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Original Research Article

Stability Indicating HPLC Method Development and Validation for the Estimation of Zonisamide in Bulk and Pharmaceutical Dosage Form

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Abstract

A simple, rapid, precise, stability indicating, isocratic reverse phase-high performance liquid chromatography method has been developed and validated for the determination of Zonisamide in bulk and pharmaceutical dosage form. The Chromatographic Separation was achieved by using Enable ODS reverse phase (250 x 4.6 mm, 5 µm particle size) C_{18}G column. Mobile phase consists of mixture of 0.1% (v/v) ortho phosphoric acid and Methanol in the ratio of 30**:**70 (v/v) and was delivered at a flow rate of 1 ml/min, while the detection was monitored at a wave length of 285 nm. The developed method showed excellent linear response (r^2>0.9999) in the range of 10-70 µg/ml. The retention time for Zonisamide was found to be 3.5 mins. The proposed method was validated as per ICH guideline and can be applied for estimation of Zonisamide in pharmaceutical dosage forms in routine analysis.

Keywords: Zonisamide, stability indicating, Validation.

INTRODUCTION

Zonisamide is a benzisoxazole derivative, chemically known as [1, 2-benzisoxazole-3-methane sulfonamide] used as an adjunctive antiepileptic in the treatment of partial seizure [1, 2]. The precise mechanism of zonisamide's antiepileptic effect remains undefined. It has been suggested that zonisamide raises the seizure threshold through action at sodium and calcium channels, stabilizing neuronal membranes and suppressing neuronal hypersynchronization [3, 4].

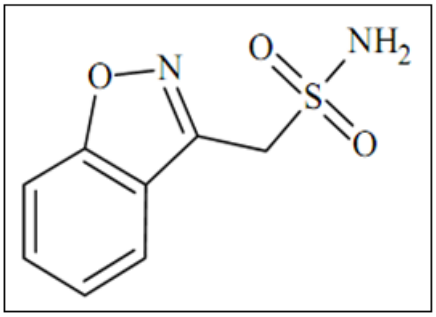

Fig-1: Chemical structure of Zonisamide

Several methods have been reported for analysis of Zonisamide using gas chromatography (GC) [5], micellar electro kinetic capillary chromatography [6-9], enzyme immunoassay, high performance liquid chromatography (HPLC) with UV detection using solid phase extraction[10]. HPLC methods for determination of impurity and degradation products for Zonisamide were also reported [11, 12]. Ion pair HPLC [13], RP-HPLC [14], stability indicating HPLC [15] and HPTLC method for simultaneous determination of lamotrigine, zonisamide and levetiracetam in human plasma [16] were also developed.

Reported methods involved complicated, time-consuming multi-step liquid-liquid extraction techniques. To the best of our knowledge, there is no work in the literature reported about fast and reliable estimation of zonisamide from pharmaceutical formulation by using RP-HPLC. The purpose of this investigation was the development of a rapid, sensitive and validated stability indicating HPLC method for quantification of zonisamide from capsule forms.

EXPERIMENTAL

Materials and Reagents

Zonisamide pure drug was supplied by Vivid labs, India as gift sample and used as such. All reagents and chemicals used were of Analytical Grade.

Instrumentation

The chromatographic system used for the method development and validation consisted of Shimadzu HPLC comprising of LC-20AD binary gradient pump, a variable wavelength programmable SPD-20A detector and an SCL 20A system controller. A Rheodyne injector 7725i fitted with a 20 µL loop was used and data were recorded and evaluated by use of LC solutions software version 5.0.

Preparation of Standard Solutions

Standard stock solution of the drug was prepared by dissolving 100 mg of zonisamide in a mixture of methanol: water (50: 50 v/v) and made up to with 100 mL with the same (1000 µg/mL). Working standard solution was prepared by diluting 10 mL of the stock solution to 100 mL with methanol: water (50: 50 v/v) (100 µg/mL).

Preparation of Sample Solution

The average weight was determined with 10 tablets, which were grounded in a mortar until fine powder. Accurately weighed amount of powder equivalent to 100 mg of Zonisamide was quantitatively transferred to a 100 ml calibrated flask and in a mixture of methanol: water (50: 50 v/v) and made up to with 100 mL with the same (1000 µg/mL). Sample solution was prepared by diluting 10 mL of the stock solution to 100 mL with methanol: water (50:50 v/v) (100 µg/mL). The solution was filtered, sonicated and the final concentration was brought to 40 µg/ml.

Chromatoraphic Conditions

The analysis was carried out using Enable ODS reverse phase (250 x 4.6 mm, 5 µm particle size) C_{18}G column. Mobile phase consists of mixture of 0.1 % v/v ortho phosphoric acid and Methanol in the ratio of 30**:**70 v/v and was delivered at a flow rate of 1 ml/min, while the detection was monitored at a wave length of 285 nm. HPLC method depends upon the nature of the sample (ionic or ionizable or neutral molecule), its molecular weight and solubility. To optimize the chromatographic conditions the effect of chromatographic variables such as mobile phase, pH, flow rate and solvent ratio were studied. The resulting chromatograms were recorded and the chromatographic parameters such as asymmetric factor, and resolution and column efficiency were calculated. The condition that gave the best resolution, symmetry and capacity factor was selected for estimation.

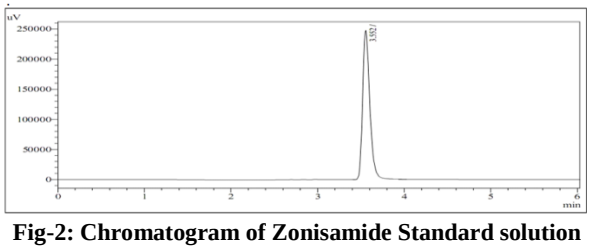

Fig-2: Chromatogram of Zonisamide Standard solution

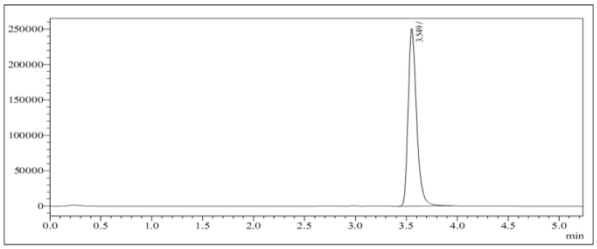

Fig-3: Chromatogram of Zonisamide Test solution

RESULTS AND DISCUSSION

Method Validation [17]

Linearity

Several aliquots of standard stock solutions of zonisamide were taken in different 10 mL volumetric flask and diluted up to the mark with mobile phase. Evaluation was performed with SPD 20AD Ultra-Violet detector at 285 nm. Peak area was recorded for all the peaks and a Calibration graph was obtained by plotting peak area versus concentration of zonisamide. The plot of peak area of each sample against respective concentration of zonisamide was found to be linear in the range of 10-70 µg/mL with correlation co-efficient of 0.9999. Linear regression least square fit data obtained from the measurements are given in Table-1. The respective slope (m), intercept (b) and correlation coefficient are given in Table-1.

Table-1: Linear regression data for calibration graph for Zonisamide by proposed method

Drug	Zonisamide
Concentration range,	10 - 70 µg/mL
Slope,	28562
Intercept,	24885
Correlation coefficient	0.9999

Precision

The precision of each method was ascertained separately from the peak area obtained by actual determination of six replicates of a fixed amount of drug. The percent relative standard deviation was calculated and was found to be 1.0948 for Zonisamide for proposed work and is presented in the Table-2.

Table-2: Precision of Zonisamide by proposed method

Mean Peak area	1448063
Std Dev	18775.31
%RSD	1.0948

Accuracy

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80 %, 100 %, and 120%) of Zonisamide bulk samples of within the linearity range were taken and added to the pre-analyzed formulation. From that percentage recovery values were calculated. The % recovery of Zonisamide was found to be 99.85%. The Data derived from % recovery of Zonisamide by proposed method was stipulated in Table-3**.**

Table-3: Data derived from Accuracy of Zonisamide by proposed method

Spiked level	Amount added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
80%	32	31.93	99.6%	
100%	40	40.01	100.2%	99.85%
120%	48	47.90	99.75%	

Assay

Sample solution of 40 µL was injected into the injector of liquid chromatograph. The retention time were found to be 3.5 mins for Zonisamide. The amount of drug present per tablet was calculated by comparing the peak area ratio of the sample solution with that of the standard solution and the % purity was found to be 99.67 for marketed formulation which is given in Table-4.

Table-4: Data derived from Assay of Zonisamide by proposed method

Brand name	Label Claim	Mean amount found	% Purity
Zonisep™ (Sunpharma)	100 mg	99.67	99.67

System Suitability Parameters

System suitability parameters can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually developed after method development and validation has been completed or The USP (2000) defines parameters that can be used to determine system suitability prior to analysis. The system suitability parameters like Theoretical plates (N), Tailing factor (T), (Table-5) were calculated and compared with the standard values to ascertain the proposed RP-HPLC method for the estimation of Zonisamide in pharmaceutical formulations.

Table-5: System suitability test parameters for Zonisamide by proposed method

Parameter	Observation
Retention time	3.5 mins
HETP	19.92
Tailing Factor	1.242
Theoretical Plates	7699.3

LOD and LOQ

ICH guideline describes several approaches to determine the detection and quantitation limits. These include visual evaluation, signal-to-noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were calculated and found to be 0.28 µg/ml and 0.84 µg/ml respectively.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the method was investigated in table 6 under a variety of conditions including changes of composition of buffer in the mobile phase and flow rate. % RSD of assay was calculated for each condition. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust.

Table-6: Data Derived from Robustness of Zonisamide by proposed method

Chromatographic Parameters		Change in %RSD
Flow rate	0.8ml	0.47%
	1.2ml	0.41%
Composition of Mobile phase	40:60	0.38%
	20:80	0.30%

Stress Degradation Studies

For Stress Degradation Analysis, 1 mL aliquots (in duplicate) of samples containing MQC level concentration are treated separately with 100 µL of 0.1N HCl (Acid stress), 0.1N NaOH (Alkaline stress), 3% v/v Hydrogen Peroxide (Oxidative Stress), for 24 Hrs. Samples for photolytic stress are placed in a transparent glass vial & placed in a UV chamber for 24 Hrs. Thermal degradation was carried out by subjecting the zonisamide sample to thermal stress at 80 ^{0}C for about 2 days. Samples are then injected for analysis. The results of analysis are then compared with similarly prepared fresh samples.

The stress studies involving light (UV) and oxidation revealed that Zonisamide was not fully degraded as shown in Table-7. However in acidic conditions (0.1N HCl), the drug was unstable and the degradation peak eluted earlier accompanied with a peak distortion and increased tailing. The % degradation was found to be 15.44%. Except for acidic conditions, the drug content was within 95–102% for all stress conditions indicating the stability and specificity of the analytical method to differentiate the degradation peaks.

Table-7: Degradation studies of Zonisamide by proposed method

Stress Degradation Parameter	% Degradation
Acid degradation	15.44%
Alkali degradation	12.9%
Oxidative degradation	7.1%
Photolytic degradation	1.6%
Thermal degradation	1.2%

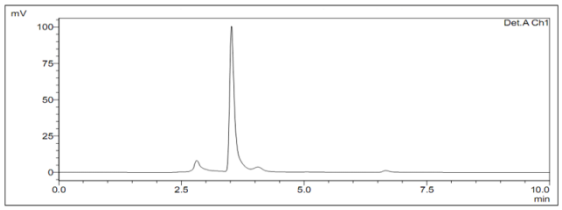

Fig-5: Chromatogram of Zonisamide showing acidic degradation by proposed method

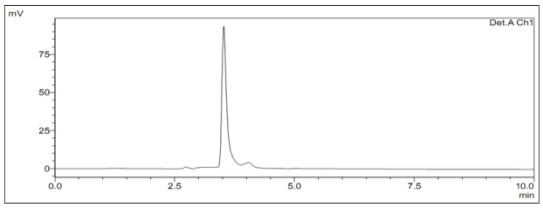

Fig-6: Chromatogram of Zonisamide showing alkali degradation by proposed method

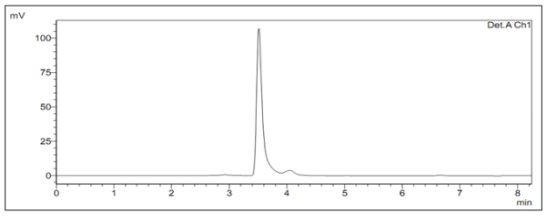

Fig-7: Chromatogram of Zonisamide showing Oxidative degradation by proposed method

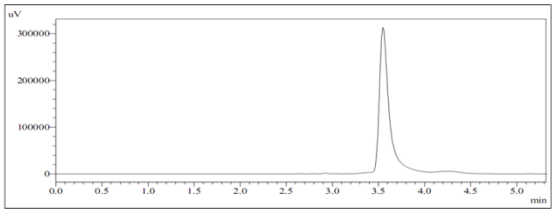

Fig-8: Chromatogram of Zonisamide showing Photolytic degradation by proposed method

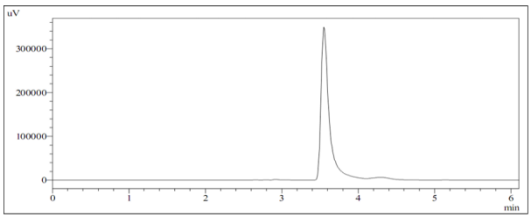

Fig-9: Chromatogram of Zonisamide showing Thermal degradation by proposed method

CONCLUSION

A validated stability-indicating RP-HPLC analytical method has been developed for the determination of Zonisamide in API and dosage form. The results of stress testing undertaken according to the International Conference on Harmonization (ICH) guidelines reveal that the method was selective and stability-indicating. The proposed method was simple, accurate, precise, specific, and has the ability to separate the drug from degradation products. In the absence of a stability indicating assay in the literature, the proposed method was suitable to use for the routine analysis of Zonisamide in either bulk API powder or in pharmaceutical dosage forms. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments such as LC–MS and or GC–MS which are complicated, costly and time consuming rather than a simple HPLC-UV method. In addition, the HPLC procedure can be applied to the analysis of samples obtained during accelerated stability experiments to predict expiry dates of pharmaceuticals. The method had proved its importance in terms of sensitivity, rapidity, economy in the stability indicating estimation of Zonisamide.

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