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# Development And Validation Of RP-HPLC Method For The Estimation Of Torasemide In Pure Form And In Pharmaceutical Dosage Forms

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## ABSTRACT

A simple Reverse phase HPLC method was developed for the estimation of torasemide in bulk and in pharmaceutical dosage forms. Torasemide was chromatographed on a reversed phase $C_{18}$ column in isocratic mode with a mobile phase comprising of Methanol and Water (pH adjusted to 2.5 with ortho phosphoric acid) in the ratio of ( 75:25 v/v). The mobile phase was pumped at a flow rate of 1ml/min and the eluents were monitored at 214nm. The calibration curve was linear in the range of 0.1 - 50µg/ml. The proposed method was found to be simple, precise and hence can be applied for routine quality control analysis of torasemide in bulk drugs and in pharmaceutical dosage forms


## KEYWORDS

RP-HPLC;
Estimation;
Torasemide;
Pharmaceutical dosage forms.

## INTRODUCTION

Torasemide (TSM)[1,2] 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. Literature survey reveals that, few chromatographic methods[3-6] have been reported for the estimation of TSM in human plasma and urine. To the best of our knowledge, there is no work in the literature reported about the estimation of TSM in pharmaceutical formulations. Hence the author has made an attempt to develop a simple, rapid, accurate and precise method for the estimation of torasemide in bulk and in pharmaceu-

tical dosage forms.

## EXPERIMENTAL

### Instrumentation

An isocratic high performance liquid chromatograph using Shimadzu LC - 10AT provided with ODS reverse phase column (250x4.6 mm ID) and supported by class - VP software was employed in the study.

### Chemicals and reagents

Torasemide was a gift sample from Dr.Reddy's labs Ltd., Hyderabad. HPLC grade Methanol (E. Merck India), Milli - Q water, ortho phosphoric acid AR grade were used for preparing the mobile phase.

### Chromatographic conditions

The mobile phase used was Methanol and Water (pH adjusted to 2.5 with ortho phosphoric acid) in the ratio of (75:25 v/v). The mobile phase was filtered through 0.45µ membrane filter and sonicated before use and then it was pumped from the solvent reservoir at a flow rate of 1ml/min and the eluents were monitored at 214nm. The run time selected was 10 min. The column was maintained at $30^0$C and the volume of each injection was 20µl. Prior to injecting solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the system.

### Assay procedure

About 100 mg of pure sample of TSM was weighed accurately and transferred to a 100mL volumetric flask and dissolved in 75ml of methanol. The solution was sonicated for 10 min and then the volume made up with a further quantity of methanol to get 1mg/mL solution. Subsequent dilutions of this solutions ranging from 0.1-50 µg/ml were made in 10ml volumetric flasks. The solutions prepared as above were filtered through 0.45µ membrane filter and then 20µl of filtrate was injected each time into the column at a flow rate of 1ml/min. Each concentration was injected six times into the column and corresponding chromatograms were obtained. Detection of the drug was performed at 214nm.From the chromatogram, the retention time and mean peak area was recorded for all the concentrations. The plot of peak area versus the respective concentrations gives the calibration curve. The regression of drug concentration over the peak area was computed using Least squares method of analysis. This regression equation was used to estimate the amount of TSM in pharmaceutical formulations.

### Estimation of TSM in Tablet dosage forms

Two commercial brands of TSM tablets were chosen for testing suitability of proposed method to estimate TSM in tablet dosage forms. For this , 20 tablets were weighed and powdered. Accurately weighed portion of tablet powder equivalent to 100mg was taken in 100ml volumetric flask and 50 ml of methanol was added , shaken well and allowed to stand for 15min with intermittent sonication to ensure complete solubility of the drug. The mixture was thoroughly mixed and made up to the mark with methanol and filtered through a 0.45µ membrane filter. From the filtrate, different aliquots were taken in separate 10ml volumetric flasks. The contents of flasks were made up to mark with methanol and mixed well. Each of the solutions (20µl) was then injected into the column. All the determinations were conducted five times and the drug content in the tablet was quantified using the regression equation obtained from the pure sample.

## RESULTS AND DISCUSSION

The development of an analytical method for the determination of drugs by HPLC has received considerable attention in recent years because of their importance in quality control of drug and drug products. The goal of this study was to develop a simple, rapid, accurate and precise HPLC method for the analysis of TSM in bulk and tablet dosage forms using most commonly employed RP C-18 column with UV detection. A typical chromatogram was shown in figure 1.

The run time of the method was set at 10min and the TSM was appeared on chromatogram at 4.25min. This indicates that the present HPLC method is rapid, which in turn shows that the method consumes less volume of HPLC solvents. When the

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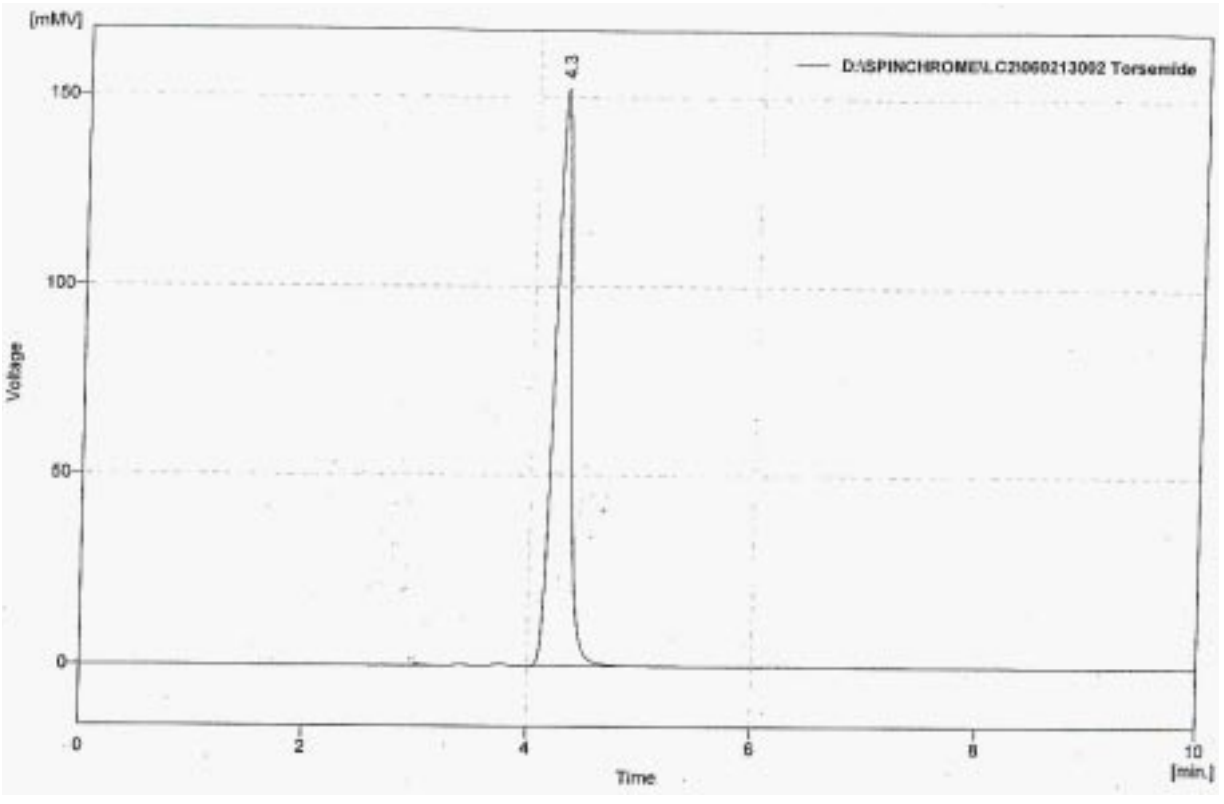


**Figure 1: Model Chromatogram for Torasemide**

same drug solution was injected six times, retention time of the drug was found to be same.

The peak areas from such different concentrations set up as above were calculated and shown in TABLE 1. A good linear relationship was observed between the concentration of the TSM and the respective peak area. The regression curve was constructed by least squares method and its mathematical expression was Y= 15264.69 X +279.16 (where Y is the peak area and X is the concentration of TSM).This regression equation was used to estimate the amount of TSM in tablet dosage forms. The intra-day and inter-day variations of the method were determined using five replicate injections of three different concentrations, which were analyzed on the same day and three different days over a period of two weeks, a low coefficient of variation was observed (TABLE 2). This shows that the present HPLC method was highly precise.

To ensure reliability and accuracy of the method recovery studies were carried out. A fixed quantity of pre analyzed sample was taken and standard was added at three different levels .The values were shown in TABLE 3. About 99.7% of TSM could be recov-

**TABLE 1: Calibration of the Proposed Method**

| Concentration (µg/ml) | Peak area* | Coefficient of variation (C.V.) |
|---|---|---|
| 0.1 | 1534 | 0.85 |
| 0.5 | 7667 | 0.28 |
| 2.0 | 30620 | 0.92 |
| 5.0 | 76537 | 0.17 |
| 10 | 152871 | 0.53 |
| 20 | 305562 | 0.15 |
| 30 | 458312 | 0.93 |
| 40 | 610866 | 0.19 |
| 50 | 763453 | 0.12 |

***Mean of six determinations**
**Regression equation from 0.1 – 50 µg/ml**
**Y= 15264.69X +279.16 (r = 0.9999)**

**TABLE 2: Precision of the Proposed Method**

| Concentration of torasemide (µg/ml) | Observed concentration of Torasemide (µg/ml) | | | |
|---|---|---|---|---|
| | Intra-day | | Inter-day | |
| | Mean (n=5) | Coefficient of variance | Mean (n=5) | Coefficient of variance |
| 10 | 9.98 | 0.82 | 9.93 | 0.91 |
| 20 | 20.05 | 0.43 | 20.03 | 0.12 |
| 40 | 40.11 | 0.18 | 40.06 | 0.25 |

**TABLE 3: Results of Recovery Study**

| Amount of drug added (µg) | Recovery from drug solution | | Recovery from tablet Formulation | |
|---|---|---|---|---|
| | Mean amount Found ( n= 5 ) | Mean % recovery | Mean amount Found (n = 5) | Mean % recovery |
| 10 | 9.97 | 99.7 | 9.99 | 99.9 |
| 20 | 20.02 | 100.1 | 20.05 | 100.2 |
| 30 | 30.05 | 100.16 | 30.10 | 100.3 |

**TABLE 4: Assay of TSM in Tablet Dosage Forms**

| Brand | Labeled amount of drug (mg) | Mean (± s.d.) amount (mg) recovered ( n= 5) | Mean (± s.d.) % of recovery (n= 5) |
|---|---|---|---|
| I | 10 | 10.01±0.08 | 100.1±0.05 |
| II | 20 | 20.03±0.12 | 100.15±0.08 |

ered from the pre analyzed samples indicating the high accuracy of the proposed HPLC method.

The HPLC method developed in the present study has also been used to quantify TSM in tablet dosage forms. TSM tablets (containing 10 and 20 mg of the drug) were quantified using the proposed analytical method and the results were given in TABLE 4. No interfering peaks were found in the chromatogram indicating that the tablet excepients did not interfere with the estimation of the drug by proposed HPLC method. The tablets were found to contain 100.1 - 100.15 % of the drug. It can be concluded that the proposed method was simple, precise and hence can be applied for routine quality control analysis of Torasemide in bulk drugs and in pharmaceutical dosage forms

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