Research Article

Determination of Zonisamide in Bulk and Pharmaceutical Formulation by UV Spectrophotometric Method

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ABSTRACT

A simple, fast and reliable UV spectrophotometric method has been developed for estimation of Zonisamide in bulk and pharmaceutical dosage form. Estimation was carried out at λmax 240 nm using 0.1 N NaOH as solvent. The linearity was observed in the range of 5-30 µg /ml with correlation coefficient (r) 0.999. The percentage recovery was found to be in range of 99.84-100.18 %. The proposed method was found to be simple, accurate, precise, reproducible and gave the acceptable recovery of the analyte which could be directly and easily applied to analysis of bulk and pharmaceutical capsule dosage form of zonisamide.

Keywords: Zonisamide, UV spectrophotometric method, Capsule dosage form.

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.

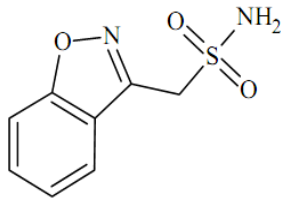

Figure 1: Chemical structure of Zonisamide

Several methods have been reported for analysis of Zonisamide using micellar electro kinetic capillary chromatography[3-5], enzyme immunoassay[6], HPLC with UV detection using solid phase extraction. HPLC methods for determination of impurity and degradation products for Zonisamide were also reported[7-8]. RP-HPLC[9-12] and HPTLC[13] method for simultaneous determination of lamotrigine, zonisamide and levetiracetam in human plasma were also developed.

Reported methods involved complicated time-consuming multi-step liquid-liquid extraction techniques. Here attempt is made to develop and validate a simple, economical, reproducible & precise UV spectrophotometric method for estimation of Zonisamide in bulk and pharmaceutical dosage form.

MATERIALS AND METHODS

Instrumentation

An ELICO UV/Visible spectrophotometer model SL 210 with 10 mm matched quartz cells was used for all spectral measurements. An electronic analytical weighing balance (1 mg sensitivity, Keeroy) used for weighing purpose.

Chemicals and Reagents

All reagents and chemicals used were of Analytical Grade. Gift sample of Zonisamide was supplied by Vivid labs, Hyd. Marketed formulation Zonisep capsules (label claim 100 mg) was procured from local market.

Preparation of standard stock solution

Accurately weighed 10 mg of Zonisamide was transferred into 100 ml volumetric flask and the content was dissolved in 0.1 N NaOH and volume was made up to the mark with 0.1 N NaOH to get a stock solution containing 100 µg/ml.

Preparation of working standard solution

From the standard stock solution 3 ml was transferred to 10 ml volumetric flask and diluting to 10 ml with 0.1 N NaOH to get a concentration of 30 µg/ml. Working standard solution of zonisamide was scanned between 200-400 nm. The wavelength maximum exhibited for zonisamide was at 240 nm (Fig.2).

Procedure for Calibration Curve

Appropriate volume of aliquot (0.5-3 ml) from standard stock solution was transferred to volumetric flask of 10 ml capacity. The volume was adjusted to the mark with 0.1 N NaOH to give solutions concentrations in the range of 5-30 µg/ml. The absorbance measurements of these solutions were carried out against 0.1 N NaOH as blank at 240 nm. Calibration curve was constructed by plotting

absorbance versus concentrations. Linear regression equation was obtained from this calibration curve.

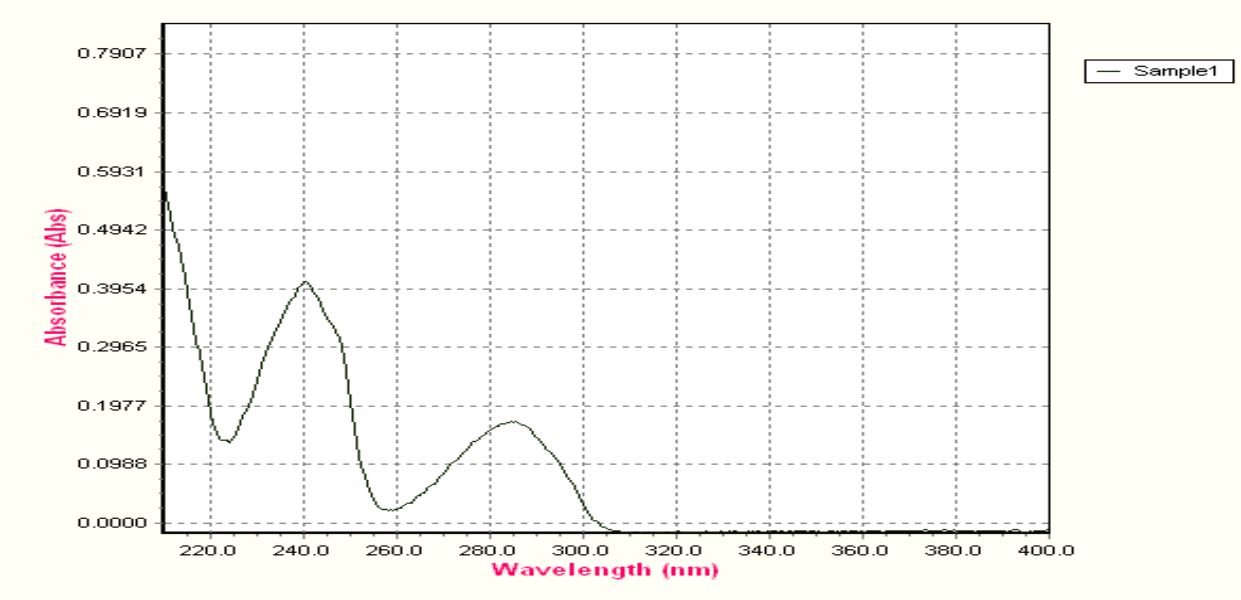

Figure 2: UV Spectra of Zonisamide

Procedure for Pharmaceutical Formulation

Ten capsules (Zonisep) were weighed and average weight was calculated. Capsule content powder equivalent to 50 mg Zonisamide was accurately weighed and transferred to 100 ml volumetric flask. To this 60 ml of 0.1 N NaOH was added and sonicated for 10 min. The flask was shaken and volume was made up to the mark with 0.1 N NaOH. The above solution was filtered through whatmann filter paper No.41. From the above solution 2 ml was transferred to 100 ml volumetric flask. Volume was made up to the mark with 0.1 N NaOH to give a solution containing 10 µg/ml. The content in the capsule was calculated from the calibration curve.

Method Validation

The developed method was validated in terms of Linearity, precision, accuracy, Limit of detection (LOD) and Limit of Quantitation (LOQ), robustness and ruggedness.

Linearity

Six points calibration curve were obtained in a concentration range from 5-30 µg/ml for Zonisamide. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was y=0.0478x+0.0136 with correlation coefficient 0.999 (Table 1 and 2, Fig. 3).

RESULTS AND DISCUSSION

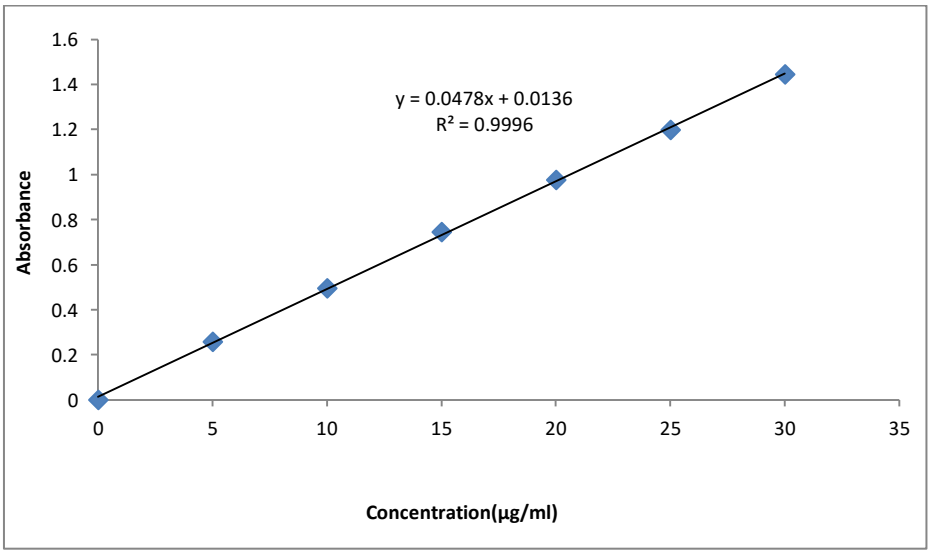

Figure 3: Calibration curve of zonisamide

Table 1: Linearity Data for Zonisamide

S. No	Concentration (µg/ml)	Absorbance
1.	5	0.2579
2.	10	0.4955
3.	15	0.7452
4.	20	0.9766
5.	25	1.1986
6.	30	1.4452

Table 2: Optical Characteristics of Zonisamide

S. No	Parameters	Method
1.	λmax (nm)	240
2.	Beers law limit (µg/ml)	5-30
3.	Sandell's sensitivity(µg/cm^2/0.001 A.U)	0.01297
4.	Molar absorptivity(L mol^{-1} cm^{-1})	1.16611 x 10^3
5.	Correlation coefficient (r)	0.9998
6.	Regression equation(Y=mX+c)	Y=0.0471X+0.0285
7.	Slope(m)	0.0478
8.	Intercept(c)	0.0136
9.	LOD (µg/ml)	0.0243
10.	LOQ (µg/ml)	0.0738
11.	Standard error of mean of Regression line	0.00494

Precision

Precision was checked in terms of repeatability, inter and intraday precision. It was expressed in percentage RSD.

Repeatability

The repeatability was evaluated by assaying six times of sample solution prepared for assay determination. Percentage RSD was calculated (Table 3).

Table 3: Repeatability

Concentration(µg/ml)	Absorbance
10	0.4850
10	0.4852
10	0.4950
10	0.4894
10	0.4906
10	0.4852
Mean	0.4884
Standard Deviation	0.00368
% RSD	0.754

Interday and Intraday precision

The intraday and interday precision study of zonisamide was carried out by estimating different concentrations of zonisamide three times on the same day (intraday precision) and on three different days (interday precision) and the results were reported in terms of Percentage RSD. (Table 4).

Table 4: Interday & Intraday Precision

S. No	Concentration (µg/ml)	Interday		Intraday	
		SD	%RSD	SD	%RSD
1	5	0.00057	1.07	0.001	1.72
2	15	0.0025	1.63	0.0015	0.98
3	25	0.003	1.17	0.0026	1.03

Accuracy

Accuracy was assessed by determination of the recovery of the method by addition of standard drug to the known amount of marketed formulation at three different concentration levels 80, 100 and 120 % taking into consideration percentage purity of added bulk drug samples. Each concentration was analyzed three times and average recoveries were measured (Table 5).

Table 5: Accuracy Studies

Name of Drug	Spiked Level	Conc. Added (µg/ml)	Conc. Recovered (µg/ml)	%Recovery±SD*
Zonisamide	80%	16	16.03	100.18%±0.0256
	100%	20	19.97	99.84%±0.0582
	120%	24	23.99	99.95%±0.0421

*Average of three determinations

Robustness

The robustness of a method is its capacity to remain unaffected by small changes in conditions. To determine the robustness of the method, the experimental conditions were deliberately altered and assay was evaluated. The effect of detection wavelength was studied at ±2 nm. For changes of conditions, the sample was assayed in triplicates. When the effect of altering one set of conditions was tested, the other conditions were held constant at the optimum values. Assay for all deliberate changes of conditions should be within 98.0–102.0 % for the proposed method (Table 6).

Table 6: Robustness Studies

Formulation	Amount of drug taken from tablet(mg)	At 238 nm	At 242 nm
		(n=3)%Assay±%RSD	(n=3)% Assay±%RSD
Zonisep(capsules)	50	99.73±0.313	99.91±0.224

Ruggedness

Ruggedness of the proposed method is determined by analysis of aliquots from homogeneous slot by two analysts using same operational and environmental conditions (Table 7).

Table 7: Ruggedness Studies

Formulation	Amount of drug taken from tablet(mg)	Analyst 1	Analyst 2
		(n=3)%Assay±%RSD	(n=3)%Assay±%RSD
Zonisep(capsules)	50	99.83±0.243	99.86±0.324

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were calculated according to below equation given by

LOD= 3.3 σ/s

LOQ=10 σ/s

Where σ is the standard deviation of y intercepts of regression lines and s is the slope of the calibration curve (Table 2).

Application of method to formulation

The proposed was applied to pharmaceutical formulation of zonisamide (Table 8).

Table 8: Assay

Formulation	Labeled Amount (mg)	Amount*Obtained(mg)	%Purity ± SD
Zonisep (Capsules)	100	99.86	99.86%± 0.685

*Average of three determinations

CONCLUSION

A simple, economic, precise & accurate UV spectrophotometric method for estimation of Zonisamide in bulk and in formulation was developed. This developed method was validated according to ICH guidelines. Beer's law was obeyed in concentration range of 5-30 µg/ml. The correlation coefficient (r^2) for Zonisamide was found to be 0.999. The % recoveries were found to be in the range of 99.84-100.18% for Zonisamide. The precision of method was determined by repeatability, intraday and interday precision whose % RSD < 1% indicates the precision of the method. The Limit of detection for Zonisamide was found to be 0.0243 µg/ml. Limit of quantitation for Zonisamide was found to be 0.0738 µg/ml. The proposed method was found to be simple, accurate, precise, and reproducible and gave acceptable recovery of the analyte which can be applied to analysis of bulk and pharmaceutical capsule formulation of zonisamide. Additionally the short analysis time and low cost is the other advantages of these methods for routine analysis. Its advantages over other existing methods are simplicity, speed and low cost.

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