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Stability Indicating HPLC Method for Determination of Torsemide in Bulk Drug and Pharmaceutical Dosage Form.

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ABSTRACT

This study describes the development and validation of stability indicating HPLC method development for torsemide. Torsemide is subjected to different stress condition as per ICH guidelines. The peak for torsemide was well resolved from peaks of degradation products, using a Ace5-C18 (250×4.6mm) column and mobile phase comprising of Acetonitrile: Water (60:40v/v), at flow rate 1ml/min. The detection was carried out at a wavelength of 270 nm. A linear response (r^2=0.996) was observed in range of 0.5-30µg/mL. The method shows good recoveries (average 99.9%) and relative standard deviation for intra and inter day were ≤ 0.38%. The method was validated for specificity and robustness also.

Keywords: Torsemide, HPLC, Validation, Stability, Degradation.

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INTRODUCTION

Torsemide (TSM) is loop diuretic and is chemically known as 3-pyridine sulfonamide N-[[(1-methylethyl) amino] - carbonyl]-4-[(3-methylphenyl) amino]. It acts by inhibiting the Na^+/ K^+/ $2Cl^-$ carrier system (via interference of the chloride binding site) in the lumen of the thick ascending portion of the loop of Henle, resulting in the decrease in reabsorption of sodium and chloride [1,2]. This drug has been approved by United States Food and Drug Administration (USFDA). Literature survey reveals that, few chromatographic methods have been reported for the estimation of TSM in human plasma and urine [3,4,5]. The spectrophotometric method for torsemide is also reported for determination of the drug in the tablet dosage form [6]. High Performance Liquid Chromatography (HPLC) is a versatile technique. According to current good manufacturing practices, all drugs must be tested with a stability-indicating assay method before release. Till date, no stability-indicating HPLC assay method for the determination of torsemide is available in the literature. It was felt necessary to develop a stability indicating Liquid Chromatography (LC) method for the determination of torsemide as bulk drug and pharmaceutical dosage form and separate the drugs from the degradation products under the International Conference on Harmonization (ICH) suggested conditions (hydrolysis, oxidations, photolysis and thermal stress) [7,8]. Therefore, the aim of the present study was to develop and validate a stability-indicating HPLC assay method for torsemide as bulk drug and in pharmaceutical dosage form as per ICH guidelines [9].

EXPERIMENTAL

Material and reagents

Torsemide was kindly provided by Cipla Ltd. Mumbai as bulk drug (purity 99.8%), and torsemide tablets, Dytor (40 mg). Acetonitrile, methanol, hydrochloric acid and sodium hydroxide pellets were obtained from Rankem Laboratories India. Hydrogen peroxide is obtained from Fischer Scientific, India. All chemicals are of HPLC grade. Milli-Q-Water was used throughout the experiment.

Chromatographic conditions

The HPLC system used was a Jasco (2000 series) system equipped with a photodiode array detector. A chromatographic column of 250 mm length and internal diameter of 4.6 mm filled with octadecyl silane Ace5-C18 (Advance Chromatography Technology, USA) stationary phase with particle size 5 µm were used. The instrumental setting was at a flow rate of 1mL /min. the injection volume was 20µL. The detection wavelength was 270 nm.

Mobile phase

The mobile phase consisted of Acetonitrile- Water (60:40, v/v) and was pumped at a flow rate of 1.0 mL/min. The mobile phase was premixed and filtered through a 0.45µ nylon filter and degassed.

Preparation of standard stock solutions

All solutions were prepared on a weight basis and solution concentrations were also measured on weight basis to avoid the use of an internal standard. Standard solution of torsemide was prepared by dissolving the drugs in the diluents and diluting them to the desired concentration. Diluent A was composed of acetonitrile and diluent B was composed of water and actonitrile in the ratios of (40:60 v/v). Approximately 5 mg of torsemide was accurately weighed, transferred in a 50 mL volumetric flask, dissolved and diluted to 50 mL with diluent A. From these stock solutions 2 mL of torsemide standard solution were transferred in a 10 mL volumetric flask and diluted with diluents B. This final solution contained 20µg /mL of torsemide.

Preparation of Sample Solutions

Ten tablets of Dytor (40mg) were finely ground using agate mortar and pestle. The ground material was extracted into diluents A by vortex mixing followed by ultra sonication. The solution was filtered through

a 0.45-micron nylon filter and an appropriate concentration of sample (20µg/mL assay concentration) was prepared in mobile phase at the time of analysis.

Procedure for forced degradation study

Stability testing is an important part of the process of drug product development. The purpose of stability testing is to provide evidence of how the quality of a drug substance or drug product varies with time under a variety of environmental conditions, for example temperature, humidity, and light, and enables recommendation of storage conditions, retest periods, and shelf life to be established. The two main aspects of drug product that play an important role in shelf-life determination are assay of the active drug and the degradation products generated during stability studies. The objective of this work was to develop an analytical HPLC procedure, which would serve as a stability-indicating method for assay of torsemide drug product. Forced degradation of the drug product was carried out under thermolytic, photolytic, acid/base hydrolytic and oxidative stress conditions.

Acidic Degradation

5 mg drug was dissolved in the 5 mL of the diluent A. 50 mL of the 0.01N hydrochloric acid was added to it. The solution was kept for 15 min. 10 mL of solution was taken from it and neutralized with 0.01 N sodium hydroxide. Then the solution was diluted with diluents B to prepare working solution of 20 µg / mL.

Alkaline Degradation

5 mg drug was dissolved in the 5 mL of the diluent A. 50 mL of the 0.01N sodium hydroxide was added to it. The solution was kept for 15 min. 10 mL of solution was taken from it and neutralized with 0.01 N hydrochloric acid. Then the solution was diluted with diluents B to prepare working solution of 20 µg / mL.

Oxidative degradation

5 mg drug was dissolved in 5 mL of diluents A. Add 50 mL of 1% H_2O_2. The solution was kept for 15 min. The solution was diluted with diluents B to prepare working solution of 20 µg / mL.

Thermal degradation

10 mg drug was kept in the hot air oven for 48 hours at 100^0 C temperature. Then the working solution was prepared using diluents A and diluents B.

Photo degradation

10 mg of drug is exposed to the short wavelength (254nm) and long wavelength (366nm) UV light for 48 hours. Then the working solution was prepared using diluents A and diluents B.

Specificity

Specificity is the ability of the method to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically, these might include degradation products, matrix etc. The specificity of the developed HPLC method for torsemide was carried out in the presence of its degradation products. Stress studies were performed for torsemide bulk drug to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress conditions exposing it with acid (0.01 N hydrochloric acid), alkali (0.01 N NaOH), hydrogen peroxide (1%), heat (100^0C) and UV light (254 and 366 nm wavelength) to evaluate the ability of the proposed method to separate torsemide from its degradation products. For light and heat study, the study period was 48 hours whereas for acid, base and for oxidation 15 min. Peak purity test for torsemide was by using PDA detector in stress samples.

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RESULT AND DISCUSSION

Optimization of chromatographic conditions

The primary target in developing this stability indicating method was to achieve the resolution Torsemide and its degradation products. To achieve the separation of degradation products we used a stationary phase C-18 and combination of mobile phase water with acetonitrile. The separation of the degradation product and torsemide was achieved on Ace5 octadecyl silane C-18 stationary phase and water and acetonitrile (40:60 v/v) as a mobile phase. The tailing factor obtained was less than 2 and retention time was also about 7.94 minute for the main peak. The chromatogram of torsemide standard drug is shown in Figure 1. The entire forced degradation products are well resolved from the main peak. The developed method was found to be specific and validated as per ICH guidelines.

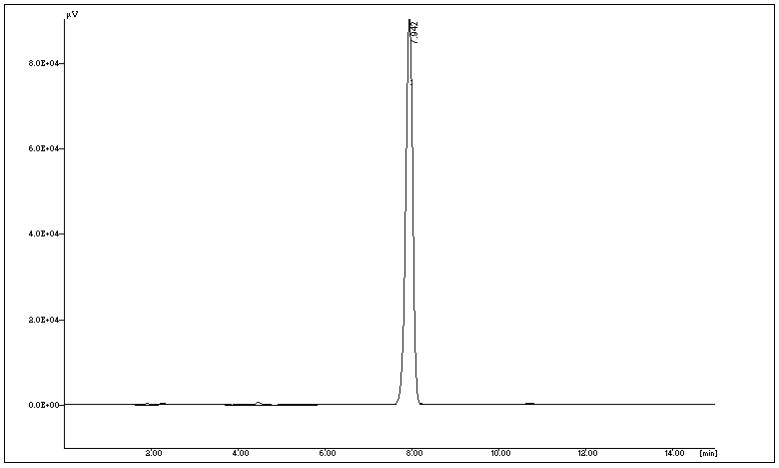

Figure 1: Chromatogram of torsemide standard drug 20µg/mL (7.942)

Result of forced degradation experiment:

Degradation was not observed for torsemide samples during stress conditions like heat, UV and light, except in base, acid and oxidation. Torsemide was degraded into acid as shown in Figure 2, base as shown in Figure 3, oxidation as shown in Figure 4 and forms polar impurities.

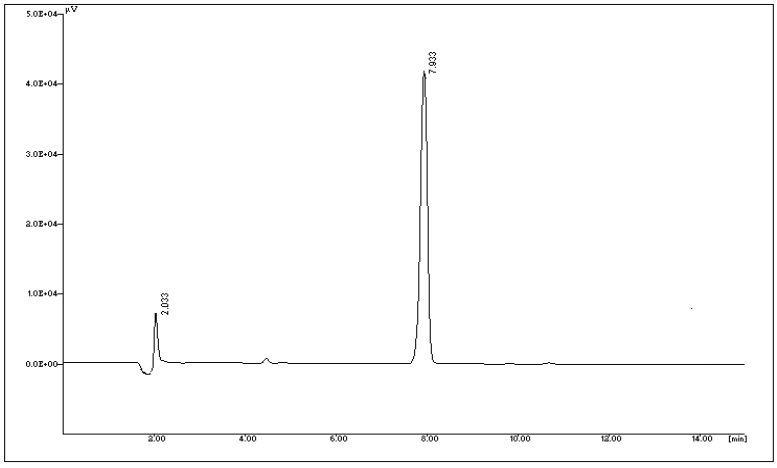

Figure 2: Chromatogram of torsemide in acid degradation [acid degraded product (2.033) and torsemide (7.933)]

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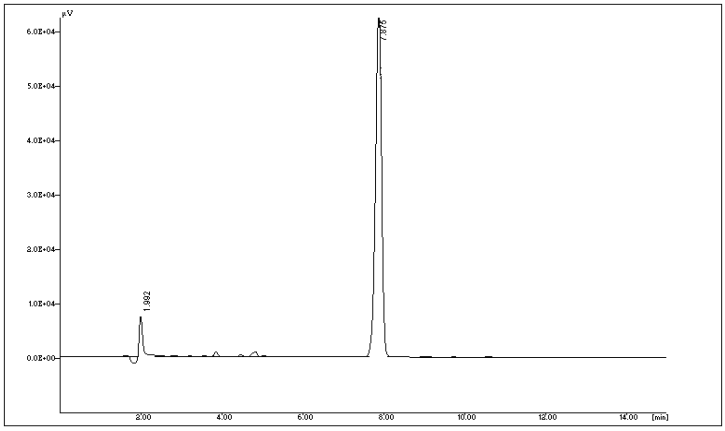

Figure 3: Chromatogram of torsemide in base degradation [base degraded product (1.992) and torsemide (7.876)]

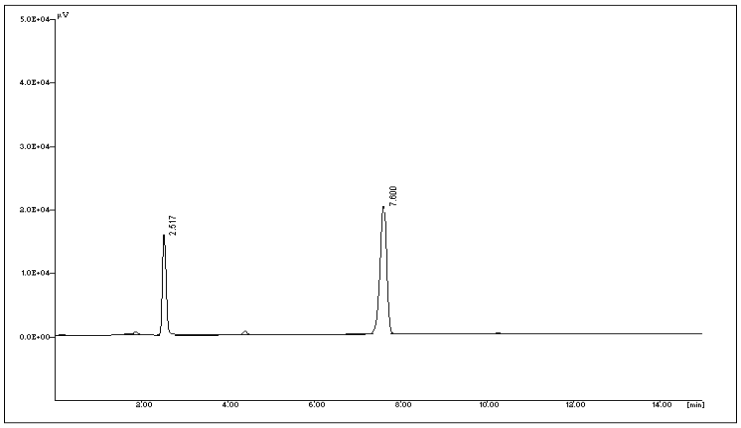

Figure 4: Chromatogram of torsemide in oxidative degradation [oxidative degraded product (2.517) and torsemide (7.600)]

In acidic condition torsemide degraded up to 8.66 %, in basic condition up to 6.18 %, in oxidative condition up to 27.19 %. In oxidative condition more degradation was found for torsemide. In thermal and photo condition no degradation was observed for torsemide. Peak purity results greater than 990 indicate that the torsemide peak is homogeneous in all stress conditions tested. The mass balance of torsemide in stress samples was close to 100%, the unaffected assay of torsemide in tablets confirms the stability indicating power of the method. The summary of the forced degradation studies is given in Table 1.

Table 1: Summary of forced degradation results

Stress Condition	Time	Assay of active Substance	% Degradation	Peak Purity[a]
Acid Hydrolysis (0.01 N HCl)	15 Min.	53.83	8.66	999
Base Hydrolysis (0.01 N NaOH)	15 Min.	89.35	6.18	999
Oxidation (1% H_2O_2)	15 Min.	66.94	27.19	999
Thermal (90^0C)	48 hours	99.65	No Degradation	999
Photo	48 hours	99.57	No Degradation	999

[a] Peak purity values in the range of 990-1000 indicate the homogenous peak

Determination of Active Ingredients in Tablets

The contents of drug in tablets were determined by the proposed method using the calibration curve. The chromatogram of the tablet sample is shown in Figure 5.

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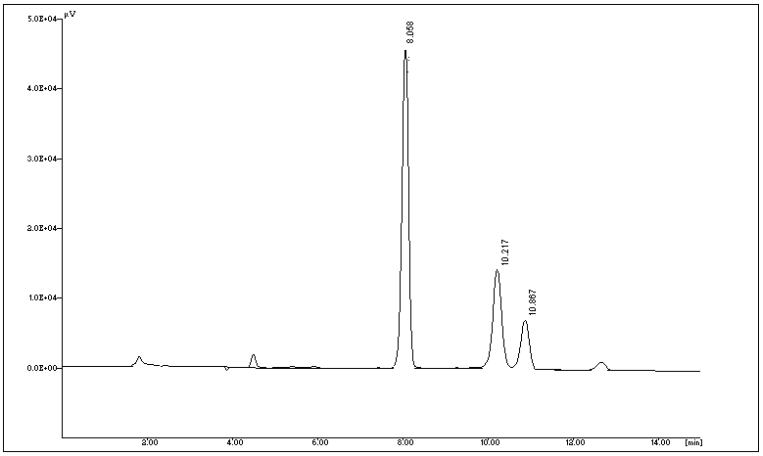
Figure 5: Chromatogram of Tablet of Torsemide 20µg/mL (8.0) and excipients (10.217 and 10.867) 3.4



Precision

Assay of method precision (intra-day precision) was evaluated by carrying out six independent assays of pramipexole test samples against reference standard, the percentage of RSD of six assay values obtained was calculated. The intermediate precision (inter-day precision) of the method was also evaluated using two different analysts, different HPLC systems and different days in the same laboratory; results are shown in Table 2.

Table 2: Result of precision of test method

Sr. no.	Assay of Torsemide as % of labeled amount	
	Analyst first Intraday precision	Analyst second Inter day precision
1	99.45	99.65
2	99.28	99.64
3	99.68	99.38
4	99.59	99.54
5	99.69	99.98
6	99.78	99.87
Mean	99.57	99.67
RSD	0.35	0.38

Recovery test or accuracy

The accuracy of the method was determined by calculating recoveries of torsemide by the standard addition method. Known amounts of standard solutions of torsemide (80, 100, and 120%) were added to prequantified sample solutions of tablets. The amounts of torsemide were determined by applying these values to the regression equation of the calibration curve. The recovery values for torsemide ranged from 99.3 to 100.6 %. The average recoveries of three levels of for torsemide were 99.9%. Results are mentioned in Table 3.

Table 3: Result of recovery tests for torsemide

Level of addition %	Ingredient	Amount added (n =3) mg	% Recovery	Average %
80	Torsemide	16	99.3	99.9
100	Torsemide	20	99.8	
120	Torsemide	24	100.6	

Linearity

Calibration curves were constructed by plotting peak areas versus concentrations of torsemide, and the regression equations were calculated. The calibration curves were plotted over the concentration range 0.5-30 (µg/mL). Accurately measured standard working solutions of torsemide (0.5, 2, 5, 10, 15, 20 and 30µg/mL) were transferred to a series of 10 mL volumetric flasks and diluted to the mark with mobile phase. Aliquots (20µL) of each solution were injected under the operating chromatographic conditions described above. The equation of the calibration curve for torsemide obtained, y = 26740x -2551, the calibration graphs were found to be linear in the above mentioned concentration. The coefficient of determination (r^2) was 0.996.

Limit of Detection and Limit of Quantification (LOD and LOQ)

The LOD was determined by the analysis of samples with known concentrations of analyte and by establishing through visual evaluation the minimum level at which the analyte could be reliably detected. The LOQ was determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte could be quantified with acceptable accuracy and precision. The LOD and LOQ for torsemide in the HPLC method was 0.021 and 0.067 µg / mL respectively.

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and the effect on resolution was recorded. The flow rate of the mobile phase was changed by 0.1 units from 0.9 to 1.1 mL/min. The effect of the mobile phase composition was studied by varying the percentage of two components while the third component was held constant. Changes in the column oven temperature, detection wavelength and injection volume were studied in the same way.

CONCLUSION

The developed method is stability indicating and can be used for assessing the stability of torsemide in bulk drugs and pharmaceutical dosage form. The developed method is specific, selective, robust, rugged and precise. This method can be conveniently used for assessing stability assay of selected substances & dissolution of tablets containing torsemide in quality control laboratory. The study showed that the drug is stable for the thermal and photo degradation conditions where as moderately degraded in acid (8.66%) and base (6.18%) conditions but highly degraded in the oxidative conditions (27.19%).

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