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

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Introduction

3-Pyridinesulfonamide, N-[[(1 methylethyl) amino] carbonyl]-4-[(3-methylphenyl) amino]-1-Isopropyl-3-[(4-m-toluidino-3-pyridyl) sulfonyl] urea, is the chemical formula for the loop diuretic medication torsemide (Figure 1). It is helpful in treating edema or hypertension brought on by hepatic, renal, or congestive heart failure.

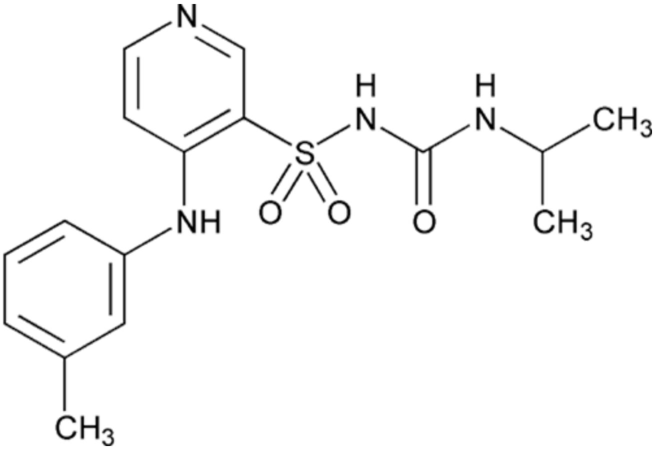

Fig 1: Torsemide

A review of the literature found information on techniques created for estimating the dosage of Torsemide in bulk, tablets, and in combination with other medicinal substances. However, no technique was created for estimating Torsemide in injection dosage form. HPLC and UV methods (2), (3), (4, 5), (7), GC-MS method (6), and conductometric method (8) are the methods that have been reported. HPLC and UV methods (9, 10), (11, 12), and HPTLC method (13), in addition to other medications, were used to estimate the amount of torsemide. The goal of the current work was to create an RP-HPLC method for estimating torsemide from injection formulation that is straightforward, accurate, robust, specific, and exact.

Methods and Material

Micro Labs, Bangalore, provided the pure drug sample (Torsemide), while Cipla Pharma, Mumbai, manufactured the injection formulation (Dytor) for the local market.

Chemicals and Reagents:

We bought analytical-grade potassium monobasic phosphate, analytical-grade methanol, analytical-grade orthophosphoric acid, and HPLC-grade water from Research-Lab Fine Chem Industries in Mumbai. Every chemical and reagent employed in the analysis was of analytical grade.

Experimental Conditions:

Using ezchrom elite software and an Agilent isocratic HPLC (LC1220) G 4286B-1220 infinite isocratic LC manual injector with a variable wavelength UV detector, quantitative HPLC was carried out. To finalize the chromatographic conditions for method development and validation of Torsemide in pharmaceutical dosage form and bulk, a number of trials were conducted. To obtain the chromatographic conditions, Zorbax C18 (250x4.6mm), 5µm was used. After setting the analytical wavelength at 288 nm, 20 µl of samples were added to the HPLC apparatus. Orthophosphoric acid was used to adjust the pH to 3.5, and the mobile phase consisted of phosphate buffer and mobile phase (50:50) at a flow rate of 1.3 mL/min. After passing through 0.41 Whatmann paper, the mobile phase was degassed using a Sonicator for five minutes.

Preparation of Standard solution:

Transfer 20 mg of precisely weighed Torsemide to a 100 mL volumetric flask, then add 50 mL of mobile phase, mix, and sonicate for 10 minutes to make up the volume (200µg/mL). To get a final concentration of 20µg/mL, transfer 1 mL of this solution to a 10 mL volumetric flask and top it off with mobile phase.

Preparation of Sample solution:

Pour around 2 mL of Torsemide injection into a 100 mL volumetric flask, then add 50 mL of mobile phase, mix, and sonicate for 10 minutes to make up the volume (200 ug/mL). To obtain 20µg/mL, transfer 1 mL of this solution to a 10 mL volumetric flask and top it off with mobile phase.

Method Validation

1. Assay: The identical protocol outlined in USP was used to conduct the assay for Torsemide. According to the USP, the percentage purity was determined using the AUC from the corresponding chromatogram (Table 1).
2. System Suitability Test: Five injections of a standard Torsemide solution at a concentration of 20 µg/mL were used to conduct the system suitability test. Table 2 provides the system suitability parameters.
3. Specificity: By injecting blank, standard, and sample solutions, the specificity investigation was conducted, and the results indicate that the standard and sample did not interfere with the blank preparation. The information is shown in Table 3.

Four. Precision: System, method, and intermediate precision studies were used to illustrate the technique's precision. Six replicate injections of the working standard solution made using the suggested procedure were used in the system precision studies, and chromatograms were captured. The area's relative standard deviation was computed and shown in Table 4. Six replicate injections of the standard solution and sample solution made using the suggested method were used in the method precision studies, and chromatograms were recorded. The region's relative standard deviation was determined and shown in Table 5. Chromatograms were recorded and tests for intermediate precision were conducted by another analyst on a different day (Table 5). The assay should be between 98 and 102% for both technique and intermediate precision.

5. Linearity: The concentration range was used to produce the standard solution for linearity. Inject 10–30 µg/mL into the chromatographic apparatus. For every drug solution concentration, the peak area was calculated and the chromatograms were created. Plotting the peak area against the torsemide concentrations yields calibration curves for the drug. Torsemide's linearity curves are displayed in Figure, and Table 6 presents the linearity data.
6. Accuracy: A known amount of Torsemide standard solution at 50%, 100%, and 150%

concentrations was spiked into the sample preparation in order to conduct the recovery investigations. At every stage of recovery, the recovery studies were conducted three times (Table 7).

7. Robustness: The method's robustness was assessed by making minor adjustments to the experimental setup, including the mobile phase's composition, pH, and flow rate, as well as the chromatographic features, including wavelength and results. Tables 8, 9, 10, and 11 exhibit the data.

Eight. Analytical solution stability: Assess the stability of the analytical solution by periodically injecting the standard preparation and sample preparation. The solution's stability is tested for 0, 3, 6, 12, 24, and 48 hours. the information shown 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.

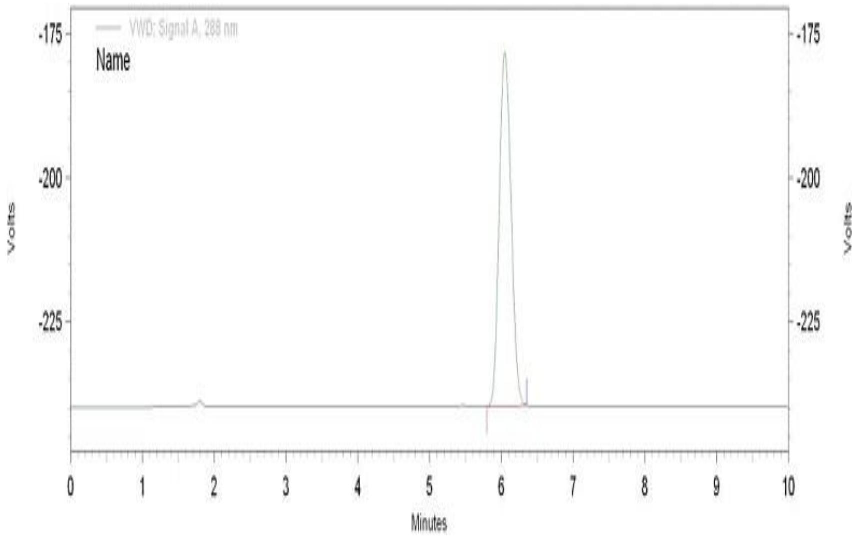

Fig. 2: Standard chromatogram

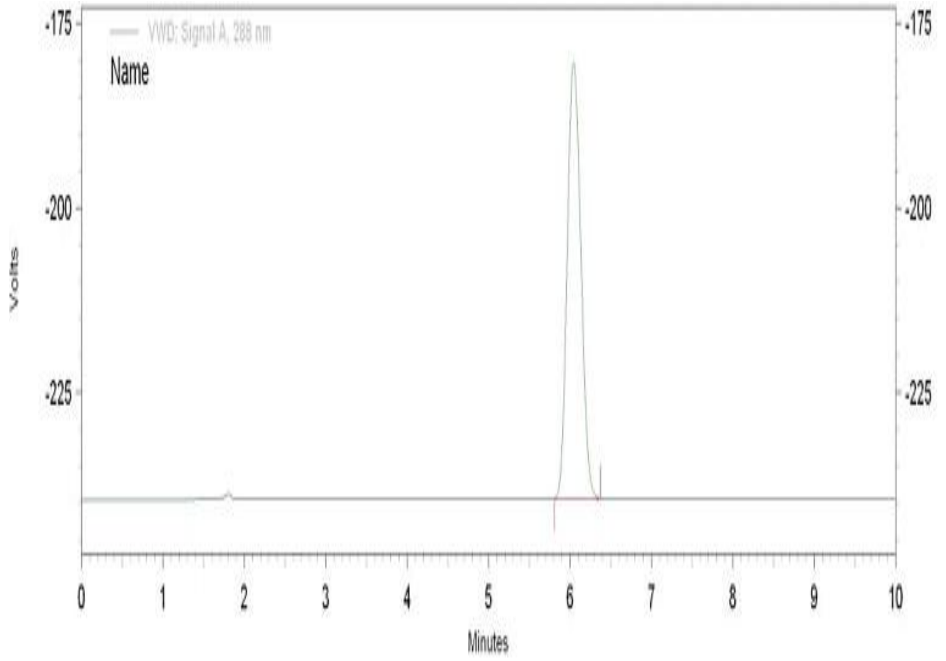

Fig. 3: Sample chromatogram

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

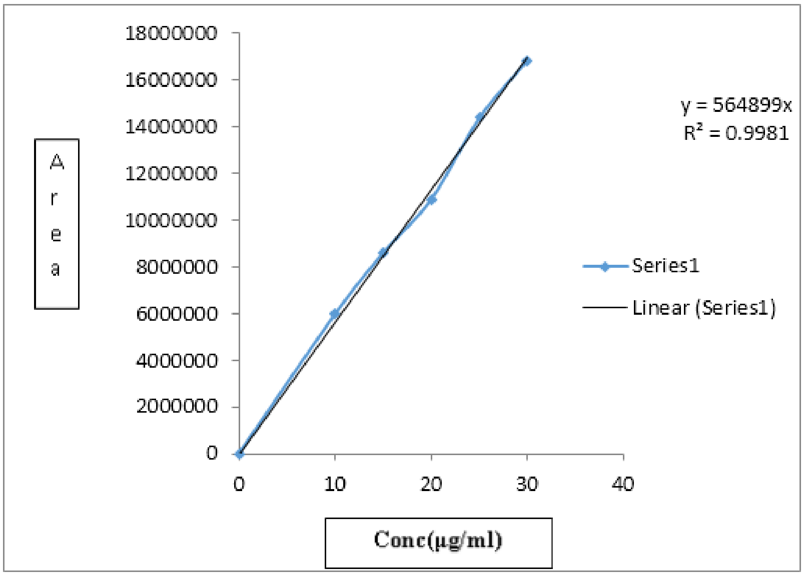

Fig 4: Linearity study of Torsemide

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**

For routine quality control testing of torsemide, the suggested RP-HPLC method was shown to be straightforward, accurate, linear, robust, exact, specific, and time-efficient.

Conclusion

Torsemide in bulk and pharmaceutical formulations, such as injection, can be routinely quantitatively analyzed using the established approach, which was found to be straightforward, accurate, exact, robust, and specific.

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