Torsemide Analysis Using Derivative Spectrophotometric Methods

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**Article History:**
Received on: 22 Aug 2020
Revised on: 20 Sep 2020
Accepted on: 22 Sep 2020

**Keywords:**
Torsemide, Spectrophotometry, Derivative, Stability

**Abstract**
An accurate stability-indicating method has been developed for the analysis of Torsemide (TOR) in bulk and pharmaceutical dosage forms. The methods used the zero-order spectrum ($0D$) of TOR aqueous solution (measured at 285 nm) and the instrumentally differentiated first ($1D$) and second ($2D$) derivative spectra (measured at 311 nm and 282 nm, respectively). The effect of light, acid (HCL) and alkali (NaOH) on the stability of TOR were studied using the new methods. ICH guidelines were used to validate the new methods. Regression analysis of Beer’s plots showed a good correlation coefficient not less than 0.998. These methods reported great inter-day and intra-day precision. Excipients interference was not detected due to the achievement of good recovery percentages (97.60 - 101.45 %, n=3). Good assay results ranged from 99.0 ± 1.7% to 100.0 ± 2.5%; the developed methods obtained n=3. The $2D$ model proved its ability to be used as a stability indication method of TOR analysis. TOR was unstable in acids and bases with or without heating. Its degradation follows the first-order kinetics. However, its aqueous solution was proved to be stable under sunlight. The established methods demonstrated good precision, sensitivity and accuracy at 95% confidence level.

**INTRODUCTION**
TOR Figure 1 is a loop diuretic used alone or in combination with other medications to treat high blood pressure and oedema. It hinders the reabsorption of chloride and sodium in the luminal membrane of the ascending limb of the loop of Henle by affecting the chloride binding site of the $1Na^+, 1K^+, 2Cl^-$ co-transport system (Friedel and Buckley, 1991; Luft, 1993).

Literature survey revealed different methods for the analysis of TOR in bulk and dosage forms, including spectrophotometric, colourimetric and chromatographic methods (Subramanian et al., 2015; Zaazaa et al., 2016). Many of the reported methods are complicated, costly and of low sensitivity.

Thus, the present study intends to develop precise and straightforward derivative spectrophotometric methods for the analysis of TOR in bulk and dosage forms and to study its stability under stress conditions.

Derivative spectroscopy involves the conversion of a normal spectrum to its first, second or higher derivative spectrum, and the average absorption spectrum is referred to as the fundamental, zero-order or $0D$ spectrum. The first derivative ($1D$) spectrum is a plot of the rate of change of absorbance with wavelength against wavelength. The second derives ($2D$) spectrum is a plot of the curvature of the $0D$ spectrum against wavelength.

The derivative spectrum shows better resolution of
overlapping bands than the fundamental spectrum and may permit the accurate determination of the \( \lambda_{\text{max}} \) of the individual bands. Secondly, derivative spectrophotometry discriminates in favour of substances of narrow spectral bandwidth against broad bandwidth substances. These advantages permit the selective determination of certain absorbing substances in samples in which non-specific interference may prohibit the application of simple spectrophotometric methods.

![Chemical structure of TOR](image)

**Figure 1: Chemical structure of TOR**

**MATERIALS AND METHODS**

**Apparatus**

UV spectrophotometric studies were carried out on Shimadzu UV-1800EN240V, double beam, (Kyoto, Japan). Sensitive balance: Kern ALS 120-4, German

**Reference standard and sample**

TOR authentic material was purchased from Sigma labs in 10 mg quantities. Examide 10 mg tablets (Torsemide 10 mg) samples were purchased from Egypt.

**Reagents**

Sodium hydroxide: BDH, Poole, England; Hydrochloric acid: BDH, Poole, England.

**Preparation of stock solutions**

**Standard stock solution**

TOR standard solution

Ten mgs of TOR standard dissolved in distilled water. Dilution was performed to obtain Solution A; 100 \( \mu \)g/ml.

**Sample stock solution**

TOR Sample solution

Ten tablets of Examide 10 mg were weighed and ground. An equivalent amount containing 10mg TOR was weighed and dissolved in 100 ml distilled water. The solution was then sonicated for 10 min and filtered (Solution B; 100 \( \mu \)g/ml).

**Procedure**

**Determination of \( \lambda_{\text{max}} \)**

A concentration of 20 \( \mu \)g/ml was obtained by dilution of the appropriate aliquot volume of solution A. This solution was then scanned in the range 200-400 nm in zero (\( 0^\text{D} \)), first (\( 1^\text{D} \)) and second (\( 2^\text{D} \)) order derivative modes, respectively.

![0D spectrum of TOR solution](image)

**Figure 2: 0D spectrum of TOR solution (20 \( \mu \)g/ml; 285 nm)**

![1D spectrum of TOR solution](image)

**Figure 3: 1D spectrum of TOR solution (20 \( \mu \)g/ml; 311nm)**

![2D spectrum of TOR solution](image)

**Figure 4: 2D spectrum of TOR solution (20 \( \mu \)g/ml; 282 nm)**

**Method validation**
Figure 5: Effect of HCL 1 M and NaOH 1 M on TOR using second derivative (20 µg/ml)

Figure 6: TOR degradation Kinetic at time intervals 10 min

Linearity
Solution A was used to prepare serial dilutions. 0D, 1D and 2D spectra of the resultant solutions were documented in the range from 240 to 400 nm. Calibration curves were constructed by plotting the mean absorbance values against concentration. Linearity data and limits of detection and quantification were then calculated (Miller and Miller, 2018)

Content uniformity
The linearity was repeated using solutions B instead of solution A and direct comparison of the absorbance of sample and standard was used to calculate the uniformity of the tablet’s solutions.

Precision
Serial dilutions from solution (concentrations of 10µg/ml, 30µg/ml and 40µg/ml) were examined three times in one day (inter-day) and three times in different days (intra-day) using the three modes (0D, 1D and 2D). Results were used to calculate the relative standard deviation (RSD %) to reflect the precision of the method.

Recovery percentage
Two ml of solution A and B were transferred into two separate 10 ml volumetric flasks. 2ml of solutions A and B were mixed in a third one, and all the three were treated as under linearity. Per cent added recovery was calculated as follows:

\[ R% = \frac{[\text{Mixture response} - \text{Sample response}/\text{Standard response}] \times 100}{\text{Sample response}} \]

Stability studies
Effect of acid and alkali on the stability of TOR solution
2 ml of solution A were transferred to five stoppered glass tubes then 1 ml of 1 M HCl was added to each tube. The volumes were then completed to 10ml with distilled water. The second derivative spectrum for the solution in the first tube was recorded. The rest four solutions were heated in a boiling water bath at 10 minutes heating time interval. Solutions were cooled, and the second derivative method was used to assess the effect of the acid on TOR degradation.

The procedure was repeated by adding 1mL of 1M NaOH instead of HCl to investigate the effect of alkali on TOR stability.

Light effect
TOR standard solution (25µg/ml) was stored in transparent glass tubes under sunlight to study the possible photodegradation using the new method.

RESULTS AND DISCUSSION

Derivative spectrophotometry is a simple, powerful technique. It is suitable for analysis of turbid solutions (Elimam et al., 2017), and it is used effectively for the assay of pharmaceutical formulations. Derivative spectrophotometry is generated by differentiation of the absorbance (A) of a sample with the wavelength (λ).

Derivative spectrophotometry also resolves the issues of drugs combination analysis, stability experiments of the drug, possible interference of drug impurities and excipients. Besides, it eases the analysis of drugs in biological fluids (Elimam et al., 2015).

The stability of a pharmaceutical product is determined by its capability to retain its efficacy, properties, and characteristics throughout its shelf life (Shantier et al., 2013). Amides containing compounds are one of the most susceptible compounds for hydrolysis.

Solvent, hydroxide ion, and many buffer species catalyze the decomposition of amides. Therefore, they are less stable in solutions (Shantier et al., 2011). It is important to investigate the chemical stability of drugs to determine its suitable storage conditions.
The zero-order spectrum of TOR showed maximum absorbance at 285 nm (Figure 2). A first and second derivatization absorption maximum was at 311 nm and 282 nm, respectively (Figures 3 and 4).

**Linearity**

The constructed calibration curves obeyed Beer’s law over the concentration range 10-50 \( \mu g/ml \). The linearity data was calculated at 95% confidence limit (Elimam et al., 2017) and summarized in Table 1.

**Precision**

The precision of the developed method was evaluated by three concentrations of TOR within the linearity range. The obtained RSD% values were within the acceptable range (0.00-2%) as stated in Table 2.

**Recovery percentage**

The freedom of tablets excipients interference was verified by recovery testing results (97.60 - 101.45 ± 2.7 %, n=3) which proves the accuracy of the new methods.

**Assay and validation**

The developed methods were then applied for the assay of pharmaceutical formulation. Excellent assay results ranged from 99.0 ± 1.7% to 100.0 ± 2.5%; n=3 was obtained for the three modes. Obtained assay result was 101.21 ± 0.95%, n=3.

Methods validity was then measured by a statistical comparison of the results obtained using the following formula (Shantier et al., 2011):

\[
t = \frac{X'}{\sqrt{\frac{N}{S}}}
\]

where \( X' \) = content % mean at 95% confidence level (99.5%), \( \mu \) = true mean \( N \) = number of measurements, \( S \) = standard deviation.

As the calculated \( t \) values ranging between 1.7 and 0.8 for the zero-order and derivative orders, respectively, at 95% confidence limit were less than tabulated one (12.706), the new methods are accurate.

**Stability Studies**

**Effect of acid and alkali**

The influence of different concentrations of acid or alkali, combined with different heating time intervals, was investigated. 1 M of either the acid or alkali without heating was found to be the condition which gave measurable degradation rate with good linearity.

The UV scanning of the \( ^2 \)D spectra of acid and alkali-treated TOR solution showed the disappearance of its peaks at 282 nm with consequent formation of two peaks at 266 nm and 292 nm (Figure 5). Heating appears to have no increase on acidic and alkaline degradation of TOR because degradation occurred in acidic and alkaline conditions did not change when heating was applied. This proves that TOR is unstable in both acidic and alkaline conditions with or without heating. Acidic and alkaline degradation of TOR was found to follow the first-order kinetic, as shown in Figure 6. A suggested
Figure 7: Proposed acidic hydrolysis pathway of TOR degradation

Figure 8: Proposed alkaline hydrolysis pathway of TOR degradation
degradation pathway of TOR by acidic and alkaline hydrolysis is shown in Figures 7 and 8 respectively.

Light effect
TOR solution remained stable upon exposure to sunlight as there was no reduction of drug absorbance or change in the derivative spectrum. This reflected the stability of TOR solution under sunlight.

CONCLUSIONS
The established methods demonstrated good precision, sensitivity and accuracy at 95% confidence level for the estimation of TOR in bulk and pharmaceutical dosage forms. Besides, the steps of the new contain no extraction step. Therefore, it can be utilized for routine analysis of the drug. On the other hand, the²D method proved its stability-indicating properties.

Conflict of Interest
The authors declare that they have no conflict of interest for this study.

Funding Support
The authors declare that they have no funding support for this study.

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