Original Article

Method Development And Validation For The Torsemide In Tablet Dosage Form By Rp-Hplc

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Abstract

For the purpose of determining Torsemide, a straight forward reverse-phase liquid chromatography method has been created and subsequently validated. In order to achieve a pH of 3.5, Ortho-phosphoric acid was used in the buffer. The mobile phase was used in a ratio of 40:60% v/v of phosphate buffer and acetonitrile. The column used was X Terra C8 (4.6 x 150 mm, 3.5 mm ID), and DAD detection at 288 nm was used at a flow rate of 0.8 ml/min. For the Torsemide assay, the technique was linear over the concentration range of 50 to 100 µg/ml. Torsemide had a retention time of 2.406 mins. The analysis's findings were verified using recovery tests and statistical analysis. Torsemide's detection limit (LOD) & quantitation limit (LOQ) and were found to be 2.91µg/ml and 10.1µg/ml, respectively. The study's findings demonstrated the usefulness of the suggested RP-HPLC method for the routine measurement of Torsemide in bulk medication and in its pharmaceutical dose form.

Keywords: Torsemide, RP-HPLC, LOD, LOQ, t- test

INTRODUCTION

A 4 amino-3 pyridine sulphonylurea derivative is where torsemide comes from (1). In terms of chemistry, it is 1-[4-(3-methyl aniline) pyridin-3-yl] sulfonyl-3-propan-2-ylurea (2-5) and is recognized by the USP (6). A member of the pyridine sulfonylurea class is of loop diuretics, torsemide. (1) High ceiling loop diuretics include torsemide. According to earlier research, its effectiveness in treating hypertension at low dosages (2.5 to 5 mg/day) and treating congestive heart failure at oral doses of 5 to 20 mg/day or increasing the excretion of urine in patients with chronic renal failure at 400 mg/day was also proven (7). These mentioned doses of Torsemide reduce blood pressure just as effectively as doses of hydrochlorothiazide (25mg) without causing dieresis (8). Torsemide is more effective than furosemide at treating edema brought on by congestive heart failure and liver cirrhosis because it causes a considerable diuresis at higher doses (10 or 20 mg) (9-12). The literature survey reveals about the reported methods of the drug in Gas chromatography (13), HPLC (14–22), and HPTLC (23-28). Using HPLC and HPTLC, the drug Torsemide was determined alone or in combination with a number of other medications (29-34). A comparison list was prepared based on the chromatographic conditions, mobile phase conditions, and retention times etc. (14-22, 24, 31, 33-35, 37-39) are shown in Table 1.

Table 1: Comparison table for the published research articles for Torsemide

Sl. No.	Analytical Method	Column	Mobile Phase Composition	pH	Retention time (min)/ Retention factor	Reference No.
1	Solid-Phase Extraction and Liquid Chromatography	Nucleosil C18	0.1 M KH_2PO_4/ 0.2 Acetonitrile (gradient)	4.5	……	14
2	HPLC-ED	µ Bondapak C18	water–acetonitrile (80:20)	3	22.5	15
3	solid-phase extraction and HPLC	LiChroCART® CN-cartridge	Perchloric acid (0.02 M) /acetonitrile (90:10)	2.5	10.30-11.40	16
4	HPLC-MS	inertsil ODS-C18	Acetonitrile, methanol and water (5:3:2)	---	1.1	17
5	HPLC	C8 analytical	acetonitrile: K phosphate buffer (2:3)	4	2.2 ± 0.2 min	18

6	RP-HPLC	(SunFire) C18	Methanol: phosphate buffer(60:40)	4	10	19
7	RP-HPLC	Luna C18	methanol: acetonitrile: phosphate buffer, (60:20:20)	3.5	3.2	20
8	RP-HPLC	C18	Acetonitrile: Phosphate Buffer (0.05M) 70:30	…..	2.4	21
9	HPLC	Bond Elut-C18 cartridges	28 mm phosphate buffer, / acetonitrile (75:25)	6.8	5.08	22
10	HPLC	Licro sphere C18 column	Methanol: Acetonitrile: Phosphate buffer (60:20:20)	6.5	5.321& 6.321	24
11	RP-HPLC	symmetry C18	acetonitrile and 0.1% Orth phosphoric acid	……	6.95	31
12	RP-HPLC	Sheisedo C18	Acetonitrile: Methanol: water (30:50:20)	3.4	2.53	33
13	HPLC (central composite design)	Shim - pack solar C18 column	acetonitrile, water and methanol (50:30:20).	…..	2.233& 4.405	34
14	RP-HPLC	µ bondapak C18 column	Acetonitrile/phosphate buffer (70:30)	2.4	6 min	35
16	RP-HPLC	Inertsil ODS 3V	Methanol/water (90:10)	3-4	8.267	37
17	HPLC	C18 silica column	Acetonitrile and Potassium dihydrogen orthophosphate buffer (40:60)	4	5±0.2	38
18	HPLC	Agilent C18 column	Potassium dihydrogen Ortho phosphate and acetonitrile (20:80)	2	2.203 & 5.490	39

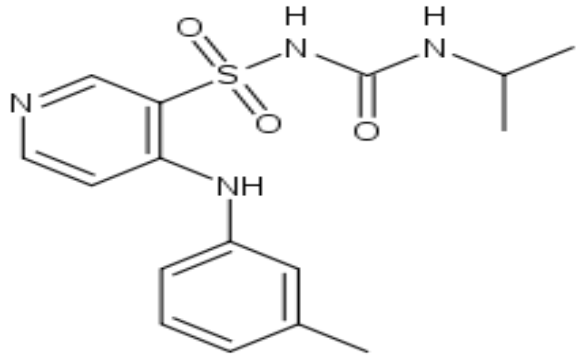

Fig 1: The chemical structure of the drug

1. EXPERIMENTAL:

Chemicals, Reagents and Instrumental Conditions: Torsemide standard bulk medication sample was given by Sun Pharmaceuticals. The Torsomide tablets were bought from a local pharmacy shop. All chemicals used for the study were HPLC grade. On an HPLC (Double pump) with auto injector and prominence diode array detector, chromatographic separation was carried out. The experiment was carried out at 288 nm. The study was conducted using a reverse phase X terra C8 (4.6 x 150mm, 3.5µm) column. The mobile phase consists of phosphate buffer and acetonitrile mixed at a ratio of 40:60 v/v with ortho-phosphoric acid to adjust the pH to 3.5 at a flow rate of 0.8 ml/min. The injection had a 20 µL volume.

Preparation of stock, working standard solutions, and sample solution

In separate 100-mL volumetric flasks, 10 mg of the medication, carefully weighed, was added to create a stock solution of Torsemide (100 µg/mL). They were dissolved in 25 ml of mobile phase, and the volume was then adjusted to the desired 100 µg/mL concentration. To get the drug's concentrations of 50, 60, 70, 80, 90, and 100 µg/ml, appropriate aliquots were pipette from the stock solution of the standard drug into a series of 10 mL volumetric flasks.

Assay of tablet:

Torsemide tablets were weighed, their average weight was calculated, and then they were ground into ~~a~~ fine powder. Weighing the powder, which is equal to 10 mg of torsemide, we transferred it to a 100 ml volumetric flask. It was dissolved in diluent, sonicated for 30 minutes, and given the necessary volume using diluent. The sample solution was then filtered using Whatmann filter paper No. 41. From the aforementioned stock solution, 0.7 ml of Torsemide was

obtained and diluted to the proper concentration in three separate 10 ml volumetric flasks. The area under the suggested optimum chromatographic conditions was then measured after injecting the sample solutions into the apparatus. The below given equation was used to estimate the concentration of the tablet sample solution. The Tablet Analysis Results are presented in Table 2.

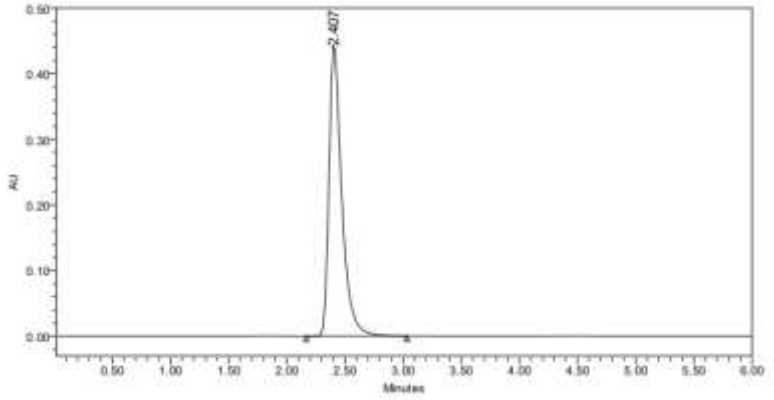

Fig:2 The representative chromatogram of tablet sample

Calculation:

$$\textbf{Assay \%} = \frac{AT}{AS} X \frac{WS}{DS} X \frac{DT}{WT} X \frac{P}{100} X \frac{Avg.Wt}{Label\ Claim} X100$$

Where:
AT = Peak Area of Torsemide sample obtained with test preparation
AS = Peak Area of Torsemide pure obtained with standard preparation
WS = Weight of working standard taken in mg
WT = Weight of sample taken in mg
DS = Dilution of Standard solution
DT = Dilution of sample solution
P = Percentage purity of working standard

Table 2: The results of tablet analysis: (Dytor®) (n=3)

Sample No	Formulation area	Found Conc. (µg/ml)	Label Claim (mg/tab)	Found concentration (mg/tablet)	% RSD
Injection 1	3371749	70.82333	20	20.22667	0.248

METHOD VALIDATION

Linearity:

According to International Conference on Harmonization (ICH) criteria, the developed approach has been validated. Torsemide working standard solution, 50–100 µg/mL in mass concentration, was introduced into the chromatographic apparatus every 20 µL. The chromatograms were created, and the peak area for each drug solution concentration was calculated. Plotting the peak area ratio vs the applied concentrations of Torsemide resulted in calibration curves for Torsemide.
The linear regression equation was found to be **Y = 47,556.15209 X - 3,385.0543, r 2 = 0.9995**

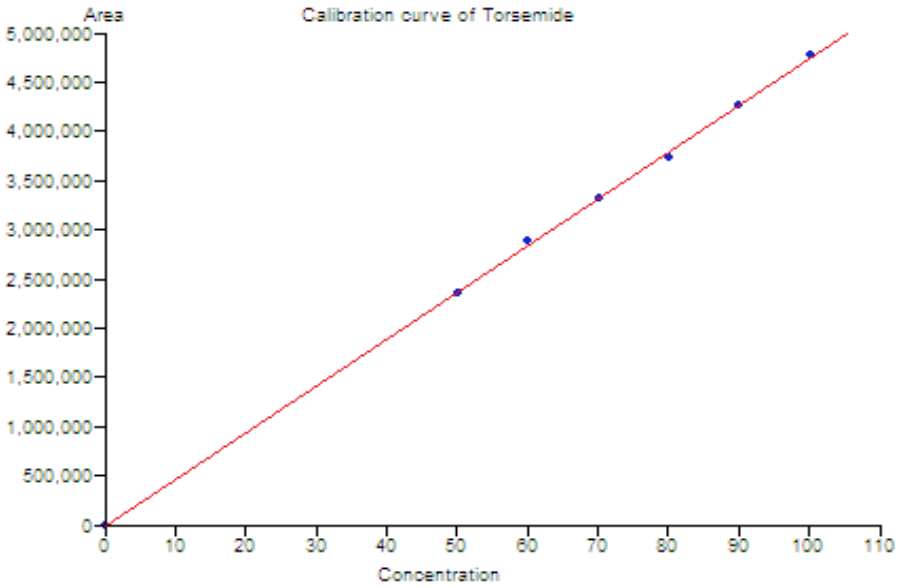

Fig 3: Calibration curve of Torsemide

Table 4: The linearity data of Torsemide

SL. No	Concentration (µg/mL)	Area
1	50	2358173
2	60	2886907
3	70	3336139
4	80	3740845
5	90	4265118
6	100	4789391

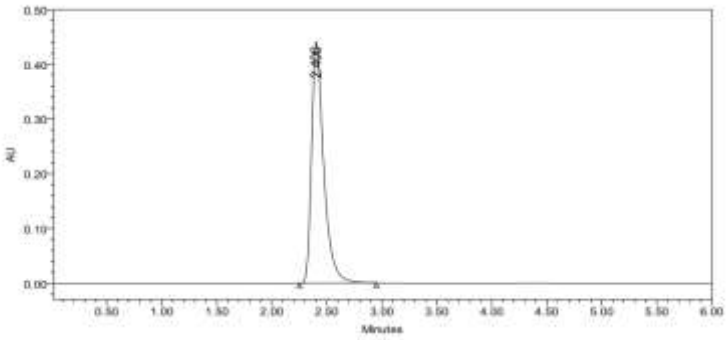

Fig 4. The chromatogram of standard Torsemide (70µg/ml)

METHOD VALIDATION:

The proposed method was validated as per ICH Q2B guidelines. **(10)**

Precision: The method's repeatability was tested by administering five replicate injections of the 70 µg/mL solution, and the % RSD was determined to be 0.30. By assessing the answer on the same day (intra-day precision) and on three other days, the variability of the procedure was investigated (inter-day precision). Results for intra-day precision (RSDs) for Torsemide were 0.30 percent at n = 5. For Torsemide, the inter-day precisions (RSDs) were 0.32 percent at n = 5.

Accuracy: The method's accuracy was examined by conducting recovery trials at various spiking levels like 50, 75 and 100%. The estimation was completed as previously mentioned. Three determinations were made and results were obtained for each level. The calculated amounts recovered and percentage recovery values are displayed in Table 5 as the results.

Table 5: Recovery Studies of Torsemide (n=6)

Formulation (µg/ml)	(%) Recovery Level	Amount of Std Drug added (µg/mL)	Amount of pure drug found (µg/ml) (n=4))	% Recovery	C.I.	% RSD	SE	t
50	50	25	24.9	99.6	99.60 ±2.2	1.414	0.704	0.568

50	75	37.5	37.66	100.426	100.426 ±1.55	0.971	0.487	0.874
50	100	50	49.91	99.835	99.835 ±0.968	0.609	0.304	0.542

SD: Standard deviation, SE: standard error, C.I.: Confidence Interval within which true value may be found at 95% confidence level = R ± ts/√n, R: Mean percent result of analysis of Recovery study (n = 4). Theoretical 't' values at 95% confidence level for n - 1 degrees of freedom t (0.05, 3) = 3.182

System suitability: System suitability test was carried out by injecting freshly prepared standard stock solution of Torsemide (70µg/ml) and the results of parameters were obtained by five replicate injections. The system suitability results are given in the Table 6.

Table 6: Results of System suitability:

Parameter	Results of Torsemide
Retention time (minutes)	2.406
USP Plate count	2415.80
Tailing factor	1.55
Repeatability (% RSD)	0.195

Specificity: The peak purity of Torsemide was assessed by comparing the retention time (R_t) of standard Torsemide. Good correlation was also found between the retention time of standard and sample of Torsemide.

Robustness: It was done by making small changes in the chromatographic conditions and found to be unaffected by small changes like ± 0.1 changes in pH and 2% change in volume of the mobile phase. All the results of the robustness parameter are less than 1% RSD. So this proposed method is robust.

LOD and LOQ: According to ICH recommendations, the limit of detection (LOD) and limit of quantification (LOQ) were estimated at 2.91 µg/ml and 10.1 µg/ml, respectively, where is the response's standard deviation (y-intercept) and S is the calibration plot's slope.

RESULTS AND DISCUSSION:

After multiple tests with different ratios of buffer and acetonitrile, such as 40:60, 50:50, 30:70, 25:75, and 35:65, and at various pH levels, such as 2, 2.45, 3, 3.5, 4, 4.5, and 2.48, the approach was finally decided upon. To obtain the optimum chromatographic peak with Rt 2.406 minutes and sensitivity, a mobile phase made up of buffer (pH 3.5) and acetonitrile in a 40:60 ratio was chosen. The experimental modalities used were satisfactorily validated using routinely established analytical techniques. Recovery studies and preliminary analysis of a standard sample were used to validate the suggested approach. The average recovery rate was found to be between 99 and 100 percent. The average recoveries from each instance's analysis were compared using the Student's t test to the theoretical value of 100 percent. Given that the calculated values are lower than the theoretical values (Table), it can be deduced that the recoveries for each analyte are in perfect accord with the calculated values. The chromatogram shows no extra peaks, indicating that the common excipients employed in the tablets did not interfere. The limit of quantification and the lower limit of detection were discovered to be 2.91 and 10.1µg/ml, respectively. This shows that the newly created HPLC method is novel, straightforward, linear, precise, reliable, sensitive, and repeatable. The routine quality control of bulk and tablet dosage forms may therefore be simply carried out using the established method.

CONCLUSIONS

The created method's precision, repeatability, and accuracy were all verified. Torsemide was shown to have an excellent linear relationship in the concentration range of 50-100 µg/mL. It was discovered that the correlation coefficient was 0.9995. The results for intra-day and inter-day precision were sufficient to show that the suggested procedure was accurate and repeatable. The assay trial demonstrated that there were no excipients interfering with the contents of Torsemide as determined in the tablet dosage form. This showed that the established HPLC method was straight forward, linear, precise, and accurate, and that it could be easily used to the routine quality control analysis of torsemide from both its pharmaceutical formulations and bulk drug.

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