Original Article

Development and validation of stability indicating UPLC assay method for ziprasidone active pharma ingredient

Abstract

Background: Ziprasidone, a novel antipsychotic, exhibits a potent highly selective antagonistic activity on D2 and 5HT2A receptors. Literature survey for ziprasidone revealed several analytical methods based on different techniques but no UPLC method has been reported so far. **Aim:** Aim of this research paper is to present a simple and rapid stability indicating isocratic, ultra performance liquid chromatographic (UPLC) method which was developed and validated for the determination of ziprasidone active pharmaceutical ingredient. Forced degradation studies of ziprasidone were studied under acid, base, oxidative hydrolysis, thermal stress and photo stress conditions. **Materials and Methods:** The quantitative determination of ziprasidone drug was performed on a Supelco analytical column (100×2.1 mm i.d., 2.7 µm) with 10 mM ammonium acetate buffer (pH: 6.7) and acetonitrile (ACN) as mobile phase with the ratio (55:45-Buffer:ACN) at a flow rate of 0.35 ml/ min. For UPLC method, UV detection was made at 318 nm and the run time was 3 min. Developed UPLC method was validated as per ICH guidelines. **Results and Conclusion:** Mild degradation of the drug substance was observed during oxidative hydrolysis and considerable degradation observed during basic hydrolysis. During method validation, parameters such as precision, linearity, ruggedness, stability, robustness, and specificity were evaluated, which remained within acceptable limits. Developed UPLC method was successfully applied for evaluating assay of Ziprasidone active Pharma ingredient.

Key words

Antipsychotic, stability indicating assay, ultra performance liquid chromatographic, validation, ziprasidone

Introduction

Ziprasidone (5-[2-[4-(1, 2-benzisothiazol-3-yl)-1-piperazinyl] ethyl]-6-chloro-1, 3-dihydro-2 H-indol-2-one) [Figure 1], a novel antipsychotic, exhibits a potent highly selective antagonistic activity on D_2 and $5HT_{2A}$ receptors. It has a high affinity for $5HT_{1a}$, $5HT_{1d}$, $5HT_{2c}$ receptor subtypes that could contribute to its overall therapeutic effect.[1,2] Ziprasidone is most effective in schizophrenia, a chronic illness that requires lifelong treatment spread over approximately 1% of the world's population.[3,4]

Literature survey for ziprasidone revealed several analytical methods based on different techniques, viz, LC-MS[5-7] assay for their quantification in plasma and brain, high performance liquid chromatography (HPLC)[8-10] method for simultaneous determination of ziprasidone in capsule formulation, and HPLC-UV[11] methods for determination ziprasidone in human plasma and urine, LC[12] with fluorescence for determination of plasma ziprasidone, and capillary zone electrophoresis[13] for determination of ziprasidone in pharmaceutical formulations.

Ultra performance liquid chromatography (UPLC) is a recent technique in liquid chromatography, which enables significant reduction in separation time and solvent consumption. Literature reports reveals that UPLC system allows about ninefold decrease in analysis time when compared with conventional HPLC system using 5 µm particle size analytical

Access this article online	
Website: http://www.cysonline.org	Quick Response Code
DOI: 10.4103/2229-5186.103097	

Sonam Mittal, Abhishek Gupta[1], Balasubramanian Narasimhan[2], Kona S. Srinivas[1], Ravi Shankar Gupta[1], Vinod Prasad Semwal[1]

Department of Pharmaceutical Chemistry, Vaish Institute of Pharmaceutical Education and Research (VIPER), Rohtak, [1]Department of Analytical Chemistry, Daiichi Sankyo India Pharma Pvt. Ltd., Gurgaon, [2]Department of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak, Haryana, India

Address for correspondence:
Mr. Abhishek Gupta,
Department of Analytical Chemistry, Daiichi Sankyo Research Centre in India, Plot-20, Sector-18, Gurgaon, Haryana, India.
E-mail: abbiotechnmr@gmail.com

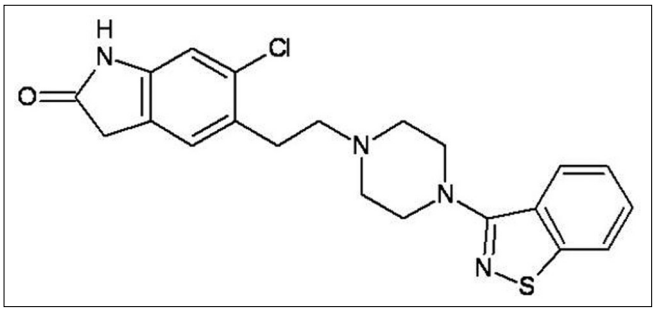

Figure 1: Chemical structure of ziprasidone

columns and about threefold decrease in analysis time in comparison to 3 μm particle size analytical column without compromise on overall separation.[14,15]

Stability testing forms an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how the quality of drug substance or drug product varies with time under the influence of variety of environmental factors such as temperature, humidity, and light and enables recommendation of storage conditions, retest periods, and shelf life to be established. The present investigation was undertaken to establish the stability indicating UPLC assay method for the estimation of ziprasidone as recommended by the International Conference on Harmonization (ICH) guidelines[16] and USP.[17]

Materials and Methods

Experimental

Chemicals and reagents

Reference standard of ziprasidone hydrochloride (Purity 99.5% Expiry June 12) was gifted by Ranbaxy Research Laboratories, Gurgaon. Acetonitrice of HPLC grade was taken form Spectrochem Labs, Mumbai, India, and ammonium acetate and hydrogen peroxide of analytical reagent grade were obtained from Qualigens, Mumbai, India. Potassium dihydrogen phosphate, triethylamine, and sodium hydroxide of laboratory reagent grade were obtained from SD fine Chemicals Ltd., Mumbai, India.

Buffer preparation

Solution of ammonium acetate (0.01 M) was prepared by dissolving about 0.77 g of ammonium acetate in 1 l of water for HPLC. The pH of this solution was adjusted to 6.7 with acetic acid. The buffer preparation was found stable with respect to pH and visual clarity for 48 h.

Chromatographic system

Analyses were performed on Acquity UPLC™ system (Waters, Milford, MA, USA), consisting of binary solvent manager, sample manager, and PDA detector. The detector was set at sampling rate of 20 points/s and filter time constant of 0.2 s. System control, data collection, and data processing were accomplished using Waters Empower™ chromatography data software. The analytical column was 100 × 2.1 mm UPLC Supelco, 2.7 μm (Merck, USA). The separation of ziprasidone was achieved by isocratic elution using acetonitrile (ACN) and acetate buffer (pH 6.7; 0.01 M). The optimized conditions were as follows: isocratic elution (55:45-Buffer:ACN) with an injection volume of 5 μl and flow rate of 0.35 ml/min at 40° C and detection wavelength of 318 nm (absorbance maxima/lambda max being 318) with back pressure of 6500 psi. The run time finalized was of 3 min.

Preparation of standard solution

Standard solution was prepared by dissolving 80 mg of standard substance in water: ACN (60: 40 v/v) mixture to obtain solution containing 80 μg/ml of ziprasidone.

Sample preparation

Sample solution (80 μg/ml) was prepared by weighing 80.0 mg of drug into 100 ml volumetric flask. Drug was dissolved into small volume of diluent, water: ACN (60: 40 v/v) and sonicated for 1 min. Then volume was made up to 100 ml with diluent. From the each stock solution, 10 ml solution was transferred to 100 ml volumetric flask and volume was made up to 100 ml to get the final sample concentration of 80 μg/ml.

Method validation

System suitability

System suitability parameters were measured so as to verify the system performance. System precision was determined on six replicate injections of standard preparations.

Specificity

Forced degradation studies were performed to demonstrate selectivity and stability indicating capability of the proposed method. The samples of ziprasidone were exposed to acidic, alkaline, thermal oxidative, and photolytic degradation conditions. All the exposed standards and samples were than analyzed by proposed method.

Linearity

Linearity was demonstrated from 70% to 130% of standard concentration using minimum six calibration level (70%, 80%, 90%, 100%, 120%, and 130%) for the compound. The method of linear regression was used for data evaluation. Peak areas of sample compound were plotted against respective concentrations.

Precision

Precision was investigated using sample preparation procedure for six real samples and analyzed by proposed method. Intermediate precision was studied using different column, performing analysis on different day and also by different analyst.

Robustness

The robustness is a measure of method capacity to remain unaffected by small but deliberate changes in

chromatographic conditions such as change in pH of buffer (±0.2 units), column temperature (±5°C), flow rate (±10%) as well as ratio of mobile phase (±2 units).

Stability of sample preparation

Stability of sample solution was established by storage of sample solution at ambient temperature for 24 h followed by its assay, which was then compared against fresh sample.

Results and Discussion

Method development

For analysis of ziprasidone, different chromatographic conditions were tried on HPLC and UPLC and results obtained were compared. Isocratic elution is simple and requires only one pump and flat baseline separation for easy and reproducible results. The results from HPLC [Figure 2b] involve few main problems: longer run time, lack of good peak shape, and low response, while with UPLC all these problems are solved and thus isocratic run using UPLC provide proper peak with a good baseline in 3 min time [Figure 2a] [Table 1, a-c] was selected for the analysis of ziprasidone.

Among various columns available for UPLC analysis, Supelco C18, 2.7 µm, (100 × 2.1) mm i.d. column was preferred, because it provides appreciable peak shape and resolution and absorbance were good. Among different mobile phase used, the mobile phase consisted of ACN and 10 mM ammonium acetate with an apparent pH adjusted to 6.7±0.1 with ammonia and acetic acid was found to be suitable for analysis of ziprasidone. Further, a flow rate of 0.35 ml/min, an injection volume of 5 µl, and UV detection at 318 nm for drug were found to be suitable for analysis. [Figure 3] indicates the peak obtained for the sample by the selected method.

Analytical parameters and validation

After satisfactory development of method, it was subjected to method validation as per ICH guidelines. The method was validated to demonstrate that it is suitable for its intended purpose by the standard procedure to evaluate adequate validation characteristics (precision, linearity, robustness, stability indicating capability).

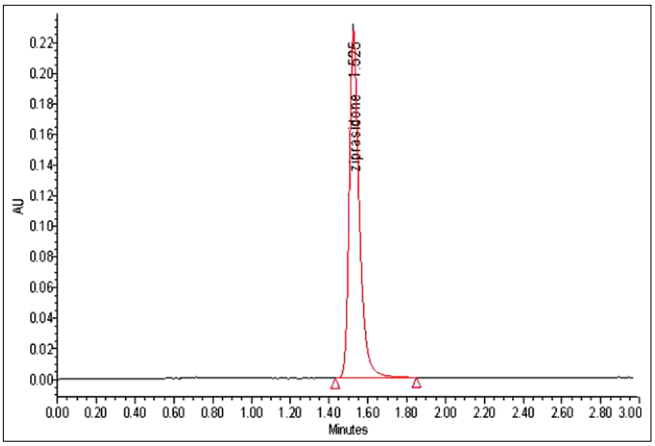

Figure 2a: UPLC chromatogram of ziprasidone

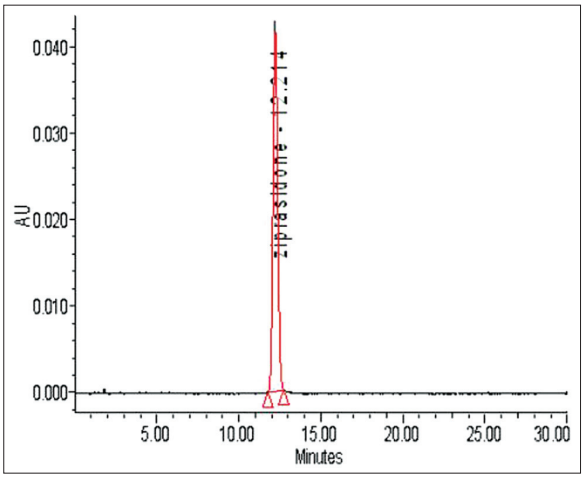

Figure 2b: HPLC chromatogram of ziprasidone

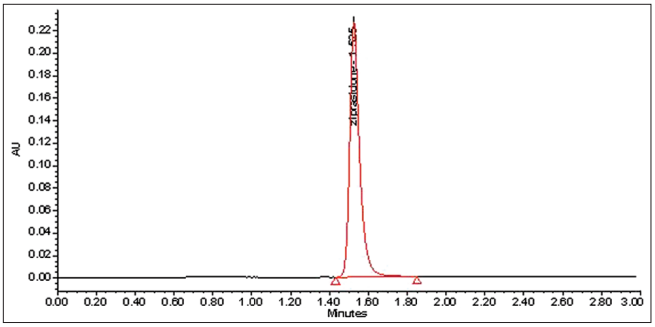

Figure 3: Typical chromatogram showing the main peak of ziprasidone

Table 1: Different chromatographic conditions applied and results obtained

S. no.	Column used	Mobile phase	Isocratic/gradient	Inj. vol.	Observation	Results
For UPLC						
a	Acquity BEH, Supelco1.2.7 µ (100×2.1) mm	Ammonium acetate (pH 6.67): ACN (70: 30)	Isocratic	5µl	Peak shape not good	Method rejected
b	Acquity BEH, Supelco, 2.7 µ (100×2.1) mm	Ammonium acetate (pH 6.67): ACN (60: 40)	Isocratic	5µl	Tailing observed	Method rejected
c	Acquity BEH, Supelco, 2.7 µ (100×2.1) mm	Ammonium acetate (pH 6.67): ACN (55: 45)	Isocratic	5µl	Peak shape good	Method accepted

System suitability

Results of other system suitability parameters such as theoretical plates, purity angle, and purity threshold are presented in [Table 2]. The data presented in [Table 2] indicated the acceptable system suitability parameters, as the % RSD is not more than 2%. Tailing factor was not more than 2 and theoretical plates are more than 1000 and purity angle was less than purity threshold.

Table 2: System suitability parameters and robustness

System suitability parameters	Robustness parameters	Ziprasidone
Column efficiency (theoretical plates)	No change (repeatability)	4635
	pH of buffer (+0.2 units)	3331
	pH of buffer (–0.2 units)	3207
	Column temperature (+5 units)	3422
	Column temperature (–5 units)	3359
	Flow (+10%)	3424
	Flow (–10%)	3513
	Organic content (+2%)	3150
	Organic content (–2%)	3907
Purity angle	No change (repeatability)	0.103
	pH of buffer (+0.2 units)	0.146
	pH of buffer (–0.2 units)	0.137
	Column temperature (+5 units)	0.175
	Column temperature (–5 units)	0.129
	Flow (+10%)	0.138
	Flow (–10%)	0.136
	Organic content (+2%)	0.151
	Organic content (–2%)	0.139
Purity threshold	No change (repeatability)	1.165
	pH of buffer (+0.2 units)	1.164
	pH of buffer (–0.2 units)	1.166
	Column temperature (+5 units)	1.151
	Column temperature (–5 units)	1.132
	Flow (+10%)	1.155
	Flow (–10%)	1.130
	Organic content (+2%)	1.139
	Organic content (–2%)	1.132

The percentage (%) RSD of area count of six replicate injections was below 2 which indicated the system precision.

Specificity

The results of forced degradation studies are given in [Table 3]. Ziprasidone was found sensitive to basic hydrolysis. The assay value was decreased to 73.52% and degradation peaks were observed in the chromatogram. Ziprasidone was found stable to acid hydrolysis yielding assay value of 101.80%. Chromatograms of base and acid degraded samples for ziprasidone are presented in [Figures 4 and 5]. Ziprasidone was sensitive to oxidative conditions and the assay value decreased to 75.66%. Chromatogram of oxidation degraded ziprasidone is shown in [Figure 6]. Ziprasidone was found to be stable under thermal and photo stress degradation studies. Peaks due to ziprasidone were investigated for spectral purity in the chromatogram of all exposed samples and standards and found to be spectrally pure.

Linearity

The response was found linear from 70% to 130% standard concentration. The correlation coefficient (R^2) was greater than 0.0.99 [Table 4 and Figure 7].

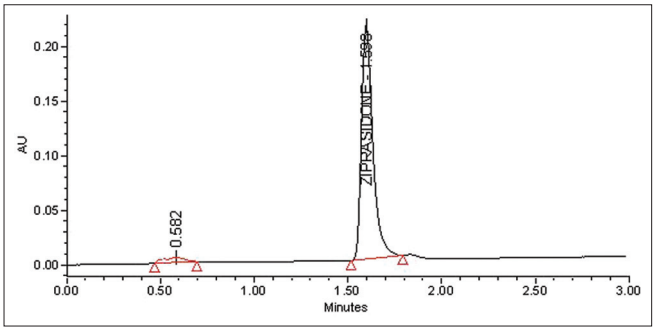

Figure 4: Chromatogram of base degraded sample of ziprasidone

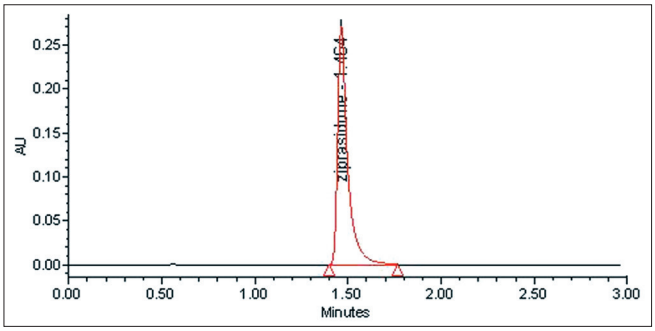

Figure 5: Chromatogram of acid degraded sample of ziprasidone

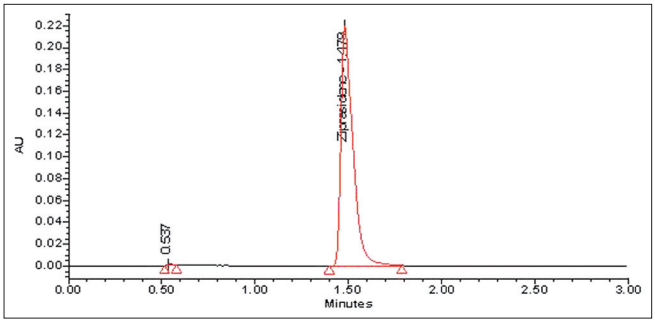

Figure 6: Chromatogram of peroxide degraded sample of ziprasidone

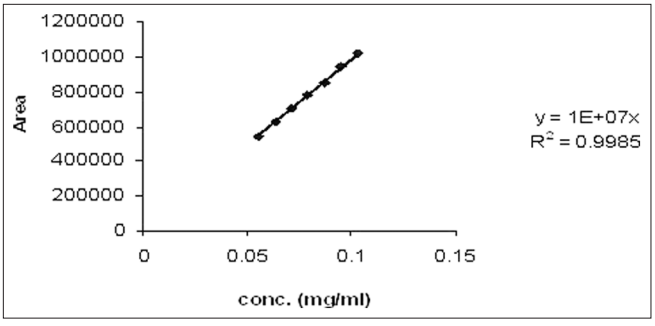

Figure 7: Linearity curve for ziprasidone

Table 3: Forced degradation data

Stress condition/solution/duration	% Assay
Alkaline degradation (0.5 ml, 10% NaOH)	94.06
Alkaline degradation (0.5 ml, 10% NaOH, 2 h)	73.52
Oxidative degradation (1 ml, 3% H2O2)	100.33
Oxidative degradation (1 ml, 3% H2O2, 60°C, 2 h)	75.66
Acidic degradation (1 ml, 10% HCl)	101.80
Acidic degradation (5 ml, 10% HCl)	101.75
Acidic degradation (1 ml, 0.05 N HCl, 0°C)	101.60
Thermal degradation (solid sample, 60°C, overnight)	100.11
Thermal degradation (solid sample, 100°C, 4 h)	100.84
Thermal degradation (solid sample, 100°C, overnight)	101.91
Photo degradation (solid sample, UV 350nm , 10 h)	100.27
Photo degradation (solid sample, 55000 lux 24 h)	100.76

Table 4: Method validation results for assay of individual compounds

Parameters	Ziprasidone
System precision (% RSD)[a]	0.46
Tailing factor	1.33
Intra-assay precision (assay)[b]	81.67
Intra-assay precision (% RSD)[c]	1.49
Inter-assay precision (assay)[b]	80.65
Inter-assay precision (% RSD)[c]	0.79
Intermediate precision—inter-analyst (% RSD)[c]	1.82
Linearity (correlation coefficient)[d]	0.999
Selectivity[e]	No interference
Stability 24 h (%)[f]	0.53

[a]Determined on six replicate injections. [b]Average of six determinations. [c]Determined on six values. [d]Six levels from 70% to 130% of standard concentration. [e]Demonstrated by forced degradation and separation of nondegradation products. [f]Correlation with freshly prepared sample

Precision

The intra-assay precision of ziprasidone was 81.67% with a % RSD of 1.49%. Inter-assay precision for ziprasidone was 80.65% with % RSD of 0.79% [Table 4]. Low values of RSD indicated that the method is precise.

Robustness

No significant effect was observed on system suitability parameters such as theoretical plates, purity angle, and purity threshold, when small but deliberate changes were made to chromatographic conditions such as change in flow rate (±10%), temperature (±5 units), pH (±0.2 units), and organic content (±2%). The results are presented in [Table 2], along with system suitability parameters of normal methodology. Thus, the method was found to be robust with respect to variability in above condition

Stability in sample solution

Sample solution did not show any appreciable change in assay value when stored at ambient temperature up to 24 h. Assay results are presented in [Table 4].

Conclusion

A novel UPLC method was successfully developed and validated for determination of ziprasidone. The total run time was 3 min, within which drug got eluted. Method validation results have proved the method to be selective, precise, accurate, robust and stability indicating. Sample solution stability was established for determination of assay and impurities. This method can be successfully applied for the routine analysis and stability study. Also, it can be used for determination of content uniformity and dissolution profiling of this product, where sample load is higher and high throughput is essential for faster delivery of results. Overall, the method provides high-throughput solution for determination of ziprasidone with excellent selectivity, precision, and accuracy.

References

1. Available from: http://en.wikipedia.org/wiki/ziprasidone [Last accessed on 2012 Jan 02].
2. Available from: www.health24.com/medical/condition-centres/77-792-815-1775,17124 [Last accessed on 2012 Jan 02].
3. Available from: www.nlm.nih.gov/medlineplus/druginfo/meds/a699062.html [Last accessed on 2012 Jan 02].
4. Available from: www.medilexicon.com/drugs/ziprasidone.php [Last accessed on 2012 Jan 02].
5. Al-Dirbashi OY, Aboul-Enein HY, Al- Odaib A. R. Rapid liquid chromatography-tandem mass spectrometry method for quantification of ziprasidone in human plasma., Biomed Chromatogr 2006;April 20(4):365-8.
6. Zhang G, Terry AV Jr, Bartlett MG. Determination of the lipophilic antipsychotic drug ziprasidone in rat plasma and brain tissue using liquid chromatography-tandem mass spectrometry. Biomed Chromatogr 2008;22:770-8.
7. Aravagiri M, Marder SR, Pollock B. Determination of ziprasidone in human plasma by liquid chromatography-electrospray tandem mass spectrometry and its application to plasma level determination in schizophrenia patients. J Chromatogr B Analyt Technol Biomed Life Sci 2007;847:237-44.
8. El-Sherif ZA, El-Zeany Badr, El-Houssini OM. Stability indicating reversed-phase high-performance liquid chromatographic and thin layer densitometric methods for the determination of ziprasidone in bulk powder and in pharmaceutical formulations., Biomed Chromatogr 2004 April;18(3):143-9.
9. Singh A, Rao BM, Deshphande GR, Sangaraju S. A Rapid Stability-Indicating LC Method for Ziprasidone Hydrochloride Chromatographia 2007;65:191-196.
10. Sudha Rani B, Reddy PV, Estimation of Ziprasidone Hydrochloride Monohydrate in Bulk and Capsules by Reverse Phase HPLC. E J Chem 2006;3:169-72.
11. Edgar PS, Lewis C, Kathryn J, Robert JF. Ther Drug Monit 2005;27:226.
12. Suckow RF, Fein M, Correll CU, Cooper TB. Determination of plasma ziprasidone using liquid chromatography with fluorescence detection. J Chromatogr B 2004;799:201-8.
13. Farina C, Kremser L, Raggi MA, Kenndler E. Determination of Ziprasidone in pharmaceutical formulations by capillary zone electrophoresis. J Pharmaceut Biomed Anal 2008;46:471-6.
14. Wren SA, Tchelitcheff P. Use of ultra-performance liquid chromatography in pharmaceutical development. J Chromatogr A 2006;1119:140-6.

15. Novakova L, Matysova L, Solich P. Advantages of application of UPLC in pharmaceutical analysis. Talanta 2006;68:908-18.
16. International Conference on Harmonization (ICH), Stability testing of new drug substances and procedures, Proceedings of the International Conference on Harmonization, Geneva, 1993.
17. The United States Pharmacopoeia, United States Pharmacopoeia convention, MD, 2003;26:1151-4.

How to cite this article: Mittal S, Gupta A, Narasimhan B, Srinivas KS, Gupta RS, Semwal VP. Development and validation of stability indicating UPLC assay method for ziprasidone active pharma ingredient. Chron Young Sci 2012; 3:286-91.

Source of Support: Nil, **Conflict of Interest:** None declared