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

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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 µg ml⁻¹ with LOD = 0.325 µg ml⁻¹ and LOQ = 0.986 µg 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 µg ml⁻¹ with LOD = 0.127 µg ml⁻¹ and LOQ = 0.387 µg 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 µg ml⁻¹ with LOD= 0.532 µg ml⁻¹ and LOQ= 1.613 µg ml⁻¹ at (275 nm) and with LOD= 0.349 µg ml⁻¹ and LOQ= 1.057 µg 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 µg ml⁻¹ with LOD= 0.345 µg ml⁻¹ and LOQ= 1.046 µg 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 µg ml⁻¹ with LOD= 0.115 µg ml⁻¹ and LOQ= 0.349 µg 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 (λ1-λ2) and 270–296 nm (λ3-λ4) of Torsemide and its degradate, using Cramer's Rule in the concentration range of 5-22.5 µg 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 acurve, 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](image-url)
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.\textsuperscript{[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\textsuperscript{®} 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 (1000μg ml\textsuperscript{-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 (50μg ml\textsuperscript{-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\textsuperscript{[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\textsuperscript{-1}).

b) Torsemide degradate working solutions (50μg ml\textsuperscript{-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 degrade are accurately transferred from their standard working solutions (50 µg ml⁻¹) into a series of 10 ml volumetric flasks then completed to volume with methanol to obtain concentration in the range of (2.5-25 µg ml⁻¹) for intact torsemide and (20 µg ml⁻¹) for torsemide degrade. 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 µg ml⁻¹ degrade, 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 (ΔP₂₇₀,₀-⁻₂₃₆,₀) against the corresponding concentrations in µg 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 (5-25 µg 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 (5-25 µg ml⁻¹) of Torsemide and aliquots equivalent to, (2.5-25 µg ml⁻¹) of its corresponding degrade from their working solutions (50µg 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 (5-25 µg ml⁻¹) of Torsemide were accurately measured and transferred from its standard working stock solution (50µg 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 μg ml⁻¹) of Torsemide were accurately measured and transferred from its standard working stock solution (50μg 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 acurve (AUC) of zero order spectra method)**:  
Aliquots equivalent to (5-22.5 μg ml⁻¹) of Torsemide were accurately measured and transferred from its standard working stock solution (50μg 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 (λ₁-λ₂) and 270-296 nm (λ₃-λ₄) of Torsemide in the concentration range of 5–22.5 μg ml⁻¹ is recorded. For Torsemide degradate Area under curve of the absorption spectra in the wavelength ranges 247-267 nm (λ₁-λ₂) and 270-296 nm (λ₃-λ₄) in the concentration range of 5–22.5 μg 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 μg/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 A₁, A₂ are the areas under curve in the range of 247-267 nm and 270-296 nm, respectively. Yₓ₁, Yₓ₂ are the absorptivity values of torsemide at (λ₁-λ₂) and (λ₃-
\( \lambda_4 \), 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 \( \mu g/mL \) 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 \( (20 \ \mu g \ ml^{-1}) \) and its oxidative degradation product \( (20 \ \mu g \ ml^{-1}) \) 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 overlap, 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 \( (5, 10, 15 \ and \ 20 \ \mu g \ ml^{-1}) \) and the divisor concentration \( (20 \ \mu g \ ml^{-1}) \) 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 ($\Delta P$ 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 ($20 \mu g$ 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 ($\lambda_1$ and $\lambda_2$) to obtain two equations \(^{[20-24]}\)

\[
CA = \frac{(AAB1 - eAB1 - mB1CB)}{mA1} \\
CB = \frac{[mA2 (AAB1-eAB1) + mA1 (eAB2-AAB2)]/ mA2mB1-mA1mB2}{mA2mB1-mA1mB2}
\]

Where $CA$, $CB$ are the concentration of component A (drug), component B (degradate); $mA1$, $mA2$ are the slope values of the drug at $\lambda_1$, $\lambda_2$; $mB1$, $mB2$ are the slope values of the degradate at $\lambda_1$, $\lambda_2$; $AAB1$, $AAB2$ are the absorbance of the binary mixture at $\lambda_1$, $\lambda_2$; $eAB1$, $eAB2$ are the sum of the intercepts of drug and degradate at $\lambda_1$ and $\lambda_2$, 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 (r² ≥ 0.9996).

According to Kaiser method[20-24], the slope values of the linear regression equations for both intact drug and its oxidative degradeate 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 bivariante determination of Torsemide and its oxidative degradeate, 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 µg 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 degradeate shows the same absorbance at these wavelengths.

**Method (F) (Area under acurve 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 degradeate. Figure (2)
Figure (2): UV- Spectra of Intact Torsemide (20µg ml\(^{-1}\)) (—) and its oxidative Degradate (20µg ml\(^{-1}\)) (.....)

Figure (3): Ratio Spectra of Torsemide (2.5–25 µg ml\(^{-1}\)) using (20µg ml\(^{-1}\)) of Torsemide Degradate as a Divisor and Methanol as a Blank.
Figure (4): Mean Centered Ratio Spectra of Torsemide (2.5 - 25 µg ml⁻¹) Using (20 µg ml⁻¹) of its Degradate as a Divisor and Methanol as a Blank

Figure (5): First-order Spectra of Intact Torsemide (20 µg ml⁻¹) (—) and its Degradation Product (20 µg 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 µg ml⁻¹. The corresponding regression equation was computed to be.
\[ \Delta P_{270-236} = 0.0255C + 0.0031 \quad (r^2 = 0.9998) \]

Where \( \Delta P \) is the amplitude difference at the selected wavelengths, \( C \) is the concentration of torsemide in \( \mu g \text{ ml}^{-1} \) 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 \( \mu g \text{ ml}^{-1} \)). The corresponding regression equation was computed to be.

\[ \text{MCN}_{250} = 0.0205C - 0.0047 \quad (r^2 = 0.9998) \]

Where MCN is the peak amplitude of the mean centered ratio spectrum curve, \( C \) is the concentration in \( \mu g \text{ ml}^{-1} \) 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 \( \mu g \text{ ml}^{-1} \). The linear regression of the graphs were as follows.

\[
\begin{align*}
A_{275} &= 0.0344C - 0.0037 \quad (r=0.9996) \\
A_{285} &= 0.0389C - 0.0085 \quad (r=0.9998)
\end{align*}
\]

Where \( C \) is the concentration of Torsemide in \( \mu g \text{ ml}^{-1} \), \( A_{275} \) 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 \( \mu g \text{ ml}^{-1} \)). The linear regression equation of the trough was.
A258.8 = 0.0073 C + 0.0017 (r² = 0.9996)

Where A is a trough height at 258.8 in cm, C is the drug concentration in μg ml⁻¹ and r² 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 (A₂₉₆.₆ and A₂₆₅.₄) was plotted against concentration in the range (5-22.5). the corresponding regression equation was computed to be:

\[(A_{296.6} - A_{265.4}) = 0.0099C - 0.0063 \text{ (r}^2=0.9998)\]

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 r² 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 μg ml⁻¹), regression equation was computed to be:

\[A_1 = 0.406C - 0.085 r^2=0.9997\]
\[A_2 = 0.956C - 0.220 r^2=1\]

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 r² 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.

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

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\textsuperscript{[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 degrade. The results are listed in table (3).

Pharmaceutical Applications
The proposed methods were applied for the determination of the studied drug in (Examide\textsuperscript{®} 10 mg) tablets, The results were validated by comparison to a previously reported method.\textsuperscript{[30]} No significant differences were found by applying student t-test and F-test at 95% confidence level\textsuperscript{[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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Ratio difference</th>
<th>Mean centering</th>
<th>Bivariate</th>
<th>First derivative</th>
<th>Dual wavelength</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>270 &amp; 236</td>
<td>250</td>
<td>275</td>
<td>285</td>
<td>258.8</td>
<td>296.6 &amp; 265.4</td>
</tr>
<tr>
<td>Linearity range</td>
<td>2.5 - 25</td>
<td>5 - 25</td>
<td>5 - 25</td>
<td>5 - 25</td>
<td>5 - 25</td>
<td>5 - 25</td>
</tr>
<tr>
<td>LOD (µg/ml\textsuperscript{1})</td>
<td>0.325</td>
<td>0.127</td>
<td>0.532</td>
<td>0.349</td>
<td>0.345</td>
<td>0.115</td>
</tr>
<tr>
<td>LOQ (µg/ml\textsuperscript{1})</td>
<td>0.986</td>
<td>0.387</td>
<td>1.613</td>
<td>1.057</td>
<td>1.046</td>
<td>0.349</td>
</tr>
<tr>
<td>Regression equation</td>
<td>0.0255</td>
<td>0.0205</td>
<td>0.0344</td>
<td>0.0389</td>
<td>0.0073</td>
<td>0.0099</td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.0031</td>
<td>-0.0047</td>
<td>-0.0037</td>
<td>-0.0085</td>
<td>0.0017</td>
<td>-0.0063</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation coefficient (r\textsuperscript{2})</td>
<td>0.9998</td>
<td>0.9998</td>
<td>0.9996</td>
<td>0.9998</td>
<td>0.9996</td>
<td>0.9998</td>
</tr>
</tbody>
</table>

\* y = a + bx \text{ where y is the response and x is the concentration.}
Table (2): Intraday and inter-day accuracy and precision for the determination of the Torsemide by the proposed methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Conc. µg.ml⁻¹</th>
<th>Intraday</th>
<th></th>
<th>Inter-day</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Found Conc. ± SD</td>
<td>Accuracy (R%)</td>
<td>Precision (RSD%)</td>
<td>Found Conc. ± SD</td>
</tr>
<tr>
<td>Ratio difference</td>
<td>10</td>
<td>10.061±0.059</td>
<td>100.614</td>
<td>0.595</td>
<td>10.087±0.022</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>14.924±0.181</td>
<td>99.494</td>
<td>1.213</td>
<td>15.081±0.163</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>24.793±0.022</td>
<td>99.173</td>
<td>0.091</td>
<td>24.976±0.271</td>
</tr>
<tr>
<td>Mean centering</td>
<td>7.5</td>
<td>7.560±0.045</td>
<td>100.811</td>
<td>0.596</td>
<td>7.517±0.079</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>14.899±0.040</td>
<td>99.329</td>
<td>0.273</td>
<td>14.789±0.130</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>25.046±0.060</td>
<td>100.185</td>
<td>0.243</td>
<td>25.003±0.028</td>
</tr>
<tr>
<td>Bivariate</td>
<td>5</td>
<td>5.04±0.039</td>
<td>100.74</td>
<td>0.768</td>
<td>4.99±0.065</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>14.98±0.059</td>
<td>99.89</td>
<td>0.396</td>
<td>14.98±0.121</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>24.91±0.090</td>
<td>99.62</td>
<td>0.362</td>
<td>24.91±0.074</td>
</tr>
<tr>
<td>First derivative</td>
<td>7.5</td>
<td>7.529±0.079</td>
<td>100.395</td>
<td>1.050</td>
<td>7.484±0.079</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>14.881±0.079</td>
<td>99.208</td>
<td>0.531</td>
<td>14.879±0.080</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>25.063±0.285</td>
<td>100.255</td>
<td>1.137</td>
<td>24.926±0.079</td>
</tr>
<tr>
<td>AUC</td>
<td>5</td>
<td>5.047±0.058</td>
<td>100.942</td>
<td>1.155</td>
<td>5.013±0.058</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>15.215±0.116</td>
<td>101.436</td>
<td>0.766</td>
<td>14.912±0.154</td>
</tr>
<tr>
<td></td>
<td>22.5</td>
<td>22.656±0.202</td>
<td>100.695</td>
<td>0.891</td>
<td>22.622±0.154</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>7.49±0.038</td>
<td>99.86</td>
<td>0.505</td>
<td>7.50±0.057</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>15.07±0.033</td>
<td>100.49</td>
<td>0.218</td>
<td>15.08±0.031</td>
</tr>
<tr>
<td></td>
<td>22.5</td>
<td>22.87±0.073</td>
<td>101.64</td>
<td>0.318</td>
<td>22.87±0.079</td>
</tr>
</tbody>
</table>
Table (3): Determination of Torsemide and its degrade in their laboratory mixtures by the proposed methods.

<table>
<thead>
<tr>
<th>Intact mg mL⁻¹</th>
<th>Degradate µg mL⁻¹</th>
<th>% degrade</th>
<th>% of intact</th>
<th>% of intact</th>
<th>Mean centered method</th>
<th>Bivariate method</th>
<th>First derivative method</th>
<th>Dual wavelength method</th>
<th>Area under curve method</th>
</tr>
</thead>
<tbody>
<tr>
<td>22.5</td>
<td>2.5</td>
<td>10</td>
<td>100.55</td>
<td>99.22</td>
<td>101.91</td>
<td>100.030</td>
<td>101.144</td>
<td>100.05</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>20</td>
<td>100.76</td>
<td>99.695</td>
<td>99.55</td>
<td>99.520</td>
<td>100.656</td>
<td>101.57</td>
<td></td>
</tr>
<tr>
<td>17.5</td>
<td>7.5</td>
<td>30</td>
<td>100.59</td>
<td>99.282</td>
<td>98.34</td>
<td>101.996</td>
<td>100.028</td>
<td>99.13</td>
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<tr>
<td>15</td>
<td>10</td>
<td>40</td>
<td>99.84</td>
<td>99.871</td>
<td>98.53</td>
<td>98.904</td>
<td>101.212</td>
<td>98.25</td>
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</tr>
<tr>
<td>12.5</td>
<td>12.5</td>
<td>50</td>
<td>99.11</td>
<td>100.434</td>
<td>99.44</td>
<td>100.054</td>
<td>98.020</td>
<td>99.30</td>
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<tr>
<td>10</td>
<td>15</td>
<td>60</td>
<td>100.35</td>
<td>101.114</td>
<td>100.780</td>
<td>99.29</td>
<td>101.53</td>
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<tr>
<td>7.5</td>
<td>17.5</td>
<td>70</td>
<td>101.91</td>
<td>100.437</td>
<td>99.178</td>
<td>100.067</td>
<td>98.03</td>
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</tr>
</tbody>
</table>

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 Method²⁰⁻²⁴ (K X 10⁶) For The Mixture of Torsemide And its oxidative Degradate By The Bivariate Method

<table>
<thead>
<tr>
<th>λ/λ</th>
<th>265</th>
<th>275</th>
<th>285</th>
<th>295</th>
<th>305</th>
<th>315</th>
<th>325</th>
</tr>
</thead>
<tbody>
<tr>
<td>265</td>
<td>0</td>
<td>182.12</td>
<td>285.72</td>
<td>302.32</td>
<td>246.32</td>
<td>152.4</td>
<td>65.36</td>
</tr>
<tr>
<td>275</td>
<td>0</td>
<td>1209.79</td>
<td>138.02</td>
<td>112.96</td>
<td>54.54</td>
<td>1.02</td>
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</tr>
<tr>
<td>285</td>
<td>0</td>
<td>41.32</td>
<td>34.46</td>
<td>2.76</td>
<td>36.28</td>
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<td></td>
</tr>
<tr>
<td>295</td>
<td>0</td>
<td>0.84</td>
<td>24.96</td>
<td>47.84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>305</td>
<td>0</td>
<td>20.76</td>
<td>39.16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>315</td>
<td>0</td>
<td>18.72</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>325</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Table (4): Results obtained by the proposed compared with a reported method³⁰ for the determination of torsemide in its pharmaceutical preparations(Examide® 10mg)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Ratio difference method</th>
<th>Mean centering method</th>
<th>Bivariate method</th>
<th>First derivative method</th>
<th>Dual wavelength method</th>
<th>Area under curve method</th>
<th>Reported method³⁰</th>
</tr>
</thead>
<tbody>
<tr>
<td>N*</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>X</td>
<td>100.132</td>
<td>99.414</td>
<td>99.178</td>
<td>100.743</td>
<td>99.05</td>
<td>99.492</td>
<td>99.838</td>
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<tr>
<td>SD</td>
<td>1.255</td>
<td>0.895</td>
<td>1.212</td>
<td>0.992</td>
<td>0.742</td>
<td>0.932</td>
<td>1.37</td>
</tr>
<tr>
<td>RSD%</td>
<td>1.253</td>
<td>0.900</td>
<td>1.218</td>
<td>0.984</td>
<td>0.749</td>
<td>0.937</td>
<td>1.37</td>
</tr>
<tr>
<td>f**</td>
<td>0.353</td>
<td>0.578</td>
<td>0.392</td>
<td>1.195</td>
<td>1.13</td>
<td>0.467</td>
<td>—</td>
</tr>
<tr>
<td>(2.306)</td>
<td>(2.364)</td>
<td>(2.306)</td>
<td>(2.364)</td>
<td>(2.446)</td>
<td>(2.364)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F**</td>
<td>1.192</td>
<td>2.342</td>
<td>1.278</td>
<td>1.908</td>
<td>3.406</td>
<td>2.16</td>
<td>—</td>
</tr>
</tbody>
</table>

* 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.

ACKNOWLEDGMENT
I am deeply thankful to ALLAH, by the grace of whom this work was realized. I wish to express my indebtedness and gratitude to staff members Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy Al-Azhar University, Cairo, Egypt for their valuable supervision, continuous guidance, and encouragement throughout the whole work.

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Spectroscopy., 2013; 104: 70–76.


