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Research Communication

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR QUANTITATIVE ESTIMATION OF TORSEMIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

***Amol. Y. Ghodke[1], Bhagwat. N. Poul[2], Mohini. B. Sorde[3]**
[1]Dept. of Pharmaceutical Analysis, [2]Dept. of Pharmacy, [3]Dept. of Quality Assurance, Maharashtra College of Pharmacy, Nilanga-413521 Dist. Latur Maharashtra, INDIA

***Corresponding Author:**
E-mail- aamolias@gmail.com

Abstract: *A simple, specific, precise and accurate Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the quantitative estimation of Torsemide in Bulk and Pharmaceutical dosage form. The proposed RP-HPLC method was carried out on Zorbax C18 (250x4.6mm), 5µm column with mobile phase phosphate buffer and methanol (50:50) (v/v). The pH of phosphate buffer was adjusted by Ortho- phosphoric acid to 3.5. The flow rate was 1.3 mL/min and the detection wavelength was 288nm. The retention time of torsemide was found at 6.0±0.2min. The method was validated for specificity, precision, accuracy, linearity and robustness. The linearity range was 10-30 µg/mL and correlation coefficient (r^2) was found to be 0.9980. The mean % recovery for Torsemide was found to be 99.80. The developed method could be employed for the routine analysis of Torsemide from different formulations and for the Torsemide calculations as well.*

Keywords: *RP-HPLC, Torsemide, Zorbax C18, specificity, linearity, precision, accuracy.*

Introduction

Torsemide is a loop 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 (**Figure 1**). It is useful in the treatment of hypertension or edema associated with congestive heart failure, renal disease and hepatic disease.

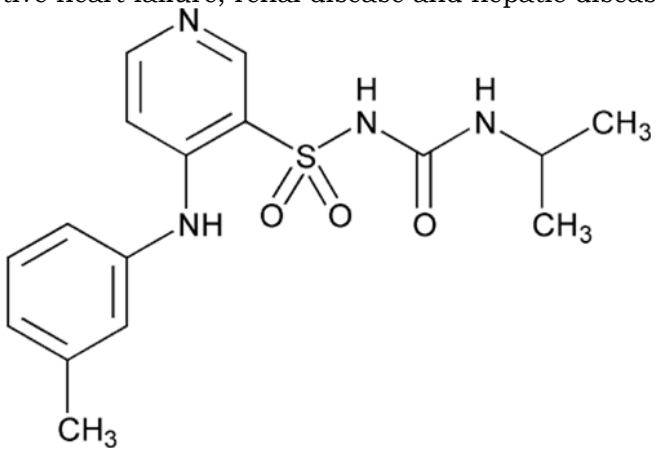

Fig 1: Torsemide

Literature survey revealed reports on methods developed for estimation of Torsemide in bulk and in tablet dosage form and in combination along with other therapeutic agents also, but no method was developed for estimation of Torsemide in injection dosage form. The reported methods are HPLC and UV method (2), (3), (4, 5), (7), GC-MS method (6), and Conductometric method (8).Torsemide along with other drugs was estimated by HPLC and UV method (9, 10), (11, 12), and HPTLC method (13). The present study was undertaken to develop simple, precise, specific, accurate and robust RP-HPLC method for the estimation of Torsemide from injection formulation.

Methods and Material

The pure drug sample (Torsemide) was gifted by Micro Labs, Bangalore and Torsemide Injection formulation (Dytor) was procured from local market (Manufactured by Cipla Pharma, Mumbai).

Chemicals and Reagents:

Potassium monobasic phosphate(Analytical grade) , Methanol(HPLC grade), Ortho Phosphoric acid(Analytical grade), and Water (HPLC grade) were purchased from Research –Lab Fine Chem Industries, Mumbai. All the reagents and chemicals used for analysis were of Analytical grade.

Experimental Conditions:

Quantitative HPLC was performed on Agilent isocratic HPLC (LC1220) with ezchrom elite software G 4286B-1220 infinity isocratic LC manual injector with variable wavelength UV detector. Several trails were carried out for finalizing the chromatographic condition for method development and validation of Torsemide in bulk and pharmaceutical dosage form. The chromatographic condition were obtained by using Zorbax C18 (250x4.6mm), 5µm. The analytical wavelength was set at 288nm and samples of 20 µl were injected to the HPLC system. The mobile phase was Phosphate buffer and Mobile phase (50:50) at a flow rate 1.3mL/min with 3.5 pH adjusted with Orthophosphoric acid. The mobile phase was filtered through 0.41 Whatmann paper and degassed for 5 min using Sonicator.

Preparation of Standard solution:

Transfer 20 mg of Torsemide, accurately weighed, to a 100-mL volumetric flask, add 50 mL of mobile phase, mix, and make up the volume with mobile phase and sonicate for 10 min. (200µg/mL). Transfer 1mL of this solution to 10 mL of volumetric flask and make up with mobile phase to get final concentration of (20µg/mL).

Preparation of Sample solution:

Transfer about 2mL of Torsemide injection to a 100-mL volumetric flask, add 50 mL of mobile phase, mix, and make up the volume with mobile phase and sonicate for 10 min. (200ug/mL). Transfer 1mL of this solution to 10 mL of volumetric flask and make up with mobile phase so as to get (20µg/mL).

Method Validation

1. **Assay:** The assay for Torsemide was performed using the same procedure given in USP. Percentage purity was calculated using AUC from the respective chromatogram as per the USP (**Table 1**).
2. **System Suitability Test:** The system suitability test was performed by using standard solution of Torsemide injecting five times of 20 µg/mL of concentration. The system suitability parameters are given in (**Table 2).**
3. **Specificity:** Specificity study was carried out by injecting blank, standard and sample solution and it shows no interference of standard and sample in the blank preparation. Data represented in **(Table 3).**
4. **Precision:** The Precision of the method was demonstrated by system precision, method precision and intermediate precision studies. In the system precision studies, six replicate injections of the working standard solution prepared as per the proposed method and chromatograms were recorded. Relative standard deviation for the area was calculated and presented in (**Table 4).** In the method precision studies, six replicate injections of the standard solution and sample solution prepared as per the proposed method and chromatograms were recorded. Relative standard deviation for the area was

calculated and presented in (**Table 5).** On another day by other analyst the test were performed for intermediate precision and chromatograms were recorded (**Table 5).** The assay calculated for both method and intermediate precision and it should be in 98 to 102%

5. **Linearity:** The standard solution for linearity was prepared in the concentration range 10 to 30µg/mL injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curves of Torsemide obtained by plotting the peak area versus the concentrations of Torsemide. The linearity curves of Torsemide shown in Figure and Linearity data presented in **(Table 6).**
6. **Accuracy:** The recovery studies were carried out by spiking known quantity of Torsemide standard solution of 50%, 100% and 150% concentration into the sample preparation**.** The recovery studies were performed three times, at each level of recovery (**Table 7**)**.**

7. **Robustness:** Robustness of the method was determined by making slight changes in the experimental conditions such as the composition of the mobile phase, pH of the mobile phase, and flow rate of the mobile phase and the chromatographic characteristics such as wavelength and results were recorded. The data is presented in **(Table 8,9,10 and 11).**

8. **Stability of analytical solution:** Evaluate the stability in analytical solution by injecting the standard preparation and sample preparation at regular interval. The stability of solution is carried out by 0, 3, 6,12,24,48 hrs. The data presented in **(Table 12).**

Results and Discussion

The Torsemide drug was analyzed by using RP-HPLC method in bulk and pharmaceutical dosage form. The aim is to develop accurate and precise method for the quantitative estimation of Torsemide in bulk and pharmaceutical dosage form. Several trails are carried out for selection of column and selection of suitable mobile phase for the method development. After trials the column used in this method Zorbax C18 (250x4.6mm), 5µm and the mobile phase is phosphate buffer and methanol (50:50). The wavelength was set at 288nm. The retention for Torsemide was found at 6.00±0.2 and the run time was 10 min. the injection volume was 20µl. **Figure 2 and 3** represents the Standard (Torsemide) and sample (Torsemide injection) chromatograms.

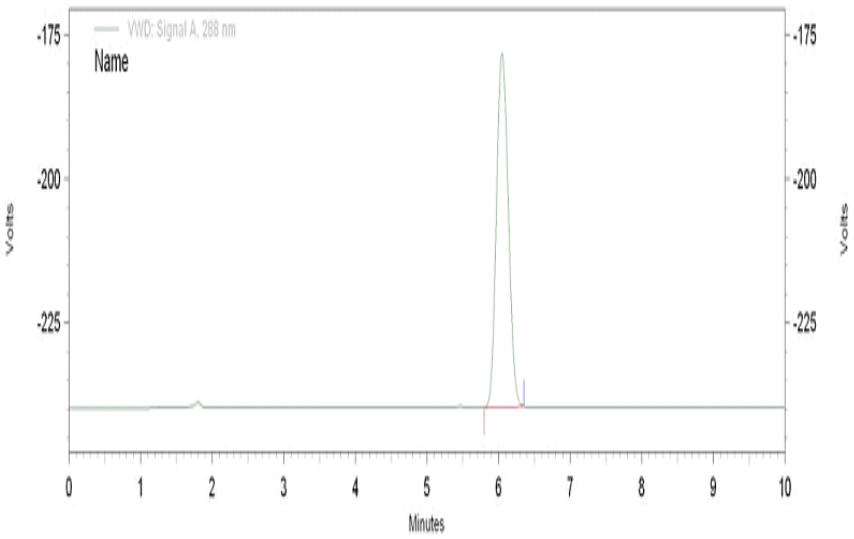

Fig. 2: Standard chromatogram

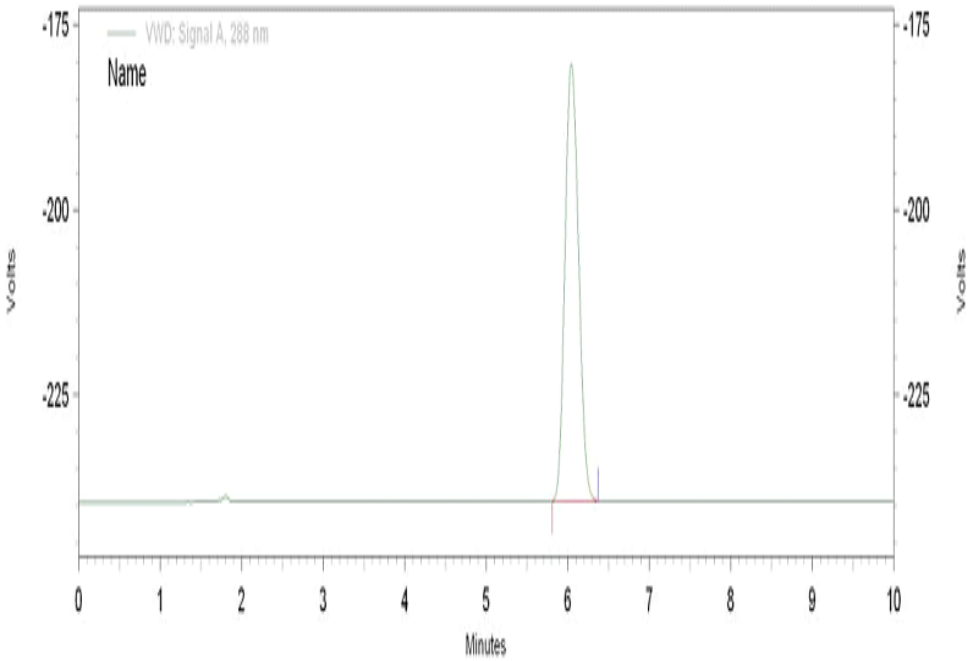

Fig. 3: Sample chromatogram

Table 1:
Result of Assay

Sample	Amount taken	Area	% Purity	% Assay
Standard drug (Torsemide)	20 mg in 100 mL	11205318	99.6	**99.96**
Sample (Torsemide injection)	2 mL in 100 mL	11246420	99.6	

The standard solution injected five times to check the instrument precision.

Table 2:
Results of System suitability test

Sr. No.	Sample	Area	RT
1	Std 01	11938508	6.153
2	Std 02	11936041	6.140
3	Std 03	11999694	6.137
4	Std 04	11940847	6.130
5	Std 05	11963856	6.120
Average		**11955789**	**6.13**
% RSD		**0.225**	**0.19**
Tailing Factor		**1.13**	
No. of Theoretical plates		**9894**	

The blank, standard solution and sample solution are injected. There should be no interferences of standard and sample in blank preparation.

Table 3:
Results of Specificity

	Area	RT
Blank	0	0
Standard(API)	11888558	6.043
Sample(injection)	11505680	6.040

The precision is done to check for the consistent results and which are in the limits. The method and intermediate precisions are showing the results within the limits.

Table 4:
Results of System Precision

Sr NO.	Sample	Area	RT
1	Std 01	11224833	6.203
2	Std 02	11323769	6.207
3	Std 03	11117444	6.207
4	Std 04	11283931	6.210
5	Std 05	11238856	6.203
6	Std 06	11693104	6.190
Average		**11313656**	**6.203**
% RSD		**1.75**	**0.11**
Tailing Factor		**1.12**	
Theoretical plates		**9307**	

Table 5:
Results of Method Precision and Intermediate Precision

Sr NO.	Method (% assay)	Intermediate (% assay)
1	99.46	100.19
2	99.64	100.02
3	100.55	98.87
4	100.12	98.71
5	100.35	100.09
6	100.37	100.19
Average	**100.08**	**99.67**
% RSD	**0.43**	**0.69**

The proposed method is linear and the range is 10µg/mL to 30µg/mL and correlation coefficient is 0.998.

Table 6:
Results of Linearity study

Concentration(µg/mL)	Area
10	5965427
15	8590425
20	10892636
25	14378766
30	16839668

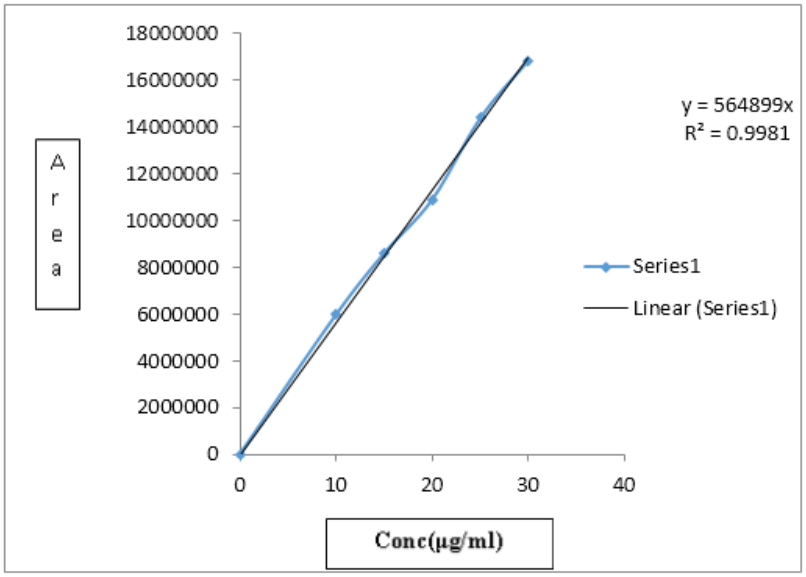

Fig 4: Linearity study of Torsemide

The accuracy of the method was determined by the recovery studies, carried out at different levels 50%, 100% and 150%.

Table 7:
Results of Accuracy

Spike Level in %	Area	Amount Added (mL)	Amt. Found (mg)	% Recovery	Mean	SD	%RSD
50%	5971068	0.01	0.00993	99.3	100.33	1.05	1.04
	6095083	0.01	0.01014	101.4			
	6028009	0.01	0.01003	100.3			
100%	11459889	0.02	0.01991	99.55	99.7	0.21	0.21
	11466165	0.02	0.01992	99.6			
	11508401	0.02	0.01999	99.95			
150%	16577207	0.03	0.02948	98.22	99.38	1.07	1.07
	16311666	0.03	0.0301	100.33			
	16248638	0.03	0.02988	99.61			

The proposed method concludes that it is robust by slight changing the parameters like flow rate, wavelength, mobile phase and change in pH and results are within limits.

Change in Flow rate:

Table 8:
Results of Robustness-Change in flow rate

Sample	As Such (1.3ml/min)	1.1 ml/min	1.5ml/min
Std 01	11224833	13316163	9886238
Std 02	11323769	13423523	9906556
Std 03	11117444	13336309	9920661
Std 04	11283931	13417327	9932057
Std 05	11238856	13402440	9927972
Average	**11237767**	**13379152**	**9914697**
% RSD	**0.69**	**0.36**	**0.18**
Tailing Factor	**1.12**	**1.05**	**1.06**
Theoretical Plates	**9212**	**7601**	**5183**

Change in Wavelength:

Table 9:

Results of Robustness-Change in Wavelength

Sample	As Such (288nm)	286 nm	290 nm
Std 01	11224833	11490993	11751979
Std 02	11323769	11571013	11868670
Std 03	11117444	11545060	11874756
Std 04	11283931	11577070	11870622
Std 05	11238856	11627238	11995227
Average	**11237767**	**11562275**	**11872251**
% RSD	**0.69**	**0.43**	**0.72**
Tailing Factor	**1.12**	**1.50**	**1.51**
Theoretical Plates	**9212**	**3866**	**4103**

Change in Mobile Phase Ratio:

Table 10:

Results of Robustness-Change in Mobile Phase Ratio

Sample	As Such (50:50)	52:48	48:52
Std 01	11224833	12085335	11239049
Std 02	11323769	12019180	11459889
Std 03	11117444	12000111	11466165
Std 04	11283931	12056448	11508401
Std 05	11238856	12017503	11489807
Average	**11237767**	**12035715**	**11432662**
% RSD	**0.69**	**0.28**	**0.96**
Tailing Factor	**1.12**	**1.12**	**1.11**
Theoretical Plates	**9212**	**9969**	**9781**

Change in pH:

Table 11:

Results of Robustness-Change in pH

Sample	As Such Buffer- 3.5	Buffer- 3.3	Buffer- 3.7
Std 01	11224833	11332679	12340637
Std 02	11323769	11344331	12085335
Std 03	11117444	11469160	12131117
Std 04	11283931	11883782	12052905
Std 05	11238856	11403750	12058893
Average	**11237767**	**11486740**	**12133777**
% RSD	**0.69**	**1.98**	**0.98**
Tailing Factor	**1.12**	**1.11**	**1.1**
Theoretical Plates	**9212**	**7095**	**7273**

The solution stability of the drug Torsemide and formulation Torsemide injection was carried out in time interval of 3,6,9,12,24 hrs and the results are found within the limits.

Table 12:

Results of Stability of Solution

Stability in hours	% Assay
0	99.23
3	101.23
6	101.55
12	101.38
24	100.23
Average	**100.72**
% RSD	**0.97**

The proposed RP-HPLC method was found to be simple, precise, specific, accurate, linear, robust and less time consuming which can be used for routine quality control test for Torsemide.

Conclusion

The developed method was found to be simple, accurate, precise, specific and robust and this method can be applied for routine quantitative analysis of Torsemide in bulk and pharmaceutical formulations like injection.

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