RESEARCH ARTICLE

A Novel Approach for Estimation of Zotepine in Tablet Dosage Form

Mukthinuthalapati Mathrusri Annapurna*, Priyanka V Deulkar

GITAM School of Pharmacy, GITAM (Deemed to be University), Visakhapatnam, Andhra Pradesh, India.

Received: 02nd September, 2023; Revised: 24th January, 2024; Accepted: 06th February, 2024; Available Online: 25th March, 2024

ABSTRACT

An antipsychotic medicine zotepine used to treat schizophrenia and mania. A novel stability-indicating method with a reversed-phase ultra-fast liquid chromatography (RP-UFLC) system has been developed to estimate zotepine in dosage forms as tablets. A Shimadzu UFLC system with a Zorbox C18 column and PDA detector was employed for the chromatographic analysis. At 1.0 mL/min flow rate, acetonitrile: 10 mM tetra butyl ammonium hydrogen sulfate (42:58) as a mobile phase. UV wavelength of detection is 221 nm. Concentration range between 0.1 and 50 µg/mL, Beer-Lambert's law was followed. The linear regression equation was y = 171935x + 11975 (r2 = 0.9999). The limit of detection (LoD) was found to be were 0.0312 µg/mL and limit of quantitation (LoQ) 0.0975 µg/mL. Method validation and a forced degradation study were carried out per ICH guidelines. The proposed approach is straightforward, reliable, accurate, and exact. It can be used to estimate the amount of zotepine in tablets.

Keywords: Stability indicating, RP-UFLC, Validation, Zotepine.

International Journal of Pharmaceutical Quality Assurance (2024); DOI: 10.25258/ijpqa.15.1.06

How to cite this article: Annapurna MM, Deulkar PV. A Novel Approach for Estimation of Zotepine in Tablet Dosage Form. International Journal of Pharmaceutical Quality Assurance. 2024;15(1):39-43.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

An antipsychotic medicine, zotepine used to treat schizophrenia and mania., its structure (Figure 1).[1] Chemical formula ($C_{18}H_{18}ClNOS$) and 331.9 g/mol molecular weight. It is a dibenzothiophene and a tertiary amino derivative. Chemically it is 2-chloro-11-(2-dimethylaminoethoxy) dibenzo[b,f]thiepine.

It acts by antagonistically binding to dopamine and serotonin receptors. Zotepine has a strong affinity for the D1 and D2 receptors also shows an effect on specific histamine receptors1. Literature reveals various techniques to quantify zotepine, such as spectrophotometry[2], high-performance liquid chromatography (HPLC)[3,4] and liquid chromatography-tandem mass spectrometry (LC-MS/MS)[5,6] are summarised in Table 1. Here novel stability-indicating reversed-phase ultra-fast liquid chromatography (RP-UFLC) method system has been developed to estimate zotepine in dosage forms as tablets. A Shimadzu UFLC system with a Zorbox C18 column and PDA detector was employed for the chromatographic analysis. At 1.0 mL/min flow rate, acetonitrile: 10 mM tetra butyl ammonium hydrogen sulphate (42:58) as a mobile phase UV wavelength of detection is 221 nm. Method validation and a forced degradation study were carried out per International Council for Harmonisation (ICH) guidelines.

MATERIALS AND METHODS

Equipment

UFLC system (Shimadzu) column Zorbox C18, detector PDA.

Chemicals and Reagents

The zotepine standard was obtained from Innovative Pharma. Tetra butyl ammonium hydrogen sulphate (Merck), acetonitrile (Merck), hydrochloric acid (Merck), sodium hydroxide (Merck), Milli Q water and hydrogen peroxide (Merck). Zotepine is available as Zotewin (Label claim 25 mg), Zotelet (Label claim 25 mg), and Zotewin (Label claim 50 mg) tablets. Prepared mobile phase used is a mixture of and acetonitrile: 10 mM tetra butyl ammonium hydrogen sulphate (42:58).

Chromatography

UFLC system (Shimadzu,) column Zorbox C18 (250 × 4.6 mm × 5 µm), detector PDA.UV detection at 221 nm. At 1.0 mL/min flow rate, Acetonitrile: 10 mM tetra butyl ammonium hydrogen sulphate (42:58) as a mobile phase, 20 µL of injection volume, and 10 minutes of run time, mobile phase used as diluent.

Working Standard Solution

Weighed 25 mg zotepine added to 25 mL volumetric flask. Added acetonitrile allows to dissolve and make up volume with acetonitrile (1000 µg/mL). These solutions were further diluted with diluent for linearity, precision, accuracy, robustness, and other study parameters and filtered using a membrane filter 0.45 µm.

Sample Preparation

Purchased twenty tablets of two distinct brands of zotepine from a nearby drugstore. Following this, the contents were

*Author for Correspondence: mmukthin@gitam.edu

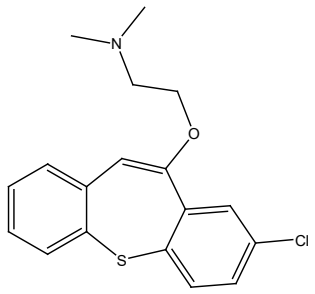


Figure 1: Chemical structure of zotepine

weighed in a 25 mL volumetric flask tablet powder equivalent to 25 mg of zotepine weighed. Added HPLC-grade acetonitrile for extraction. Sonicated to dissolve. Volume made up with diluent. These solutions 10 µg/mL were injected in UFLC (n = 3), area under the curve noted down from representative chromatograms and mean area calculated. The percentage of purity of zotepine in the tablet formulations was calculated from the linear regression equation.

Method Optimization

by applying the above-mentioned chromatographic conditions in UFLC system, 20 µg/mL zotepine standard solution was injected. System suitability parameters meet acceptance criteria. The chromatograms of the blank and API zotepine (Rt: 3.608 minutes) are shown in Figures 2 and 3.

Method Validation

In order to validate the method for system applicability, linearity, precision, accuracy, robustness, and limit of detection (LoD) and limit of quantification (LoQ), Q2(R1)7 of ICH guidelines followed.

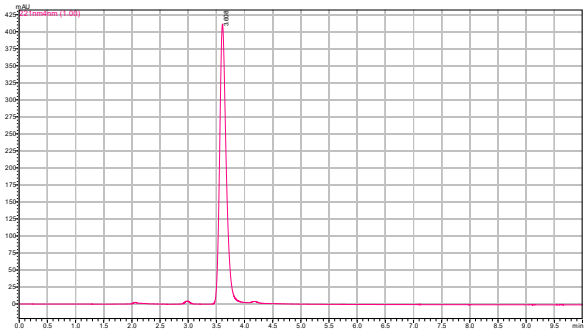


Figure 3: Chromatogram of zotepine API (20 µg/mL)

Suitability of system

The determination of system appropriateness characteristics involved injecting 20 µL of the standard solution into five replicates. Every parameter, including the tailing factor, theoretical plates, and RSD, satisfies the approval criteria.

Linearity

Linearity was performed by injecting (n = 3) the drug solutions (0.1-50 µg/mL) in to the UFLC system and thereby noting the mean peak area obtained for the respective chromatograms. Calibration curve was drawn by plotting the concentration of zotepine drug concentration on the x-axis and the corresponding mean peak area on the y-axis. S/N ratio was used to calculate the LoD and LoQ where LoQ is ten times of that of S/N ratio and LoD is 3.3 times S/N.

Table 1: Analytical methods for the estimation of zotepine

Method	*Reagent/max (nm)*	*Linearity (µg/mL)*	*Reference*
Spectrophotometry	HCl Sodium acetate buffer (pH 4.0)/261	0.1–50	2
HPLC	TFA: Methanol: ACN (5: 40: 55)/265	2.5–25	3
HPLC	ACN:KH_2PO_4 (55:45)/264	5–50	4
LC-MS/MS/QTOF	TFA: ACN/254	0.1–250	5
LC-EC/ESI-MS/MS	10 mM Am formate with 10% ACN: 10 mM Ammonium formate with 90% ACN/253	0.01–2	6
UFLC	Mix of acetonitrile:10 mM Tetra butyl ammonium hydrogen sulphate (42:58)/221	0.1–50	Present method

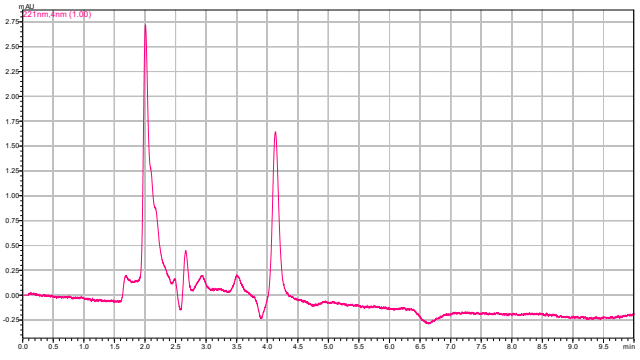


Figure 2: Chromatogram of blank

Table 2: Linearity data

Conc. (µg/mL)	*Mean peak area**	*%RSD*
0.1	17995	0.21
0.5	87579	0.35
1	185692	0.25
2	345871	0.22
5	864978	0.51
10	1758642	0.44
20	3527044	0.32
30	5140298	0.57
40	6846512	0.42
50	8625974	0.61

*Mean of three replicates

Table 3: Precision studies

Intraday precision			*Interday precision*		
Conc. (µg/mL)	*Mean peak area**	*%RSD*	*Conc. (µg/mL)*	*Mean peak area**	*%RSD*
10	1754983.5	0.148	10	1754046.5	0.0671
20	3430398	0.035	20	3428755.33	0.0204
40	6846238	0.014	40	6842854.33	0.0237

*Mean of three replicates
Mean of three days replicates*

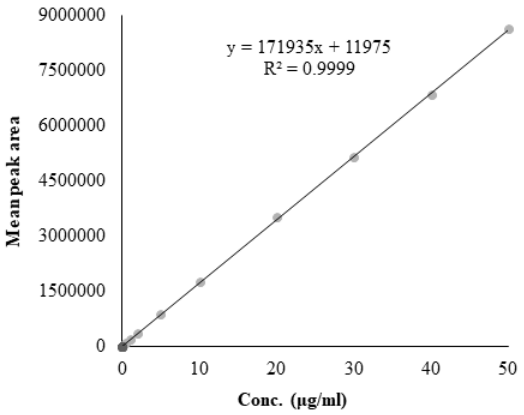


Figure 4: Zotepine calibration curve

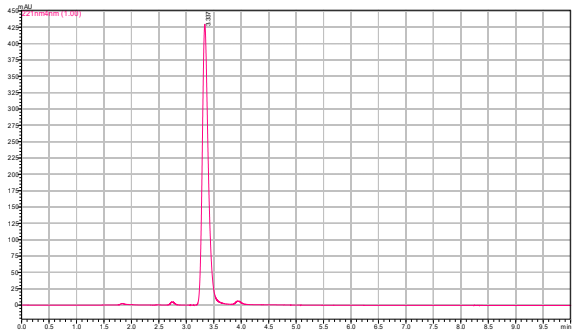


Figure 5: Chromatogram of zotepine tablet formulation (20 µg/mL)

Precision

The precision study was carried out on the same day with concentrations of 10, 20 and 40 µg/mL. For intraday and inter-day precision study on three consecutive days. The peak area values (n = 3) of the chromatograms were recorded. The %RSD of mean peak area value were determined.

Accuracy

Determined the %recovery at three levels 50, 100, and 150%. Zotepine standard 10 µg/mL solution has been spiked to form of 5, 10 and 15 µg/mL concentrations solution by injecting these solutions at (n = 3) times the %recovery was noted.

Robustness

Small changes in the method has been done to optimize chromatographic conditions. Like flow rate (± 0.1 mL/min; i.e., 0.9, and 1.1 mL/min), mobile phase composition (TBHS: Acetonitrile 58: 42) (± 2 % v/v; 56:44 and 60:40), wavelength of detection (± 2 nm; 223 and 219 nm), pH (± 0.1; 3.30 and 3.50).

Table 4: Accuracy

Conc. spiked (µg/mL)	*Formulation (µg/mL)*	*Total Conc. (µg/mL)*	**Conc. obtained (µg/mL)*	*%Recovery*
5 (50%)	10	15	14.97	99.80
	10	15		
	10	15		
10 (100%)	10	20	19.91	99.55
	10	20		
	10	20		
15 (150%)	10	25	24.92	99.68
	10	25		
	10	25		

*Mean of three replicates

Table 5: Robustness study

Parameter	*Condition*	**Mean peak area*	**Mean Area (% RSD)*
Flow rate (± 0.1 mL/min)	0.9	3392541	3419589 (0.85)
	1.0	3427044	
	1.1	3439182	
Detection wavelength (± 2 nm)	223	3430685	3428728.33 (0.03)
	221	3427044	
	219	3428456	
Mobile phase composition Tetra butyl ammonium hydrogen sulphate: Acetonitrile (± 2 %, v/v)	56:44	3428947	3427993.33 (0.61)
	58:42	3427044	
	54:46	3427989	
pH (± 0.1)	3.50	3428546	3428465 (1.02)
	3.40	3427044	
	3.30	3429805	

*Mean of three replicates

Table 6: Zotepine tablets assay

S. No.	*Brand name*	*Label claim (mg)*	**Obtained amount (%w/w)*	*Recovery%**
1	Brand 1	25	24.91	99.64
2	Brand 2	25	24.89	99.56

*Mean of three replicates

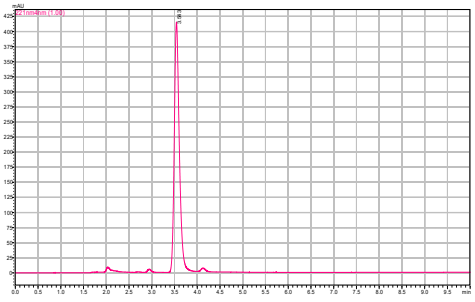
Stress Degradation Studies

To determine zotepine's (20 µg/mL) stability under the specified conditions, stress degradation studies[8] were carried out. The process of acidic degradation involved heating a

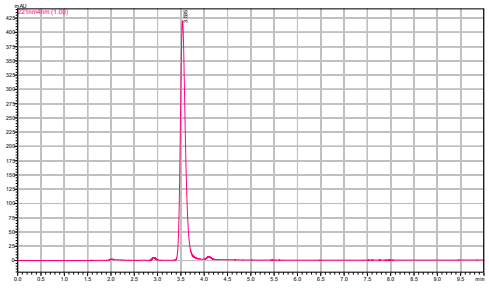
Table 7: Stress degradation study of zotepine

Stress condition	*R_t (min)*	*Mean peak area*	*%Recovery*	*%Drug degradation*	*Theoretical plates (>2000)*	*Tailing (<1.5)*	*Resolution (>2)*
Standard drug	3.608	3427044	100	0	4853.89	1.299	-
Basic	3.535	3226919	94.16	5.84	5425.67	1.315	-
Acidic	3.543	3224706	94.10	5.90	5185.07	1.296	-
Oxidation	2.700 3.556	2268536	66.19	33.81	4590.42	1.308	3.935
Thermal	3.522	3274576	95.55	4.45	5114.24	1.261	-

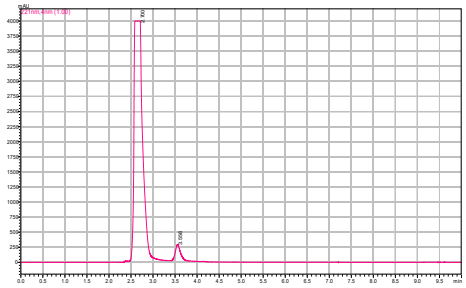
*Mean of three replicates



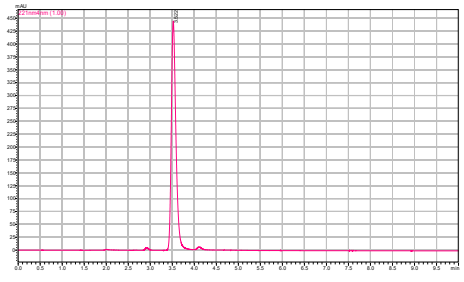
Acidic degradation



Basic degradation



Oxidative degradation



Thermal degradation

Figure 6: Zotepine (20 µg/mL) stress degradation studies representative chromatograms of during

solution of zotepine (20 µg/mL) in a water bath for 30 minutes at 60ºC added 0.1 N HCl 1-mL. After cooling and neutralizing it with 1-mL of 0.1 N NaOH, the makeup volume with diluent and 20 µL was injected into the UFLC system. The process of alkaline degradation involved heating a solution of zotepine (20 µg/mL) for 30 minutes at 60ºC in a water bath with 1-mL of 0.1N NaOH. After cooling, it was diluted with diluent and neutralized with 0.1 N HCL 1-mL, then injected 20 µL into the UFLC system.

Oxidative degradation involved heating a solution of zotepine (20 µg/mL), adding 1-mL hydrogen peroxide 30% in a water bath for 30 minutes at 60ºC with solution, after cooling down diluted with mobile phase, injection volume of 20 µL injected to UFLC system.

Assay of Zotepine Tablets

Zotepine is available as Zotewin (Label claim 25 mg), Zotelet (Label claim 25 mg), and Zotewin (Label claim 50 mg) tablets. Twenty tablets of zotepine were obtained from the pharmacy store. Weighed accurately and powdered then in 25 mL volumetric flask weighed powder equivalent to 25 mg of zotepine. Added acetonitrile sonicate to dissolve. Make up with diluent (10 µg/mL). It was injected into the UFLC (n = 3). The percent purity of zotepine in the tablet formulations was calculated.

RESULTS AND DISCUSSION

Novel RP-UFLC method put forth to estimate the amount of zotepine present in tablet dosage forms.

System Suitability

System suitability was assessed in order to confirm its functionality. Five injections of the standard solution 20 µL were injected and the chromatograms were recorded. System suitability, like RSD, resolution, theoretical plates, and tailing factor, is observed to meet acceptance criteria.

Method Validation

Zotepine exhibits linearity within the 0.1 to 50 µg/mL concentration range, as indicated by Table 2 percentage RSD (0.21–0.61). Linear regression equation yielded y = 171935x + 11975, with a correlation coefficient of 0.9999, as illustrated in Figure 4. Determined LOD 0.0312 and LOQ 0.0975 µg/mL. According to Table 3, the percentage RSD for intraday and interday in precision studies was found to be 0.014 to 0.148 and 0.0204 to 0.0671, respectively. This value is less than 2.0, suggesting that the method is precise. According to Table 4

accuracy, the recovery was found to be 99.55 to 99.80%, and the relative standard deviation (RSD) was 0.08 to 0.88, or less than 2%, suggesting that the method is accurate. Less than 2% was found in the robustness study's %RSD, which was 0.03 to 1.02. This suggests that the method is robust Table 5.

Zotepine Tablets Assay

The suggested optimized method used for quantification of zotepine in two different brands of 25 mg zotepine tablets. The percentage of recovery was found to be 99.56 to 99.64, as indicated in Table 6. The chromatogram of Zotepine tablet formulation was shown in Figure 5.

Stress Degradation Studies

zotepine eluted at 3.608 minutes in standard, theoretical plates 4853.89 (> 2000) and tailing factor 1.299 (< 1.5) zotepine has demonstrated 5.84% degradation during the basic degradation. Theoretical plates 5425.67, tailing factor 1.315, RT 3.535 minutes. It exhibits 5.90% degradation during the acidic degradation process. Theoretical plates are 5185.07, tailing factor is 1.296, and RT is 3.543 minutes. During oxidative degradation, zotepine RT 3.556 minutes, with a degradant peak occurring at 2.700 minutes. The analysis reveals 33.81% degradation of theoretical plates 4590.42; the tailing factor is 1.308, with a resolution 3.935. During the thermal degradation process, zotepine was eluted at 3.522 minutes and demonstrated approximately 4.45% degradation theoretical plates 5114.24, tailing factor 1.261. The method is said to be specific and selective as zotepine does not interfere with any other degradant peaks.[9-11] System suitability meets acceptable criteria as shown in (Table 7). Representative chromatograms of stress degradation studies are shown in Figure 6.

CONCLUSION

An innovative liquid chromatographic method, which is stability indicating by using reverse phase ultrafast, has been developed to assess zotepine quantitatively in tablet dosage forms. ICH rules were adhered to during method validation. The separate degradation peaks from the primary analyte peak demonstrate the method specificity recommended approach. This approach is user-friendly, robust, accurate, and precise. It may be used to estimate the dosage of zotepine in tablets. According to the approved requirements, the system's suitability characteristics are met.

ACKNOWLEDGEMENT

The authors gratefully acknowledged the zotepine gift samples provided by Innovative Pharma. A conflict of interest does not exist.

REFERENCES

1. Harada T and Otsuki S. Antimanic effect of Zotepine. Clinical Therapeutics. 1986; 8 (4): 406-414.
2. Mathrusri Annapurna M, Krishna Chaithanya R and Kumar JSP. New spectrophotometric techniques for the quantification of Zotepine - An antipsychotic drug. Journal of Chemical and Pharmaceutical Sciences. 2015; 8(3): 515-518.
3. Manjula Devi AS and Ravi TK. Validated UV spectrophotometric and HPLC methods for quantitative determination of zotepine. Research Journal of Pharmacy and Technology. 2012; 5(3): 342-345.
4. Shruthi K, Sridhar T, Raj Kumar V and Venumadhav N. Development and validation of stability indicating RP-HPLC method for the estimation of neuroleptic drug zotepine in bulk and tablet dosage form. International Journal of Pharmacy and Pharmaceutical Sciences. 2014; 6 (Suppl 2): 456-459.
5. Srinivas R, Talluri MVN, Naveen R, Divya CH and Raju B. Selective separation, detection of zotepine and mass spectral characterization of degradants by LC–MS/MS/QTOF. Journal of Pharmaceutical Analysis, 2013; 4(2): 107-116.
6. Kazuyoshi N, Issey O, Hideya K, and Ryuichi A. Application of on-line electrochemistry/electrospray/tandem mass spectrometry to a quantification method for the antipsychotic drug Zotepine in human serum. The Japan Society for Analytical Chemistry, 2009; 25(10): 1197-1201.
7. ICH Validation of analytical procedures: Text and methodology Q2 (R1), International Conference on Harmonization (2005).
8. ICH Stability testing of new drug substances and products Q1A (R2), International Conference on Harmonization (2003).
9. Bhilare NV, Marulkar VS, Kumar D, Chatap VK, Patil KS, Shirote PJ. An insight into prodrug strategy for the treatment of Alzheimer's disease. Medicinal Chemistry Research. 2022 Mar;31(3):383-99. https://doi.org/10.1007/s00044-022-02859-1
10. Bhakre H, Agrawal A, Chatap VK. Formulation, Development and Evaluation of Highly Oxidative Degradative Drug Molecule Injectable Dosage form by Lyophilisation Techniques. International Journal of Drug Delivery Technology. 2023;13(4):1378-1384.
11. Chatap V, Choudhari G, Jain P, Bhat MR. Synthesis and Characterization of Hydroxypropyl Sesbania Galactamannan Seed Gum for Pharmaceutical Application. International Journal of Pharmaceutical Quality Assurance. 2023;14(2):303-309. DOI: 10.25258/ijpqa.14.2.11