



## RP-HPLC Determination of Torsemide in Pharmaceutical Formulation by Liquid Chromatography

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### ABSTRACT

A high-performance liquid chromatographic method for the determination of Torsemide is described. The assay uses a reversed-phase gradient system and UV-detection. The chromatographic separation was carried out on a  $\mu$ Bondapak C<sub>18</sub> column with a mobile phase consisting of Acetonitrile/Phosphate Buffer 0.05M (pH 2.4) in ratio of 70/30. The method was validated and found to be linear in the range of 50-100  $\mu$ g/ml. The retention time of Torsemide was  $6.00 \pm 0.20$  min. The chromatograms showed good resolution and no interference with impurity. The mean recovery of the Torsemide was found to be above 99.9%. Both accuracy and precision data showed good reproducibility. The linearity range was found to be 50-100  $\mu$ g/ml with coefficient of variation of 0.998 at calibration point. The limit of detection and limit of quantization for Torsemide were found to be 148.1ng and 448.9ng respectively. These results suggested that the analytical method was linear, precise, and accurate.

**Keywords:** HPLC, Acetonitrile, isocratic, Torsemide.

### 1. 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<sup>[1-4]</sup>. Torsemide is useful in the treatment of mild-to-moderate hypertension in doses of 2.5 to 5 mg given once daily<sup>[5]</sup>. These doses lower blood pressure as effectively as 25 mg of hydrochlorothiazide but without producing diuresis<sup>[6]</sup>. 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<sup>[7-9]</sup>.

Recently a formulation of torsemide has been launched in market. In this formulation, torsemide shows a synergistic effect with other combination. torsemide was determined by several methods for the analysis including gas chromatography (GC)<sup>[10]</sup>, liquid chromatography with UV detection (LC-UV)<sup>[11]</sup>, HPTLC<sup>[12,13]</sup> derivative spectrophotometric<sup>[12,13]</sup>. Torsemide was determined with or without combination of several drugs by HPLC,

spectrophotometrically and HPTLC<sup>[14]</sup> but literature survey revealed that no HPLC method has been reported yet for single estimation for Torsemide. The present study was aimed to develop a simple, rapid, precise, accurate, and selective chromatographic method for estimation of Torsemide oxalate in bulk and dosage forms with the use of buffer in the mobile phase in short duration.

### 2. METHODS AND MATERIAL

Bulk sample of Torsemide was obtained from Ranbaxy Laboratories, Devas, India. The commercial samples of tablet containing 10 mg of torsemide were purchased from local market. Acetonitrile (HPLC Grade), Water (HPLC Grade), Monobasic Potassium phosphate (AR Grade), orthophosphoric acid (AR Grade) were purchased from RANCHEM (India). Milli-Q water was used throughout the experiment. Quantitative HPLC was performed on isocratic HPLC of Waters consisting of 510 HPLC Pump, manual with 20 $\mu$ L sample injection loop and UV 481 detector. Chromatographic conditions were obtained using a  $\mu$ Bondapak column (C<sub>18</sub> 250mm x 4.6mm

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5 $\mu$ m). The analytical wavelength was set at 288 nm and samples of 20 $\mu$ l were injected to HPLC system. The mobile phase was Acetonitrile and Phosphate Buffer (0.05 M) in ratio of 60:40 (pH 2.4) at a flow rate of 1ml/min. The mobile phase was filtered through 0.22 $\mu$ m filter and degassed for 10 minutes by sonication.

#### Preparation of standard stock solutions

Accurately weighed 10 mg of Torsemide was transferred to a 10 ml volumetric flask, sufficient amount of acetonitrile was added to dissolve it followed by addition of 3 ml of 0.05M phosphate buffer (pH 2.4). Then volume was made up to 10 ml (stock A; 1000  $\mu$ g/ml) with acetonitrile. 1ml of stock A was taken into 10 ml volumetric flask and further diluted up to 10 ml with acetonitrile (stock B; 100  $\mu$ g/ml). Aliquots of stock B were further diluted up to 10 ml to get concentration of 10, 20, 30, 40, 50, 60, 70, 80, and 90  $\mu$ g/ml.

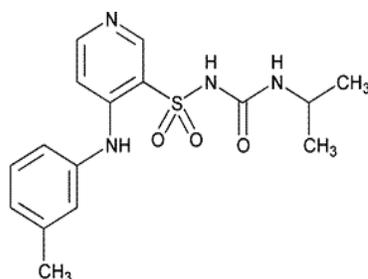


Figure 1: Structure of Torsemide

#### Sample preparation

Twenty tablets were weighed and content emptied. The average weight was determined. It was finely powdered and mixed thoroughly. Accurately weighed tablet powder equivalent to 10 mg of Torsemide was transferred to a 10 ml volumetric flask, sufficient amount of acetonitrile was added to dissolve it followed by addition of 3 ml of 0.05M phosphate buffer (pH 2.4). Then volume was made up to 10 ml (stock A; 1000  $\mu$ g/ml) with acetonitrile. 1ml of stock A was taken into 10 ml volumetric flask and further diluted up to 10 ml with acetonitrile (stock C; 100  $\mu$ g/ml). Aliquots of stock C were further diluted up to 10 ml to get concentration of 10, 20, 30, 40, 50, 60, 70, 80, and 90  $\mu$ g/ml.

#### Determination of Assay

Twenty tablets (Dytor 10mg) manufactured by Cipla, were weighed and content emptied. The average weight was determined. It was finely powdered and mixed thoroughly. Accurately weighed tablet powder equivalent to 10 mg of Torsemide was transferred to a 10 ml volumetric flask, sufficient amount of acetonitrile was added to dissolve it followed by addition of 3 ml of 0.05M phosphate buffer (pH 2.4) and filtered by 0.25 micron membrane filter. Then volume was made up to 10 ml (stock A; 1000  $\mu$ g/ml) with acetonitrile. 1ml of stock A was taken into 10 ml volumetric flask and further diluted

up to 10 ml with acetonitrile. Five replicates of sample (100  $\mu$ g) in equal volume (20 $\mu$ L) were injected separately into the stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured. The amount of drug present per tablet was calculated by comparing a sample peak with that of standard solution. The amount of drug present per tablet was calculated by comparing a sample peak with that of standard solution shown in Table No.1.

Formulation	Label Claim gm/tab	Amount found gm/tab $\pm$ RSD*	%Assay* $\pm$ RSD*
Torsemide	0.0100	0.0096 $\pm$ 0.47	98.87 $\pm$ 0.47

Table 1: Assay Result of Dosage Form

\*denotes average of five determinations (n=5)

#### Method Validation

##### Specificity

Subjecting the drug solution in triplicate and retention time and peak area were measured followed by injecting the sample solution of the same concentration in triplicate.

##### Linearity

The standard curve was prepared in the concentration range 50 to 100 $\mu$ g/mL for Torsemide. The linearity of these methods was evaluated by linear regression analysis, using least square analysis method and data is shown in Table 2.

S.No.	Parameter	
1	Calibration range ( $\mu$ g/ml)	50 to 100
2	Correlation Coefficient (r2) $\pm$ S.D*	0.9998
3	Retention time (min.) $\pm$ S.D*	6.208 $\pm$ 0.004
4	Resolution factor	1.8
5	Tailing factor*	1.0183 $\pm$ 0.007
6	Asymmetry	0.98 0.97
7	No. of theoretical plate*	2893 $\pm$ 63.996
8	Limit of quantification (ng/ml)	0.4489
9	Limit of Detection	0.1481
10	Precision (RSD*, %) Intraday (n=5)	0.026
11	Robustness (RSD*, %)	0.792

Table 2: Validation and System Suitability Parameters

\* Mean of five determinations (n=5).

##### Limit of Detection and Limit of Quantitation

The LOD and LOQ were determined for HPLC method. The limits were determined based on the standard deviation amongst response and slope of the curve at lowest concentrations.<sup>[15, 16]</sup> The standard deviation of y-intercepts of regression lines were determined and kept in the following equation for the determination of detection limit and quantification limit. Detection limit = 3.3  $\sigma$ /s; quantification limit = 10  $\sigma$ /s; Where  $\sigma$  is the standard deviation of y intercepts of regression lines and s is the slope of the calibration curve. The result is shown in Table No. 2

### Precision

The precision studies were performed by repeatability studies. Standard solutions were prepared and were injected in triplicate. The response of each injection was measured and the precision was calculated using  $\pm$  S.D and % RSD equations. The % RSD values for repeatability precision study should be  $\leq$  1%, which confirm that method is sufficiently precise. The result is shown in Table No. 2

### Robustness

The robustness was performed by adding 50, 100, and 150 % of the pure drug with the formulation taken for analysis in two different mobile phase compositions of 60/40 and 80/20. The % recovery was calculated for each added concentration in both mobile phases. The result is shown in Table No. 2

### System Suitability

System suitability tests were performed as per the USP 31 to confirm the suitability and reproducibility of the system. The test was carried out by injecting 20 $\mu$ L standard solutions of torsemide 100  $\mu$ g/mL. This was repeated five times. The RSD values of Torsemide were  $\pm$ 0.46. The RSD values were found to be satisfactory and meeting the requirements of USP 31. Theoretical plates, tailing factor were determined and are presented in Table

### Accuracy

The accuracy of the developed method was determined by recovery studies. The recovery studies are usually made by spiking the known amount of pure drug with the formulation. It is usually done by adding 50, 100, and 150 % of the pure drug with the formulation taken for analysis and result were discussed in Table No. 3.

S. No.	% Drug Added	% Drug Recovered $\pm$ % RSD*	% Recovery $\pm$ % RSD*
1	80	79.13 $\pm$	98.90
2	100	99.85 $\pm$	99.85
3	120	119.99 $\pm$	99.44

Table 3: Recovery Studies of Torsemide in Dosage Form

### 3. RESULTS

Optimization of the mobile phase was performed based on resolution; asymmetric factor and peak area obtained for Torsimide for develop a suitable and robust LC method for the determination of torsemide different mobile phases and columns were employed to achieve the efficient separation and resolution. The criteria employed for selecting the mobile phase for the analysis of the drugs were cost involved and time required for the analysis. Attempts with traditional reverse phase columns presented poor peak symmetry and tailing problem. Most of the separation methods in literature overcame these problems by use of buffers in mobile phase<sup>[17-20]</sup>. The proposed method was able to selectively

separate torsemide in a short chromatographic run with the use of buffer mobile phase. The retention time is 6.00  $\pm$  0.20 min. The chromatogram is shown in (Figure 2).

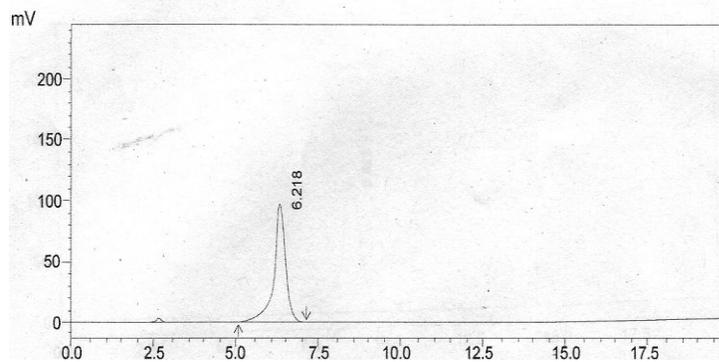


Figure 2: Chromatogram of Torsemide

### 4. DISCUSSION

The proposed method for quantitative determination of Torsemide in pharmaceutical formulation is efficient and sensitive. The excipients of the commercial sample analyzed did not interfere in the analysis, which proved the specificity of the method for these formulations. The HPLC method was found to be simple, rapid, precise, accurate, and sensitive. Its advantages over other existing methods are its low-cost and less time consuming. This method can be used for routine quality control of Torsemide in commercial samples.

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### 6. REFERENCES

- Gennaro A.R. Remington, The Science and Practice of Pharmacy, Lippincott Williams and Wilkins, Pennsylvania; 21 ed.1, 537-544, 2005.
- Stenlake J B, Beckett A.H. Practical Pharmaceutical Chemistry, 4 ed. CBS Publishers and Distributors; 2, 85-170, 2002.
- British Pharmacopoeia, Published by The stationary office under the license from the controller of Her Majesty's Stationary Office for the Health Dept; 2058-2059 2007.
- Dunn C J, Fitton A, Brogden R N."Torsemide. An update of its pharmacological properties and therapeutic efficacy". Drugs 1995; 49: 1, 121-42.
- Gүүл, S J, Jounela A J. The efficacy and tolerability of enalapril with a formulation with a very low dose of hydrochlorothiazide in hypertensive patients resistant to enalapril monotherapy. Am J Hypertens 1995; 8: 727.
- Neutel J M. Metabolic manifestations of low-dose diuretics. Am. J Med. 1996; 101:71, S-82S.
- Ellison D H. The physiologic basis of diuretic synergism: its role in treating diuretic resistance. Ann Intern Med 1991; 14: 886-894.
- Dirks JH, Sutton RAL. Diuretics: physiology, pharmacology, and clinical use. WB Saunders: 1986.

9. Martin S. Continuous infusion of loop diuretics: Pharmacodynamic concepts and clinical applications. *Clin. Trends in pharmacy Practice* 1994; 8:10-15.
10. Sharma K, Gupta S, Sharma Y. estimation of spironolactone and torsemide in combined tablet dosage form using by multicomponent mode of analysis, *pharmatutor-art-1180*.
11. Khan I J, Loya P, Saraf M N. A simplified HPLC method for quantification of torsemide from human plasma and its application to a bioequivalence study *Indian J Pharm Sci.* 2008; 70: 4, 519-22.
12. B Adelaida, A Gamboa ragon, N A Julio, C M Cecilia, A R Villagra, Lopez lara M, Edda C V L, Quantitative Analysis of Torsemide in human plasma by High Performance Liquid Chromatography with ultraviolet detection, *Rev Mex Patol Clin.* 2011; 58: 41, 95-200.
13. Sharma S, Sharma M C, Kohli D V, Chaturvedi S C. Isocratic reverse phase HPLC estimation method of Torsemide and Spironolactone in pharmaceutical combined dosage form, *Optoelectron Adv Mat* 2010; 4: 2, 234 – 237.
14. Sharma S, Sharma MC, Kohli D V, Sharma A D. Development and Validation of TLC-Densitometry Method for Simultaneous Quantification of Montelukast Sodium and Levocetirizine Dihydrochloride Pharmaceutical Solid dosage form, *Der Pharmacia Lettre* 2010; 2: 1, 489-494
15. Code Q<sub>2</sub>A-Text on Validation of Analytical Procedure Step-3 Consensus Guideline, ICH Harmonised Tripartite Guideline: 1994.
16. Code Q<sub>2</sub>B- Validation of Analytical Procedure Methodology Step-4 Consensus Guideline, ICH Harmonised Tripartite Guideline : 1994.
17. Sharma B.K., *Instrumental Methods of Chemical Analysis*, 24th ed. Goel Publishing House: 3-20, 286-385, 2005
18. Skoog D A, West D M, F J, Hollar S R. Crouch, *Fundamental of Analytical Chemistry*, 8<sup>th</sup> ed. Cengage Learning India Private Limited: 815-995; 2008.
19. Williams D A, Lemke T L, *Foye's Principles of Medicinal Chemistry*, 6th ed. Lippincott Williams and Wilkins: 732; 2007.
20. Fillic D, Dumic M, Klepic B, Danilovski A, Tudja M. *Polymorph V of Torasemide*; 2007.

Conflict of Interest: None Declared