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DEVELOPMENT AND VALIDATION OF SIMPLE UV SPECTROSCOPIC METHOD FOR ESTIMATION OF ZONISAMIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Zonisamide is a benzisoxazole derivative, used as an adjunctive antiepileptic in the treatment of partial seizure. A Simple, economic, reproducible & precise spectroscopic method has been developed for estimation of Zonisamide in bulk and pharmaceutical dosage form. Estimation was carried out at λ_{max} 240nm using methanol as solvent in stock solution and subsequent dilution with distilled water. The linearity was observed in the range of 1-40 µg /ml with correlation coeffient (r^2) 0.999.The percentage recovery was found to be in range of 99-100%. The proposed method was validated as per ICH guideline and can be applied for estimation of Zonisamide in pharmaceutical dosage forms in routine analysis.

KEY WORDS

Zonisamide, spectroscopic method, methanol, distilled water, routine analysis

INTRODUCTION

Zonisamide is a benzisoxazole derivative, used as an adjunctive antiepileptic in the treatment of partial seizure[1, 2]. Zonisamide may be a carbonic anhydrase inhibitor although this is not one of the primary mechanisms of action. Zonisamide may act by blocking repetitive firing of voltage-gated sodium channels leading to a reduction of T-type calcium channel currents, or by binding allosterically to GABA receptors[3]. It is official in US pharmacopoeia[4]. Various UV spectrophotometric methods for estimation of zonisamide based on condensation[5], complex and redox reaction[6] were developed. Several methods have been reported for analysis of Zonisamide using gas chromatography (GC) [7], micellar electrokinetic capillary chromatography[8,9,10], enzyme immunoassay[11], high performance liquid chromatography (HPLC) with UV detection using solid phase extraction[12]. HPLC methods for determination of impurity and degradation products for Zonisamide were also reported[13, 14]. Ion pair HPLC[15], RP-HPLC[16], stability indicating HPLC[17], LC method[18] and HPTLC method for simultaneous determination of lamotrigine, zonisamide and levetiracetam in human plasma[19] were also developed. So, here we have developed simple, economic, precise & accurate method for the quantitative estimation of Zonisamide in bulk drug and its pharmaceutical dosage form.

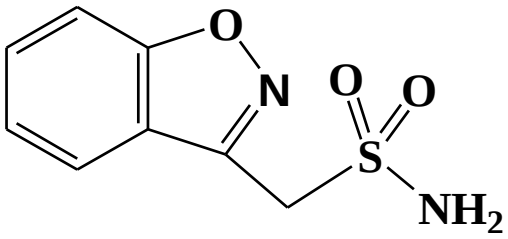

Figure 1: Chemical structure of Zonisamide

MATERIALS AND METHODS

Instrumentation

A SHIMADZU 1800 UV-VISIBLE spectrophotometer with 1.0 cm matching quartz cells were used for absorbance measurements. The UV spectra were recorded over the wavelength 400-800 nm.

Chemicals and Reagents

All reagents and chemicals used were of Analytical Grade. Gift sample of Zonisamide was supplied by BDR lifesciences, Baroda. Marketed formulation was procured from local market.

Preparation of standard stock solution

Accurately weighed 10 mg of Zonisamide was transferred into 100 ml volumetric flask and dissolved in methanol. Sonicate for 5 min and diluted up to the mark with methanol to get a stock solution containing 0.1mg/ml of Zonisamide (100 µg/ml Zonisamide)

Selection of Analytical Wavelength

Different aliquots like 0.1 ml, 1 ml, 2 ml, 3 ml, and 4 ml were withdrawn from the standard stock solution in to 10 ml volumetric flasks and dilute upto mark with water to produce range of concentrations of 1 – 40 µg/ml of Zonisamide and spectrum was recorded between 200-400 nm. The absorbances of solution were measured at 240 nm for the estimation of drug by proposed method.

Validation of proposed method

The proposed method was validated according to the ICH Guideline Q2 (R1)[20]. Method was validated in terms of Linearity, precision, Accuracy, Limit of detection (LOD) and Limit of Quantification (LOQ). Accuracy can be expressed as percentage recovery of the known amount of the standard drugs added to the known amount of the pharmaceutical dosage form. The precision (R.S.D) was expressed with respect to the repeatability, intra-day and inter-day variation in the expected drug concentrations. After validation, the developed methods have been applied to pharmaceutical dosage form.

Calibration curve (Linearity)

Appropriate volume of aliquot from Zonisamide standard stock solution was transferred to volumetric flask of 10 ml capacity. The volume was adjusted to the mark with distilled water to give solutions containing 1-40 µg/ml of Zonisamide. Calibration curve was constructed by plotting absorbance versus concentrations for drug. Linear regression equation was obtained from this calibration curve. The Beer-Lambert's concentration range was found to be 1-40 µg/ml for Zonisamide.(**Figure 3**)

Precision

Precision was checked in terms of repeatability, inter and intraday precision. Relative standard deviation was calculated and was within limit (Not more than 2%).

Repeatability

The repeatability was evaluated by assaying 6 times of sample solution prepared for assay determination. Relative standard deviation (R.S.D) was calculated.

Interday and intraday precision

The intraday and interday precision study of Zonisamide was carried out by estimating different concentrations of Zonisamide (1, 20, 40 µg/ml) 3 times on the same day and on 3

different days and the results were reported in terms of Relative standard deviation.

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 3 different concentration levels 80, 100 and 120% taking into consideration percentage purity of added bulk drug samples. Each concentration was analyzed 3 times and average recoveries were measured.

Determination of 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 according to below equation given by ICH guideline.

$$LOD = 3.3\ \sigma/S$$

$$LOQ = 10\ \sigma/S$$

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

Determination of Zonisamide from Capsule dosage form

Sample Preparation

Powder of 10 capsules (Zonisep) was weighed and mixed. The net content of capsule was found. Capsule content powder quantity equivalent to 50 mg Zonisamide was accurately weighed and transferred to volumetric flask of 100 ml capacity. 60 ml of methanol was transferred to this volumetric flask and sonicated for 10 min. The flask was shaken and volume was made up to the mark with methanol. The above solution was filtered through whatman filter paper. From this solution 2 ml was transferred to volumetric flask of 100 ml capacity. Volume was made up to the mark with distilled water to give a solution containing 10µg/ml Zonisamide. The resulting solution was analyzed by proposed method. The quantitation was carried out by keeping these values to the straight line equation of calibration curve.

RESULTS AND DISCUSSION

A simple, economic, precise & accurate method for estimation of Zonisamide in bulk and in formulation was developed. This developed method was validated according to ICH guidelines. The summary of validation parameters for proposed method was given in **Table 1**.The spectrum of different concentration of Zonisamide reveals that drug gives maximum absorbance at 240 nm wavelength (**Figure 2**). So, 240 nm wavelength was used for detection of Zonisamide.

Linearity was assessed for Zonisamide by plotting calibration curve of the absorbance at selected wavelength versus concentration range 1-40 µg/ml. The correlation coefficients (r^2) for Zonisamide was found to be 0.9999 (**Figure 3**). The following equation for straight line was obtained for Zonisamide.

$$Y = 0.0456x + 0.0006$$

The % recoveries were found to be in the range of 99.85-100.63% for Zonisamide (**Table 5**). The precision of method was determined by repeatability, intraday and interday precision and was expressed as the R.S.D. (**Table 1, 3, 4**), which indicate good method precision. The Limit of detection for Zonisamide was found to be 0.28µg/ml. Limit of quantification for Zonisamide was found to be 0.85 µg/ml (**Table 1**). Spectrophotometric method was successfully applied to Zonisamide capsule dosage form. The results are shown in **Table 6**.

Table 1: Summary of Validation Parameters of proposed Spectroscopic Method

Parameter	Zonisamide
Accuracy (% Recovery)	99.85-100.62%
Repeatability (n=6) (R.S.D)	0.79
Interday precision (n=3) (R.S.D)	0.70
Intraday precision (n=5) (R.S.D)	1.24
Limit of detection µg/ml (LOD)	0.28
Limit of quantification µg/ml (LOQ)	0.85

Table 2: Statistical Data of Zonisamide

Parameter	Zonisamide
Analytical Wavelength	240 nm
Range	1-40 µg/ml
Slope	0.0456
Intercept	0.0006
Regression Coefficient	0.999
Standard deviation of slope	0.000239
Standard deviation of intercept	0.000838
Repeatability (R.S.D.)	0.79

Table 2: Linearity Data of Zonisamide

Sr no.	Concentration µg/ml	Mean ± S.D	R.S.D
1	1	0.043±0.0008	1.91
2	10	0.456±0.0006	0.14
3	20	0.914±0.0036	0.39
4	30	1.376±0.0070	0.51
5	40	1.821±0.0073	0.40

Table 3: Intraday precision Data of Zonisamide (n=5)

Sr no.	Concentration µg/ml	Mean ± S.D	R.S.D
1	1	0.055±0.0008	1.51
2	20	0.914±0.0022	0.24
3	40	1.826±0.0062	0.34

Table 4: Interday precision Data of Zonisamide (n=3)

Sr no.	Concentration µg/ml	Mean ± S.D	R.S.D
1	1	0.057±0.001	1.75
2	20	0.913±0.010	1.18
3	40	1.796±0.0195	1.08

Table 5: Accuracy Data of Zonisamide (n=3)

Name of Drug	Level	Amount of drug in powder(mg)	Amount of Standard Spiked (mg)	Actual amount (µg/ml)	Average of Amount Recovered (Mean)(µg/ml)	Recovery (%) (mean ± S.D)	RSD
Zonisamide	80%	50	40	18	18.02	100.11±0.306	0.30
	100%	50	50	20	20.12	100.63±0.660	0.65
	120%	50	60	22	21.96	99.85±0.511	0.51

Table 6: Assay Results of Marketed Formulation (n=3)

Formulation	Actual concentration (µg/ml)	Amount obtained (µg/ml)	% Zonisamide ± S.D
Capsule (Zonisep)	10	9.90	99.06±0.89

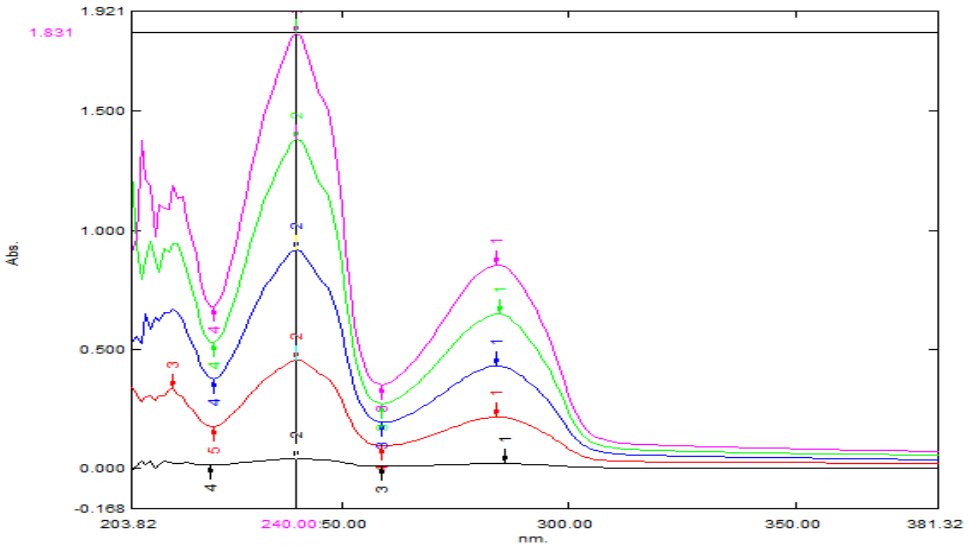

Figure 2: Spectra of Zonisamide of different concentration at 240 nm.

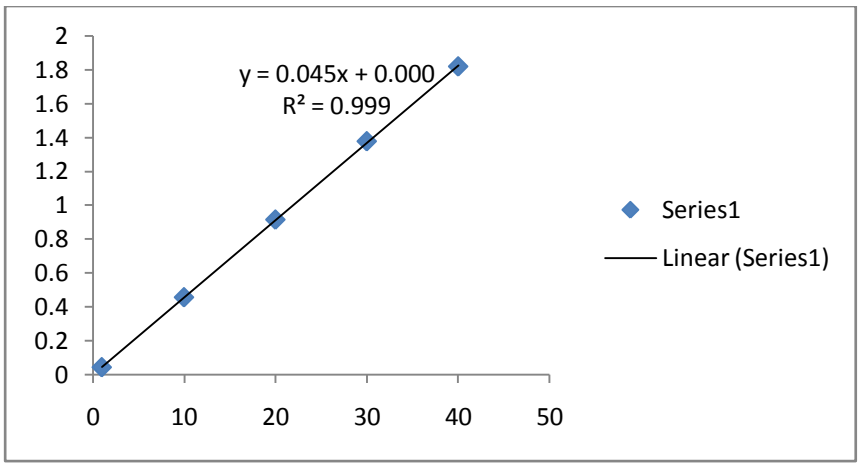

Figure 3: Calibration curve of Zonisamide standard solutions at 240 nm.

CONCLUSION

The proposed UV spectroscopic method provides simple, precise, accurate and reproducible quantitative analysis for determination of Zonisamide in pharmaceutical formulation. The method was validated as per ICH guidelines in terms of linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ). The proposed method can be used for routine analysis and quality control assay of Zonisamide in pharmaceutical dosage form.

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REFERENCES

1. Zonisamide (Zonegran) Verdict and summary, Midlands's therapeutics review and advisory committee , (March 2007)
2. Angus A., L James W., Zonisamide – a review of experience and use in partial seizures, Neuropsychiatric Dis. Treat, 2(3):269–280 (Sep 2006)
3. United State Pharmacopoeia, Volume III, 34th edition, United State Pharmacopoeial Convention, Rockville, 4638, (2011)
4. Ashok reddy S., chandrashekar kb, Spectrophotometric determination of Zonisamide bulk and in tablet dosage form by using p-dimethylaminobenzaldehyde, International journal of advances in pharmaceutical research, 2(11):582-6, (2011)
5. Sudha Lakshmi P. and Rambabu C., Assay of Zonisamide by Redox and Complex Reactions, Journal of Pharmacy Research, 4(9): 3222-3223, (2011)
6. Greiner E, Sosanko S, Darla R, Lower MA, Matthew D. Drug Monitoring: Simultaneous Analysis of Lamotrigine, Oxcarbazepine, 10-Hydroxycarbazepine, and zonisamide by HPLC-UV and a Rapid GC Method Using a Nitrogen-Phosphorus Detector for Levetiracetam. J Chromatographic Sci. ,45:616–22, (2007)
7. Thormann W, Theurillat R, Wind M, Kuldvee R. Therapeutic drug monitoring of antiepileptics by capillary electrophoresis characterization of assays via analysis of quality control sera containing 14 analytes. J Chromatographic Sci. ,924:429–37, (2001)
8. Kataoka Y, Makino K, Oishi R. Capillary electrophoresis for therapeutic drug monitoring of antiepileptics. J Electrophoresis.,19:2856–60, (2005)
9. Kazutaka M, Goto Y, Sueyasu M, Futagami K, Kataoka Y, Ois R. Micellar electrokinetic capillary chromatography for therapeutic drug monitoring of zonisamide. J Chromatogr B Biomed Sci Appl. ,695:417–25, (1997)
10. Kalbe K, Nishimura S, Ishii H, Sunahara N, Kurooka S. Competitive binding enzyme immunoassay for zonisamide, a new antiepileptic drug, with selected paired-enzyme labeled antigen and antibody. J Clin Chem. ,36:24–7, (1990)
11. Yamashita S, Furuno K, Kawasaki H, Gomita Y, Yoshinaga H, Yamatogiand Y, et al. Simple and rapid analysis of lamotrigine, a novel antiepileptic, in human serum by high-performance liquid chromatography using a solid-phase extraction technique. J Chromatogr B Biomed Sci Appl.,670:354–7, (1998)
12. Vijayakumar E., Dhore D., Kumar M., HPLC Method for Simultaneous Determination of Impurities and Degradation Products in Zonisamide, Indian Journal of Pharmaceutical science, 71(5): 521–526, (2009)
13. Maryam H., Alipour E., Arezou F., Determination and Validation of Zonisamide and its Four Related Substances by HPLC and UV-Spectrophotometry, Indian Journal of Pharmaceutical science, 72(3): 302–306, (2010)
14. Ananda reddy K., Determination of zonisamide in capsule dosage form by using RP-HPLC, Int. J. Chem. Sci. , 9(4): 1698-1704, (2011)
15. Udaykumar Rao B., Nikalje P., Determination of Furosemide and Zonisamide as a Drug Substance and in Dosage Form by Ion Pair –Reversed Phase Liquid Chromatographic Technique, Journal of Applied Pharmaceutical Science ,02 (05): 94-99, (2012)
16. Rao D., Chakravarthy i., Kumar S., Stability Indicating HPLC Method for the Determination of Zonisamide as Bulk Drug and in Pharmaceutical Dosage Form, Chromatographia , 64(5-6):261-66, (2006)
17. Dny; eo B. Pathare, Ashok S. Jadhav; Murlidhar S. Shingare, Stability indicating LC method, Chromatographia, 66 (11-12): 945-947, (2007)
18. Development and validation of an analytical method based on high performance thin layer chromatography for the simultaneous determination of lamotrigine, zonisamide and levetiracetam in human plasma, Journal of pharmaceutical and biomedical analysis,763-70
19. ICH Harmonized Tripartite Guideline, Q2 (R1) Validation of Analytical Procedures, current step 4 version, Parent guideline dated 6 November (1996)

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