WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 8, 1049-1070. Research Article ISSN 2277– 7105

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING METHOD FOR ASSAY DETERMINATION OF ZIPRASIDONE HCL BY USING RP-UPLC ANALYTICAL METHOD

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Article Received on
24 February 2018,
Revised on 15 March 2018,
Accepted on 05 April 2018,
DOI: 10.20959/wjpr20188-11902

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ABSTRACT

Method development, validation and stability indicating RP-UPLC method for Assay determination of Ziprasidone Hcl in pharmaceutical products. This method has high degree of performance to separation and quantification of Ziprasidone even in the presence of its impurities. This efficient Separation can be achieved by using new Waters Acquity CSH Phenyl hexyl with dimensions (100mm x 2.1 mm, 1.7µm). The buffer used in this method KH_2PO_4 at pH 2.5 and Acetonitrile in the ratio of (70:30 v/v) with a flow rate of 0.3 ml/min and the absorbance was monitored at 209 nm. The total run time was 3.0 min. The correlation coefficient of the method shows good linear relationship with 0.9999. The limit of detection and quantification are determined for Ziprasidone HCl 0.01µg/ml and 0.03µg/ml. The signal to noise ratio has been observed 4 and 15 for LOD & LOQ respectively. The precision of the method is less than 0.60% and the % recovery of Ziprasidone HCl is between 99.8 – 100.9%. When the drug is subjected to different stress conditions and the resulting degradation products obtained were not interfere during the determination of Ziprasidone HCl.

KEYWORDS: Ziprasidone HCl, RP-UPLC, Method development, Validation, Stability-indicating.

INTRODUCTION

Ziprasidone hydrochloride is an antipsychotic drug that chemically differs from phenothiazine or butyro phenone antipsychotic agents. Ziprasidone Hcl is chemically Known as 5-[2-[4-(1,2-benzothiazol-3-yl)piperazin-1-yl]ethyl]-6-chloro-1,3-dihydro-2H-indol-2-onehydrochloride (Figure:1) and is used for the treatment of schizophrenia, mania and mixed states associated with bipolar disorder. Gennaro et.al[1], Brunton et.al[2] reported that the Ziprasidone Hcl act as a potent selective antagonist activity for the serotonin type 2 (5-HT2), dopamine type 2 (D2), 1 and 2 adrenergic and H1 histaminergic receptors. The methods reported in the literature revealing that determine Ziprasidone Hcl in bulk and its pharmaceutical dosage forms includes RP-HPLC P.Sudha Rani et.al(ODS C18 column used and sample monitored at awavelength 314 nm)[3], Kareti Srinivasa Rao et.al(Linchospher RP18,250×4.6×5µ, Ammonium Acetate used as a buffer and LOD& LOQ values are 0.46,1.0µg/ml)[4], Ravisankar et. al(Welchrom C18 250×4.6×5µ and LOD & LOQ values are 0.112, 0.339µg/ml respectively)[5], Srivastava Pratibha et.al(Hypersil C8 150×4.6×5µ, flow rate is 1.8ml/min and LOD, LOQ values are 0.60, 1.83µg/ml respectively)[6], Chudasama et.al(YMC C18 150×4.6×3µ column used and run time 5min, tailing of the peak is 1.65)[7], Yuri et.al (Method development techniques)[8], Palvovic et.al(Spherisorb ODS-1 250×4.6×5 µ column used and KH_2PO_4+acetonitrile are used in the ratio of 80:20)[9], Prasanthi et.al (Phenomonex C18 250×4.6×5µ column used and flow rate is1.5ml/min, LOD&LOQ values are 0.30, 0.90µg/ml)[10], Piyush Trivedi et.al(Zorbax SB-C8 50×4.6×3.5µ,LOD and LOQ values are not established)[11], Zhang.G et.al(Eclipse XDBC8 150×4.6×5µ and LOQ value is 2µg/ml).[12] The separation and detection of Ziprasidone HCl by using LCMS/MS Zhang.G et.al(Eclipse XDBC8 150×4.6×5µ and % recovery one of the analyte was 74.8%).[13] Reverse phase UHPLC Daniel oakowiecki et.al (BEH Phenyl 50×2.1×1.7µ and LOQ Value was 0.25µg/ml)[14], Mia Summers and Kenneth et.al (Acquity UPLC CSH C18 50×2.1×1.7µ and Linearity was found to be 0.993).[15] Based on the above HPLC literature review revealed that some of the methods have longer run time, high sample volume, flow rate, LOD and LOQ are higher side and 5 impurities are not separated in these methods. UPLC method was developed only for the related substances of Ziprasidone HCl. The research work carried out by using Waters Acquity CSH Phenyl hexyl (100mm x 2.1 mm, 1.7µm) dimensions column not yet used in any other methods. Based on these limitations our aim to develop a method and validation for ziprasidone hcl by using RP-UPLC with column new Waters Acquity CSH Phenyl hexyl (100mm x 2.1 mm, 1.7µm) dimensions. This work describes the validation parameters stated by the International Conference on Harmonization (ICH)[16-19] guidelines

which includes specificity, precision, linearity, accuracy, stability of analytical solution, robustness and system suitability. Product information given in table-1.

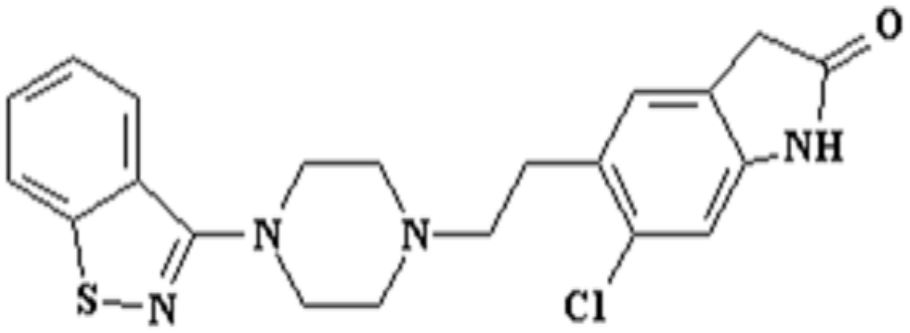


Fig: 1 Chemical structure of Ziprasidone HCl.

Table: 1

Product Name	**Ziprasidone HCl**
Chemical Name	5-[2-[4-(1,2-benzisothiazol-3-yl)piperazin-1-yl]- ethyl]-6-chloro-1, 3-dihydro-2h-indol-2-one hydrochloride
CAS Reg.No:	146939-27-7
Molecular Formula	$C_{21}H_{21}ClN_4OS.HCl.H_2O$
Molecular Weight	467.42

EXPERIMENTAL

Chemicals and reagents

Samples of Ziprasidone Hclwere contributed from Aurobindo pharma Ltd, Pydibhimavaram, India. HPLC grade Acetonitrile, AR grade KH_2PO_4 and Orthophosphoric Acid were purchased from Merck india Ltd, Mumbai. High pure water was prepared by using Millipore Milli-Q water purification system. New Waters Acquity CSH Phenyl hexyl (100 x 2.1 mm, 1.7 µm) column (Part no. # 186005407) and waters Acquity BEH(50mm×2.1mm,1.8µm)was procured from Waters India Ltd, India, and Bangalore.

Instrumentation (Apparatus)

Acquity-RP-UPLC system equipped with an LC pump (model ACQ-BSM) used for method development and validation its contain, an online degasser, auto sampler (model ACQ-SM) with thermostat, and detector (TUV) (model ACQ-TUV). The data was acquired, monitored and processed by using Empower3 software. Design expert version 9 (Stat-Ease Inc., Minneapolis, USA) was used for the optimizing chromatographic conditions. The buffers p^H was monitored by using Metrohm 780 p^H meter and weights taken by using the Sartorius CPA225D balance. In this research work we have attempted various chromatographic

columns for method development. After number of trails we have been selected Waters Acquity CSH Phenyl hexyl (100 x 2.1 mm, 1.7 µm) column based on its performance.

Chromatographic conditions

The chromatographic separations were performed by using New Waters Acquity CSH Phenyl hexyl (100 x 2.1 mm, 1.7 µm) (Part no. # 186005407). The p^H of the buffer KH2PO4 was adjusted to p^H 2.5 by addition of orthophosphoric Acid whereas the mobile phase contains buffer (p^H 2.5) and Acetonitrile in the ratio of (70:30). The flow rate of the mobile phase is 0.3 mL/min. The column temperature was maintained at 30°C and the absorption was measured at 209nm. The total Run time of the method was found to be 3.0min and the injection volume was 2µL. Mobile phase used as a diluent. Mobile phase Filter through 0.22µ finer porosity membrane filter. The chromatographic conditions were given in table-2.

Table: 2

Instrument	:	RP-UPLC make by Waters
Mode of analysis	:	Isocratic
Flow rate	:	0.3 mL/min
Detector wave length	:	209 nm
Column temperature	:	30^0C
Injection volume	: 2.0µL	
Column	:	WatersAcquityCSHPhenylhexyl (100x2.1mm, 1.7µ
Run time	:	3.0 min
Sample Manager Temp	: 25°C	

Inference

Results from the table-2 indicated that the eluting run time was completed within 3 minutes compared with other HPLC methods for assay determination in the literature. Moreover the injection volume of the sample required for RP-UPLC method is 2 µL is sufficient but in the case of HPLC methods it needs about 20 µL.

Table: 3

S. No.	NAME	RT
1	Ziprasidone HCl	~1.45

Preparation of standard and sample solutions

Preparation of standard solution

Weighed and transferred accurately 25 mg of Ziprasidone Hcl working standard into a 100 mL clean, dry volumetric flask added 50 mL of mobile phase and sonicate to dissolve. Make up to volume with mobile phase. Diluted 5 mL of this solution to 50 mL with mobile phase. Filter through 0.22µ finer porosity membrane filter.

Preparation of sample solution

Weighed and transferred accurately 25 mg of Ziprasidone Hclworking standard into a 100 mL clean, dry volumetric flask add 50 mL of mobile phase and sonicate to dissolve. Make up to volume with mobile phase. Diluted 5 mL of this solution to 50 mL with mobile phase. Filter through 0.22µ finer porosity membrane filter.

Calculation of Ziprasidone Hcl Assay

The API samples assay was calculated by the following equation

$$\frac{AT}{AS} \times \frac{WS}{100} \times \frac{5}{50} \times \frac{100}{WT} \times \frac{50}{5} \times P \times \frac{100}{(100 - W.C)}$$

Where AT is peak average area due to Ziprasidone Hcl in the sample preparation, AS is Average peak area due to Ziprasidone Hclin the standard. WS is the weight of Ziprasidone Hclstandard taken in mg, WT is the Weight of the sample taken in mg, P is the Potency of the Ziprasidone Hclworking/reference standard. W.C is the water content of the Ziprasidone HCl.

RESULTS AND DISCUSSION

Method development and Optimization of RP-UPLC conditions

The RP-UPLC conditions were optimized by using trials with different columns, several mobile phase compositions; flow rate and p^H were studied. The Analytical method development and validation play an important role in the determination of Assay in pharmaceutical products. In this method less quantity of solvents are used and total consumption of solvents is not more than 0.9ml per run time. One of the principles of green chemistry is prevention of waste. Further advantages of RP-UPLC method is able to increase the speed, sensitivity and resolution compared to HPLC methods. By considering above

aspects, RP-UPLC instrument is a most suitable technique for the assay determination of Ziprasidone HCl.

Selection of stationary phase

Based on the structure, molecular weight of API and impurities present in the products C18 columns like Waters BEH C18 column were initially screened for the separation. But these columns failed to provide acceptable separation and peak shape. For also several other C18 columns with other stationary phases are screened for separation but a remarkable selectivity was achieved with New Waters Acquity CSH Phenyl hexyl (100mm x 2.1 mm, 1.7 µm) partial size was finalised. This column was not yet used in any other methods.

Waters Acquity BEH C18 column

The trifunctionally bonded BEH Column particle gives a widest usable p^H range i.e's 1-12range, superior low p^H stability and ultra low column bleed for high sensitivity applications.

Waters Acquity CSH Phenyl hexyl column:

This column are used to provide an alternative selectivity and are a valuble tool for method development. The trifunctionally bonded C_6 phenyl ligand is a robust and low bleed sorbents that selectively retains polyaromatic compounds through Π-Π interactions.

Table: 4 Stationary phase Information.

Brand	**Acquity UPLC**	**Particle size(dp)**	**1.7 µm**
%Carbon Load	14	Particle Substrate	Hybrid
Bonding Technology	Phenyl-Hexyl	Pore size	130Å
Chemistry	Phenyl	Silanol Activity	Low
Endcapped	yes	Surface Area	185
ID	2.1 mm	Technology	CSH
Length	100mm	USP Classification	L11
Particle Shape	Spherical	Units in package	1/pkg
Mode	Reversed-phase	p^Hrange	1-11

Selection of Mobile phase

As one of the objectives of the method is to develop a different buffer, i.e's ortho phosphoric acid+water, potassium dihydrogen orthophosphate water buffers were evaluated. It is observed that in potassium dihydrogen ortho phosphate buffer is a promising candidate for efficient separation of impurities. The organic modifiers, methanol and acetonitrile were

uased at different composition conditions. Based on the results, acetonitrile was finalised as a organic modifier.

Selection of Diluent

Ziprasidone Hcl was practically insoluble in isopropyl alcohol, tetrahydrofuran, water, methanol and methylene chloride. But It is soluble in acetonitrile and stable for at least 24 hrs at 25°C. Hence, acetonitrile was selected as a diluent.

Experimental design for optimising flow rate, buffer concentration and column temperature

In initial method development trials with one factor at a time (OFAT) variation revealed that the flow rate and column temperature and composition of organic modifier had significant impact on selectivity. Since optimising the chromatographic parameters with OFAT approach consumes lot of time and does not provide the design space, a design of experiments (DoE) was used for optimising these chromatographic parameters. The design space defines the experimental region in which changes to method parameters will not significantly affect the quality and results. As working within the design space is not considered as a change, the scientist can have freedom to operate the method at different chromatographic condition.

Based on the analysis, it was understood that, to obtain good analyte peak shape, column temperature and flow rate are monitored at 30°C and 0.3 mL/min respectively.

Table: 5 Optimized chromatographic conditions.

Instrument	:	RP-UPLC make by Waters
Mode of analysis	:	Isocratic
Flow rate	:	0.3 mL/min
Detector wave length	:	209 nm
Column temperature	:	30^0C
Injection volume	: 2.0µL	
Column	:	WatersAcquityCSHPhenylhexyl (100x2.1mm, 1.7µm)
Run time	:	3.0 min
Sample Manager Temp	: 25°C	

METHOD VALIDATION

The developed analytical method was subjected to validation with respect to various parameters such as linearity, limit of quantification (LOQ), limit of detection (LOD),

accuracy, precision, recovery studies, specificity and reproducibility as per the ICH guidelines.

SYSTEM SUITABILITY

In the optimized RP-UPLC conditions, system suitability parameters were evaluated for Ziprasidone Hcl(Fig. 2). Tailing factor for Ziprasidone Hclwas not more than 2.0. The USP plate count for Ziprasidone Hclis not less than 2000. % RSD of the five injections was not more than 1.0%. The results are summarised in Table: 6

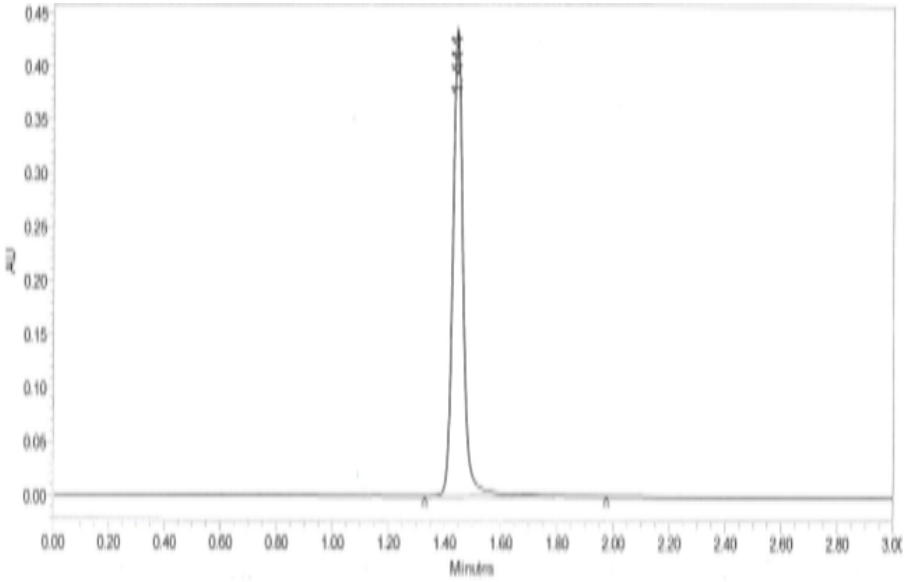


Fig: 2 System suitability chromatogram.

Table: 6

Parameters	Ziprasidone HCl	Limit
Tailing factor	1.25	NMT 2.0
USP Plate count	8288	NLT 2000
Retention time (min)	1.44	~1.45

SPECIFICITY

The specificity of the proposed method was demonstrated by interference study. It was found that presence of some common exicipients did not interferences at the retention time of Ziprasidone HCl. Thus the developed method can be successfully applied for determination of Ziprasidone Hcl in bulk drug form.

Blank and Impurity interference

The blank, sample enriched with impurities were prepared and injected in RP-UPLC. No interference was observed at any of the peaks of interested blank.

Blank chromatogram

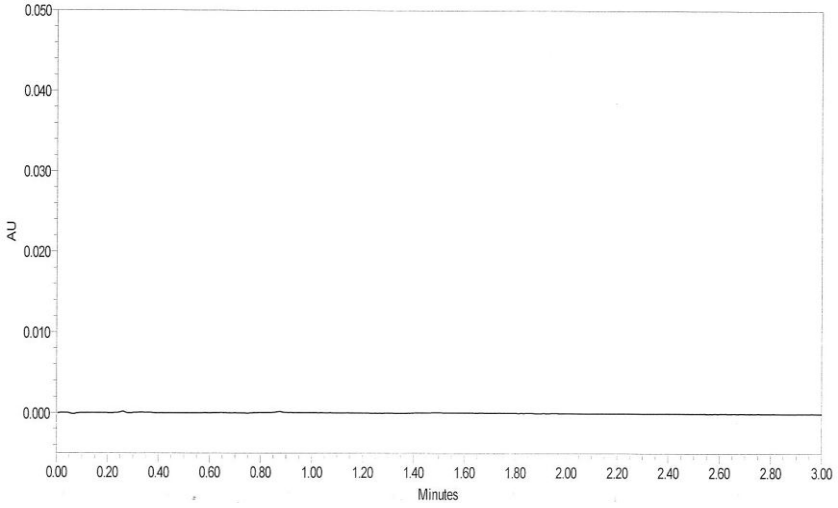


Fig: 3

Ziprasidone Hcl impurities (A,B,C)

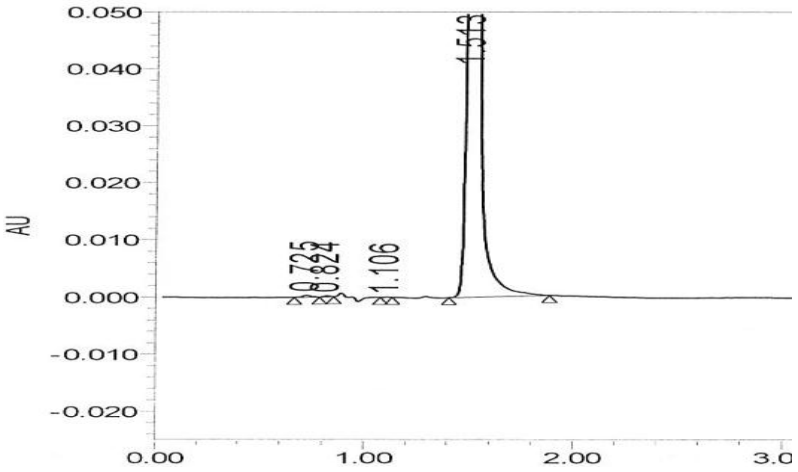


Fig:4

Ziprasidone Hcl impurities (D,E)

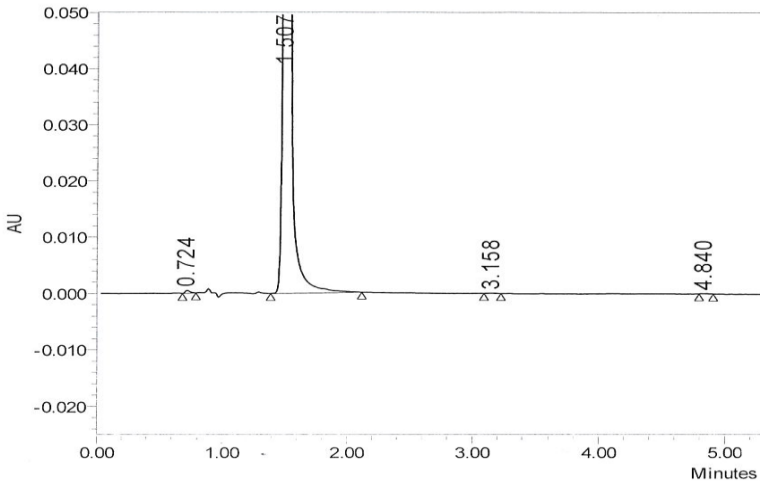


Fig: 5

Forced degradation/ Stress study

The stress studies for Ziprasidone Hcl were performed at concentration 25.0µg/mL to provide an indication of the stability indicating property and specificity of the proposed method. The stress studies were performed on API samples to provide an indication and identification of the generated degradents of the drug substance. Intentional degradation was attempted with stress conditions of acid (5N HCl for 60min), base (5N NaOH for 60min), oxidation (30% H_2O_2 for 60min), Photolytic (exposure to sunlight at window shade 48hours i.e. equal to watt hours/square meter and 1.2 million lux hours) and thermal (80 °C for 24hours) to evaluate the ability of the proposed method to separate the impurities of Ziprasidone Hclfrom its degradation products shown in Figure: 6 to 11. The results and system suitability parameter are summarised in Table: 7.

Undegraded Sample chromatogram.

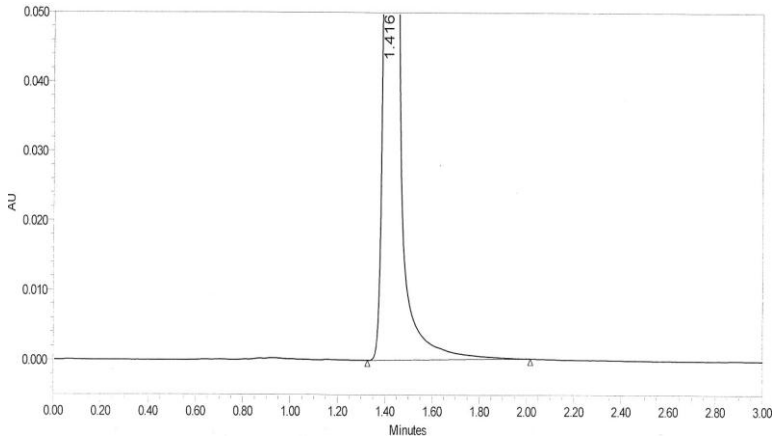


Fig: 6.

Sample chromatogram of peroxide degradation

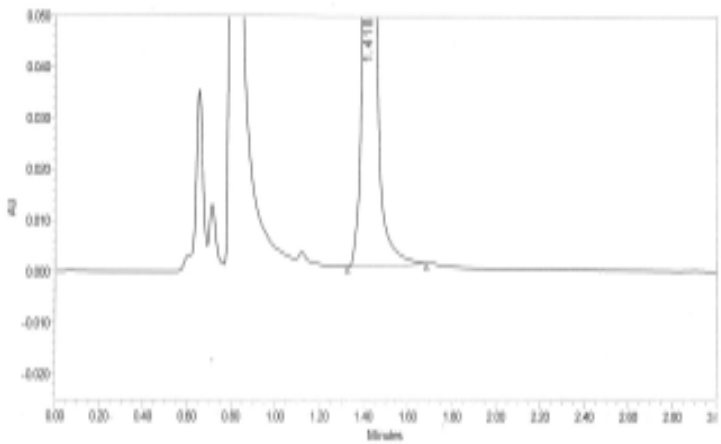


Fig: 7.

Sample chromatogram of Thermal degradation

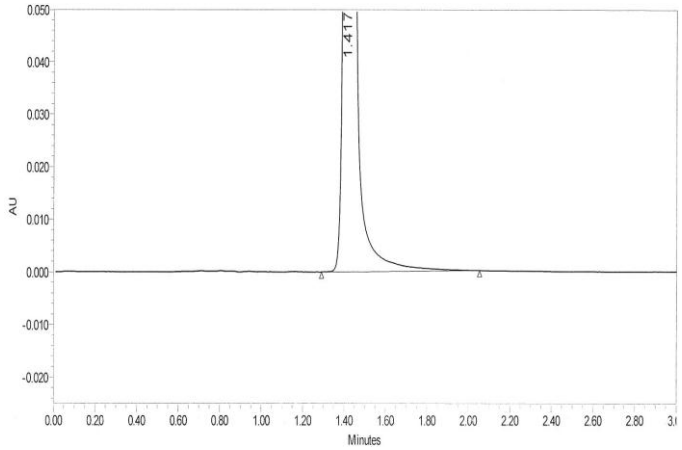


Fig: 8.

Sample chromatogram of Photo degradation

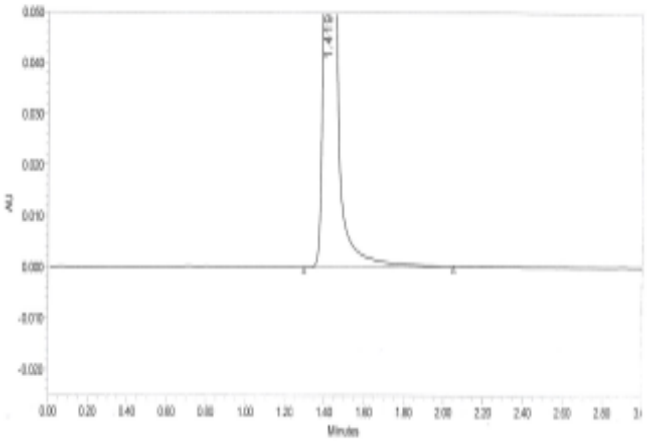


Fig: 9.

Sample chromatogram of Acid degradation

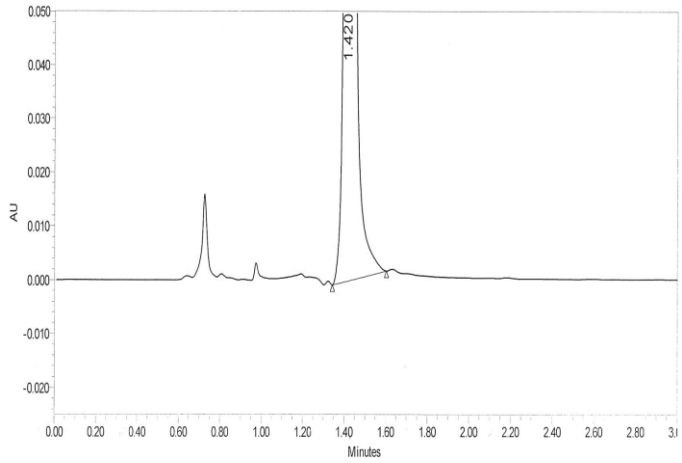


Fig: 10

Sample chromatogram of Base degradation

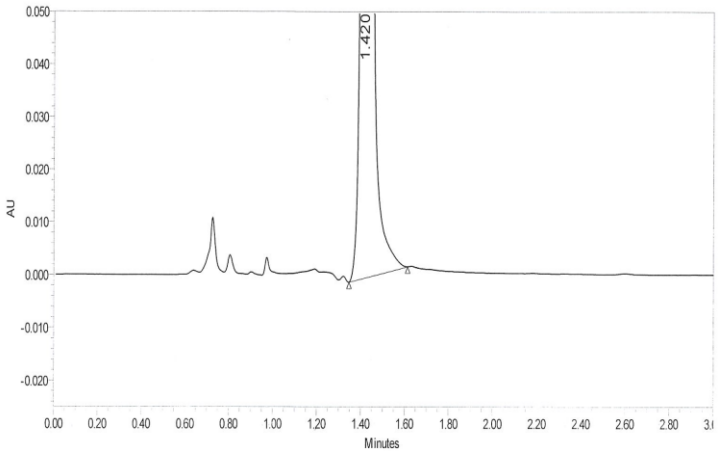


Fig: 11

Table: 7

Parameters	Initial SST	Bracketing	Limit
Tailing factor	1.25	1.25	NMT 2.0
USP Plate count	8577	8670	NLT 2000
Retention time min	1.47	1.47	~1.45

Degradation study				
S.No	Condition	Time	% Degradation	
			% Assay	%Degradation
1	Undegraded sample	Fresh	100%	NA
2	5N HCL added sample	60min	94.5%	5.5%
3	5N NaOH added sample	60min	99.0%	1.0%
4	30% H2O2 added sample	60min	86.9%	13.1%
5	Thermal 80°C heated sample	24 hrs	100.1	Nil
6	Photo Degradation sample	48 hrs	100.0	Nil

Inference

Based on the above results from the table it is revealed that in the oxidation degradation process high degradation products are obtained than that of the undegarded sample.

LINEARITY

Linearity for Ziprasidone Hcl Assay

The calibration curve was plotted over the concentration range of 0.03 to 37.4 µg/ml of Ziprasidone HCl. The calibration curves were prepared by plotting the peak area versus the concentration and analyzed through linear regression (Figure 12). The linearity was observed in the expected concentration range, demonstrating its suitability for analysis.The system suitability and results are summarised in Table: 8.

Table: 8 Linearity (Correlation coefficient).

Parameters	**Initial SST**	**Bracketing**	**Limit**
Tailing factor	1.21	1.21	NMT 2.0
USP Plate count	8477	8395	NLT 2000
Retention time min	1.45	1.45	~1.45
Precision			
Injection-01	1116200		%RSD NMT 1.0%
Injection-02	1122103		
Injection-03	1129794		
Injection-04	1122896		
Injection-05	1119248		
Mean	1122048		
SD	5068		
RSD	0.45		

S.No	**Concentration(µg/ml)**	**Response(Area)**
1	37.5	1671359
2	30.0	1332716
3	25.0	1120531
4	20.0	886311
5	12.5	552550
6	7.5	322053
7	2.5	108422
8	1.25	44158
9	0.5	17789
10	0.125	3647
11	0.03	1162
12	0.01	367
Average	11.40708333	505088.75
Standard Deviation	13.45149393	600938.67
%RSD	117.9223	118.9768
Slope	44680.7306152	
Intercept	-4699.7693482	
Correlation	0.9999679	
Ziprasidone Hcl Linearity Calculation sheet		

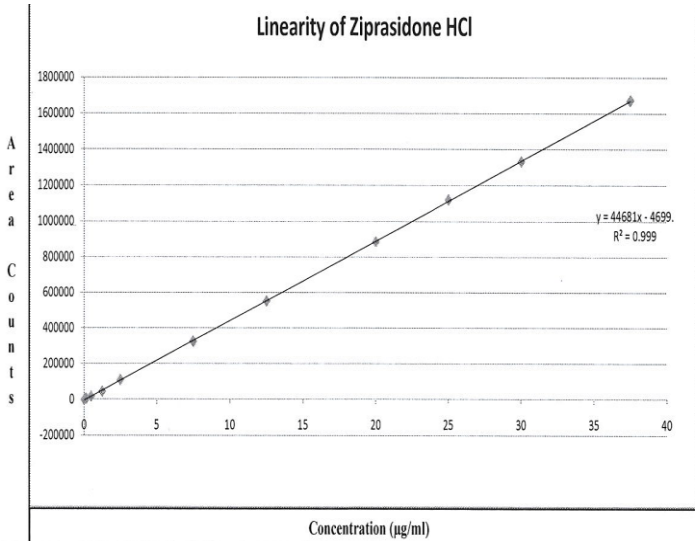


Fig: 12.

Inference

Based on correlation coefficient values from table-6 were within the Acceptance criteria.

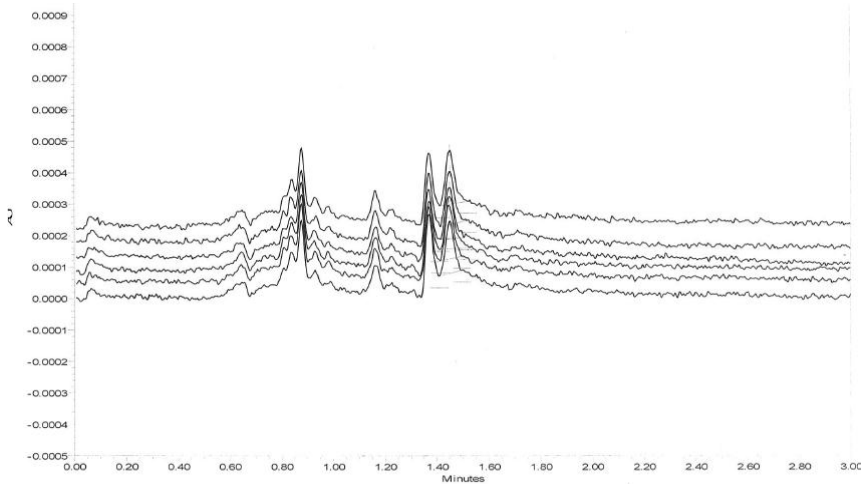
LIMIT OF DETECTION AND LIMIT QUANTIFICATION

The limit of detection (LOD) and limit of quantitation (LOQ) were established for Ziprasidone Hclby diluting the standard stock solution. The concentration at 0.01µg/ml,0.030µg/ml LOD and LOQ for Ziprasidone HCl. The signal to noise ratios was found to more than 4 and 15 respectively for the analyte. Hence these concentrations were finalised as LOD and LOQ concentrations. Further precision was found to be 9.02% at LOD level for analyte and the results are summarised in Table: 9. Overlay chromatograms of LOD & LOQ are represent below.

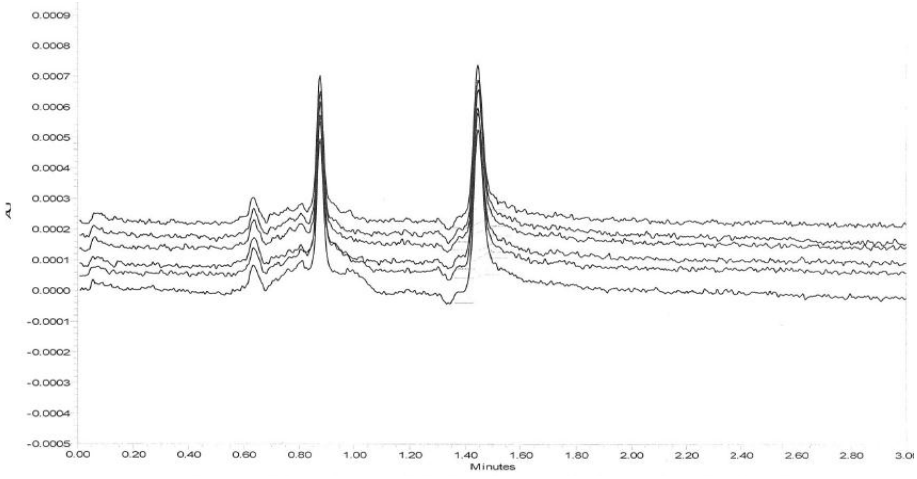
Table: 9 LOD and LOQ Precision.

Parameters	**Initial SST**	**Bracketing**	**Limit**
Tailing factor	1.22	1.21	NMT 2.0
USP Plate count	8449	8459	NLT 2000
Retention time min	1.45	1.45	~1.45
Precision			
Injection-01	1126048		%RSD NMT 1.0%
Injection-02	1124482		
Injection-03	1121624		
Injection-04	1119556		
Injection-05	1119002		
Mean	1122142		
SD	3064		
RSD	0.27		

Ziprasidone HCl	LOQ (%RSD NMT 10.0%)	LOD (%RSD NMT 33.0%)
Injection-1	1121	422
Injection-2	1219	422
Injection-3	1215	333
Injection-4	1238	422
Injection-5	1194	394
Injection-6	1201	377
Average	1198	395
Standard Deviation	40.70	35.65
%RSD	3.39	9.02



Fig; 13 Overlay Chromatogram of LOD.



Fig; 14 Overlay Chromatogram of LOQ.

ACCURACY

Accuracy for Assay of Ziprasidone HCl

The accuracy of the method was determined by calculating recoveries of Ziprasidone Hclby the standard addition method. Known amounts of standard solutions of Ziprasidone Hcl(80, 100, and 120 % level) were added to previously analyzed sample solutions of bulk drug form.

The percentage of recoveries was calculated. The percentages of recoveries were between 98.0 to 102.0. The results are summarised in the following Table: 10.

Table: 10 Accuracy and SST parameters.

Parameters	**Initial SST**	**Bracketing**	**Limit**
Tailing factor	1.25	1.25	NMT 2.0
USP Plate count	8705	8729	NLT 2000
Retention time min	1.48	1.48	~1.45
Precision			
Injection-01	1134282		RSD (NMT 1.0%)
Injection-02	1129642		
Injection-03	1128688		
Injection-04	1133649		
Injection-05	1135023		
Mean	1132257		
SD	2884		
%RSD	0.25		

% of Drug Added	**Spiked Conc. (W/W)**	**Recovered Conc. (W/W)**	**% Recovery (Criteria 98.0 to 102.0)**
Ziprasidone Hcl at 80%	81.9	81.7	99.8
Ziprasidone Hcl at 80%	81.5	81.8	100.4
Ziprasidone Hcl at 80%	81.5	81.9	100.5
Ziprasidone Hcl at 100%	102.8	103.5	100.7
Ziprasidone Hcl at 100%	102.9	103.8	100.9
Ziprasidone Hcl at 100%	103.0	103.6	100.6
Ziprasidone Hcl at 120%	123.4	123.4	100.0
Ziprasidone Hcl at 120%	123.0	123.5	100.3
Ziprasidone Hcl at 120%	123.0	123.4	100.3

Inference

Results from the table-8, it is illustrated that recovered concentration of spiked samples was found to be within the acceptance criteria i.e. 98.0 to 102.0.

PRECISION

The precision of the method was demonstrated by system precision and method precision.

System precision

System precision for assay was demonstrated by injecting standard solution under the same operating conditions. The peak areas of Ziprasidone Hcl were measured and the % RSD was found to be 0.19%. The results are summarised in Table: 11.

Table: 11.

Parameters	Initial SST	Bracketing	Limit
Tailing factor	1.25	1.25	NMT 2.0
USP Plate count	8577	8670	NLT 2000
Retention time min	1.47	1.47	~1.45
System precision			
S.No	**Area**		**Criteria % RSD**
Injection-1	1134776		**(NMT1.0%)**
Injection-2	1132244		
Injection-3	1132010		
Injection-4	1130212		
Injection-5	1129286		
Mean	1131695		
SD	2097		
%RSD	0.19		

Inference

Results from the table-9, it is illustrated %RSD of standard was found to be within the acceptance criteria i.e. NMT 1.0%.

Method precision

Method precision for analyte was demonstrated by preparing six samples at spec level. These solutions were injected along with a standard solution of Ziprasidone Hcl prepared at spec level. The relative standard deviation of analyte content obtained from all six preparations results was found to be 0.36%. The results are summarised in Table: 12.

Table: 12

Method precision			
Ziprasidone HCl	**Assay result**	**% RSD**	**Criteria % RSD**
Sample-01	100.9	0.36%	NMT 1.0%
Sample-02	99.9		
Sample-03	100.1		
Sample-04	100.2		
Sample-05	100.1		
Sample-06	100.5		

Inference

Results from the table-10, it is illustrated that %RSD of results precision was found to be within the acceptance criteria i.e. NMT 1.0%.

Intermediate precision (ruggedness)

Intermediate precision for analyte was demonstrated by preparing six different samples at spec level by different analyst and different day. These solutions were injected along with a standard solution of Ziprasidone Hclprepared at spec level. The relative standard deviation of analyte content obtained from all six preparations results was found to be 0.41%. The sst parameters and results are summarised in Table: 13.

Table: 13

Parameters	**Initial SST**	**Bracketing**	**Limit**
Tailing factor	1.25	1.25	NMT 2.0
USP Plate count	8288	8255	NLT 2000
Retention time min	1.42	1.42	~1.45
Precision			
Injection-01	1127311		RSD (NMT 1.0%)
Injection-02	1127751		
Injection-03	1127741		
Injection-04	1123239		
Injection-05	1126205		
Mean	1126449		
SD	1902		
%RSD	0.17		
Intermediate precision			
Ziprasidone HCl	**Assay result**	**% RSD**	**Criteria % RSD**
Sample-01	98.9	0.41%	NMT 1.0%
Sample-02	99.4		
Sample-03	98.9		
Sample-04	99.5		
Sample-05	99.9		
Sample-06	99.7		
Cumulative RSD for precision			
Analyst-01	**Analyst-02**	**Cumulative (12 Results)**	**Criteria % RSD**
0.36%	0.41%	0.59%	NMT 1.0

Inference

Sample Results from the table-10, 11 it is observed that cumulative RSD's were found to be within the limit i.e. NMT1.0%.

FILTER COMPATIBILITY

Filter compatibility to the sample is concluded from the recovery study indicated that there is no absorption of these component to filter.

SOLUTION STABILITY

The standard and samples solutions were kept on room temperature at 25°c and injected the aged samples (Every one hour) into the RP-UPLC. The peak areas corresponding to Ziprasidone Hcl were measured. Calculated the similarity factor and found that the values are below 1.0% RSD. Thus indicates the sample and standard solutions are stable for at least 24 hrs when stored on room temperature condition. Sst parameters are shown below table-14.

Table 14

Parameters	Initial SST	Bracketing	Limit
Tailing factor	1.27	1.22	NMT 2.0
USP Plate count	8611	8657	NLT 2000
Retention time min	1.47	1.45	~1.45

METHOD ROBUSTNESS

Method robustness was performed by applying small changes in the ratio of mobile phase, injection volume, and column temperature, p^H and flow rate. The results of change in ratio of mobile phase, column temperature, wavelength, and injection volume are shown in Table 13. The flow rate 0.33– 0.27 mL/min, buffer pH 2.31 to 2.72,wavelength 206 to 212nm and Column temperature 25°C to 35°C and Study of the mobile phase composition 75:25, 65:35. Based on this, the method is proved to be robust and can easily be implemented in quality control laboratories for the regular analysis of Ziprasidone Hcl samples with great confidence.

Table 15

Method Robustness				
Chromatographic Parameter	**Condition**	**Retention time(RT) ~1.45**	**Theoretical plates(N) NLT 2000**	**Tailing(T) NMT 2.0**
Wavelength(nm)	212	1.48	8630	1.27
	206	1.49	8721	1.27
Temperature(°C)	25°C	1.49	8501	1.28
	35°C	1.48	8782	1.27
Acetonitrile (%)	75:25×	2.76	11231	1.18
	65:35	1.02	7670	1.29
Flow rate(ml/min)	0.33	1.35	8266	1.26
	0.27	1.64	9073	1.26
pH variation	2.31	1.52	8938	1.27
	2.72	1.53	8910	1.27

Inference

Solvent composition is more critical based on above data. Measure mobile phase composition accurately**.**

DISCUSSION

The purpose of the present work was to develop a short, robust, RP-UPLC method for the accurate quantitation of Ziprasidone Hclmentioned in the European pharmacopeia and United States pharmacopeia. This method is developed such that it can work for both drug substance and drug product. As mentioned in the introduction section, several reports are available for quantification of Ziprasidone HCl. The European pharmacopeia method, the only chromatography method reported Assay method utilises the spherical octayl silyl silica 150x4.6mm and 5µwith an Isocratic mode of elution and 20µL injection volume. The elution time is approximately 15 min. The United States pharmacopeia method, the only chromatography method reported Assay method utilises the spherical octayl silyl silica 150x4.6mm and 5µ with an Isocratic flow mode 1.5ml/min and 20µL injection volume. The elution time is approximately 15 min.

The developed method was successfully validated for both drug substance and drug product as per the ICH guideline. The proposed method is much superior to reported methods in terms of solvent consumption, run time, instrumental technique (RP-UPLC), selectivity, and applicability to Assay analysis, applicability to drug substance and drug product.

APPLICATION TO PHARMACEUTICAL INDUSTRY:

This work will help industry to develop, manufacture and launch the product in a fast and economical way which in turn reduces the cost of the medicine and help the patient to avail quality, innovative and affordable medicine.

CONCLUSION

A stability indicating RP-UPLC Assay method has been developed for determination of Ziprasidone Hcl. Developed method is proved to be robust using the experimental design, this method can be successfully implemented in the quality control lab for the routine analysis of this product. Further this RP-UPLC method was successfully validated as per ICHQ2 (R1) guideline and proved to be precise, linear, sensitive, accurate, and robust. This method is short and simple, hence implementation of this method in quality control and analytical development labs can yield high throughput. As low amounts of solvents are

required, implementation of this method will be eco friendly. This is the first RP-UPLC Assay method that can accurately quantitative the Ziprasidone HclAssay.

ACKNOWLEDGEMENTS

The authors are heartily thankful to acknowledge Aurobindo Pharma Ltd and Andhra University for providing all the facilities to carry out the research work.

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