as diluent.

Mass-spectrometry conditions

Liquid chromatography-mass spectrometry (LC-MS) analysis was carried out using Waters Quattro Micro LC-MS/MS coupled with Wates Alliance 2695 separation module. Ziprasidone degradation samples were subjected to LC-MS analysis. The optimized MS tune parameters for LC/MS studies were Capillary Voltage; 3KV, Cone Voltage: 25V, Source

temp.:100°C, Desolvation temp.: 400°C.

Preparation of solutions

Preparation of standard solutions

A stock solution of ziprasidone (5.0mg mL^{-1}) was prepared by dissolving appropriate amount in the diluent. Working solutions of 500 and 50µg mL^{-1} were prepared from above stock solution for related substances determination and assay determination respectively. A stock solution of impurities (mixture of IMP-1, IMP-2, IMP-3 and IMP-4) at a concentration of 0.5 mg mL^{-1} was also prepared in diluent.

Preparation of sample solutions

Transferred about 100mg of ziprasidone sample into 200mL volumetric flask, dissolved in, and diluted to volume with the diluent.

Preparation of assay solutions

Transferred about 10mg of ziprasidone sample into 200mL volumetric flask, dissolved in, and diluted to volume with diluent.

Analytical method validation

The developed chromatographic method was validated for selectivity, linearity, range, precision, accuracy, sensitivity, robustness and system suitability.

Specificity/application of stress (Forced degradation study)

Selectivity of the developed method was assessed by performing forced degradation studies. The terms selectivity and specificity are often used interchangeably. Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. Stress testing of the drug substance can help to identify the likely degradation products, which can in turn help to establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used.

The specificity of the developed LC method for ziprasidone was determined in the presence of its impurities namely IMP-1, IMP-2, IMP-3, & IMP-4 and degradation products. Forced degradation studies were performed on ziprasidone to provide an indication of stability indicating property and specific-

ity of the proposed method[14,15]. The stress conditions employed for degradation study includes light (carried out as per ICH Q1B), heat (100°C), acid hydrolysis (0.1N HCl), base hydrolysis (0.1N NaOH), water hydrolysis and oxidation (5 % H$_2$O$_2$). For heat and light studies, study period was 10 days where as for base and oxidation, it was at 80°C for 1 h and 0.5 h respectively. And for acid it was at 80°C for 4 h. Peak purity of stressed samples of ziprasidone was checked by using a Aquity photo diode array detector (PDA) from Waters. The purity angle within the purity threshold limit demonstrates the analyte peak homogeneity.

Assay studies were carried out for stressed samples against the qualified reference standard and the mass balance (% assay + % of degradation products + % of impurities) was calculated. Assay analysis was also performed on some batch samples.

Analytical method validation

Precision

The precision of the related substance method was checked by injecting six individual preparations of (500µg mL^{-1}) ziprasidone spiked with 0.15 % each IMP-1, IMP-2, IMP-3 and IMP-4. The % RSD of area for each IMP-1, IMP-2, IMP-3 and IMP-4 calculated. Precision study was also determined by performing the same procedures on a different day (Interday precision).

The intermediate precision (ruggedness) of the method was also evaluated using different analyst, different column and different instrument in the same laboratory.

Assay method precision was evaluated by carrying out six independent assays of test sample of ziprasidone against qualified reference standard. The % RSD of six assay values obtained was calculated. The intermediate precision of the assay method was evaluated by different analyst and by using different instrument from the same laboratory.

Sensitivity

Sensitivity was determined by establishing the Limit of detection (LOD) and Limit of quantitation (LOQ) for IMP-1, IMP-2, IMP-3 and IMP-4 estimated at a signal-to-noise ratio of 3:1 and 10:1 respectively, by

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injecting a series of dilute solutions with known concentration. The precision study was also carried out at the LOQ level by injecting six individual preparations of IMP-1, IMP-2 IMP-3 and IMP-4, calculated the % RSD for the areas of each impurity.

Linearity

Linearity test solutions for assay method were prepared from stock solution at five concentration levels from 50 to 200 % of assay analyte concentration (25, 37.5, 50, 75 and 100µg mL^{-1}). The peak area versus concentration data was collected and performed regression analysis by the method of least squares. The Correlation coefficient, Slope & y-intercept values were calculated from the calibration plot obtained.

Linearity test solutions for related substance method were prepared by diluting the impurity stock solution to the required concentrations. The solutions were prepared at seven concentration levels. From LOQ to 200 % of the permitted maximum level of the impurity (i.e. LOQ, 0.015 %, 0.0375 %, 0.075%, 0.15 %, 0.225 % and 0.3 % was subjected to linear regression analysis with the least square method. Calibration equation obtained from regression analysis was used to calculate the corresponding predicted responses. The Correlation coefficient, Slope & y-intercept values were calculated from the calibration plot obtained.

Accuracy

The accuracy of the assay method was evaluated in triplicate by standard addition procedure with five known concentration levels from 50 to 200 % of assay analyte concentration (25, 37.5, 50, 75 and 100µg mL^{-1}). For each concentration, three sets were prepared and injected in triplicate. The percent recoveries of added drug substance at each concentration were calculated.

The bulk sample shows the presence of IMP-1 at a level of 0.01 %, IMP-2 at a level of 0.01 %, IMP-3 at a level 0.02 % and single unknown impurity at a level of 0.04 %, it shows a total of 0.15 % of unknown impurities (limit: not more than 0.15 % for known impurities, not more than 0.1 % for single unknown impurity, for total impurities the limit is 0.50 %). The study was carried out in triplicate at 0.075

%, 0.1125 % 0.15 %, 0.225 % and 0.3 % of the analyte concentration (500µg mL^{-1}). The percentage of recoveries for IMP-1, IMP-2, IMP-3 and IMP-4 at each concentration level were calculated.

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately changed and the resolution (R_s) between ziprasidone, IMP-1, IMP-2, IMP-3 and IMP-4 were evaluated. The flow rate of the mobile phase was 0.2mL min^{-1}. To study the effect of flow rate on the developed method, 0.05 units of flow changed (i.e. 0.15 and 0.25mL min^{-1}). The effect of column temperature on the developed method was studied at 33°C and 37°C instead of 35°C and use of a column from a different batch. In the all above varied conditions, the components of the mobile phase were held constant.

Solution stability and mobile phase stability

The solution stability of ziprasidone in the assay method was carried out by leaving the test solutions of samples in tightly capped volumetric flasks at room temperature for 48 h. The same sample solutions were assayed 6 h interval up to the study period against freshly prepared standard solution. The mobile phase stability was also carried out by assaying the freshly prepared sample solutions against freshly prepared reference standard solutions 6 h interval up to 48 hrs. Mobile phase prepared was kept constant during the study period of mobile phase stability. The % RSD of assay of ziprasidone was calculated for the study period during mobile phase and solution stability experiments.

The solution stability of ziprasidone and its related impurities were carried out by leaving spiked sample solution in tightly capped volumetric flask at room temperature for 48 h. Content of IMP-1, IMP-2 ,IMP-3 and IMP-4 were determined every 6 h interval up to the study period.

Mobile phase stability was also carried out for 48 h by injecting the freshly prepared sample solutions for every 6 h interval. Content of IMP-1, IMP-2, IMP-3 and IMP-4 was checked in the test solutions. Mobile phase prepared was kept constant during the study period.

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TABLE 1 : System suitability report

Compound	RRT	USP Resolution (R_S)	USP Tailing factor (T)	No of theoretical plates USP tangent method (N)
IMP-1	0.45		1.2	2530
Ziprasidone	1.0	21.4	1.1	71463
IMP-2	1.08	4.9	0.9	68262
IMP-3	1.44	17.6	1.1	64883
IMP-4	1.57	5.4	1.1	59238

RESULTS AND DISCUSSION

Method development and optimization

All the impurities and ziprasidone solutions were prepared in diluent at a concentration of 100μg mL^{-1} and scanned in UV-Visible spectrometer; all the four impurities and ziprasidone were having UV maxima at around 254nm. Hence detection at 254 nm was selected for method development purpose.

The main target of the chromatographic method is to get the separation of closely eluting peaks namely IMP-2 and ziprasidone. Before switching over the method to the UHPLC, initial trials were taken with HPLC system by using different mobile phase combinations and different stationery phases. Though we were able to achieve the required separation of the impurities IMP-1, IMP-3 & IMP-4, the resolution between ziprasidone and the IMP-2 were not achieved. So the method was transferred to UHPLC system and initial trials were taken with Acquity 50 mm column. Ammonium acetate was taken as the buffer and different trials were taken by varying composition of buffer and acetonitrile in isocratic mode. The composition, pH, and flow rate of the mobile phase were changed to optimize the separation conditions. The effect of pH on analyte elution was related to the degree of ionization. A pH of 5.0 was regarded as optimum because at this pH the analyte peak was sharp and well resolved. However the separation of IMP-2 from the ziprasidone peak was not satisfactory. So it was decided to go for gradient mode and different gradient compositions were tried before optimizing the final method. So once the separation of IMP-2 and ziprasidone were achieved, the stressed sample of ziprasidone was injected to check the stability indicating power of the method. However it was found the the base degraded impurity and the IMP-1 was
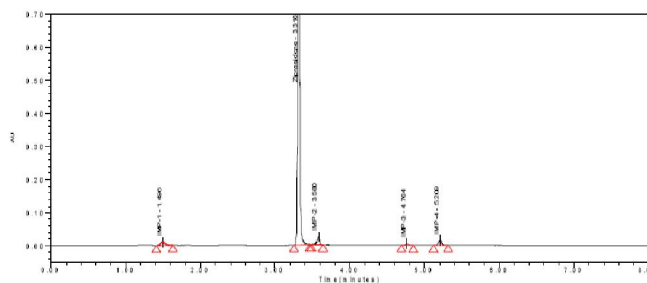

Figure 2 : Typical chromatograms of ziprasidone spiked sample (System suitability)

co eluted with this condition. So changed the column to 100mm keeping all other parameters same and when the stressed samples are injected with this condition, all process impurities as well as the degradation impurities were well separated from each other, with minimum resolution of 3.0 between IMP-2 and the ziprasidone peak. When ziprasidone sample spiked with all the impurities (system suitability solution) was injected the resolution (R_s) between all the impurities and ziprasidone was >2.0, the symmetry of ziprasidone peak was also very good.

The satisfactory chromatographic separation (retention time of ziprasidone is ~3.3 min and the resolution (R_s) between all the impurities was >2) was achieved on Aquity UPLC BEH C18 100 × 2.1 mm with 1.7μm particles, using 0.01M Ammonium acetate pH 5.0 as mobile phase A and Acetonitrile as solution B with a flow rate of 0.2mL min^{-1}. The HPLC gradient program was optimized as: (time (min) / % solution B: 0/40, 1.0/40, 2.0/80, 5.5/80, 6.0/40, 8.0/40. The column temperature as maintained at 35°C and the detection was monitored at a wavelength of 254nm. The injection volume was 1μL. Buffer and Acetonitrile (60:40, *v/v*) was used as diluent. In the optimized gradient conditions ziprasidone, IMP-1, IMP-2, IMP-3 and IMP-4 were well separated with a resolution (R_s) of greater than 2 and the typical retention times of IMP-1, IMP-2, IMP-3, IMP-4 and ziprasidone were about 1.5, 3.6, 4.8, 5.2 and 3.3 min respectively. The system suitability results were given in (TABLE 1) and the developed UHPLC method was found to be specific for ziprasidone and its impurities namely IMP-1, IMP-2 and IMP-3 and IMP-4 (TABLE 1). Peak purity of stressed samples of ziprasidone was checked by using Aquity Photo diode array detector of Waters (PDA). The purity angle within the purity threshold limit obtained in all

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TABLE 2 : Summary of forced degradation results

Stress condition	Time	% Assay of active substance	% Mass Balance = (% of assay + % of degradation products + % of impurities)	Remarks
Acid hydrolysis(0.1N HCl) (reflux at 80°C)	4 h	99	99.9	No major degradation
Base hydrolysis (0.1N NaOH) (reflux at 80°C)	1 h	76.5	99.8	Major degradation with the formation of Impurity with RRT 0.55
Oxidation (5 % H_2O_2) (reflux at 80°C)	0.5 h	69.5	99.7	Major degradation with the formation of Impurity with RRT 0.49
Water hydrolysis (reflux at 100°C)	2 h	99.5	99.8	No degradation observed
Light (photolytic degradation)	10 days	100	100	No degradation observed
Thermal (100°C)	10 days	100	99.9	No degradation observed

TABLE 3 : Results of Linearity study for impurities and drug substance, Linearity data of ziprasidone Impurities

Impurity	Calibration equation	r2	Range (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)
IMP-1	y = 323629x+323.8	0.999	0.04-1.5	0.01	0.04
IMP-2	y = 350874x--487	0.999	0.05-1.5	0.015	0.05
IMP-3	y = 96532x+218	0.998	0.10-1.5	0.025	0.1
IMP-4	y = 325833x+904	0.999	0.04-1.5	0.01	0.04
Assay	y = 4421x--7801	0.999	25-100	-	

TABLE 4 : Results of robustness study

Sr. No	Parameter	Variation	Resolution (R_s) between Imp-2 and Ziprasidone
1	Temperature (± 2°C of set temperature)	(a) At 33°C	4.9
		(b) At 37°C	4.7
2	Flow rate (± 25% of the set flow)	(a) At 0.15 mL min⁻¹	4.9
		(b) At 0.25 mL min⁻¹	4.8

stressed samples demonstrates the analyte peak homogeneity. All stressed samples of ziprasidone (heat (100C), acid hydrolysis (0.1N HCl), base hydrolysis (0.1N NaOH), water hydrolysis and oxidation (5 % H_2O_2)) were analyzed for extended run time of 30 min to check the late eluting degradants.The typical chromatogram of System suitability shown in figure 2.

The proposed method is applied for the related substances and assay analysis of 3 different batches of ziprasidone. The assay results and the % of impurities obtained were within the specification limit. The mass balance (% of assay + % of degradation products + % of impurities) of stressed samples was close to 99.8 % (TABLE 2). The assay of ziprasidone is unaffected in the presence of IMP-1, IMP-2, IMP-3 and IMP-4 and its degradation products confirming the stability indicating power of the developed method.

Results of forced degradation studies

Degradation behavior

Stress studies on ziprasidone under different stress conditions suggested the following degradation behavior. Ziprasidone was stable to Acid stress, Photolytic & thermal stress. Substantial degradation of the drug was observed under base & oxidative hydrolytic conditions.

Degradation in basic solution

When exposed to 0.1N NaOH at 80°C for 1 h, prominent degradation was observed (~23.45 %) with the major degradant as the formation of an impurity eluting at RRT of about 0.55 with 19.2 % (Figure 3(a)). The mass spectrum of the major degradant at RRT 0.55 is shown in figure 3(b).

Oxidative conditions

The drug when exposed to 5 % Hydrogen peroxide at 80°C for 0.5 h, shown prominent degradation (~31.0 %) With the formation of a degradant at RRT of about 0.49 as the major degradant with 20.5 %. (Figure 3(c)). The mass spectrum of the major degradant at RRT 0.49 is shown in figure 3(d).

Peak purity test results derived from PDA detector, confirmed that the ziprasidone peak was homogeneous and pure in all the analyzed stress samples. No degradants were observed after 10 min in the extended runtime of 30 min of all the ziprasidone samples.

Identification and characterization of the major degradants by LC-MS/MS

From the above stress studies, an unknown major degradant was observed at about retention time of 1.6 min (RRT 0.49) under peroxide stress. The positive ion mode ESI spectrum of the unknown impurity at RRT 0.49 has displayed the protonated molecular ion peak
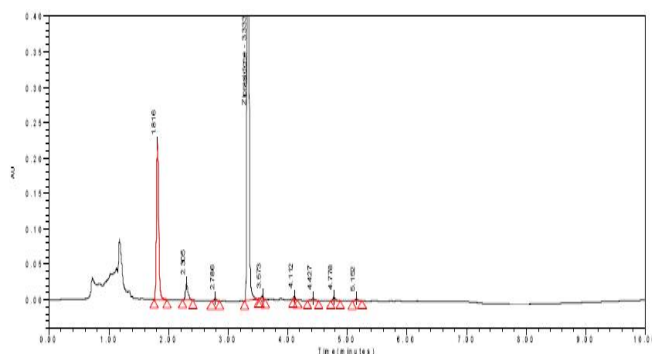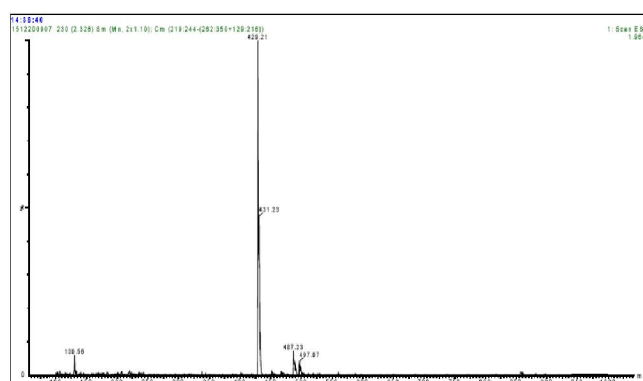
Figure 3(a) : Ziprasidone base degradation sample 0.1N NaOH (1 h ,80 C), Total degradation: 23.45% % Major dégradant (RRT 0.55 = 19.2 %)
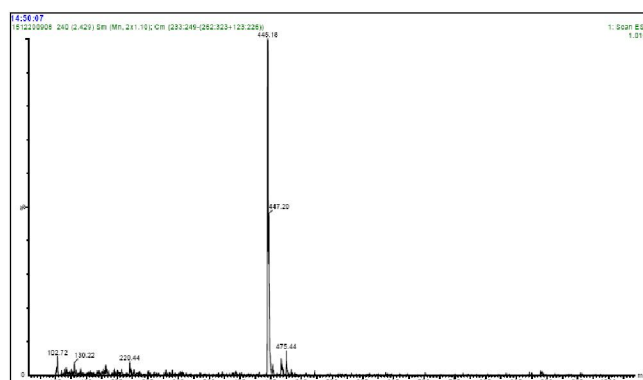
Figure 3b : Mass spectrum of base degradant at RT of 1.815 (RRT 0.55)
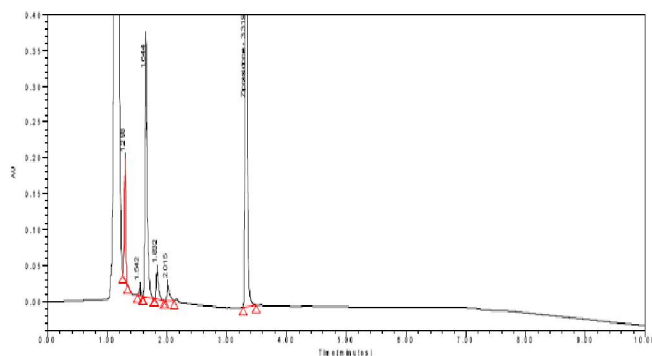
Figure 3c : **Ziprasidone peroxide degradation sample 5 % Peroxide (0.5 h, 80°C), Total degradation: 31 %, Major degradant (RRT 0.49 = 20.5 %)**

Figure 3d : Mass spectrum of peroxide degradant at RT of 1.644 (RRT 0.49)

Figure 3 : Typical chromatograms of stressed ziprasidone samples

Compound	Structure	Chemical name	Mol. Form.	Mol. eight
Peroxide degradant (Imp-RRT 0.49)		5-(2-(4-(1,2-benzisothiozole-3-yl) 1-piperazinyl) –1-hydroxy ethyl)-6-chloro-1, 3-dihydro-2H-indole-2-one	$C_{21}H_{21}ClN_4O_2S$	428
Base degradant (Imp-RRT 0.55)		5-(2-(4-(1,2-benzisothiozole-3-yl) 1-piperazinyl) –1-hydroxy ethyl)-3-oxo-6- chloro-1, 3-dihydro-2H-indole-2-one	$C_{21}H_{19}ClN_4O_3S$	444

Figure 4 : Proposed chemical structures for the degraded impurities

as base peak at m/z 429, confirming the Molecular weight of peak as m/z 428 which is 16 mass units higher than that of ziprasidone (m/z 412). The possibility of oxidation fulfills the addition of 16 mass units to the molecular mass of ziprasidone.Hence, the proposed impurity is a oxidize product of ziprasidone is as shown in figure 4.

An unknown degradant was observed at about retention time of 1.8 min (RRT 0.55) under base stress and was found to be a major degradant. The positive ion mode ESI spectrum of the unknown impurity at RRT

0.55 has displayed the protonated molecular ion peak as base peak at m/z 445, confirming the Molecular weight of peak as m/z 444 which is 32 mass units higher than that of ziprasidone (m/z 412). Hence the proposed impurity is a hydrolysed product of ziprasidone, and the proposed structure may be as shown in figure 4.

Method validation

Precision

The % RSD of assay of ziprasidone during assay

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method precision study was 0.3% and the % RSD of area of IMP-1, IMP-2, IMP-3 and IMP-4 in related substance method precision study was within 2.0%. Confirming the good precision of the developed analytical method.

The % RSD of assay results obtained in intermediate precision study was within 0.3 % and the %RSD for IMP-1, IMP-2, IMP-3 and IMP-4 were well within 2.0 %, confirming the ruggedness of the method (TABLE 3).

Sensitivity

The limit of detection of IMP-1, IMP-2, IMP-3 and IMP-4 were 0.002, 0.003, 0.005 and 0.002% (of analyte concentration, i.e.500μg mL^{-1}) respectively for 1μL injection volume. The limit of quantitation of IMP-1, IMP-2, IMP-3 and IMP-4 were 0.008, 0.01, 0.02 and 0.008% (of analyte concentration, i.e. 500μg mL^{-1}) respectively for 1μL injection volume. The precision at LOQ concentration for IMP-1, IMP-2, IMP-3 and IMP-4 were below 2%.

Linearity

Linear calibration plot obtained by the least square regression analysis for assay method was obtained over the calibration ranges tested, i.e. 25-100μg mL^{-1} and the correlation coefficient obtained was greater than 0.999. The Slope and the Intercept value obtained from the linear regression graph is as shown in (TABLE 3). The result shows an excellent correlation existed between the peak area and concentration of the analyte in the range 50-200 % of analyte concentration.

Linear calibration plot for related substance method was obtained over the calibration ranges tested, i.e. LOQ to 0.3 % for IMP-1, IMP-2, IMP-3 and IMP-4. The correlation coefficient obtained was greater than 0.999 for all four impurities. The Slope and the Intercept value obtained from the linear regression graph was as shown in (Table 3). The result shows an excellent correlation existed between the peak area and concentration of IMP-1, IMP-2, IMP-3 and IMP-4 in the range LOQ to 200 % of the permitted maximum level of the impurity.

Accuracy

The percentage recovery of ziprasidone in bulk drug samples ranged from 99.2 to 101.5 %. The percentage

recovery of IMP-1, IMP-2, IMP-3 and IMP-4 in bulk drug samples ranged from 99.2 to 102.4 %.

Robustness

Close observation of analysis results for deliberately changed chromatographic conditions (flow rate, and column temperature) revealed that the resolution between closely eluting impurities, namely IMP-2 and ziprasidone was always greater than 2.0, illustrating the robustness of the method (TABLE 4).

Solution stability and mobile phase stability

The % RSD of assay of ziprasidone during solution stability and mobile phase stability experiments was within 1.0. No significant changes were observed in the content of IMP-1, IMP-2, IMP-3 and IMP-4 during solution stability and mobile phase stability experiments. The solution stability and mobile phase stability experiments data confirms that sample solutions and mobile phase used during assay and related substance determination were stable up to the study period of 48 h.

CONCLUSION

The gradient UHPLC method developed for quantitative assay and related substance determination of ziprasidone in bulk drug is precise, accurate and specific. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method is stability indicating and can be used for the routine analysis of production samples and also to check the stability of ziprasidone samples.

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REFERENCES

[1] G.J.Marek, L.L.Carpenter, C.J.McDougle, L.H.Price; Neuropsychopharmacology, **28**, 402-12 **(2003)**.

[2] T.F.Seeger, P.A.Seymour, A.W.Schmidt, S.H.Zorn, D.W.Schulz, L.A.Lebel, S.McLean, V.Guanowsky, H.R.Howard, J.A.Lowe; J.Pharmacology Exp. Ther., **275(1)**, 101-13 **(1995)**.

[3] C.Hiemke, A.Dragicevic, G.Grunder, S.Hatter, J.Sachse, I.Vernaleken, M.J.Muller; Ther.Drug Monit., **26**, 156-160 **(2004)**.

[4] Manickam Aravagiri, R.Stephen Marder, Bruce Pollock; J.Chromatogr.B, **847(2)**, 237-244 **(2007)**.

[5] R.F.Suckow, M.Fein, C.U.Correll, T.B.Cooper; J.Chromatogr.B, **799(2)**, 201-208 **(2004)**.

[6] O.Y.Al-Dirbashi, H.Y.Aboul-Enein, A.Al-Odaib, M.Jacob, M.S.Rashed; Biomed.Chromatogr., **20(4)**, 365-8 **(2006)**.

[7] Guodong Zhang, V.Alvin Terry, Jr., G.Michael Bartlett; Biomed.Chromatogr., **22(7)**, 770-778.

[8] Guodong Zhang, V.Alvin Terry, Jr., G.Michael Bartlett; J.Chromatogr.B, **858(2)**, 276-281 **(2007)**.

[9] Z.A.El-Sherif, B.El-Zeany, O.M.El-Houssini, M.S.Rashed, H.Y.Aboul-Enein; Biomed.Chromatogr., **18**, 143-149 **(2004)**.

[10] A.Singh, B.M.Rao, S.Sangaraju, G.R.Deshpande, Srinivas; Chromatogr., **65**, 191-196 **(2007)**.

[11] ICH Stability Testing of New Drug Substances and Products Q1A (R2) International Conference on Harmonization, IFPMA, Geneva, **(2003)**.

[12] ICH Guidelines on Validation of Analytical Procedures, Text and Methodology Q2 (R_1), FDA, Published in the Federal Register, **60**, 1126 **(1995)**.

[13] USP; United States Pharmacopoeia, 32nd Edn. United States Pharmacopeial Convention, Rockville, **(2009)**.

[14] M.Bakshi, S.Singh; J.Pharm.Biomed.Anal., **28**, 1011-1040 **(2002)**.

[15] Drug Stability Principles and Practices, 3rd Edition, Edited by Jens.T.Carstensen, C.T.Rhodes; **(2000)**.