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Development of Stability-Indicating Spectrophotometric Methods for the Analysis of Zonisamide in Bulk and Dosage Form

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ABSTRACT

The objective of this research was to develop simple, sensitive and stability-indicating zero (^{0}D), first (^{1}D) and second (^{2}D) order derivative spectrophotometric methods for the analysis of zonisamide (ZON) in bulk and dosage forms. The original UV spectrum (zero-order) of ZON aqueous solution was measured at 284 nm against its blank. This spectrum was differentiated instrumentally to generate the first and second derivative spectra which were measured at 271+ 295 nm and 302+ 284 nm, respectively. The developed methods were validated as per ICH guidelines. Also the absorbance ratio between ZON absornance at 239 nm and 284 nm was determined. ZON degradation behavior in both acidic and alkaline media was investigated using first and second derivative spectroscopic methods. ZON obeyed beers law over the concentration ranges (10 – 60) µg/ ml for ^{0}D and ^{1}D and (20-100) µg/ ml for ^{2}D. The correlation coefficient (r) was found to be (0.999 for ^{0}D, 0.999 for ^{1}D and 0.9989 for ^{2}D). The detection and quantitation limits were found to be (LOD= 2.08 for ^{0}D, 1.38 for ^{1}D and 9.53 for ^{2}D) µg/ ml; LOQ=6.93 for ^{0}D, 4.62 for ^{1}D and 31.8 for ^{2}D) µg/ ml. The precision of the developed methods were generally very good as RSD% values were ≤ 5%. The zero-order derivative spectrum of ZON shows two sharp bands at 239 nm and 284 nm. The ratio between the absorbance at these wavelengths was found to be in the range (1.9–2.3) which can be used for qualitative analysis of ZON. Regarding ZON stability profile, it showed that the drug is unstable under acidic and alkaline conditions as it undergo degradation following the first order kinetics and it was found to be unstable in outdoor conditions also.The statistical validation at 95% confidence level proves the sensitivity, precision, accuracy and the stability-indicating properties of the developed methods.

Introduction

Zonisamide (ZON) is a benzisoxazole derivative (sulfonamide derivative); structurally it is 1, 2-benzoxazol-3-ylmethanesulfonamide (Fig. 1). It has the molecular formula ($C_8H_8N_2O_3S$), used as an adjunctive antiepileptic in the treatment of partial seizure. The exact method by which ZON exerts its anticonvulsant effect is unknown. It appears then, that ZON does not potentiate the synaptic activity of GABA. ZON also serves as a weak inhibitor of carbonic anhydrase [1].

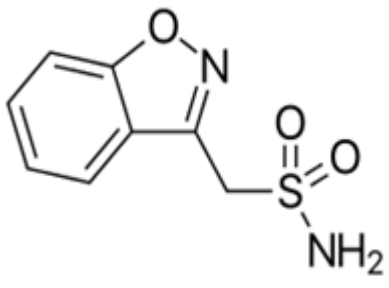

Fig 1. Chemical structure of ZON.

The stability of a drug substance or drug product is defined as its capacity to remain within established specifications, i.e. to maintain its identity, strength, quality, and purity until the retest or expiry date. Stability testing of an active substance or finished product provides evidence of how the quality of a drug substance or drug product varies with time under a variety of environmental conditions [2-5]. Development of stability-indicating methods using stress degradation is recommended by International Conference of Harmonization [6]. Drug stability studies results are very important in drug self-life estimation and the effect of degradation products on decreasing efficacy and causing toxicity. It can guide drug design, formulation and drug analysis [7].

In addition to the official HPLC method, there are some other reported methods used for the assay of ZON In bulk and pharmaceutical formulations or biological fluids reviewed from the literature. Several methods have been reported for the analysis of ZON. Those methods include gas chromatography (GC) [9], micellar electro kinetic capillary chromatography [9-10] enzyme immunoassay [12], high performance liquid chromatography (HPLC) with UV detection using solid phase extraction [13] and HPLC method only for ZON by different conditions [13-19]. No derivative

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spectrophotometric method have been published till date for the analysis and of ZON, also no literature is reported for stability studies by derivative UV spectrophotometric method. Therefore it is deemed useful to develop a simple and accurate derivative spectrophotometric method for the analysis and stability studies of ZON.

Experimantal

Instrumentation

UV spectrophotometric studies were carried out on Shimatzu UV- 1800EN240V, double beam, (Kyoto, Japan). The operating conditions were as follow:

- Wavelength range: 200-400 nm.
- Scan speed: Medium, 0.2 nm/s.

Sensitive balance: Kern ALS 120-4, Germany

Reference, sample and reagents

ZON reference standard was purchased from Lab CO in UAE (98%). ZON Capsules (Zonisep®) 25 mg were kindly provided by a colleague from India. All solutions were prepared using methanol as solvent.

Standard stock solution

Standard stock solution was prepared by dissolving 0.01 g of ZON standard in 100 ml methanol (Solution A; 100 µg/ml).

Sample solution

A quantity of the capsules powder containing 0.01 g (0.03832 g) was dissolved in 20 ml methanol and transferred to 100 ml volumetric flask. The volume was then completed to mark with methanol and the solution was sonicated for 10 min and filtered (Solution B; 100 µg/ml).

Procedures

Determination of λmax

The standard stock solution of ZON was diluted to obtain a concentration of 40µg/ml. The solution was scanned vs blank within the range 200-400 nm at 0D, 1D and 2D order derivative modes, respectively.

Absorbance Ratio determination for identification of ZON in bulk and dosage forms

Solution A and B were diluted to obtain serials dilution 20, 40, 60, 80 and 100 µg/ml. The resulted solutions were scanned within the range 200-400 nm.

Method validation

Linearity

Serial dilutions were made from both ZON standard and sample solutions (100µg/ml) by transferring accurately measured volumes (2, 4, 6, 8 and 10 ml) into a set of 10 ml volumetric flasks. The volumes were then completed to mark with methanol. The first order derivative and the second order derivative spectra were recorded over the range 200-400 nm. The procedure was repeated three times. The mean absorbance values were plotted against concentration to construct the calibration curves. The regression data was calculated for the mean calibration curves.

Limit of detection and quantification were determined from the calibration curve using adopted formulae (20)

LOD = 3.3 SB/Slope LOQ = 10SB/Slope

Where SB is the standard deviation $s_{y/x}$ calculated from the regression analysis data.

Content uniformity

The procedure under linearity was repeated using solution B instead of solution A. the content uniformity of the capsules solution was evaluated by the direct comparison of sample/standard absorbance values or linearity data.

Precision

Serial dilutions from solution A were carried out to obtain concentrations of 20 µg/ml, 40 µg/ml and 60 µg/ml of ZON. These solutions were scanned at three modes (0D, 1D and 2D) three times within the same day (inter-day) and at three consecutive days (intra-day). The results obtained were used to evaluate the precision of the developed method in terms of relative standard deviation values (RSD **%).**

Recovery percentage

The freedom of interference by the capsule excipients was confirmed by results obtained for recovery testing of added amount of authentic ZON to the sample solution in the ratio of 1:1. Two ml of each solution A and B were transferred to separate stoppered glass tubes. Another 2 ml of solution B was mixed with 2 ml of solution A in a third tube. The above solutions were scanned at the three modes. The recovery percentage was determined using the following equation (20)

Percent Recovery = [(Amix- Asam)/Astd] ×100

Where Amixis the absorbance of mixture; Asam is the absorbance of sample, Astdis the absorbance of the standard.

Effect of Acid on the stability of ZON solution

Two ml of solution B were transferred to four stoppered glass tubes then 1 ml of 1 M HCL was added to each tube. The volumes were then completed to 10 ml with methanol. The first and second derivative spectra for the solution in the first tube were recorded. The rest three solutions were heated in a boiling water bath for 5, 15 and 25 min respectively. The solutions were then cooled. The effect of the acid on the degradation of ZON with and without heating was monitored by the first and second derivative spectrophotometric methods.

Effect of Alkali on the stability of ZON solution

The above procedure described above for the effect of acid on ZON stability was followed using 0.1 M NaOH instead of 1M HCl.

Light effect

20 µg/ml of ZON in methanol was prepared in 25 ml flask. The absorbance of this solution was measured at zero time and then exposed to sunlight for 6 hours. The solution was then scanned to evaluate the effect of light on ZON stability.

Results and Discussion

Determination of λmax

The zero order derivative spectrum of ZON showed absorption maximum at 239nm (possible transition due to π-π*) and 284 nm (possible transition due to n-π*) (Fig. 2). First and second derivatization of the resultant spectrum showed bands at 271nm, 295 nm and 302nm, 284 nm respectively. (Fig.3 and 4)

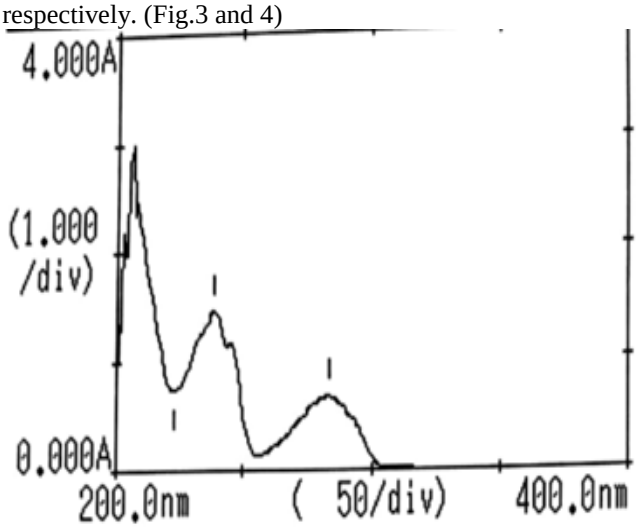

Fig.2. UV spectrum of ZON solution 40 µg/ml; 239, 284 nm.

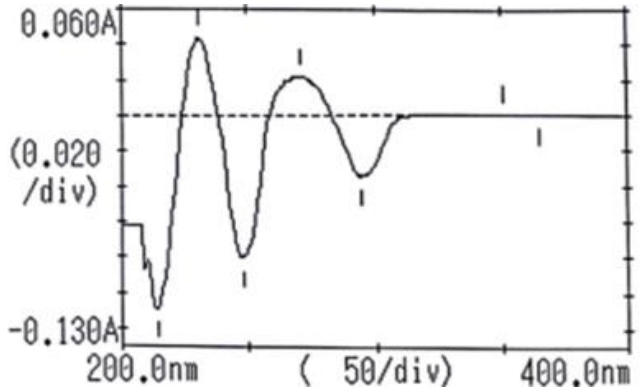

Fig.3. First derivative spectrum of ZON solution 40 µg/ml.

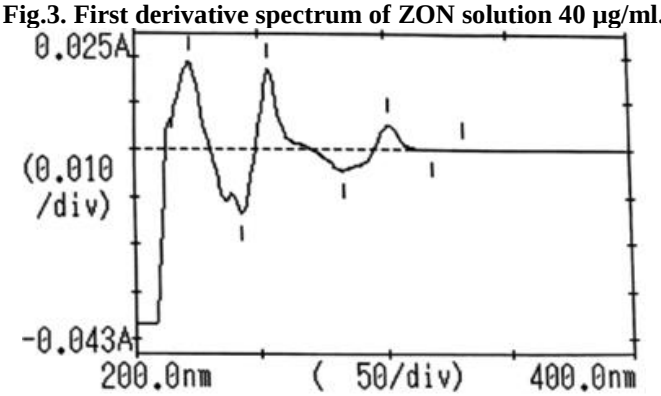

Fig. 4. Second derivative spectrum of ZON solution 40µg/ml.

Absorbance Ratio determination for identification of ZON in bulk and dosage forms

The zero-order derivative spectrum of ZON showed two sharp bands at 239 nm and 284 nm (fig 2). The ratio between the absorbance at these wavelengths using the below formula was found to be in the range between 1.9 –2.3 for all solutions.

Absorbance Ratio = Absorbance at 239nm / Absorbance at 284nm

Linearity

The calibration curves, relating the ZON concentration in a range 10- 60 µg/ml to the mean absorbance values, were constructed for the three modes. Linearity was found to obey Beer's law with a good correlation coefficient (not less than 0.998). The regression analysis data was calculated at 95% confidence level for the developed method using the following formula (20)

$$y = (b \pm ts_b)\, x + (a \pm ts_a)$$

Where b is the slope, a is the intercept, S_b is the standard deviation of the slope, S_a is the standard deviation of intercept, t is the t-value at 95% confidence level for (n–2) degrees of freedom.

The results obtained for linearity data of the developed methods are summarized in table 1, which reflected the accuracy and consistency of these curves.

Table 1. Linearity data of the developed methods (n =3).

Parameter	^{0}D	^{1}D	^{2}D
λ_{max}	284 nm	271+295 nm	302+284 nm
Concentration range	10 - 60 µg/ml	10-60 µg/ml	20-100µg/ml
Slopes ± SD	$0.0163 \pm 1.24 \times 10^{-3}$	$1.64 \pm 8.28 \times 10^{-5}$	$2.55 \times 10^{-4} \pm 7.48 \times 10^{-5}$
Intercept ± SD	-0.0109 ± 0.0483	$5.3 \times 10^{-4} \pm 3.24 \times 10^{-3}$	$2.55 \times 10^{-4} \pm 4.9 \times 10^{-3}$
lOD	2.08 µg/ml	1.38 µg/ml	9.53 µg/ml
LOQ	6.93 µg/ml	4.62 µg/ml	31.8 µg/ml
R	0.999	0.999	0.9989

Assay and validation

The developed methods were applied for the drug uniformity testing in ZON capsule. Good assay results ranged from 98.4% to 107.3% n=3 were obtained.

Precision

The inter-day and intra-day precision was studied at all modes. RSD% values were found to be within 0.00-3.86% (inter-day) and 0.00-4.6% (intra-day). The precision of the developed methods were generally satisfactory as RSD% values were ≤5%.

Recovery percentage

The accuracy of the developed methods at the three modes and freedom of interference by ZON capsule excipients were confirmed by good results of recovery testing (101.9 ± 1.7 %, n=3)

Effect of Acid on the stability of ZON solution

The effect of different acid concentrations with different heating time intervals on the stability of ZON were investigated using the developed methods. 1ml of 1M HCl with 5minutes heating time interval was found appropriate to study its effect. The degradation rate was calculated by plotting the log % remained drug vs time (Fig. 5). The degradation process was found to be a first order reaction.

Effect of Alkali on the stability of ZON solution

ZON peak and concentration were found to decrease upon treating with 1ml of 0.1M NaOH and heating for 5 minutes interval. The percentage of degradation was found to be up to 88% in 25 minutes. The degradation process followed first order kinetics due to the obtained linear plot of log % remained drug vs heating time (Fig. 6).

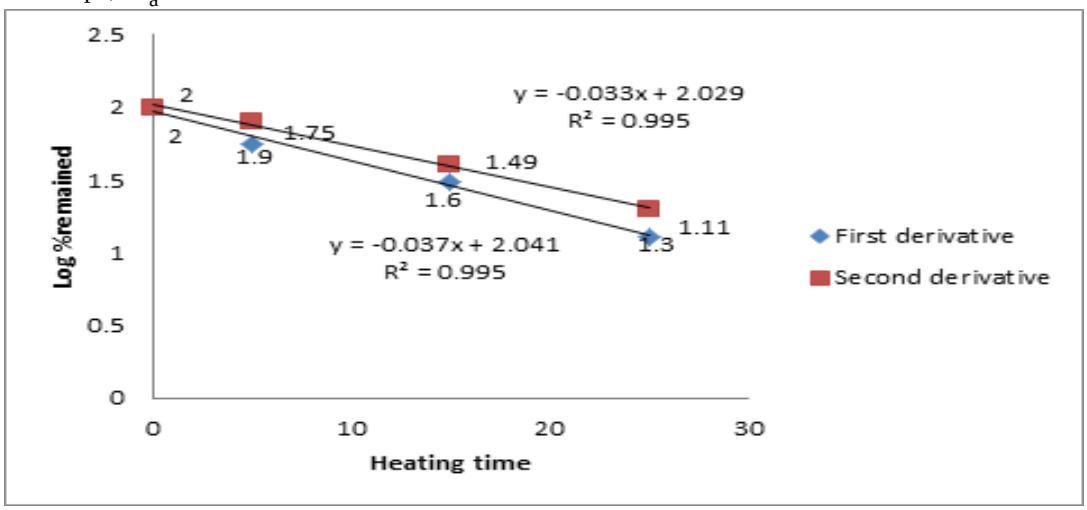

Fig 5.Effect of 1 M HCl on ZON degradation at 100 °C using first and second derivative methods.

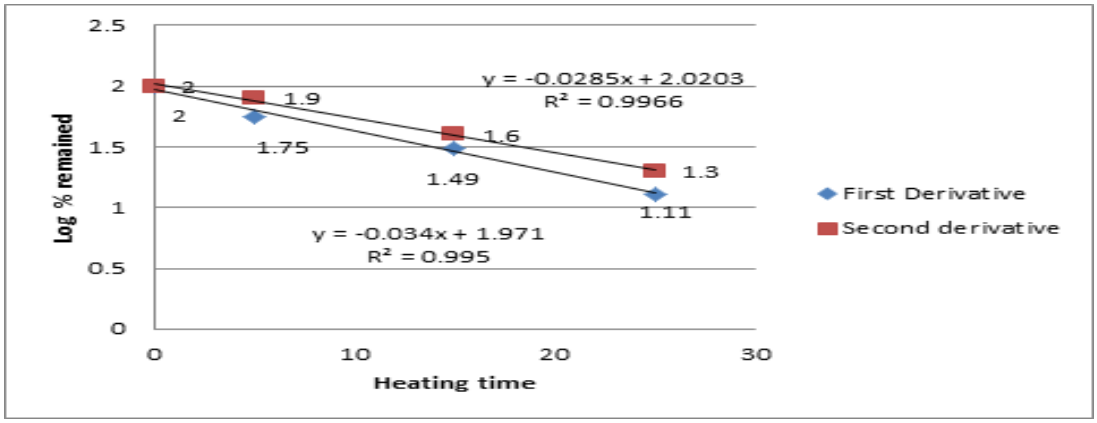

Fig 6. Effect of 0.1 M NaOH on ZON degradation at 100 °Cusing first and second derivative methods.

Effect of light

Sharp decrease in ZON solution concentration occurred upon exposure to sunlight for 6 hours. The concentration at 1D, 2D modes was decreased to almost half of the initial concentration (40%). However, no further concentration reduction occurred until 14 days in outdoor condition. This reflects the instability of ZON to light.

Conclusion

The developed spectrophotometric methods proved to be simple, stability indicating, rapid, accurate and precise for the determination of ZON in bulk and dosage forms. In addition, the procedure of the developed method does require neither extraction step nor chemicals and thus can be used for routine analysis of the drug.

The results of the zero-order method (0D) reflected good precision. However, its direct application is expected to be limited for samples free from irrelevant absorption; on the other hand ^{1}D and ^{2}D methods proved their stability-indicating properties. Results showed that the drug is unstable under acidic and alkaline conditions as it undergoes degradation following the first order kinetics and it was found to be unstable in outdoor conditions also. The method seems to be suitable for the quality control in pharmaceutical industry because of its simplicity and selectivity.

Conflict of Interests

All authors have none to declare

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