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Research Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR THE ESTIMATION OF NEUROLEPTIC DRUG ZOTEPINE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

Objective: The objective of the method was to develop a simple, rapid, efficient, cost effective and reproducible, stability indicating reverse phase high performance liquid chromatography method (RP-HPLC) for the quantification of zotepine in bulk and pharmaceutical dosage form.

Methods: The RP-HPLC analysis was carried out on an enable C_{18} G column with a mobile phase of acetonitrile and potassium dihydrogen ortho phosphate buffer 10 mM (pH adjusted to 3.0 with ortho phosphoric acid) in the ratio of 55:45 v/v. The analyte was detected at 264 nm using UV detector. The method was validated in terms of linearity, accuracy, precision, LOD, LOQ and robustness as per ICH guidelines.

Results: The method was found to be linear in the range of 5-50 µg/ml. Limit of detection and limit of quantitation was found to be 24 and 250 ng/ml respectively. Recovery was found to be in the range 99.7-100.02% and precision less than 1%. The developed method was successfully applied for the estimation of zotepine in marketed table formulation (Sirilept®) and percentage assay was found to be 99.62%. Forced degradation studies were performed under different conditions. The drugs were degraded in acidic, basic and oxidative conditions. The peaks of degraded products were well resolved from the actual drug. The results obtained prove that the developed method is a stability indicating method.

Conclusion: The developed RP-HPLC method was simple, rapid, accurate, precise and stability indicating for the quantification of zotepine in bulk and tablet dosage form.

Keywords: Zotepine; RP-HPLC; Validation; Stability indicating.

INTRODUCTION

Stability indicating method is used to evaluate the ability of analytical method to estimate the analyte and its degradation products without any interference from the degraded products generated by forced degradation studies [1].

According to ICH and FDA guidelines forced degradation studies are conducted to investigate the stability indicating power of the developed analytical method [2].

The developed method is expected to allow the analysis of individual degraded products. Zotepine, 2-[(8-chloro dibenzo (b,f) thiepin-10-yl)oxy]-N,N-dimethylethanamine, Fig. 1 is an atypical antipsychotic used in the treatment of acute and chronic schizophrenia which has high affinity for serotonin $5HT_{2A}$ and $5HT_{2C}$ receptors and dopamine D_1, D_2, D_3 and D_4 receptors. It acts as an antagonist at central dopamine (D_1 and D_2) receptors. It also binds to serotonin, adrenergic (α_1), and histamine (H_1) receptors, and inhibits noradrenaline reuptake [3-5]. It has fewer adverse effects than conventional antiphsycotics [6]. Research studies on zotepine and its adverse reactions related to metabolic effects and movement disorders are available [7].

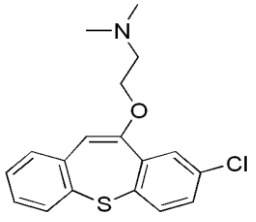

Fig. 1: **Structure of zotepine**

On extensive survey of literature, very few stability indicating RP-HPLC methods using UV technique have been reported for the estimation of zotepine in bulk and pharmaceutical formulation [8-9]. Hence an attempt was made for the development and validation of stability indicating RP-HPLC method for the estimation of zotepine. Forced degradation studies were performed to prove that the developed method is stability indicating.

MATERIALS AND METHODS

Chemicals

Zotepine was obtained as gift sample from Symed laboratories, Hyderabad. Methanol and acetonitrile of HPLC grade were obtained from Sigma Aldrich Chemicals limited, Maharastra. Potassium dihydrogen ortho phosphate and ortho phosphoric acid of analytical grade were purchased from Finar chemicals limited, Mumbai. Marketed tablet formulation (Sirilept®) tablets manufactured by Sun Pharmaceuticals limited, Hyderabad were purchased.

HPLC system

The HPLC system used was LC 20AT Shimadzu system with LC Solutions software. The system was equipped with LC 20AT prominence pump and UV detector. The chromatographic separation was carried out on enable C_{18} G column (150×4.6 mm, 5 µ). Elution was performed with a mobile phase containing acetonitrile and potassium dihydrogen ortho phosphate (pH 3.0; 10 mM) (55:45 v/v). pH was adjusted to 3.0 by ortho phosphoric acid. Mobile phase was freshly prepared and filtered through 0.45 µm membrane filter and degassed prior to the analysis.

Preparation of standard stock solution

Standard stock solutions were prepared by transferring 100 mg of zotepine in to 100 ml volumetric flask. Methanol was added to the flask to dissolve the sample and the volume was made upto the mark. From this solution 10 ml was transferred into 100 ml volumetric flask and the volume was made upto the mark with mobile phase. From this solution working standards of required concentrations were prepared.

Preparation of sample solution

Twenty tablets were weighed and powdered. Tablet powder equivalent to 25 mg of zotepine was weighed and transferred into 100 ml volumetric flask. 50 ml of mobile phase was added and sonicated for 5 min. Finally the volume was made upto the mark with mobile phase and filtered through 45 µm membrane filter.

From this solution working standards of different concentrations were prepared for chromatographic analysis.

Validation of the method

The developed method was validated according to ICH guidelines in terms of specificity, sensitivity, sensitivity, linearity, LOD, LOQ, accuracy, Precision and robustness [10-13].

Specificity

Specificity was established by complete separation of analyte in the presence of tablet excipients and without interferences at the retention time of zotepine.

Linearity

Linearity was established by least squares linear regression analysis of calibration curve. Linearity was determined in the range of 5-50 µg/ml.

LOD and LOQ

LOD and LOQ were determined by using the following formulae,

$LOD = \frac{3.3 \times SD}{slope}$; $LOD = \frac{10 \times SD}{slope}$

Where SD is the standard deviation of response

Precision and accuracy

Precision and accuracy of the method was monitored for three days. Accuracy of the method was calculated by recovery studies at three levels by standard addition method. For intraday precision nine determinations of three concentrations were analysed on the same day. For interday precision nine determinations of three concentrations were analysed for three consecutive days.

Robustness

Robustness of the method was investigated under a variety of conditions like change in pH of mobile phase (±0.2), flow rate (±0.2 ml/min) and wavelength (±2 nm), change in column temperature (±2^0 C). In each variation analysis was made in three replicates and %RSD of peak areas were determined.

Forced degradation studies

In order to establish whether the developed method is stability indicating zotepine (API) was stressed under various conditions (acid, base, oxidation and thermal) to perform forced degradation studies.

Acid degradation studies

Zotepine standard solution of concentration 1000 µg/ml was prepared with mobile phase and treated with 5 ml of 1N HCl. The resultant solution was analysed for every 24 h after prior dilution.

Alkaline degradation studies

Zotepine standard solution of concentration 1000 µg/ml was prepared with mobile phase and treated with 5 ml of 1N NaOH. The resultant solution was analysed for every 24 h after prior dilution.

Oxidation studies

Zotepine standard solution of concentration 1000 µg/ml was prepared with mobile phase and treated with 5 ml of 3% H_2O_2. The resultant solution was analysed for every 24 h after prior dilution.

Thermal stress studies

Zotepine powder was exposed to dry heat at 60^0 C. The powder was removed for every 24 h and diluted as mentioned above and analysed.

RESULTS AND DISCUSSION

Method development

Initially wavelength was selected for the method development and different compositions, pH and flow rate of the mobile phase were tried during method development. The 264 nm was selected for the current method since at this wavelength zotepine can be selected with high sensitivity. In the course of optimizing the composition of mobile phase, acetonitrile in combination with various buffers like phosphate and acetate with varying pH values were tried. After a series of preliminary experiments it was concluded that potassium dihydrogen ortho phosphate buffer resulted in better peak shape. Peak with good shape and symmetry was observed by the mobile phase consisting of acetonitrile - potassium dihydrogen ortho phosphate (pH 3.0, 10 mM) (45:55, v/v) set at a flow rate of 1ml/min. This composition was used for further studies Fig. 2.

Method validation

Specificity

The specificity of the method is shown in Fig. 3 where zotepine was eluted completely without any interference from tablet excipients at its retention time.

This shows that the excipients of tablets did not interfere the analyte elutio

Linearity

The calibration curve was constructed between peak area and respective concentrations. The calibration curve was linear over the range of 5-50 µg/ml. Correlation coefficient was found to be 0.997. The regression equation for calibration curve was found to be y= 59138x + 19600. Results of linearity are shown in Table 1 and Fig. 4

LOD and LOQ

LOD and LOQ were determined by the earlier mentioned equations. LOD was found to be 24 ng/ml and LOQ was found to be 250 ng/ml.

Precision and Accuracy

Precision of the method was determined in terms of intraday and interday precision. The % RSD obtained for intraday and interday precision was less than 2%. Accuracy was calculated by recovery studies at three levels viz 50%, 100% and 150% by standard addition method. The percentage recovery was found to be 99.70%. The values of intra and interday precision are shown in Table 2 and accuracy results are shown in Table 3.

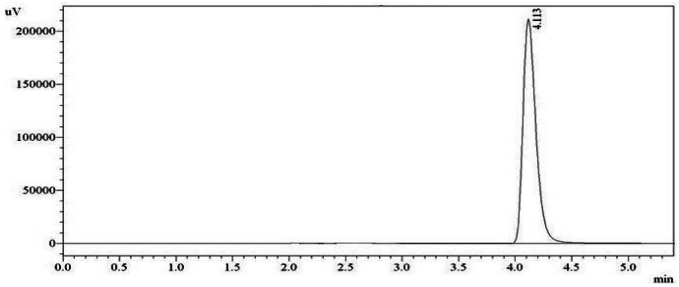

Fig. 2: Chromatogram showing estimation of zotepine (Rt 4.1)

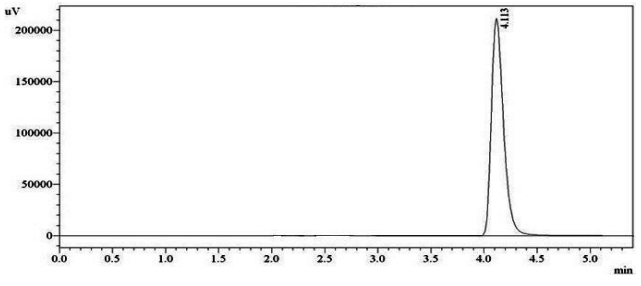

Fig. 3: Chromatogram of sample solution (marketed formulation)

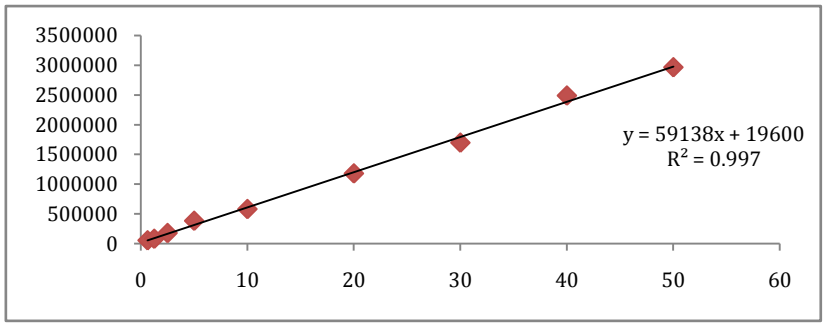

Fig. 4: Calibration curve

Table 1: Analytical performance parameters for linearity

Parameter	Zotepine
Linear range	5-50 µg/ml
Slope	59138
r^2	0.997

Table 2: Intraday and Interday precision results

Concentration (µg/ml)	%RSD	
	Intra day	Inter day
5	0.8	0.6
20	0.4	0.3
50	0.7	0.4

Table 3: Accuracy results Table 3: Accuracy results

	Spike level	Amount present (µg/ml)	Amount recovered (µg/ml)	% Recovery
1.	50%	10	9.97	99.70
2.	100%	20	19.98	99.90
3.	150%	30	30.06	100.02

Table 4: Robustness testing of the method

Factor		% RSD
pH of mobile phase	2.8	0.4
	3.0	0.2
	3.2	0.6
Flow rate (ml/min)	0.8	0.7
	1.0	0.5
	1.2	0.8
Wavelength (nm)	262	0.7
	264	0.2
	266	1.3
Column temperature	28	0.7
(⁰ C)	30	0.2
	32	0.5

Robustness

Robustness of the method was determined by deliberately changing parameters like flow rate, pH of mobile phase and wavelength and temperature of the column. Samples were analysed in triplicates and %RSD was calculated from peak areas. Results of robustness are summarized in Table 4

Forced degradation studies

All the stressed samples in acid, alkaline and oxidative degradation studies were decomposed to 14, 10 and 9% respectively.

No decomposition was seen on exposure of solid drug to dry heat. The peaks of degraded products were well separated from the analyte peak with good resolution Fig. 5 (a, b, c) which indicates that the developed method is stability indicating. The forced degradation studies data are summarized in Table 5.

Assay

The validated method was applied to the determination of zotepine in commercially available Sirilept® tablets. Chromatogram obtained by assay of tablets shows in Fig. 6. The percentage assay was found to be 99.62%. The results of assay indicate that the developed method is selective without interference from excipients of table.

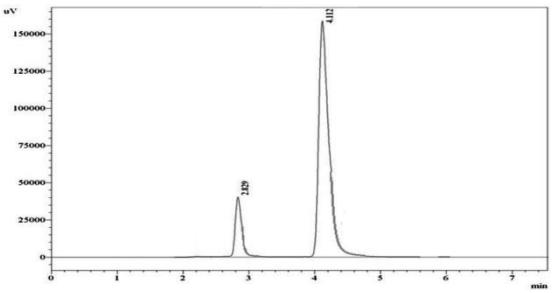

Fig. 5a: Chromatogram showing acid degradation of zotepine (an additional peak is observed at Rt 2.8 which is the peak of degraded product of zotepine)

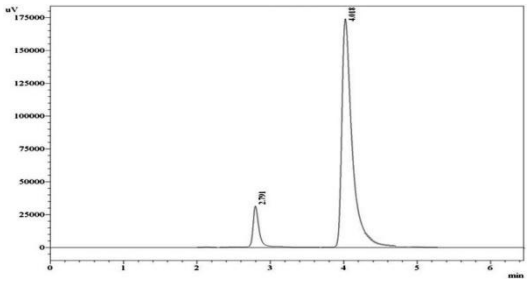

Fig. 5b: Chromatogram showing alkaline degradation of zotepine (an additional peak is observed at Rt 2.7 which is the peak of degraded product of zotepine)

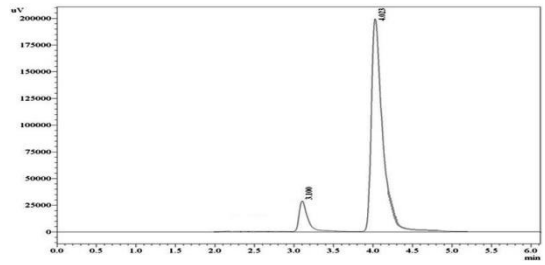

Fig. 5c: Chromatogram showing oxidative degradation of zotepine (an additional peak is observed at Rt 3.1 which is the peak of degraded product of zotepine.

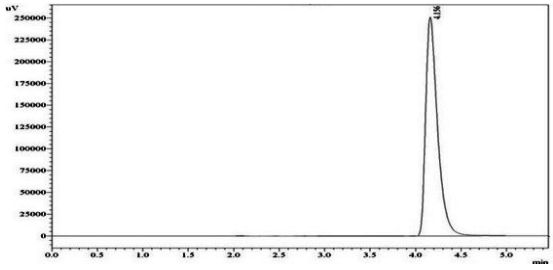

Fig. 6: Chromatogram showing analysis of Sirilept® tablets

Table 5: Data of forced degradation studies

S. No.	Stress condition	Time	Degradation (%)
1	Acid hydrolysis (1N HCl)	24 h	14
2	Base hydrolysis (1N NaOH)	48 h	10
3	Oxidative degradation (3% H_2O_2)	3 h	9
4	Thermal degradation (60^{0}C)	7 days	stable

CONCLUSION

A simple, sensitive, specific, accurate and precise stability indicating RP-HPLC method was developed and validated for the routine analysis of bulk and tablet dosage form of zotepine. The method is sensitive enough for the detection of analyte in pharmaceutical formulation when compared to the research works found in the literature. The results of forced degradation studies reveal that the method is stability indicating.

The proposed method has the capability to separate the analyte from their degradation products obtained during forced degradation studies and excipients found in tablets. The method can be employed for the routine analysis of zotepine.

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