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Development and Validaton of Reverse Phase HPLC Method for Estimation of Ziprasidone Hydrochloride in Bulk Form

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Abstract: The chromatographic method validation for the test procedure of related substances for Ziprasidone Hydrochloride was simple, reliable, sensitive and less time consuming. Weigh accurately about 60mg of Ziprasidone hydrochloride working standard in a 100ml volumetric flask. Dissolve and dilute to the volume with the diluents and mix. Pipette out 5.0ml of the solution in a 100ml volumetric flask and dilute to the volume with the diluent. Further dilute 5ml of this solution to 50ml with diluent, and mix (3ppm).

- ***Test Preparation:***

Weigh accurately about 75mg of sample in a 50ml volumetric flask. Sonicate to dissolve and dilute to volume with the diluent and mix (1500ppm).

- ***System Suitability Preparation:***

Weigh accurately about 37.5mg of Ziprasidone hydrochloride working standard and 2.5mg of impurity-C working standard in a 25ml volumetric flask. Dissolve and dilute to volume with the diluent and mix (1500ppm of Ziprasidone hydrochloride and 100ppm of impurity-C).

- ***Procedure:***

Inject diluent as a blank followed by single injection of system suitability preparation into above chromatographic system. Record the chromatograms and measure the system suitability parameters. Limits are as below; the resolution between peaks due to Ziprasidone and impurity –C should not be less than 2. If system suitability passes, then make three replicate injections of standard preparation and record the chromatograms. Relative standard deviation for the area of three replicate injections of standard preparation should not be more than 5%. If relative standard deviation passes, then make single injection of test preparation and record the chromatograms.

Keywords: *Validation, Chromatogram, Ziprasidone, Mobile Phase, Replicate Injections.*

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I. INTRODUCTION

Pharmaceutical chemistry is a branch of science that makes use of general laws of chemistry in detail about the study of drugs i.e. their properties, chemical nature, composition, structure, the physical and chemical properties of the drugs, the methods of quality control and the condition of their storage and deals with the study of influence of the drug on organisms. The family of drugs may be broadly classified as 1. Pharmacodynamic agents and 2. Chemothera peutic agents.

Pharmacodynamic agents refer to a group of drugs, which stimulate or depress various functions of body to provide some relief to the body in case of body abnormalities without curing the disease. They are mainly used in the case of non-infectious diseases to correct the abnormal body functions. These agents have no action on infective organisms, which cause various diseases. Non-selective central nervous system (C.N.S) modifiers (depressants or stimulants), adrenergic stimulants and blocking agents, cholinergic and cholinergic blocking agents, cardiovascular agents; diuretics, antihistaminic

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agents and anti-coagulating agents are some of the examples of this group [1-2].

Chemotherapeutic agents which are selectively more toxic to the invading organisms without harmful effect to the host. Some of the examples of this group are organo metallic agents, anti malarial, anti bacterial, (sulfa drugs and antibiotics), anti protozoals, anti fungal agents, anti helminthes, anti tubercular agents and anti neoplastics etc.

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles (general and specific) relating to individual drugs, and published in the form of a book called a pharmacopoeia (e.g. I.P, U.S.P, B.P and Martindale extra pharmacopoeia, M.E.P). Pharmaceutical analysis deals not only with medicaments (drug and formulation), but also with their precursors i.e. with the raw material whose degree of purity, which in term decides the quality of medicaments. The quality of a drug each determined, after establishing its authenticity, which is carried by testing its purity and the quality of the pure substance in a drug and its formulation.

The methods of estimation of drugs are divided in to physical, chemical and physico-chemical and biological ones. Among these physic-chemical and physical methods include determination of solubility, transparency or degree of turbidity, color, density or specific gravity (for liquids), moisture content and melting, freezing and boiling points. Physico-chemical methods are employed to study the physical phenomenon that occurs because of chemical reactions. Among the physico-chemical methods the most important are optical (refractometry, polarimetry, emission and the fluorescence methods of analysis, photometry including photocolorimetry and Spectro fluorometry covering UV-visible and I.R regions, nephelometry and turbidimetry), electrochemical (potentiometry, colorimetry, amperometry and polarography) and chromatographic methods like (column, paper, thin layer, gas liquid, high performance liquid) etc. method involving nuclear reactions such as nuclear magnetic resonance (N.M.R) and paramagnetic resonance (P.M.R) are becoming more and more popular. The combination of liquid chromatography with mass spectroscopy is one of the most powerful tools available, the chemical methods include the gravimetric and volumetric procedures, which are based on complex formation, acid-base reactions, precipitations and redox reactions, titration in non-aqueous media and complexometric titrations are various types are widely used in pharmaceutical analysis. Several drugs introduced in to the market have been increasing at an alarming rate. These drugs may be either new entities or partial structural modifications of the existing drugs and which are included in one or few pharmacopoeias only, after a time lag because of several reasons. Under these conditions, standards and analytical procedures for these drugs may not be available in official books or may be inaccessible. Hence it becomes necessary to develop newer analytical methods for such drugs. In this context, pharmaceutical analysis plays an important role in the quality assurance and quality control of bulk drug samples and pharmaceutical formulations. The fundamental principle of operation of spectrophotometer is based on that when a light of definite wavelength passed through a cell containing solution or solvent some of the light is absorbed by the solution or solvent and remaining light is transmitted. The transmitted light falls on the detector that transforms the radiant energy to electrical energy measured by galvanometer. The purpose of HPLC method is to enable quantification of a drug compound under a variety of physical, chemical and photochemical stress conditions. It is required that the method is stable specific for the drug, i.e. the drug peak is separated from all degradation product peaks. Also, the linearity and the minimum level of quantification of the method should be established[3-6].

➢ *Validation of HPLC Method:*

Method validation is performed to ensure that an analytical methodology is accurate, specific, reproducible and rugged over specified range that an analyte will be analyzed. Method validation provides an assurance of reliability during normal use, and is sometimes referred to as “the process of providing documented evidence that the method does what it is intended to do”. The objective of the analytical procedure should be clearly understood since this governs the validation characteristics, which need to be evaluated. Typical validation characteristics, which should be considered, are System suitability, Detection of limit (LOD), Precision, Specificity, Accuracy, Linearity, Robustness and Ruggedness. The terms are referred to as “analytical performance parameters”, or sometimes as “analytical figure of merit”. Most of these terms are familiar and are used daily in the laboratory[7-8].

➢ *Drug Profile*

- *Ziprasidon Hydrochloride*

✓ Molecular Formula : $C_{21}H_{21}ClN_4OS$
✓ IUPAC Name : 5-[2-[4-(1, 2-benzisothiazol-3-yl)-1-Piperazinyl] ethyl]-6-chloro-1, 3-dihydro-2H-indol-2-one

➢ *Plan of Work*

- The present study includes the analytical method validation to quantify Ziprasidone Hydrochloride and Galantamine Hydrobromide.
- Standard analytical procedures for these drugs or formulations may not be available and if available may not suit to our actual conditions of use. So, it is required to develop newer analytical methods, which are accurate, precise, specific, linear, simple and rapid.
- The modern method of choice of assays is High – Pressure Liquid Chromatography (HPLC), which requires highly sophisticated equipment, trained personal, high purity chemicals and proper maintenance.

The various steps that are involved in here these are:

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II. MATERIALS AND METHODS

➢ *Materials Used:*

Table 1 Reagent and Chemical Used

Reagent and Chemical used			
S.No	**Reagents**	**Grade**	**Supplier**
1.	Potassium dihydrogen phosphate	AR-Grade	Merck
2.	Sodium phosphate monobasic	ACS-Grade	Merck
3.	Potassium hydroxide	AR-Grade	Merck
4.	Phosphoric acid	AR-Grade	Merck
5.	Triethyl amine	HPLC-Grade	Merck
6.	Acetic acid	AR-Grade	Merck
7.	Acetonitrile	HPLC-Grade	Ranken
8.	Methanol	HPLC-Grade	Merck
9.	Water	HPLC-Grade	Ranken

Table 2 Instruments & Equipment Used

Instruments & Equipment used:	
HPLC	**Water alliance**
Sonicator	Bandelin
Centrifuge Apparatus	Heraeus Biofuge Primo
Analytical Balance	Metler
HPLC Column	C-8

III. METHODOLOGY

A. Validation of Related Substances Method:

➢ *Ziprasidone Hydrochloride*

- *Preparation of Buffer:*

Prepare a homogeneous mixture of aqueous 0.05M potassium dihydrogen phosphate. Filter through 0.2 µ membrane filter.

- *Preparation of Mobile Phase:*

✓ *Mobile Phase-A:*

Prepare a homogeneous mixture of buffer and methanol (67:33).Adjust P^H of the mixture to 3.0±0.05 with phosphoric acid. Sonicate and degas.

✓ *Mobile Phase-B:*

Prepare a homogeneous mixture of buffer, methanol and Acetonitrile (40:05:55). Adjust P^H of the mixture to 6.0±0.05 with KOH solution. Sonicate and degas.

➢ *Standard Preparation:*

Weigh accurately about 60mg of Ziprasidone hydrochloride working standard in a 100ml volumetric flask. Dissolve and dilute to the volume with the diluents and mix. Pipette out 5.0ml of the solution in a 100ml volumetric flask and dilute to the volume with the diluent. Further dilute 5ml of this solution to 50ml with diluent, and mix (3ppm).

➢ *Test Preparation:*

Weigh accurately about 75mg of sample in a 50ml volumetric flask. Sonicate to dissolve and dilute to volume with the diluent and mix (1500ppm).

➢ *System Suitability Preparation:*

Weigh accurately about 37.5mg of Ziprasidone hydrochloride working standard and 2.5mg of impurity-C working standard in a 25ml volumetric flask. Dissolve and dilute to volume with the diluent and mix (1500ppm of Ziprasidone hydrochloride and 100ppm of impurity-C).

➢ *Procedure:*

Inject diluent as a blank followed by single injection of system suitability preparation into above chromatographic system. Record the chromatograms and measure the system suitability parameters. Limits are as below; the resolution between peaks due to Ziprasidone and impurity –C should not be less than 2. If system suitability passes, then make three replicate injections of standard preparation and record the chromatograms. Relative standard deviation for the area of three replicate injections of standard preparation should not be more than 5%. If relative standard deviation passes, then make single injection of test preparation and record the chromatograms[9-11].

- *Chromatographic System:*

✓ Instrument: HPLC equipped with pump, UV detector and recorder.
✓ Column: Agilent Zorbax R_x C-8(4.6mm X 150mm) 5 µm.
✓ Flow rate: 1.5ml /minute.
✓ Detector: UV at 229nm.
✓ Injection Volume: 10 µl
✓ Column Temperature: 30° C

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➢ *Gradient Programming:*

Table 3 Gradient Programming

Time(min)	Mobile phase-A%	Mobile phase-B%
0.01	100	0
15.00	100	0
20.00	85	15
30.00	85	15
40.00	55	45
55.00	40	60
65.00	25	75
70.00	20	80
71.00	100	00
75.00	Stop	Stop

➢ *Approximate Relative Retention Times:*

Table 4 Approximate Relative Retention Times

Peak name	RRT	RF
Ziprasidone	1.0	
Impurity-A	About 0.22	0.75
Impurity-B	About 0.7	1.00
Impurity-C	About 0.84	1.00
Impurity-D	About 1.84	1.4
Impurity-E	About 2.18	0.56
Choloroacetyl oxyindole	About 0.48	0.7

Approximate retention time for Ziprasidone peak is about 21.5mins.

- *Calculation for Ziprasidone:*

Calculate all individual known and unknown impurities by following formula:

% impurity = AT×WS×DT×P×F×100/AS×WT×DS×100

Where –

✓ AT= Area of impurity peak from test chromatogram.
✓ AS= Average area of Ziprasidone peak from standard chromatograms.
✓ WT= Weight of test taken in mg.
✓ WS= Weight of standard taken in mg.
✓ DS= Dilution of standard preparation.
✓ DT= Dilution of test preparation.
✓ P= Purity of working standard.
✓ F= Response factors for respective impurities.

Disregard all peaks below 0.05%.

Total impurities = Sum of all impurities.

➢ *Determination of Specificity:*

- *Preparation:*

Prepare system suitability and standard solution and test solution as per method of analysis. Prepare individual known impurities standard solution and test solution spiked with all known impurities (spiked test solution). Inject gradient blank, blank and system suitability solution in single, three replicate injections of standard solution, test solution and all individual known impurity standard solutions in single and then spiked test solution in single. Evaluate the system suitability parameters from system suitability chromatogram.

➢ *Determination of Precision:*

- *System Precision:*

✓ *Preparation:*

Prepare system suitability and standard solution as per method of analysis. Prepare spiked test solution. Inject blank, system suitability solution, spiked test solution in single and three replicate injections of standard solution. Evaluate the system suitability parameters from system suitability chromatogram[12].

- *Method Precision:*

✓ *Preparation:*

Prepare system suitability and standard solution as per method of analysis. Prepare spiked test solution. Inject blank, system suitability solution, spiked test solution in single and six replicate injection of standard solution. Evaluate the system suitability parameters from system suitability chromatogram.

- *Intermediate Precision:*

✓ *Preparation:*

Carry out method precision by different analyst on different HPLC system, using different column on another

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day. Calculate the percentage of known impurities, any other impurity and total impurities for all six tests separately. Then calculate average and percentage of RSD of individual known impurities, any other impurity and total impurities for twelve test preparations (six from method precision and six from intermediate precision).

- *Determine The Limit of Detection (LOD) AND LIMIT OF QUANTIFICATION (LOQ):*

Prepare system suitability and standard solution as per method of analysis. Prepare spiked test solution. Inject blank, system suitability solution, spiked test solution in single and three replicate injections of standard solution. Evaluate the system suitability parameters from system suitability chromatogram[13].

- *Limit of Detection (LOD):*

✓ *Procedure:*

Determine the limit of detection by the analysis of standard with known concentration of standard and known impurities and by establishing the minimum level at which the respective peak can reliably detected visually. For determination of LOD inject lowest concentration 0.01% of test concentration. If at these concentrations respective peak is not detected visually, then inject above concentration till the respective peaks are visually detected.

Confirm the LOD by injecting standard and known impurities standard solution in six replicates at LOD level.

- *Limit of Quantification (LOQ):*

✓ *Procedure:*

Determine the limit of quantification by the analysis of standard with known concentration of standard and known impurities and by establishing the minimum level at which the respective peak can be quantified with acceptable accuracy and precision. Consider 0.04% of test concentration is the minimum concentration level for determination of LOQ.

Confirm LOQ by injecting standard and known impurities standard solution in six replicate at LOQ concentration. Calculate the %RSD of area of six replicate injections[14-16].

- *Determination of Linearity:*

✓ *Procedure:*

In continuation with above experiment prepare standard solutions (for standard and all individual known impurities) from concentration LOQ level to 150% of the specification limit (use minimum six concentration level). Perform three replicate injections at each concentration and average the peak areas. Plot area on Y-axis and concentration on X-axis. Evaluate correlation coefficient. Slope and Y-intercept from each linearity graph. Report correlation coefficient, Y-intercept and slope.

- *Determination of Accuracy:*

✓ *Procedure:*

In continuation of linearity experiment, prepare the test solution as per the method of analysis and inject in triplicate. Prepare the test solution and spiked the known impurities at LOQ level, at 100% level and at 150% level in triplicate at each concentration level and injected in single. Area and linearity graph(Y-intercept and slope used for accuracy calculation).

- *Calculate Amount Recovered by Using Following Formula:*

Corrected area – intercept of linearity curve/ slope of linearity curve

- *Use Amount Recovered for Calculation of Accuracy:*

Amount recovered × 100 / amount added

- *Test Solution:*

✓ *Preparation:*

Establish the stability of test sample in diluent in auto sampler for a period of 24 hrs. Prepare system suitability and standard solution as per method of analysis. Prepare spiked test solution. Inject blank, system suitability solution and spiked test solution in single and three replicate injections for standard solution. Evaluate the system suitability parameters from system suitability chromatogram.

Prepare the test preparation as per method of analysis and keep in an auto sampler at 10˚C. Inject this solution into the HPLC system over a period of about 24 hrs. Plot areas of impurity on Y-axis against time on X-axis. Draw the linear trend line.

Use following formula for calculation of %Δ (difference)-

%Δ= time in hrs × slope × 100 / Y-intercept

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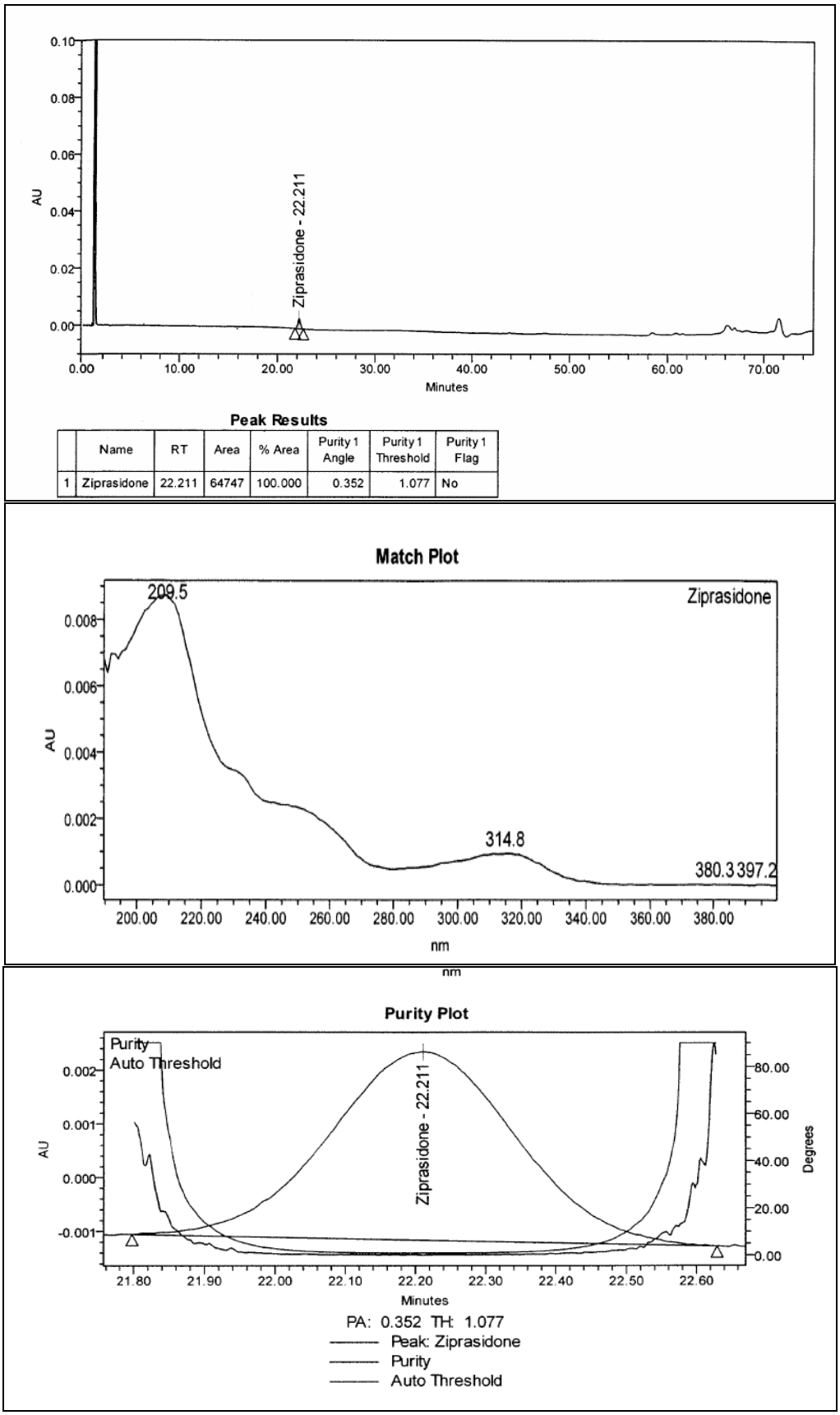
➢ *Ziprasidone Hydrochloride:*

- *Standard Solution Chromatogram*



	Name	RT	Area	% Area	Purity 1 Angle	Purity 1 Threshold	Purity 1 Flag
1	Ziprasidone	22.211	64747	100.000	0.352	1.077	No

Fig 1 Standard Solution Chromatogram

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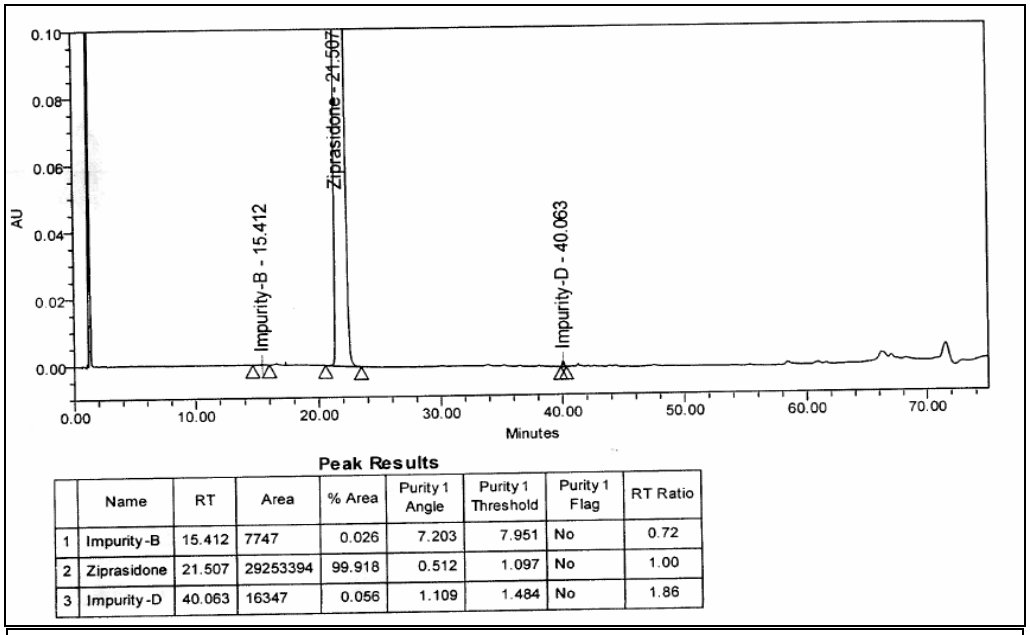
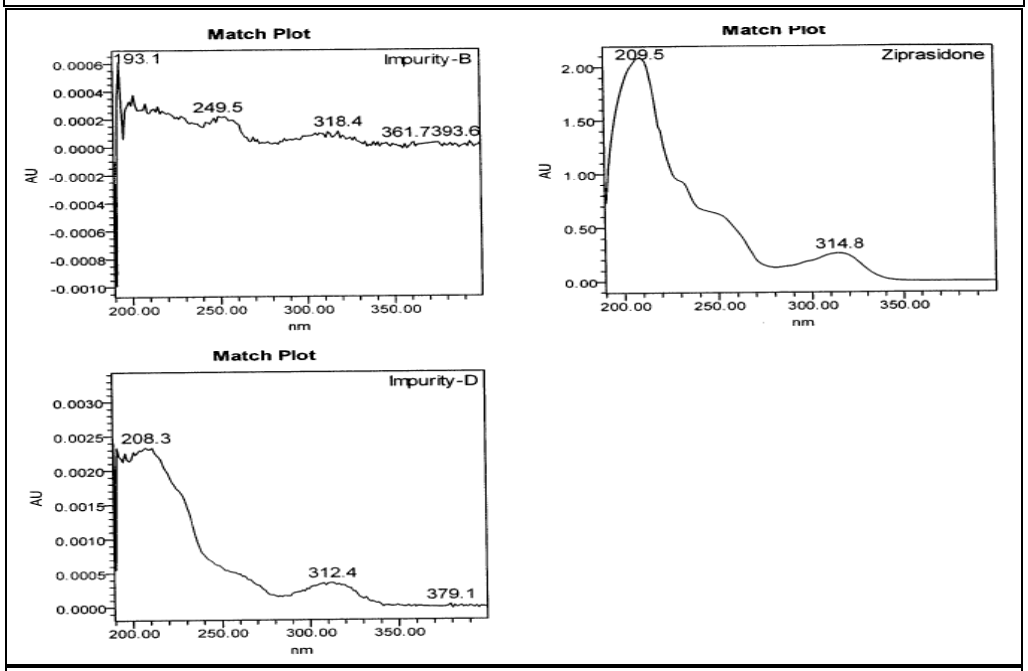
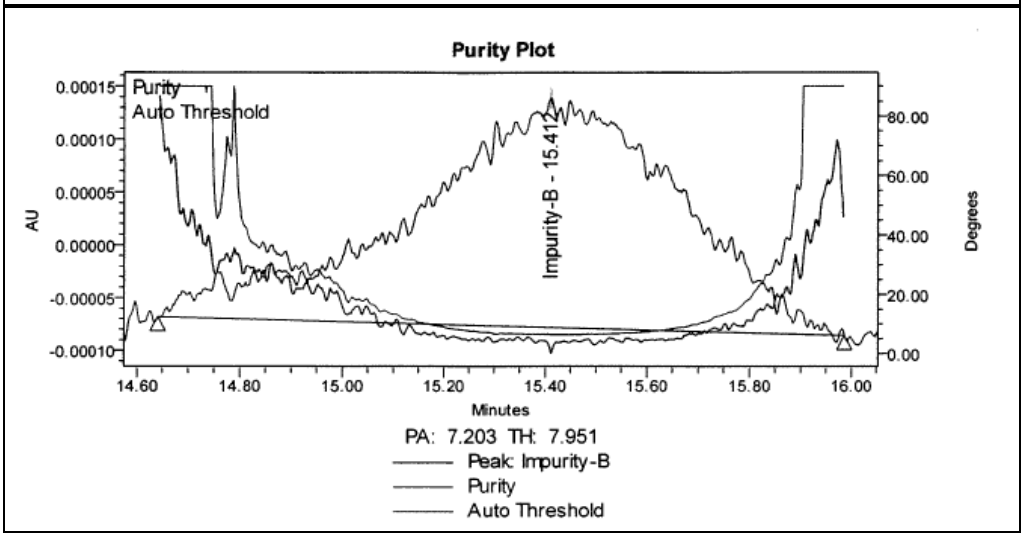
- *Test Solution Chromatogram*



Peak Results

	Name	RT	Area	% Area	Purity 1 Angle	Purity 1 Threshold	Purity 1 Flag	RT Ratio
1	Impurity-B	15.412	7747	0.026	7.203	7.951	No	0.72
2	Ziprasidone	21.507	29253394	99.918	0.512	1.097	No	1.00
3	Impurity-D	40.063	16347	0.056	1.109	1.484	No	1.86





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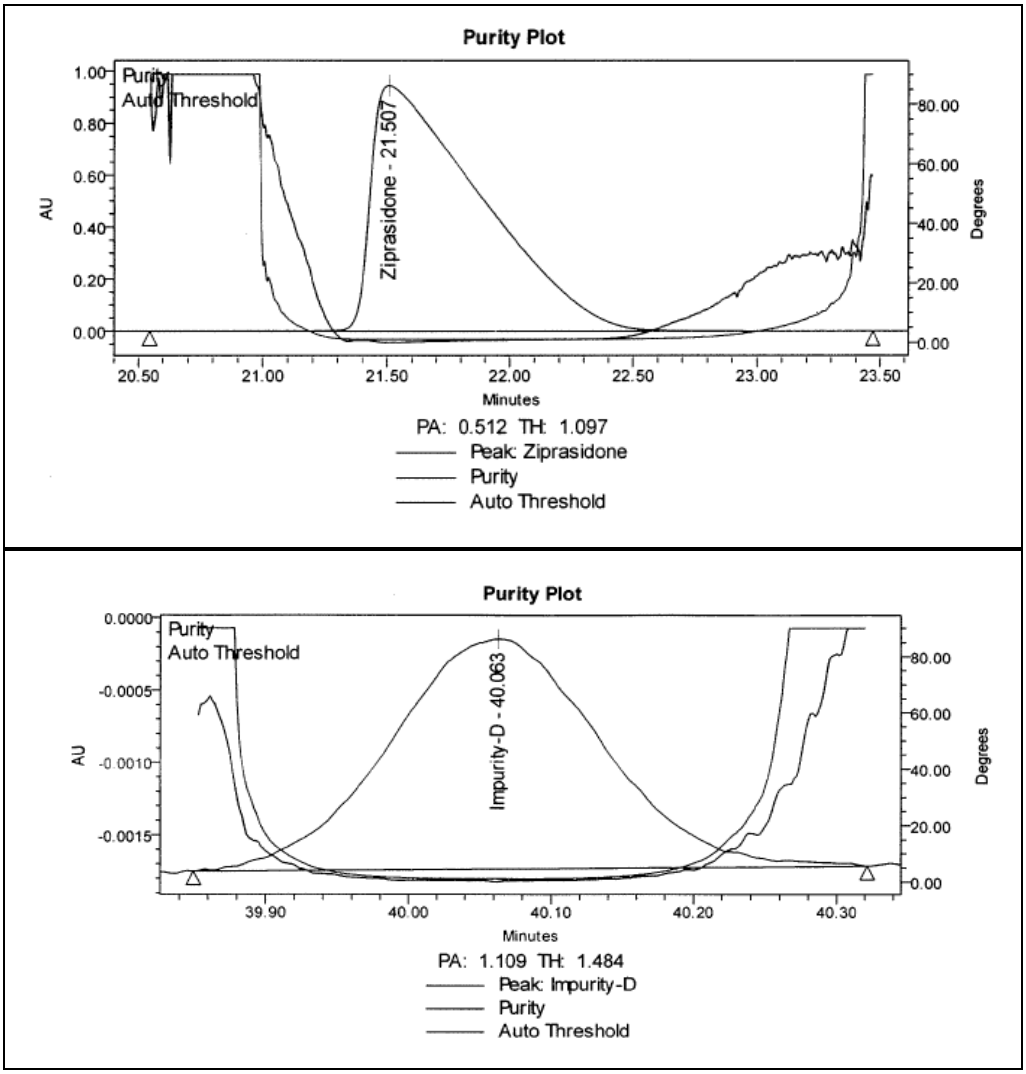

Fig 2 Test Solution Chromatogram

- *Method Precision:*

✓ *Preparation:*

Prepared system suitability solution and standard solution as per method of analysis. Prepared spiked test solution. Injected blank, system suitability and spiked test solution in single and six replicate injections of standard solution.

Table 5 Evaluated the System Suitability Parameters from System Suitability Chromatogram.

System suitability		
System Suitability Parameters	**Observations**	**Acceptance Criteria**
Resolution between Ziprasidone related compound –C and Ziprasidone peak	4.12	Not less than 2.0
Conclusion: System Suitability Passes.		
Standard replicate injections		
Injection No.	**Peak area**	**Acceptance criteria**
1	66952	%RSD: Not More than 5.0
2	65018	
3	65633	
4	65766	
5	65948	
6	66474	
Average	65965	
% RSD	1.02	

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Table 6 Evaluated the System Suitability Parameters from System Suitability Chromatogram

System suitability		
System Suitability Parameters	**Observations**	**Acceptance Criteria**
Resolution between Ziprasidone related compound –C and Ziprasidone peak	3.75	Not less than 2.0
Conclusion: System Suitability Passes.		
STANDARD REPLICATE INJECTIONS		
Injection No.	**Peak area**	**Acceptance criteria**
1	73200	%RSD: Not more than 5.0

Method precision is carried out by different analyst on different HPLC system, using different column on another day. Calculated the % of individual known impurity, any other impurity and total impurities for all six test preparations separately. Then calculated average and % RSD of all individual known impurities, any other impurity and total impurities for 12 test preparations. (Six from method precision and six from intermediate precision)[17].

Table 7 Condition I: (Analyst I , HPLC I, Column I, DAY I)

CONDITION I: (ANALYST I , HPLC I, COLUMN I, DAY I)								
Peak Name	**Test 1**	**Test 2**	**Test 3**	**Test 4**	**Test 5**	**Test 6**	**Average**	**% RSD**
Ziprasidone Related compound-A	ND	ND	ND	ND	ND	ND	NA	NA
Chloroacetyl Oxyindole	ND	ND	ND	ND	ND	ND	NA	NA
Ziprasidone Related compound-B	BQL	BQL	BQL	BQL	BQL	BQL	NA	NA
Ziprasidone Related compound-C	ND	ND	ND	ND	ND	ND	NA	NA
Ziprasidone Related compound-D	0.072	0.074	0.074	0.076	0.075	0.076	0.075	2.67
Ziprasidone Related compound-E	ND	ND	ND	ND	ND	ND	NA	NA
Acceptance criteria: % RSD Should not be more than 20.0 for impurity less than 0.1% and 10.0 for Impurity more than 0.1%								

Table 8 Condition II: (Analyst II , HPLC II, Column II, DAY II)

CONDITION II: (ANALYST II , HPLC II, COLUMN II, DAY II)								
Peak Name	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6	Average	% RSD
Ziprasidone Related compound-A	ND	ND	ND	ND	ND	ND	NA	NA
Chloroacetyl Oxyindole	ND	ND	ND	ND	ND	ND	NA	NA
Ziprasidone Related compound-B	BQL	BQL	BQL	BQL	BQL	BQL	NA	NA
Ziprasidone Related compound-C	ND	ND	ND	ND	ND	ND	NA	NA
Ziprasidone Related compound-D	0.061	0.063	0.063	0.064	0.066	0.066	0.064	3.13
Ziprasidone Related compound-E	ND	ND	ND	ND	ND	ND	NA	NA
Acceptance criteria: % RSD Should not be more than 20.0 for impurity less than 0.1% and 10.0 for Impurity more than 0.1%								

➢ *Limit of Detection (LOD) and Limit of Quantitation (LOQ):*

Prepared system suitability solution and standard solution as per method of analysis. Prepared spiked test solution. Injected blank, system suitability and spiked test solution in single and three replicate injections of standard solution**.**

Table 9 Evaluated the System Suitability Parameters from System Suitability Chromatogram

System suitability		
System Suitability Parameters	**Observations**	**Acceptance Criteria**
Resolution between Ziprasidone related compound –C and Ziprasidone	3.97	Not less than 2.0
Conclusion: System Suitability Passes.		
Standard replicate injections		

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Injection No.	Peak area	Acceptance criteria
1	67298	%RSD: Not More than 5.0
2	66912	
3	67119	
Average	67110	
% RSD	0.29	
Peak Name	**Retention time in spiked test solution**	**Resolution**
Ziprasidone Related compound-A	4.819	NA
Chloroacetyl Oxyindole	11.003	13.61
Ziprasidone Related compound-B	16.084	6.33
Ziprasidone Related compound-C	18.840	2.96
Ziprasidone	22.029	3.78
Ziprasidone Related compound-E	47.748	38.87

- *Preparation:*

The limit of detection is determined by analysis of standard with known concentration of Ziprasidone and its known impurities and by establishing the minimum level at which the respective peak is reliably visually detected. For determination of LOD, lowest concentration injected is 0.01% of test concentration. If all these peaks are not visually detected at this concentration (0.01%), then above 0.01% concentration solutions are injected till the impurity peaks are visually detected. LOD values are given in the following table LOD is confirmed by injecting Ziprasidone and impurities standard solution in six replicates at LOD level[18].

➢ *Limit of Quantitation (LOQ):*

The limit of Quantitation is determined by the analysis of standard with known concentration of Ziprasidone and its known impurities and by establishing the minimum level at which the impurity can be quantified with acceptable accuracy and precision 0.04% is the minimum concentration level considered for determination of LOQ.

Table 10 The LOQ Level in Ppm and Percentage is Given in LOQ Table.

LOQ		
Name	**LOQ in PPM**	**LOQ in %**
Ziprasidone Related compound-A	0.6	0.04
Chloro acetyl Oxyindole	0.6	0.04
Ziprasidone Related compound-B	0.6	0.04
Ziprasidone Related compound-C	0.6	0.04
Ziprasidone	0.6	0.04
Ziprasidone Related compound-E	0.6	0.04

LOQ is confirmed by injecting Ziprasidone and its known impurities standard solution in Six replicate at LOQ concentration. %RSD of area of six replicate injections is calculated.

Table 11 Precision at LOQ

PRECISION AT LOQ								
STD Name	**Inj-1**	**Inj-2**	**Inj-3**	**Inj-4**	**Inj-5**	**Inj-6**	**Average**	**% RSD**
Ziprasidone Related compound-A	16125	16246	16195	16429	16047	16237	16213	0.80
Chloro acetyl Oxyindole	16912	17151	17326	17266	17171	17231	17176	0.84
Ziprasidone Related compound-B	10778	10830	11025	11070	10657	11004	10894	1.50
Ziprasidone Related compound-C	9047	8865	8985	8760	8861	8884	8900	1.14
Ziprasidone	11974	12008	11989	12140	12053	12233	12066	0.84
Ziprasidone Related compound-E	23609	23403	23752	23979	23759	23218	23620	1.16
Acceptance criteria: RSD Should not be more than 10.0 %.								

- *Conclusion:* % RSD of area of six replicate injections is below 10.0.Method is validated for precision at LOQ level.

- *Determination of Linearity:*

✓ *Preparation:*

In continuation with the above experiment prepared standard solutions for Ziprasidone and all known impurities from concentration LOQ level to 150 % of the specification limit. Three replicate injections are performed at each concentration and the peak areas are averaged Calculated correlation coefficient and linear equation. Plotted area on Y-axis and concentration on X-axis.

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Linear regression analysis will demonstrate the acceptability of the method for quantitative analysis over the full spectrum of the concentration range.

Table 12 Linearity of Ziprasidone Related Compound -A

Conc.(%) of spec. level	Conc. in ppm	Peak areas	Average Peak areas
LOQ level 0.04	0.600	16461	16543
		16581	
		16588	
50	0.761	21147	21105
		20941	
		21227	
75	1.157	30804	30829
		30936	
		30746	
100	1.523	40364	40481
		40574	
		40504	
125	1.918	51303	51522
		51601	
		51663	
150	2.284	60664	60674
		60833	
		60524	

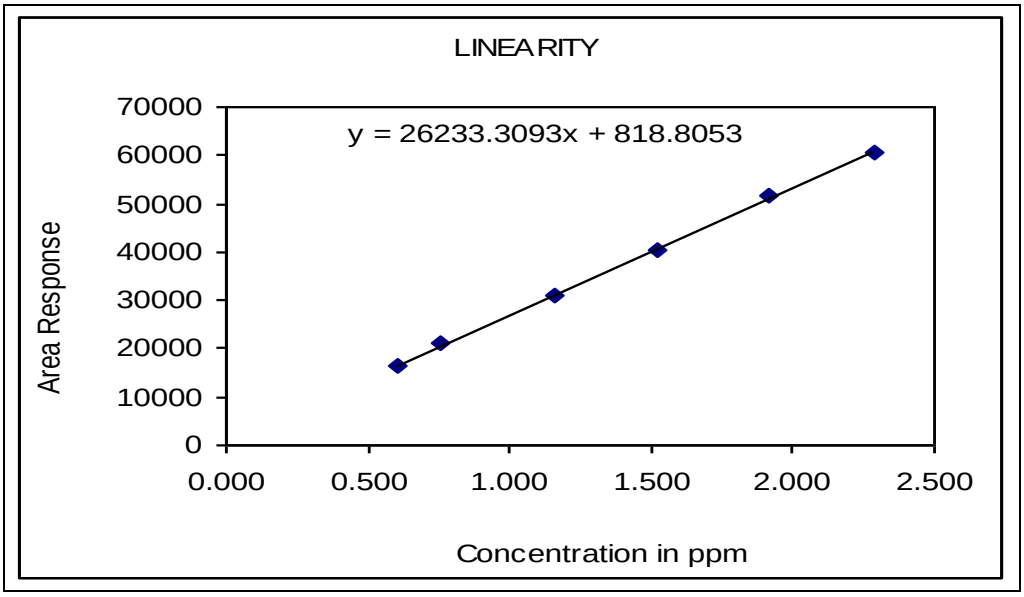

Fig 3 Linearity

Coefficient of correlation: 0.9998 (Not less than 0.990)

- *Conclusion:*
  Correlation coefficient is above 0.99; Method is validated for linearity from LOQ level to 150% of specification level.

Table 13 Linearity of Ziprasidone Related Compound –B

Conc.(%) of spec. level	Conc. in ppm	Peak areas	Average Peak areas
LOQ level 0.04	0.600	10812	10364
		9979	
		10302	
50	1.513	26918	26690
		26594	
		26557	
75	2.300	39055	39117

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		39496	
		38800	
100	3.025	50979	51053
		51072	
		51108	
125	3.812	63335	63419
		63103	
		63819	
150	4.538	74655	75117
		75857	
		74838	

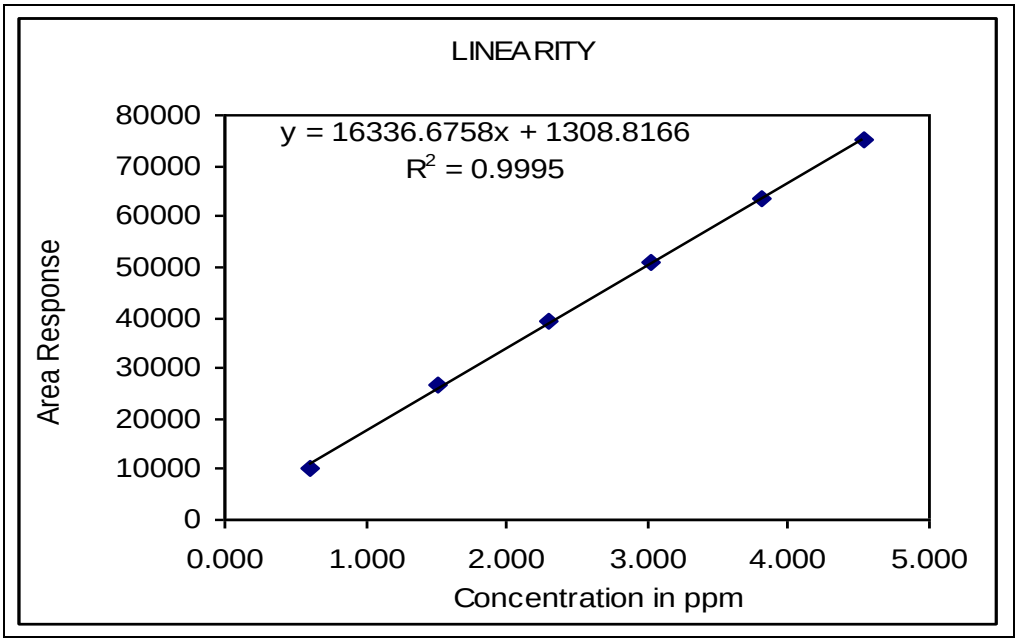

Fig 4 Concentration in ppm

Correlation coefficient: 0.9998 (Not less than 0.990)

- *Conclusion:*
  Correlation coefficient is above 0.99; Method is validated for linearity from LOQ level to 150% of specification level.

Table 14 Summarized System Suitability

Validation Parameter	**Resolution between Abacavir Sulfate related compound –B and Abacavir Sulfate related compound -C peak**
Specificity	2.95
System Precision	2.91
Method Precision	2.93
Intermediate Precision	2.95
Linearity & Accuracy	2.91
Ideal Condition	3.04
Flow rate +0.2 ml/min	2.86
Flow rate -0.2 ml/min	3.13
Acetonitrile Variation +5%	2.45
Acetonitrile Variation -5%	3.29
pH change +0.2 units	2.64
pH change -0.2 units	2.86
Buffer concentration(ADP) +10%	3.06
Buffer concentration(ADP) -10%	2.60
Buffer concentration(TAHS) +10%	2.77
Buffer concentration(TAHS) -10%	3.01
Spiked test solution stability	3.05

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Test solution stability	3.03
Filter paper interference	3.05
Bench top mobile phase stability 72.0 hrs	2.99
Degradation study	

*ADP- ammonium Dihydrogen Phosphate

*TAHS-Tetra butyl Hydrogen sulphate

➢ *Validation Summary*

Table 15 Validation Summary

Validation Parameter	RC-A	RC-B	RC-C	Abacavir sulfate	O-Pyrimidinederivative
Specificity (Peak Purity)	Peak purity pass	Peak purity pass	Peak purity pass	Peak purity pass	Peak purity pass
	Peak purity pass	Peak purity pass	Peak purity pass	Peak purity pass	Peak purity pass
System Precision					
50% (%RSD)	0.19	**0.59**	**0.45**	**0.39**	**2.43**
100% (%RSD for Area)	0.11	**0.50**	**0.10**	**0.35**	**0.82**
100%(%RSD for RT)	0.32	**0.16**	**0.15**	**0.11**	**0.05**
150% (%RSD)	0.29	**0.27**	**0.26**	**0.54**	**1.03**
Method Precision					
Analyst-1 (%RSD)	NA	NA	NA	NA	NA
Analyst-2 (%RSD)	NA	NA	NA	NA	NA
Intermediate Precision (%RSD)	NA	NA	NA	NA	NA
LOD (ppm)	0.1	0.1	0.1	0.1	0.1
LOQ (ppm)	0.4	0.4	0.4	0.4	0.4
Precision at LOQ level (%RSD)	0.12	1.19	0.53	0.47	1.48
Linearity (Correlation coefficient)	0.999	0.999	0.997	0.999	0.999
Accuracy					
Accuracy at LOQ Level	108.9	84.8	83.6	NA	104.3
Accuracy at 100%	87.5	93.2	91.4	NA	99.9
Accuracy at 150%	93.6	94.6	91.7	NA	99.3
Robustness	System suitability passes**.**				
Degradation Study (Peak purity)					
Condition	**RC-A**	**RC-B**	**RC-C**	**Abacavir sulfate**	**O-Pyrimidinederivative**
Acidic	Peak purity pass	NA	NA	Peak purity pass	Peak purity pass
Alkali	Peak purity pass	NA	NA	Peak purity pass	Peak purity pass
Oxidation	Peak purity pass	NA	NA	Peak purity pass	Peak purity pass
Reduction	Peak purity pass	NA	NA	Peak purity pass	Peak purity pass
Heating (solid)	Peak purity pass	NA	NA	Peak purity pass	Peak purity pass
Heating (solution)	Peak purity pass	NA	NA	Peak purity pass	Peak purity pass
UV (solid)	Peak purity pass	NA	NA	Peak purity pass	Peak purity pass
UV (solution)	Peak purity pass	NA	NA	Peak purity pass	Peak purity pass

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IV. CONCLUSION

The chromatographic method validation for the test procedure of related substances for Ziprasidone Hydrochloride was simple, reliable, sensitive and less time consuming. The advantage of the present test procedure is that it does not require any complicated mobile phase and it is a simple isocratic method. The present method can be confidently used for rapid and precise estimation of Ziprasidone Hydrochloride. Especially this procedure can be a major interest in pharmaceutical analysis, since it offers a distinct quality control in the test procedure of assay of Ziprasidone Hydrochloride tablet forms. This method may be recommended for routine and quality control analysis of the investigated drug. The developed related substances method was validated for parameters such as System suitability, Linearity, Precision, Accuracy, Ruggedness and Robustness. Hence this method is suitable, Linear, Accurate, Robust for the related substances of Ziprasidone Hydrochloride. The present work shows, a validated, highly sensitive method for determination of Ziprasidone Hydrochloride.

REFERENCES

[1]. R. Chatwal, & SHAM K Anand, "Instrumental Methods of Chemical Analysis", 9th edition, Himalaya pub. House Publisher, 2002.
[2]. http://www.analyse-it.com/products/clinical/method-comparision.html.
[3]. D.M Wagrowski- Diehl; E.S Grumbach; P.C.Iraneta, "Development of New HPLC column for the Retention and Separation of Highly Polar Compounds", Presentation at Pittsburgh Conference 2002.
[4]. Validation of analytical procedure, Methodology, ICH Harmonized tripartite Guidelines. 108, 1996.
[5]. Michael W Dong, ''Modern HPLC for Practical Scientists." Wiley-International Publishers.
[6]. U.S. Pharmacopoeia 23, pp 946-949, 1590-1591, U.S. Pharmacopoeia Convention, 1995.
[7]. R.J. Hamilton and Swell, Introduction to HPLC. 2nd edition, 2-9.
[8]. Sharma, B.K "Instrumental Methods of Chemical Analysis", 19th edition, Goel Publisher. Houses, Meeru, 2000.
[9]. K.D Tripathi MD "Essentials of Medical Pharmacology", 5th, Jaypee Brothers Medical Publishers (p) Ltd. New Delhi.
[10]. http://www.md.ucl.ac.be/
[11]. www.sciencedirect.com
[12]. www.drreddys.com
[13]. www.expressindia.com
[14]. www.informed.org
[15]. Goodman & Gilman. The Pharmacological Basis of Therapeutics. 11th edition.
[16]. Martindale. The Extra Pharmacopoeia. 31st ed. Pharmaceutical press (London), 2002.
[17]. Goodman and Gilman. The pharmacological basis of therapeutics. 10th ed. McGraw-Hill (New York), 2001.
[18]. Pankaj K, Choudhary P, Development and validation of spectrophotometric method for the estimation of Ziprasidone HCl, Oriental Journal of Chemistry 2006; 21:1.