VALIDATED STABILITY INDICATING SPECTROPHOTOMETRIC METHODS FOR QUANTITATIVE DETERMINATION OF ANTICONVULSANT DRUG ZONISAMIDE IN THE PRESENCE OF ITS OXIDATIVE DEGRADATION PRODUCT[**]

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Four simple, reproducible and rapid stability-indicating spectrophotometric methods were developed and efficiently applied for the estimation of zonisamide (anticonvulsant drug) in the presence of its oxidative degradation product. The degradation pathway was clearly illustrated and the structure of the degradation product was elucidated by infrared and mass spectroscopy. The proposed methods were the direct spectrophotometric method (D^0), the first derivative method (D^1), the ratio difference method (RD) and, finally, the first derivative of the ratio spectra method ^{1}DD. Good sensitivity and high reproducibility were obtained via the developed methods. Linearity ranges were 5–40 μg/mL for the first method and 2–40 μg/mL for the other three methods. Mean recovery percentages of the methods were 100.12±0.590 for D^0, 99.97±0.606 for D^1, 99.88±0.546 for RD, and 99.94±0.619 for ^{1}DD. The specificity of the methods was investigated by analyzing synthetic mixtures of different percentages of zonisamide and its oxidative degradation product. The proposed methods were validated in accordance with ICH guidelines. Statistical analysis showed no significant differences in comparison with the official method.

Keywords: *zonisamide, stability-indicating, first derivative, ratio difference, derivative ratio.*

СПЕКТРОФОТОМЕТРИЧЕСКИЕ МЕТОДЫ КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ ПРОТИВОСУДОРОЖНОГО ПРЕПАРАТА ЗОНИСАМИДА В ПРИСУТСТВИИ ПРОДУКТА ЕГО ОКИСЛИТЕЛЬНОЙ ДЕГРАДАЦИИ

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Для оценки противосудорожного препарата зонисамида в присутствии продукта его окислительного разложения разработаны четыре простых и воспроизводимых спектрофотометрических метода. Проиллюстрирован путь деградации, структура продукта деградации установлена с помощью инфракрасной и масс-спектроскопии. Предложены прямой спектрофотометрический метод (D^0), метод первой производной (D^1), метод разности отношений (RD) и метод первой производной отношений спектров (^{1}DD). Методы показали хорошую чувствительность и высокую воспроизводимость, диапазоны линейности 5–40 мкг/мл для первого метода и 2–40 мкг/мл для трех других методов, средние проценты восстановления: 100.12±0.590 для D^0, 99.97±0.606 для D^1, 99.88±0.546 для RD и 99.94±0.619 для ^{1}DD. Методы применены для анализа синтетических смесей с разным содер-

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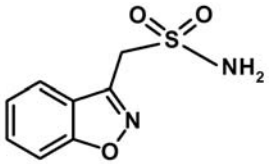
жанием зонисамида и продукта его окислительного разложения. Предложенные методы проверены в соответствии с рекомендациями ICH. Статистический анализ не выявил существенных различий по сравнению с официальной методикой.

Ключевые слова: *зонисамид, показатель стабильности, первое производное, разность соотношений, соотношение производных.*

Introduction. Zonisamide (ZNS) (chemical name is 1,2-benzisoxazole-3-methanesulfonamide [1])



is a sulfonamide with anticonvulsant properties that has been used as adjunctive therapy with antiepileptic drugs for recurrent seizures and it has a dual mechanism of either blocking the firing of voltage-gated Na-channels or allosterically binding to GABA receptors [2, 3].

Several methods have been published for the quantitative determination of ZNS in both pharmaceutical dosage forms and biological fluids such as the spectrophotometric method [4], HPLC [5, 6], HPTLC [7], and the electroreduction method [8]. Few stability-indicating HPLC methods have been published [9, 10]. To the best of our knowledge, none of the two published methods has clarified the degradation pathway of ZNS and, up till now, neither of them used spectrophotometric methods for its determination in the presence of its degradation products.

The aim of this work is to elucidate the ZNS degradation product structure by IR and mass spectroscopy and, thereafter, clarify the degradation pathway of ZNS. Furthermore, to develop valid, simple and economic effective stability indicating spectrophotometric methods to be an alternative to the costly traditional HPLC.

Experimental. ZNS was kindly supplied by Mash premiere for Pharmaceutical Industries & Cosmetics (Cairo, Badr city, Egypt); its purity was found to be 99.87±0.806 according to the official method [1]. Convagran® hard gelatin capsules manufactured by Mash premiere for Pharmaceutical Industries & Cosmetics (Badr city, Egypt) were purchased from the local market in different concentrations of 25 mg/capsule batch No. M2012417, 50 mg/capsule batch No. M2013617 and 100 mg/capsule batch No. M2000918.

The degradation product was prepared as detailed later in the procedure. All chemicals used throughout this study were of analytical grade–methanol (Merck, Germany), 30% hydrogen peroxide (Adwic, Egypt), NaOH and acetic acid (El NASR Pharmaceutical Chemicals Co., Abu-Zabaal, Cairo, Egypt), hydrochloric acid 37% (Honeywell, USA), chloroform (Sigma-Aldrich, USA) and distilled water prepared in-house by the Aquatron water still A8000 system.

Stock standard solutions of ZNS and its degradation product (1 mg/mL) were prepared in methanol. Working standard solutions of ZNS and its degradation product (100 μg/mL) were prepared from their corresponding stock standard solutions in methanol.

*Procedure: to prepare ZNS degradation product. T*wenty-five milligrams of ZNS pure powder was weighed into a 25-mL volumetric flask, and the least amount of methanol necessary to dissolve ZNS was added. The volume was then completed with 10% H_2O_2 and then refluxed for 16 h. The solution was examined at intervals to check complete degradation on a TLC plate using chloroform–methanol–acetic acid (8:1.5:0.5 by volume) as a developing system. The produced ZNS-degraded solution was purified by methanol many times and then the powder obtained was used for structure elucidation by IR and mass spectroscopy.

Construction of calibration curves. Aliquots equivalent to 20–400 μg of ZNS were transferred from its working standard solution (100 μg/mL) into a series of 10-mL volumetric flask, and then volumes were completed to the mark with methanol. Zero-order absorption spectra were scanned from 200–400 nm using methanol as the blank and saved in computer.

For *direct spectrophotometric method (D^0):* ZNS absorbance values were recorded at 283.5 nm and plotted against their corresponding concentrations range 5–40 μg/mL, then the regression equation was computed.

For *first derivative spectrophotometric method (D^1):* ZNS first derivative spectra were obtained using $\Delta\lambda = 8$ and scaling factor 100. The calibration curve was assessed by plotting peak amplitudes at 295.3 nm against their corresponding concentrations 2–40 μg/mL and then the linear regression equation was computed.

For *ratio difference method (RD):* Ratio spectra were obtained via dividing the saved zero-order absorption spectra of ZNS 2–40 μg/mL by the spectrum of its oxidative degradation product (20 μg/mL). The difference of peak amplitudes at 221.8 and 245.8 nm was calculated. The calibration curve was constructed and the linear regression equation between ZNS concentrations and the difference in peak amplitudes $\Delta P_{221.8–245.8nm}$ were computed.

For *first derivative of the ratio spectra method (^{1}DD):* First derivative of ZNS stored ratio spectra were obtained using $\Delta\lambda = 4$ and scaling factor 10. The peak amplitude of the obtained spectra was measured at 249.2 nm. The calibration curve was constructed between peak amplitude and the corresponding concentrations 2–40 μg/mL, and then the regression equation was computed.

Laboratory-prepared mixtures were prepared by mixing different ratios of the intact drug and its oxidative degradation product and then their spectra were scanned between 200 and 400 nm. The procedures under construction of the calibration curve of each method were followed. Concentrations of ZNS were obtained from the linear regression equation of each method.

Ten hard gelatin capsules of Convagran® of each dosage (25, 50, and 100 mg) were opened and emptied to be mixed separately. Then, into three separate volumetric flasks of volumes (25, 50, and 100 mL), an equivalent amount of mixed powder was accurately weighed to get the standard stock solution of 1 mg/mL each. Methanol was added to complete volumes to the mark and then all flasks were sonicated for 20 min. The solutions were filtered separately and working stock solutions of 100 μg/mL were prepared in 100-mL volumetric flasks each. Then the procedures were completed as described under each method.

Results and discussion. The concept of forced degradation studies emerged in the field of pharmaceutical research to determine the intrinsic stability of the drug in order to prove the quality of the medicinal products under the influences of environmental factors. Degradation studies are used to set a shelf life for the product, suitable packaging materials, and for determining the right environment for storage [11]. Various analytical techniques can be used to estimate the structure of the expected degradation products such as those used in this study—namely mass and IR spectroscopy [12, 13].

ZNS was subjected to different stress conditions with different concentrations of alcoholic HCl and alcoholic NaOH to obtain any potential degradation by following (ICH) guidelines Q1A (R2) [14]. ZNS showed its stability even when refluxed with alcoholic HCl or NaOH of different concentrations (0.1, 1, 2, and 5 N) separately for up to 12 h. Only oxidative degradation was observed when ZNS refluxed with 10% hydrogen peroxide for 16 h at 80°C. Complete degradation was confirmed by testing the solution every 2 h on a TLC plate using chloroform–methanol–acetic acid (8:1.5:0.5 by volume) as a developing system. The observed spot, indicating the occurrence of degradation, was presented as the R_f value different from the R_f value of the intact drug (Fig. 1).

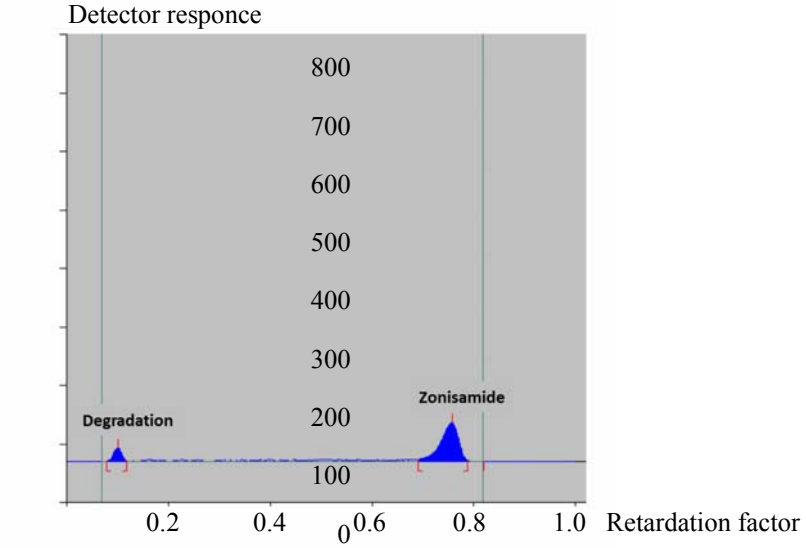


Fig. 1. TLC densitogram of ZNS and its oxidative degradation product using chloroform: methanol: acetic acid 8:1.5:0.5 by volume at 240 nm.

IR and mass spectroscopy were used to elucidate the structure of the degradation product; IR spectrum of the oxidative degradation product is marked by the appearance of C=O and alcoholic OH groups' bands at 1712 and 3000–3500 cm^{-1}, respectively. Moreover, the prominent existence of the sulfonamide group– before and after degradation oxidative condition—appeared as a characteristic band at 1338 cm^{-1} in both spectra (see Figs. 2a,b).

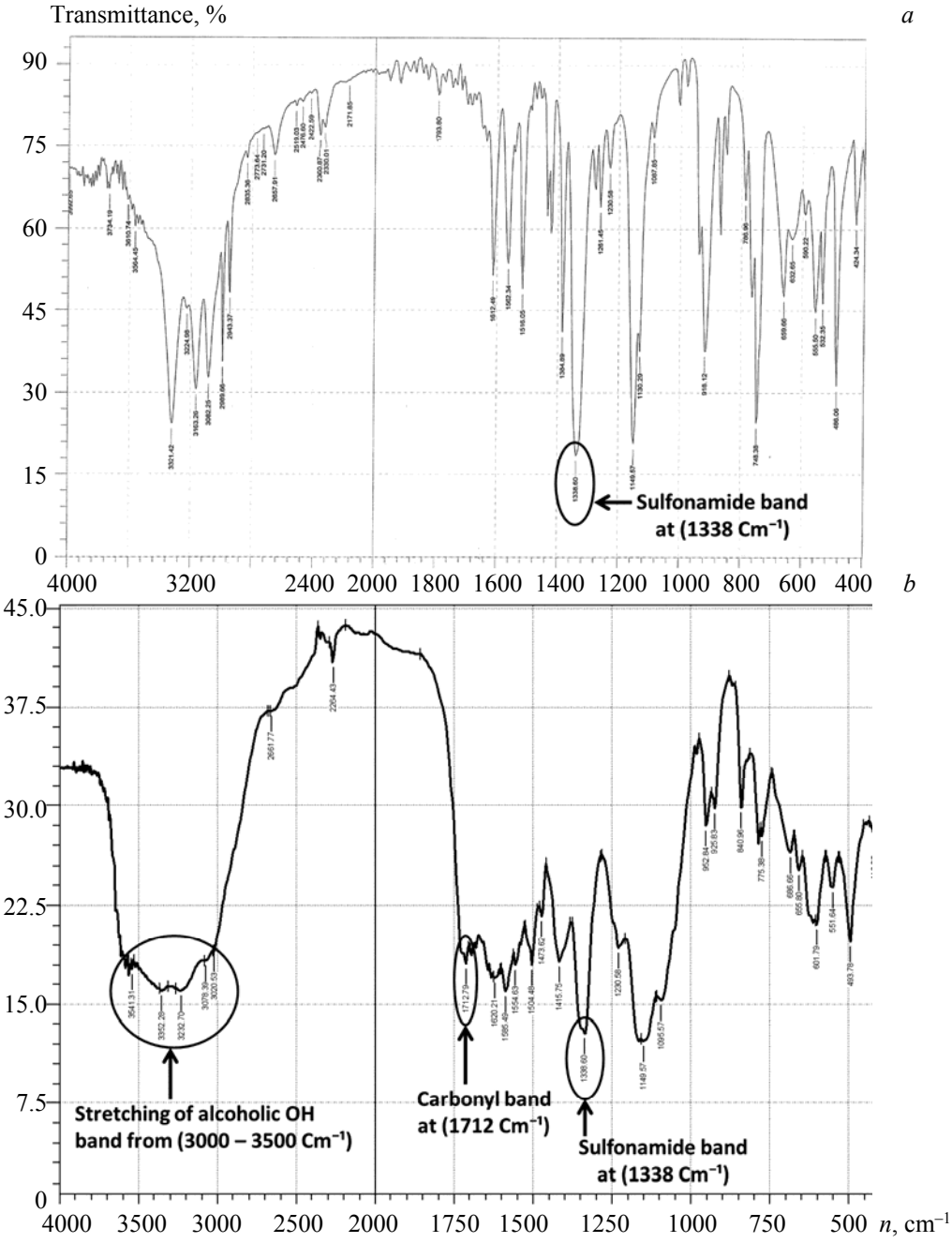


Fig. 2. IR spectrum of (a) pure intact ZNS and (b) its oxidative degradation product.

An electrospray ionization mass spectrometry ESI-MS scan proved the predicted degradation pathway as ZNS negative ion ESI-MS with an exhibited peak at $m/z = 210.88$ (Fig. 3a) and the degradation product positive ion ESI-MS scan revealed a peak at $m/z = 177$ (Fig. 3b). The suggested pathway is represented in Fig. 4.

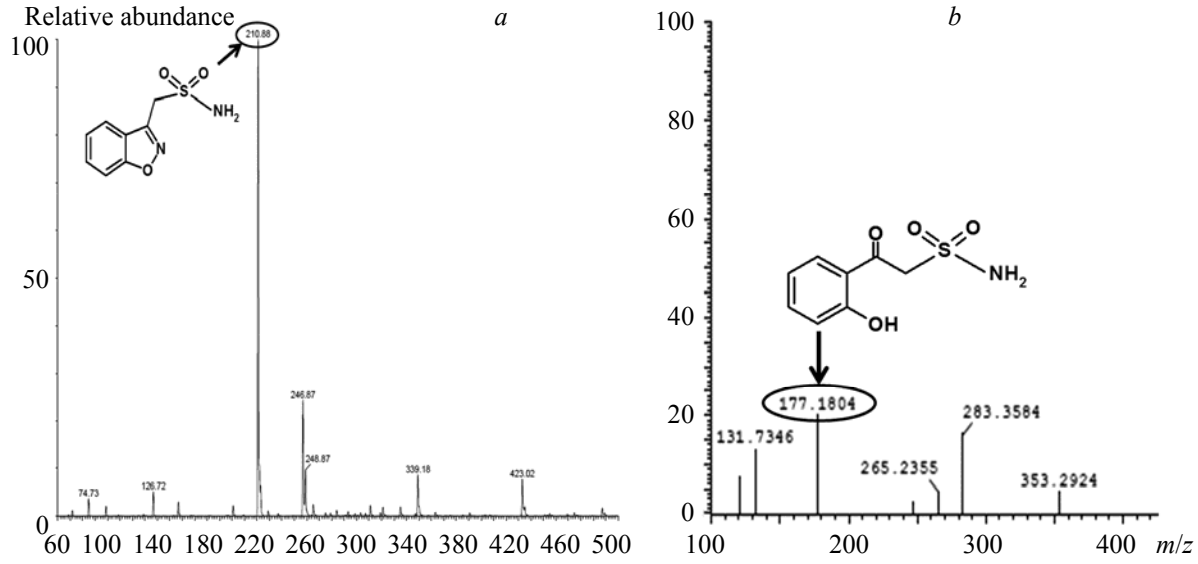


Fig. 3. Negative ESI-MS of (a) ZNS and positive ESI-MS of (b) ZNS oxidative degradation product, m/z = 177.18.

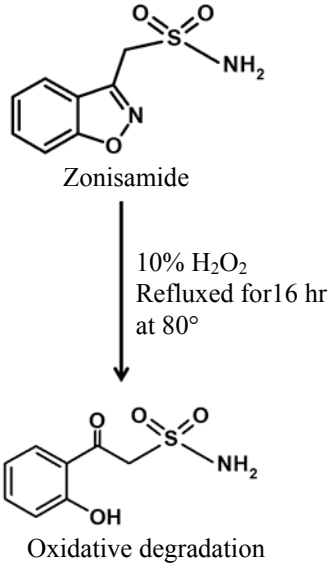


Fig. 4. Suggested pathway for oxidative degradation of ZNS.

Severe overlap was observed between zero-order absorption spectra of ZNS and its oxidative degradation all over the wavelengths except between 260 and 400 nm, allowing classical direct spectrophotometric method to be performed at 283.5 nm (Fig. 5). Simplicity is the main advantage of direct spectrophotometric method [15, 16]. A calibration graph was constructed in the range of 5–40 μg/mL by plotting each absorbance against its corresponding concentration. The linear regression equation was calculated and found to be

$$y = 0.0188x - 0.0006 \text{ and } r = 0.9999.$$

First derivative spectrophotometric method (D^1) [17, 18] was performed to solve the severe overlap between the spectra of ZNS and its oxidative degradation product. The linear relationship was formed between peak amplitude and the corresponding concentrations. The chosen wavelength was (295.3 nm) using $\Delta\lambda$ = 8 nm and scaling factor 100 (Fig. 5b). Therefore, a calibration graph was constructed with a linear regression equation of

$$y = 0.1242x - 0.0071,\ r = 1,$$

where y is the peak amplitude, multiplied by (–1), x is the corresponding concentration in μg/mL, and r is the correlation coefficient.

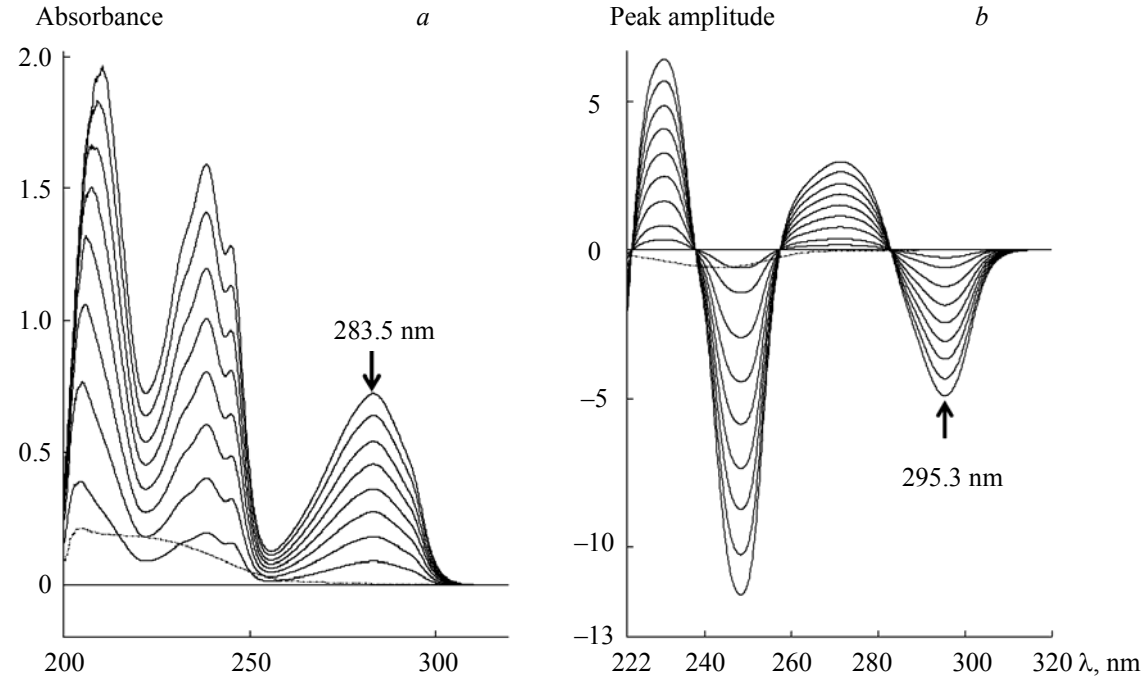


Fig. 5. (a) Zero-order absorption spectra 5–40 μg/mL of pure ZNS and 20 μg/mL of its oxidative degradation product using methanol as a blank. (b) First derivative spectra 2–40 μg/mL of pure ZNS and first derivative spectrum 20 μg/mL of its oxidative degradation product using methanol as a blank.

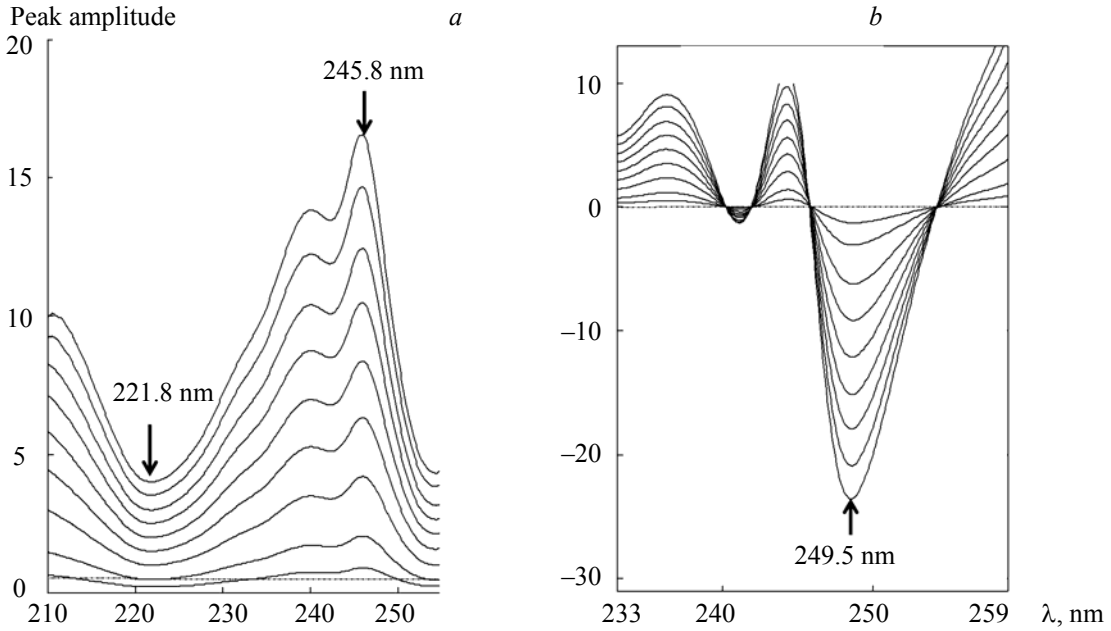


Fig. 6. (a) Ratio spectra 2–40 μg/mL of pure ZNS and 10 μg/mL of its oxidative degradation product using the spectrum 20 μg/mL of oxidative degradation product as a divisor and methanol as a blank. (b) First derivative of ratio spectra 2–40 μg/mL of pure ZNS and 10 μg/mL of its oxidative degradation product using spectrum 20 μg/mL of oxidative degradation product as a divisor using methanol as a blank.

The ratio difference method (RD) has the advantage of removing any contribution from overlapping substance by canceling the whole spectrum of the degradation product [18, 19]. Differences of peak amplitude of the degradation product at any two wavelengths were zero; as its ratio spectrum was a straight line parallel to *x*-axis. Four different divisors with different concentrations 10, 20, 40, and 60 μg/mL of the deg-

radation product were tried; however, minimum noise was observed by using the spectrum (20 μg/mL) degradation product as a divisor. Good reproducibility and maximum selectivity were obtained when the differences in amplitudes were recorded at λ_1 and λ_2 (221.8 and 245.8 nm) (Fig. 6a). A calibration graph was between ΔP and the corresponding concentrations 2–40 μg/mL. The linear equation was calculated and found to be

$$y = 0.3179x + 0.1179,\ r = 1.$$

The first derivative of ZNS saved ratio spectra in the range 2–40 μg/mL were obtained using $\lambda = 4$ and a scaling factor of 10 [20] (Fig. 6b). The first derivative of the ratio spectra method shows good sensitivity and high reproducibility. A calibration graph was constructed representing the relationship between the peak amplitude at 249.5 nm and its corresponding concentrations. Linear regression equation was calculated and found to be

$$y = 0.5091x + 0.0303,\ r = 0.9999,$$

where y is the peak amplitude, multiplied by (–1), x is the corresponding concentration in μg/mL, and r is the correlation coefficient. Validation was performed for all the suggested methods as per (ICH) Q2B guidelines [21]. Linear relationship was obtained for all methods in the range of 2–40 μg/mL ZNS except that the (D^0) method was obtained in the range of 5–40 μg/mL, both ranges achieved satisfactory results with good recoveries (Table 1).

TABLE 1. Validation Parameters of the Proposed Methods for the Determination of Zonisamide Pure Samples

Parameter	D^0	D^1	RD	^1DD
Linearity				
Slope	0.0188	0.1242	0.3179	0.5091
Intercept	–0.0006	–0.0071	0.1179	0.0303
Correlation coefficient, r	0.9999	1	1	0.9999
Range, μg/mL	5–40	2–40	2–40	2–40
Precision, RSD %				
Repeatability*	1.057	0.935	0.549	0.789
Intermediate precision**	0.842	1.068	0.921	0.993
Accuracy, mean ± SD	100.12±0.590	99.96±0.606	99.89±0.546	99.94±0.619

*The intra-day ($n = 3$), RSD of three different concentrations 10, 15, 20 μg/mL of ZNS triplicate analysis within the day.
**The inter-day ($n = 3$), RSD of three concentrations 10, 15, 20 μg/mL of ZNS triplicate analysis/day on three successive days by the suggested spectrophotometric methods.

The procedures under linearity were repeated three times using different concentrations to study the accuracy of the suggested methods. Satisfactory results with good recoveries were illustrated in Table 1. Three concentrations (10, 15, 20 μg/mL) of ZNS were repeated 3 times intra-daily using the suggested methods. The repeatability results showed low deviations as it was illustrated in Table 1. The aforementioned concentration of ZNS was repeated in three successive days. Mean recoveries and relative standard deviation (RSD) were calculated and satisfactory results were illustrated in Table 1.

Different synthetic laboratory mixtures containing different percentages of ZNS and its oxidative degradation product were analyzed using the suggested spectrophotometric methods to evaluate their specificity [22]. Acceptable mean recoveries and RSD% were achieved. ZNS could be determined in the presence of up to (80%) of its degradation product in (D^0), while it was determined in the presence of up to (90%) of the degradation product for all other methods (D^1, RD, and ^1DD). Results were illustrated in (Table 2).

The suggested spectrophotometric methods were effectively applied for the determination of ZNS in Convagran® capsules. The validity of the methods was further assessed by applying the standard addition technique. Satisfactory results were illustrated in (Table 3). No considerable difference was observed in the statistical comparison between the results obtained from the proposed methods of the pure drug samples and the official method [1] (Table 4).

TABLE 2. Determination of Zonisamide in Laboratory-Prepared Mixtures with the Proposed Spectrophotometric Methods

Degradation product, %	D^0	D^1	RD	^1DD
5	100.73	101.25	101.33	100.65
10	99.97	99.81	99.96	99.25
20	100.50	101.42	101.66	100.59
30	100.04	100.39	100.66	99.71
40	100.53	101.86	101.30	101.09
50	99.63	99.92	100.04	98.91
60	100.60	101.69	101.90	100.27
70	98.67	101.24	101.36	99.38
80	98.80	99.90	99.66	101.17
90	116.92*	102.44	100.21	100.46
Mean	99.94	100.99	100.81	100.15
SD	0.769	0.928	0.798	0.790
RSD%	0.769	0.919	0.792	0.789

*Rejected values.

TABLE 3. Quantitative Determination of Zonisamide in Convagran® Capsule with the Proposed Spectrophotometric Methods and Results of Application of Standard Addition Technique

Convagran® capsules 25 mg ZNS/capsule B.N. M2012417			D^0	D^1	RD	^1DD
Found*% ± SD			99.22±0.671	100.37±0.112	99.46±0.727	101.61±0.362
Standard addition technique	Taken, µg/mL	Added, µg/mL	Recovery%			
	15	10	100.67	99.20	98.27	99.56
		15	99.25	98.85	99.43	100.60
		20	100.98	99.63	98.86	98.80
	Mean ± SD		100.30±0.921	99.23±0.394	98.85±0.581	99.65±0.904
Convagran® capsules 50 mg ZNS/capsule B.N. M2013617			D^0	D^1	RD	^1DD
Found*% ± SD			99.34±1.192	101.63±0.791	99.66±1.047	100.73±0.668
Standard addition technique	Taken, µg/mL	Added, µg/mL	Recovery%			
	15	10	100.22	99.28	98.17	98.75
		15	99.44	99.40	99.60	98.24
		20	99.76	100.12	98.70	99.15
	Mean ± SD		99.81±0.394	99.60±0.453	98.82±0.723	98.71±0.458
Convagran® capsules 100 mg ZNS/capsule B.N. M2000918			D^0	D^1	RD	^1DD
Found*% ± SD			99.79±0.905	101.20±0.225	98.50±0.454	100.77±0.232
Standard addition technique	Taken, µg/mL	Added, µg/mL	Recovery%			
	15	10	100.45	99.56	98.13	99.72
		15	99.44	100.67	99.11	98.91
		20	99.44	100.44	98.09	100.78
	Mean ± SD%		99.77±0.582	100.22±0.587	98.44±0.580	99.80±0.939

*Average of three determination.

TABLE 4. Statistical Analysis of the Results Obtained with the Proposed Methods and the Reported Method for the Determination of Zonisamide in Pure Powder

Method	D^0	D^1	RD	^1DD	Official method*
Mean	100.12	99.96	99.89	99.94	99.87
SD	0.590	0.606	0.546	0.619	0.806
Variance	0.348	0.367	0.298	0.383	0.649
n	8	9	9	9	7
Student's *t*-test**	0.692 (2.16)	0.256 (2.14)	0.059 (2.14)	0.197 (2.14)	
F-value**	1.86 (4.21)	1.77 (4.15)	2.18 (4.15)	1.69 (4.15)	

*Official HPLC method using C18 column, acetonitrile:methanol:1.36 g/L phosphate buffer (1:1:8 by volume) as a mobile phase and UV detection at 240 nm.
**Figures between parentheses represent the corresponding tabulated values of *t* and *F* at $p = 0.05$.

Conclusions. The forced degradation study showed that zonisamide is only liable to oxidative degradation. IR and mass spectroscopy were utilized to elucidate zonisamide degradation structure which has not been published before. The suggested stability-indicating spectrophotometric methods are feasible and reliable for quantitative analysis of zonisamide in pharmaceutical formulations and in the presence of its oxidative degradation product. Also, the developed methods are favorable alternatives to expensive commonly used HPLC methods. Moreover, the proposed D^1, RD, and ^1DD methods are more sensitive than D^0 despite its simplicity. As all of the proposed methods require no expensive chemicals or sophisticated instruments, their application is allowed in routine quality control analysis of zonisamide.

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