



STABILITY-INDICATING SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF TORSEMIDE IN PURE FORM AND PHARMACEUTICAL PREPARATION

Khalid Abdel-Salam M. Attia, Nasr M. El-Abasawy and Rady F. Abdul-Kareem*

Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Al-Azhar
University, 11751, Nasr City, Cairo, Egypt.

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*Correspondence for

Author

Dr. Rady F. Abdul-Kareem.

Pharmaceutical Analytical
Chemistry Department,
Faculty of Pharmacy, Al-
Azhar University, 11751,
Nasr City, Cairo, Egypt.

ABSTRACT

Six simple, rapid, sensitive, accurate and precise stability-indicating spectrophotometric methods were developed for the determination of Torsemide in bulk powder and in pharmaceutical preparation. **Method (A)**, Ratio difference method; is based on measuring the difference in the amplitude of intact Torsemide in presence of its degradation product at two different wavelengths, this is done at 236 nm and 270 nm in the range of 2.5 – 25 $\mu\text{g ml}^{-1}$ with LOD = 0.325 $\mu\text{g ml}^{-1}$ and

LOQ = 0.986 $\mu\text{g ml}^{-1}$, **Method (B)**, Mean centering method; the method was applied for analysis of Torsemide in presence of its degradation product this is done at 250 nm in the range of 5-25 $\mu\text{g ml}^{-1}$ with LOD = 0.127 $\mu\text{g ml}^{-1}$ and LOQ = 0.387 $\mu\text{g ml}^{-1}$, **Method (C)** Bivariate method, is used for the determination of intact Torsemide in presence of its degradation product at 275&285 nm in the range of 5-25 $\mu\text{g ml}^{-1}$ with LOD= 0.532 $\mu\text{g ml}^{-1}$ and LOQ= 1.613 $\mu\text{g ml}^{-1}$ at (275 nm) and with LOD= 0.349 $\mu\text{g ml}^{-1}$ and LOQ= 1.057 $\mu\text{g ml}^{-1}$ at (285 nm) ., **Method (D)**, First derivative of zero order spectra method (^1D); is used for the determination of intact Torsemide in presence of its degradation product at 258.8 nm in the range of 5-25 $\mu\text{g ml}^{-1}$ with LOD= 0.345 $\mu\text{g ml}^{-1}$ and LOQ= 1.046 $\mu\text{g ml}^{-1}$, **Method (E)**, Dual wavelength method (DW) ; is based on measuring the difference in absorbance of intact Torsemide in presence of its degradation product at two different wavelengths in which degradate absorbance difference at these wavelengths is zero, this is done at 265.4 nm and 296.6 nm in the range of 5-22.5 $\mu\text{g ml}^{-1}$ with LOD= 0.115 $\mu\text{g ml}^{-1}$ and LOQ= 0.349 $\mu\text{g ml}^{-1}$, **Method (F)**, Area under curve (AUC) of zero order spectra method ; in which selection of

the wavelength ranges which give good selectivity and percentage recovery 247–267 nm (λ_1 - λ_2) and 270–296 nm (λ_3 - λ_4) of Torsemide and its degradate, using Cramer's Rule in the concentration range of 5–22.5 $\mu\text{g ml}^{-1}$ for each drug and its degradate with LOD=0.108 and LOQ=0.329. The obtained results were statistically compared with those of the reported method by applying t-test and F-test at 95% confidence level and no significant difference was observed regarding accuracy and precision.

KEYWORDS: Torsemide, Ratio difference, Mean centering, Bivariate, First derivative of zero order spectra, Dual wavelength, Area under a curve, pure form, pharmaceutical preparation.

INTRODUCTION

Torsemide is the (Figure 1) loop type diuretic drug, chemically it is *3-Pyridinesulfonamide, N-[(1-methylethyl) amino]carbonyl]-4-[(3-methylphenyl)amino]-1-Isopropyl-3-[(4-m-toluidino-3-pyridyl)sulfonyl urea*.^[1-4] Torsemide is useful in the treatment of mild-to-moderate hypertension in doses of 2.5 to 5 mg given once daily.^[5] These doses lower blood pressure as effectively as 25 mg of hydrochlorothiazide but without producing diuresis.^[6] Higher doses of torsemide (10 or 20 mg) are associated with significant diuresis and are more effective than Furosemide in treating edema associated with congestive heart failure and cirrhosis of the liver.^[7-9]

Torsemide was determined by several methods for the analysis including gas chromatography (GC)^[10], liquid chromatography with UV detection (LC-UV)^[11], HPTLC, derivative spectrophotometric^[12,13,14] and fluorimetry.^[14] Torsemide was determined with or without combination of several drugs by HPLC, spectrophotometrically and HPTLC^[15]

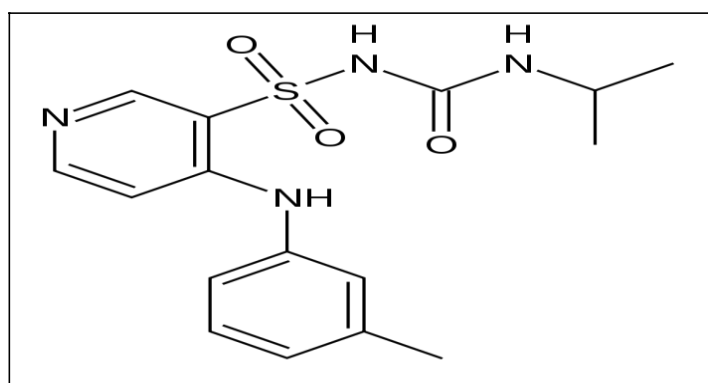


Figure 1: chemical structure of Torsemide

The aim of this work was to develop and validate a simple, rapid, reliable and precise UV spectrophotometric methods for analysis of Torsemide in bulk and tablet formulation in presence of its oxidative degradation product without previous separation by UV–VIS spectrophotometry with satisfactory statistical validation measures.^[16,17]

MATERIALS AND METHODS

Apparatus

- Shimadzu UV-Vis. 1650 Spectrophotometer (Japan).
- Hot plate (Torrey pines Scientific, USA).
- Jenway, 3510 pH meter (Jenway, USA).

Materials and Reagents

- Torsemide was kindly provided by Apex Pharma-Egypt and certified to contain 99.70%. (Batch number: TM 0040612
- Examide[®] tablets: batch number: MT3090713, manufactured by Apex Pharma-Egypt Company. Each tablet was labeled to contain 10 mg of Torsemide.
- Hydrogen peroxide 30 % aqueous solution and methanol (El Nasr Co., Egypt).

Standard Solution

(a) Torsemide stock solution ($1000\mu\text{g ml}^{-1}$). It was freshly prepared daily by transferring 0.1 g of TOR into a 100 mL volumetric flask, then dissolving in 50mL methanol and the volume was completed with methanol. The stability of the prepared solution was studied, and it has been found to be stable with no apparent degradation at least for 24 h at 25 °C.

(b) Torsemide working solutions ($50\mu\text{g ml}^{-1}$). It was freshly prepared by suitable dilution from its stock solution using methanol as diluent [5ml from stock solution add to 100 ml methanol.

Preparation of the degradation product^[18]

- a) Torsemide degradate stock solution is prepared by transfer 0.1 g of Torsemide to a 250 ml conical flask containing 50 ml of 3% Hydrogen peroxide. reflux for 2 h and cool to room temperature. evaporate to dryness , the residue was extracted three times with 25 ml methanol ,filtered into 100 ml volumetric flask then the volume was adjusted to the mark by the same solvent .The obtained solution was claimed to contain (1 mg ml^{-1}).
- b) Torsemide degradate working solutions ($50\mu\text{g ml}^{-1}$). It was freshly prepared by suitable dilution from its stock solution using methanol as diluent.

Construction of the Calibration Curves (General Procedures): Method A (Ratio difference method): Aliquots portion in the range (0.5 – 5 ml) Torsemide and (4 ml) Torsemide degradate are accurately transferred from their standard working solutions ($50 \mu\text{g ml}^{-1}$) into a series of 10 ml volumetric flasks then completed to volume with methanol to obtain concentration in the range of ($2.5\text{--}25 \mu\text{g ml}^{-1}$) for intact torsemide and ($20 \mu\text{g ml}^{-1}$) for torsemide degradate. The spectra of the prepared standard solutions are scanned from 200 - 400 nm and stored in the computer. For the determination of Torsemide in presence of its degradation product, the stored spectra of Torsemide are divided by the spectrum of $20 \mu\text{g ml}^{-1}$ degradate, to obtain the ratio spectra.

The calibration curve was constructed by plot the amplitudes of difference of the ratio spectra at 236.0 and 270.0 nm ($\Delta P_{270.0-236.0}$) against the corresponding concentrations in $\mu\text{g ml}^{-1}$ of Torsemide, the regression equation was derived.

Method B (Mean Centering method)

The ratio spectra obtained as before were mean centered using MATLAB. The calibration curve was constructed relating the amplitudes of the mean centered values to the corresponding concentrations of Torsemide equivalent to ($5\text{--}25 \mu\text{g ml}^{-1}$) at 250 nm the regression equation was derived.

Method C (Bivariate Method): Into two separate sets of 10 ml volumetric flasks, aliquots equivalent to ($5\text{--}25 \mu\text{g ml}^{-1}$) of Torsemide and aliquots equivalent to. ($2.5\text{--}25 \mu\text{g ml}^{-1}$) of its corresponding degradate from their working solutions ($50 \mu\text{g ml}^{-1}$) were transferred. The flasks were completed to volume with methanol. The spectra of Torsemide and its degradation product were recorded between 200 and 400 nm and stored on a computer. Absorbance values were measured for both Torsemide and its degradation product at the optimum wavelengths found by the Kaiser method (275 and 285 nm) then the regression equation was derived.

Method D (First derivative of zero order spectra method (1D))

Aliquots equivalent to ($5\text{--}25 \mu\text{g ml}^{-1}$) of Torsemide were accurately measured and transferred from its standard working stock solution ($50 \mu\text{g ml}^{-1}$) into a set of 10-ml volumetric flasks and the volumes were completed to the mark with methanol. The zero order (0D) absorption spectrum of each solution was recorded against methanol as a blank, then the first derivative (1D) spectra were computed using scaling factor = 10 and $\Delta\lambda = 4$

nm. The peak trough heights at 258.8 nm were recorded, plotted each against its corresponding concentration, and the regression parameters were computed.

Method E (Dual wavelength method (DW):

Aliquots equivalent to (5-22.5 $\mu\text{g ml}^{-1}$) of Torsemide were accurately measured and transferred from its standard working stock solution (50 $\mu\text{g ml}^{-1}$) into a set of 10 ml volumetric flasks and the volumes were completed to the mark with methanol.

The spectra of the prepared standard solutions are scanned from 200 - 400 nm and the absorbance of these solutions was measured at 265.4 and 296.6 nm. Calibration curve was constructed by plotting difference in absorbance ($A_{296.6}$ and $A_{265.4}$) versus drug concentration, and the regression parameters were computed.

Method (F) (Area under curve (AUC) of zero order spectra method):

Aliquots equivalent to (5-22.5 $\mu\text{g ml}^{-1}$) of Torsemide were accurately measured and transferred from its standard working stock solution (50 $\mu\text{g ml}^{-1}$) into a set of 10-ml volumetric flasks and the volumes were completed to the mark with methanol.

Area under curve of the absorption spectra in the wavelength ranges 247-267 nm (λ_1 - λ_2) and 270-296 nm (λ_3 - λ_4) of Torsemide in the concentration range of 5–22.5 $\mu\text{g ml}^{-1}$ is recorded. For Torsemide degradate Area under curve of the absorption spectra in the wavelength ranges 247-267 nm (λ_1 - λ_2) and 270-296 nm (λ_3 - λ_4) in the concentration range of 5–22.5 $\mu\text{g ml}^{-1}$ is also recorded.

Then the absorptivity 'Y' values of each were calculated where Y = the recorded area under curve of each component (from 247 to 267 nm or 270 to 296 nm)/concentration of the component (in $\mu\text{g/mL}$). The concentrations of the studied components in the prepared solutions were determined by applying Cramer's rule (Gabriel Cramer 1704-1752) and matrices in the following equations:

$$A_1 = Y_{x1}C_x + Y_{z1}C_z \quad (\lambda_1 - \lambda_2)$$

$$A_2 = Y_{x2}C_x + Y_{z2}C_z \quad (\lambda_3 - \lambda_4)$$

Where A_1 , A_2 are the areas under curve in the range of 247-267 nm and 270-296 nm, respectively. Y_{x1} , Y_{x2} are the absorptivity values of torsemide at (λ_1 - λ_2) and (λ_3 -

λ_4), respectively. Y_{Z1} and Y_{Z2} are the absorptivity values of the degradate at ($\lambda_1 - \lambda_2$) and ($\lambda_3 - \lambda_4$), respectively. C_X and C_Z are the concentrations in $\mu\text{g/mL}$ of torsemide and its degradate, respectively.

Analysis of pharmaceutical preparation (sample)

Ten **Examide® 10 mg** tablets were accurately weighed and finely powdered, then a quantity equivalent to 10 mg of Torsemide was extracted three times with 25 ml methanol by mixing well for 10 minutes then filtered through whatman filter paper No. 41 into 100 ml volumetric flask. Filter paper was washed with methanol, adding washings to the volumetric flask and the volume was made up to the mark with methanol to obtain a concentration of (0.1 mg ml^{-1}). The sample solution was analyzed using the procedure described under methods A,B,C,D,E,F.

RESULTS AND DISCUSSION

Spectral Characteristics

The zero order (0D) absorption spectra of Torsemide ($20 \mu\text{g ml}^{-1}$) and its oxidative degradation product ($20 \mu\text{g ml}^{-1}$) were recorded against methanol as blank over the range of 200 – 400 nm. (Fig.2)

For method A (Ratio difference method)^[19-20]

The zero-order absorption spectra of Torsemide (Fig.2) show an overlapping, so we develop a spectrophotometric method which allow the determination of the drug in presence of its degradate without previous separation.

In this method, the absorption spectra of the drug were divided by a suitable absorption spectrum of the degradate (divisor) to get the ratio spectra. Different concentrations of divisor (Torsemide degradate) are used (5, 10, 15 and $20 \mu\text{g ml}^{-1}$) and the divisor concentration ($20 \mu\text{g ml}^{-1}$) of Torsemide degradate is found the best regarding average recovery percent. The difference in peak amplitudes between two selected wavelengths in the ratio spectra is proportional to the concentration of the drug without interference from its divisor (Fig.3). The method comprises two critical steps, the first is the choice of the divisor. The selected divisor should compromise between minimal noise and maximum sensitivity. The second critical step is the choice of the wavelengths at which measurements are recorded. Any two wavelengths can be chosen provided that they exhibit different amplitudes in the ratio spectrum and good linearity is present at

each wavelength individually. The selected wavelengths are 236 and 270 nm (ΔP 270-236 nm) which gave the best results.

For method B (Mean centering method)^[20]

In this method, the absorption spectra of the drug were divided by a suitable absorption spectrum of the degradate (divisor) to get the ratio spectra (Fig.3). The best divisor concentration was ($20 \mu\text{g ml}^{-1}$) of Torsemide degradate. The obtained ratio spectra were mean centered using MATLAB and the concentration of Torsemide was determined by measuring the amplitude at 250 nm (Fig. 4).

For method C (Bivariate method)^[20-24]

Bivariate calibration spectrophotometric method is a direct method which has been proposed for the resolution of binary mixtures. The principle of bivariate calibration is the measurement of two components (A and B) at two selected wavelengths (λ_1 and λ_2) to obtain two equations^[20-24]

$$CA = (AAB1 - eAB1 - mB1CB)/mA1$$

$$CB = [mA2 (AAB1 - eAB1) + mA1 (eAB2 - AAB2)] / mA2mB1 - mA1mB2$$

Where C_A , C_B are the concentration of component A (drug), component B (degradate); m_{A1} , m_{A2} are the slope values of the drug at λ_1 , λ_2 ; m_{B1} , m_{B2} are the slope values of the degradate at λ_1 , λ_2 ; $AAB1$, $AAB2$ are the absorbance of the binary mixture at λ_1 , λ_2 ; $eAB1$, $eAB2$ are the sum of the intercepts of drug and degradate at λ_1 and λ_2 , respectively.

This simple mathematic algorithm allows the resolution of the two components by measuring the absorbance of their mixture at the two selected wavelengths and using the parameters of the linear regression functions evaluated individually for each component at the same wavelengths.

In order to apply the bivariate method in the resolution of Torsemide and its oxidative degradate, the absorbance of the two component at seven different selected wavelengths was recorded in the region of overlapping; 265, 275, 285, 295, 305, 315 and 325 nm. The calibration curve equations and their respective linear regression coefficients were obtained directly with the aim of ensuring that there was a linear

relationship between the absorbance and the corresponding concentration. All of the calibration curves at the selected wavelengths showed a satisfactory linear regression coefficient ($r^2 \geq 0.9996$).

According to Kaiser method^[20-24], the slope values of the linear regression equations for both intact drug and its oxidative degradate at the selected wavelengths were used to calculate the sensitivity matrices K to find out the optimum pair of wavelength at which the binary mixtures were recorded, table (4). For the bivariate determination of Torsemide and its oxidative degradate, 275 and 285 nm were found to give the maximum value of K and thus can be used for the analysis; Calibration graphs were constructed for the determination of Torsemide by the proposed procedure, where Beer's law was obeyed in the range of 5-25 $\mu\text{g ml}^{-1}$.

For method D (First derivative of zero order spectra method(¹D))^[25]

It is clear from the spectra in figure (2) that, there is a band overlapping between the drug and its degradation product. Such overlapping was eliminated by the first derivative (¹D) scanning of torsemide and its degradation product in methanol, torsemide has a trough at 258.8 nm which shows no interference from the degradation product.

Thus, it would be possible to adopt the (¹D) spectrophotometry at 258.8 nm for direct determination of torsemide in presence of its degradation product as seen in Figure (5).

Method (E), Dual wavelength method (DW)^[20]

The most striking feature of the dual wavelength method is its simplicity and rapidity. From the overlay spectra of torsemide and its degradation product shown in Figure (2), two wavelengths 265.4 nm and 296.6 nm were selected for the estimation of intact torsemide in presence of its degradation product as the oxidative degradate shows the same absorbance at these wavelengths.

Method (F) (Area under curve method (AUC))^[26-27-28-29]

In the area under curve method (AUC), selection of wavelength ranges is an important step during method optimization hence it affects selectivity of the method. Different wavelength ranges were tested where the wavelength ranges of 247-267 nm and 270-296 nm were the most suitable ranges regarding selectivity for the drug and the degradate. Figure (2)

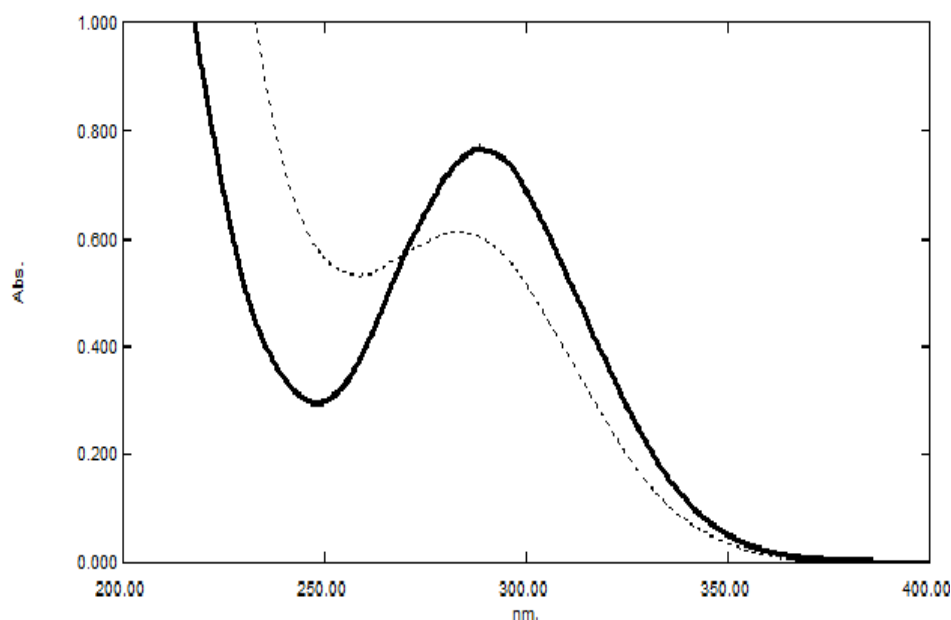


Figure (2): UV- Spectra of Intact Torsemide ($20\mu\text{g ml}^{-1}$) (—) ,and its oxidative Degradate ($20\mu\text{g ml}^{-1}$) (.....)

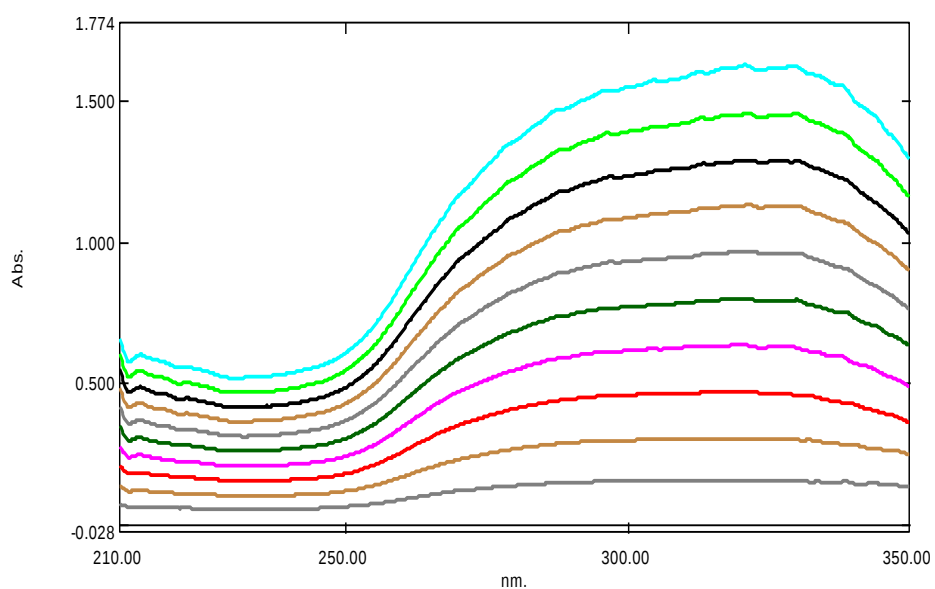


Figure (3): Ratio Spectra of Torsemide ($2.5 - 25 \mu\text{g ml}^{-1}$) using ($20\mu\text{g ml}^{-1}$) of Torsemide Degradate as a Divisor and Methanol as a Blank.

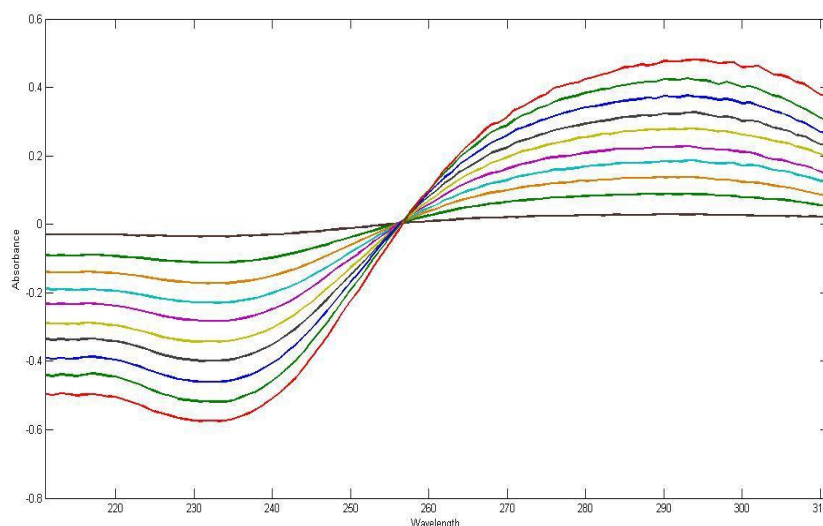


Figure (4): Mean Centered Ratio Spectra of Torsemide ($2.5 - 25 \mu\text{g ml}^{-1}$) Using ($20 \mu\text{g ml}^{-1}$) of its Degradate as a Divisor and Methanol as a Blank

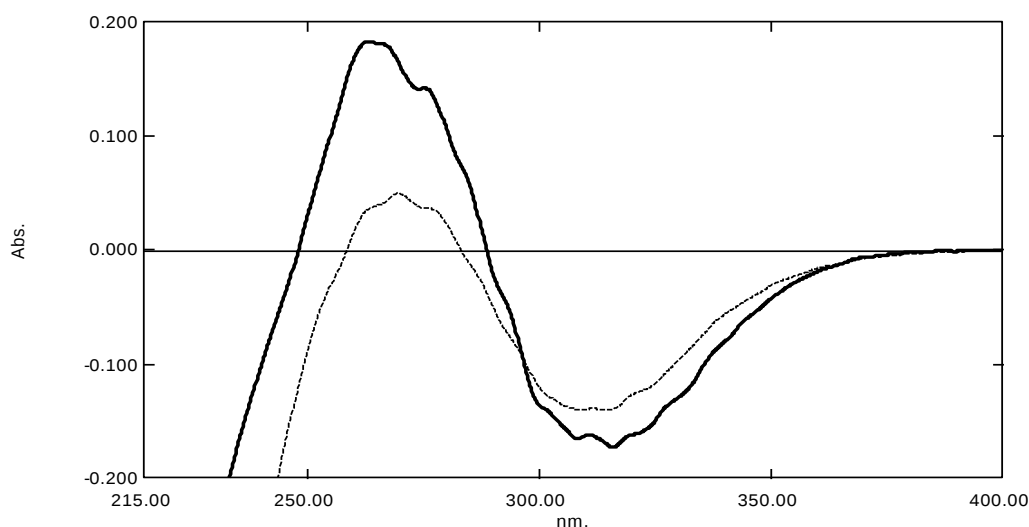


Figure (5): First-order Spectra of Intact Torsemide ($20 \mu\text{g ml}^{-1}$) (—) and its Degradation Product ($20 \mu\text{g ml}^{-1}$) (.....) in Methanol.

Validation of the methods

Linearity and range

-For ratio difference method

Linear correlation was obtained between the differences in amplitudes at 270 and 236 nm, against the corresponding concentration of Torsemide. Good linearity is obtained in the concentration range of $2.5 - 25 \mu\text{g ml}^{-1}$. The corresponding regression equation was computed to be.

$$\Delta P_{270-236} = 0.0255C + 0.0031 \quad (r^2 = 0.9998)$$

Where ΔP is the amplitude difference at the selected wavelengths, C is the concentration of torsemide in $\mu\text{g ml}^{-1}$ and r^2 = the correlation coefficient as shown in table (1).

For mean centering method

Linear correlation was obtained between the mean centered values at 250 nm, against the corresponding concentration of Torsemide. Good linearity is obtained in the concentration range of (5 - 25 $\mu\text{g ml}^{-1}$). The corresponding regression equation was computed to be.

$$MCN_{250} = 0.0205 C - 0.0047 \quad (r^2 = 0.9998)$$

Where MCN is the peak amplitude of the mean centered ratio spectrum curve, C is the concentration in $\mu\text{g ml}^{-1}$ and r^2 = the correlation coefficient, as shown in table (1).

For Bivariate method

For the bivariate determination of Torsemide and its oxidative degradate, 275 and 285 nm were found to give the maximum value of K and thus can be used for the analysis. Calibration graphs were constructed for the determination of Torsemide by the proposed procedure, where Beer's law was obeyed in the range of 5-25 $\mu\text{g ml}^{-1}$. The linear regression of the graphs were as follows.

$$A_{275} = 0.0344 C - 0.0037 \quad (r=0.9996)$$

$$A_{285} = 0.0389 C - 0.0085 \quad (r=0.9998)$$

Where C is the concentration of Torsemide in $\mu\text{g ml}^{-1}$, A_{275} is the absorbance values at 275 nm and A_{285} is the absorbance values at 285 nm and r^2 is the correlation coefficient, as shown in table (1)

For first derivative method (1D)

At the described wavelength linear relationship was obtained between the trough in cm and the torsemide concentration in the range (5-25 $\mu\text{g ml}^{-1}$). The linear regression equation of the trough was.

$$A_{258.8} = 0.0073 C + 0.0017 \quad (r^2 = 0.9996)$$

Where A is a trough height at 258.8 in cm, C is the drug concentration in $\mu\text{g ml}^{-1}$ and r^2 is the correlation coefficient, as shown in table (1)

For dual wavelength method:

At selected two wavelengths (296.6 & 265.4), the difference in absorbance at these wavelengths ($A_{296.6}$ and $A_{265.4}$) was plotted against concentration in the range (5-22.5). the corresponding regression equation was computed to be.

$$(A_{296.6} - A_{265.4}) = 0.0099C - 0.0063 \quad (r^2 = 0.9998)$$

where ($A_{296.6} - A_{265.4}$) is the difference in absorbance at 296.6 and 265.4 nm, C is the concentration of torsemide and r^2 is the correlation coefficient, as shown in table (1).

For Area Under Curve method(AUC)

at selected area under curves which give linear relationship against concentration (5-22.5 $\mu\text{g ml}^{-1}$), regression equation was computed to be:

$$A_1 = 0.406C - 0.085 \quad r^2 = 0.9997$$

$$A_2 = 0.956C - 0.220 \quad r^2 = 1$$

where A_1 & A_2 are area under curves at wavelength ranges (247-267) and (270-296) respectively, C is the concentration of torsemide and r^2 is the correlation coefficient, as shown in table (1)

Limits of detection and quantitation

The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated according to ICH guidelines^[16] from the following equations.

$$\text{LOD} = 3.3 N / B \quad \text{LOQ} = 10 N / B$$

Where N is the standard deviation of the intercept and B is the slope of the corresponding calibration curve.

LOD and LOQ values of Torsemide for each method were listed in table (1).

Accuracy and precision

According to the ICH guidelines^[16], three replicate determinations of three different concentrations of the studied drug in pure form within their linearity ranges were performed in the same day (intra-day) and in three successive days (inter-day) for each method. Accuracy as recovery percent (R%) and precision as percentage relative standard deviation (RSD%) were calculated and results are listed in table (2).

Specificity

The specificity of the proposed methods were assured by applying the laboratory prepared mixtures of the studied drug and its degradate. The results are listed in table (3).

Pharmaceutical Applications

The proposed methods were applied for the determination of the studied drug in (Examide[®] 10 mg) tablets, The results were validated by comparison to a previously reported method.^[30] No significant differences were found by applying student t-test and F-test at 95% confidence level^[17], indicating good accuracy and precision of the proposed methods for the analysis of the studied drugs in their pharmaceutical dosage form (table 4).

Table (1): Regression parameters for the determination of Torsemide by the proposed methods

Parameters	Ratio difference	Mean centering	Bivariate		First derivative	Dual wavelength	AUC
Wavelength (nm)	270&236	250	275	285	258.8	296.6&265.4	247 -267
Linearity range (μgml^{-1})	2.5-25	5-25	5-25		5-25	5-22.5	5-22.5
LOD (μgml^{-1})	0.325	0.127	0.532	0.349	0.345	0.115	0.108
LOQ (μgml^{-1})	0.986	0.387	1.613	1.057	1.046	0.349	0.329
Regression equation *	0.0255	0.0205	0.0344	0.0389	0.0073	0.0099	0.406
Slope (b)	0.0031	-0.0047	-0.0037	-0.0085	0.0017	-0.0063	-0.085
Intercept (a)							
Correlation coefficient (r^2)	0.9998	0.9998	0.9996	0.9998	0.9996	0.9998	0.9997

* $y = a + bx$ where y is the response and x is the concentration.

Table (2): Intraday and inter-day accuracy and precision for the determination of the Torsamide by the proposed methods

Method	Conc. $\mu\text{g}.\text{ml}^{-1}$	Intraday			Inter-day		
		Found Conc. $\pm$ SD	Accuracy (R%)	Precision (RSD%)	Found Conc. $\pm$ SD	Accuracy (R%)	Precision (RSD%)
Ratio difference	10	10.061$\pm$0.059	100.614	0.595	10.087$\pm$0.022	100.875	0.224
	15	14.924$\pm$0.181	99.494	1.213	15.081$\pm$0.163	100.540	1.082
	25	24.793$\pm$0.022	99.173	0.091	24.976$\pm$0.271	99.905	1.087
Mean centering	7.5	7.560$\pm$0.045	100.811	0.596	7.517$\pm$0.079	100.237	1.051
	15	14.899$\pm$0.040	99.329	0.273	14.789$\pm$0.130	98.595	0.880
	25	25.046$\pm$0.060	100.185	0.243	25.003$\pm$0.028	100.015	0.115
Bivariate	5	5.04$\pm$0.039	100.74	0.768	4.99$\pm$0.065	99.83	1.296
	15	14.98$\pm$0.059	99.89	0.396	14.98$\pm$0.121	99.89	0.811
	25	24.91$\pm$0.090	99.62	0.362	24.91$\pm$0.074	99.62	0.298
First derivative	7.5	7.529$\pm$0.079	100.395	1.050	7.484$\pm$0.079	99.786	1.056
	15	14.881$\pm$0.079	99.208	0.531	14.879$\pm$0.080	99.208	0.541
	25	25.063$\pm$0.285	100.255	1.137	24.926$\pm$0.079	99.707	0.317
Dual wavelength	5	5.047$\pm$0.058	100.942	1.155	5.013$\pm$0.058	100.269	1.163
	15	15.215$\pm$0.116	101.436	0.766	14.912$\pm$0.154	99.416	1.034
	22.5	22.656$\pm$0.202	100.695	0.891	22.622$\pm$0.154	100.546	0.682
AUC	7.5	7.49$\pm$0.038	99.86	0.505	7.50$\pm$0.057	99.99	0.763
	15	15.07$\pm$0.033	100.49	0.218	15.08$\pm$0.031	100.50	0.203
	22.5	22.87$\pm$0.073	101.64	0.318	22.87$\pm$0.079	101.65	0.343

Table (3): Determination of Torsemide and its degradate in their laboratory mixtures by the proposed methods.

Intact mg mL ⁻¹	Degradate µg mL ⁻¹	% degradate	Ratio difference method	Mean centering method	Bivariate method	First derivative method	Dual wavelength method	Area under curve method
			% of intact	% of intact	%of intact	% of intact	% of intact	% of intact
22.5	2.5	10	100.55	99.22	101.91	100.030	101.144	100.05
20	5	20	100.76	99.695	99.55	99.520	100.656	101.57
17.5	7.5	30	100.59	99.282	98.34	101.996	100.028	99.13
15	10	40	99.84	99.871	98.53	98.904	101.212	98.25
12.5	12.5	50	99.11	100.434	99.44	100.054	98.020	99.30
10	15	60	100.35	101.114		101.780	99.29	101.53
7.5	17.5	70	101.91	100.437		99.178	100.067	98.03
Mean % ± SD			100.45 ±0.86	100.008 ±0.688	99.56 ±1.421	100.209 ±1.221	100.06 ±1.126	99.69 ±1.435

Table (4): Values of The Sensitivity Matrix determinants Calculated according to Kaiser's Method^[20-24] (K X 10⁶) For The Mixture of Torsemide And its oxidative Degradate By The Bivariate Method

λ/λ	265	275	285	295	305	315	325
265	0	182.12	285.72	302.32	246.32	152.4	65.36
275		0	1209.79	138.02	112.96	54.54	1.02
285			0	41.32	34.46	2.76	36.28
295				0	0.84	24.96	47.84
305					0	20.76	39.16
315						0	18.72
325							0

Table (4): Results obtained by the proposed compared with a reported method^[30] for the determination of torsemide in its pharmaceutical preparations(Examide[®] 10mg)

Parame ters	Ratio difference method	Mean centering method	Bivariate method	First derivative method	Dual wavelength method	Area under curve method	Reported method ^[30]
<i>N</i> *	5	5	5	5	5	5	5
<i>X</i>	100.132	99.414	99.517	100.743	99.05	99.492	99.838
<i>SD</i>	1.255	0.895	1.212	0.992	0.742	0.932	1.37
RSD%	1.253	0.900	1.218	0.984	0.749	0.937	1.372
<i>t</i> **	0.353 (2.306)	0.578 (2.364)	0.392 (2.306)	1.195 (2.364)	1.13 (2.446)	0.467 (2.364)	—
<i>F</i> **	1.192 (6.388)	2.342 (6.388)	1.278 (6.388)	1.908 (6.388)	3.406 (6.388)	2.16 (6.388)	—

* No. of experimental.

** The values in the parenthesis are tabulated values of *t* and *F* at (*p*= 0.05).

CONCLUSION

The proposed methods are simple, rapid, accurate and precise and can be used for the determination of Torsemide in pure form and in its pharmaceutical dosage form as well as in presence of its degradation product.

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