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**QUANTITATIVE DETERMINATION OF TRIMETAZIDINE
DIHYDROCHLORIDE FROM PHARMACEUTICAL FORMS BY REVERSED
PHASE – HPLC**

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ABSTRACT

An economic, simple, selective, accurate and reproducible high-performance liquid chromatographic (HPLC) method for the analysis of Trimetazidine Dihydrochloride in pharmaceutical formulations was developed and validated in the present study. The mobile phase consisted of 50:50 % v/v Methanol : Phosphate buffer (0.02 mol/l potassium dihydrogen phosphate). It was found that the retention time of Trimetazidine Hydrochloride was 4.650 ± 0.04 min. HPLC analysis of Trimetazidine Dihydrochloride was carried out at a wavelength of 240 nm with a flow rate of 1.0 mL/min. The linear regression analysis data for the calibration curve showed a good linear relationship with a regression coefficient of 0.999 in the concentration range of 17.5 – 140 $\mu\text{g/mL}$.

The linear regression equation was $y = 26516.1X - 347.4$. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 7 and 14 ng, respectively. The developed method was validated for accuracy, precision, detection and quantification limits as per the ICH guidelines. The wide linearity range, accuracy, sensitivity, short retention time, and composition of the mobile phase indicated that this method is better for the quantification of trimetazidine hydrochloride.

Keywords: Trimetazidine Dihydrochloride, Tablets, Validation, Estimation, RP-HPLC

INTRODUCTION

Trimetazidine dihydrochloride, an anti-ischemic metabolic agent, improves myocardial glucose utilization through the inhibition of fatty acid oxidation, and it can be used as a vasodilator in angina pectoris and in ischemia of neurosensorial tissues as in Meniere's disease [1-8]. Chemical structure of Trimetazidine Dihydrochloride

(TMZ), 1-(2, 3, 4-Trimethoxybenzyl) piperazine dihydrochloride is shown in **Figure 1**.

Several methods like HPLC with electrochemical detection [9], HPLC with UV-detection [10], HPTLC [11], GC-MS [12], UV spectrophotometric method [13], voltammetry [14], slow injection chemiluminescence [15] and LC-MS [16-17] have been reported for the quantitative determination of Trimetazidine Dihydrochloride in biological fluids and pharmaceutical preparations. Nowadays, RP-HPLC is using routine analytical technique, which gives a possibility for developing a simple, rapid, cost effective and reproducible method for assay of Trimetazidine Dihydrochloride in its dosage. Therefore, the aim of the current work is to develop a simple RP-HPLC method for analysis of Trimetazidine Dihydrochloride in tablet formulations.

MATERIALS AND METHODS

Reagents

All chemicals and reagents were used of HPLC. TMZ used was standard reference compound according to European Pharmacopoeia. Tablet formulation containing TMZ 35 mg was obtained commercially. HPLC grade Methanol was procured from Merck Ltd. All other chemical reagents were of analytical grade.

Instrumentation

A Shimadzu HPLC system was utilized consisting of the following components: quaternary pump LC – 20 AD, vacuum degasser unit DGU – 20 A₅ and a UV/VIS variable detector SPD – 20 A. Separation was carried out on a LiChrosorb C 8 column (125 x 4 mm, particle size 5 µm) under reversed phase partition chromatographic conditions. The equipment was controlled by a PC with properly installed chromatographic software.

Chromatographic Conditions

The mobile phase was a 50:50 % v/v Methanol:Phosphate buffer (0.02 mol/l potassium dihydrogen phosphate). The mobile phase was filtered through 0.45 µm membrane filter and degassed by using a sonicator for about 10 min before use. The sample solutions were also filtered using 0.45 µm membrane filters. The mobile phase was delivered isocratically at a flow rate 1.0 ml/min. The column was maintained at 40°C temperature. The injection volume was a 20 µl and the total run time was 6 minutes. The detection was carried out at 240 nm. Typical chromatogram is given in **Figure 2**.

Preparation of the Standard Solution

Accurately weighed quantity of 35 mg TMZ is transferred in 100 ml volumetric flask, dissolved with 50 ml distilled water and

made up with distilled water having the concentration of 350 µg/ml of TMZ. From TMZ solution, 10 ml was taken and diluted to 50.0 ml with distilled water to reach a concentration range 70 µg/ml.

Sample Preparation

Twenty tablets were accurately weighed (to obtain the average mass of one tablet) then finally powdered and weight equivalent to 35 mg of TMZ was weighed and transferred into a 250 ml volumetric flask. Approximately 150 ml of distilled water was added and the mixture was sonicated for 10 minutes. The mixture was then diluted to volume with distilled water. The solution was then filtered off through a 0.45 µ filter paper discarding the first few ml of filtrate. From the sample solution by further dilutions were prepared the required working solution with concentration 70 µg/ml.

Placebo Solution Preparation

The placebo solution is prepared by using 170 mg of placebo, dissolved in 150 ml distilled water and the mixture was sonicated for 10 minutes. The mixture was diluted to 250.0 ml with distilled water and mixed well. The solution was then filtered off through a 0.45 µ filter paper discarding the first few ml of filtrate. This solution was injected to HPLC system.

RESULTS AND DISCUSSION

Validation of the Method and Procedures

All of the analytical validation parameters for this proposed method were determined according to ICH guidelines as follows:

Selectivity

The selectivity of the method was evaluated with regard to interference due to the presence of any other excipients. This shows that drug was clearly separated from its excipients. Thus, the HPLC method presented in this study was found to be selective, depicted in **Figure 3**.

Limit of Detection and Quantification

The detection limit (LOD) is the lowest amount of analyte in the sample, which can be detected but not necessarily quantified as an exact value. The quantification limit (LOQ) is the lowest amount of analyte in the sample, which can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ are calculated as given in **Table 1**.

Linearity

Calibration standards at five levels were prepared by appropriately mixing and further diluting stock standard solutions in the concentration range of 17.5 – 140 µg/ml. Samples in triplicates were made for each concentration, and peak areas were plotted against the corresponding concentrations to obtain the calibration graph. The regression equation was derived using mean peak area concentration data, and the concentration of the unknown was computed from the

regression equation. Hence, the regression line relating standard concentrations of drug using regression analysis showed linearity in the studied range. The results are given in **Table 1**.

Precision

Three injections of same concentration were given on the same day, and these studies were also repeated on different days to determine inter-day precision. Assay $\mu\text{g/ml}$ and RSD values obtained indicate a valid method. The results obtained for the evaluation of precision of the method are compiled in **Table 2**.

Accuracy

The accuracy of the method was validated by recovery studies and was found to be significant under specification limits. The percent standard error which is an indicator of accuracy is ≤ 1.0 and is indicative of high accuracy. The calculated percent of relative standard deviation (% RSD) can be considered to be satisfactory. The percentage RSD value was $< 1\%$. The results obtained for the evaluation of accuracy are given in **Table 3**.

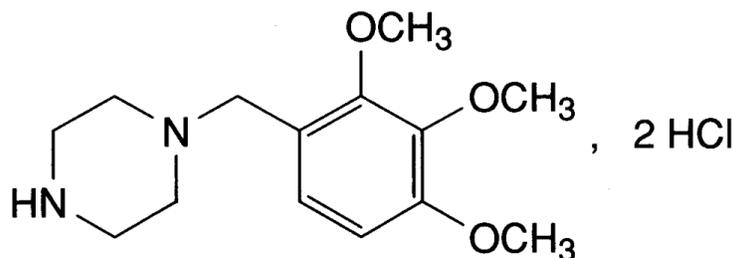


Figure 1: Chemical Structure of TMZ

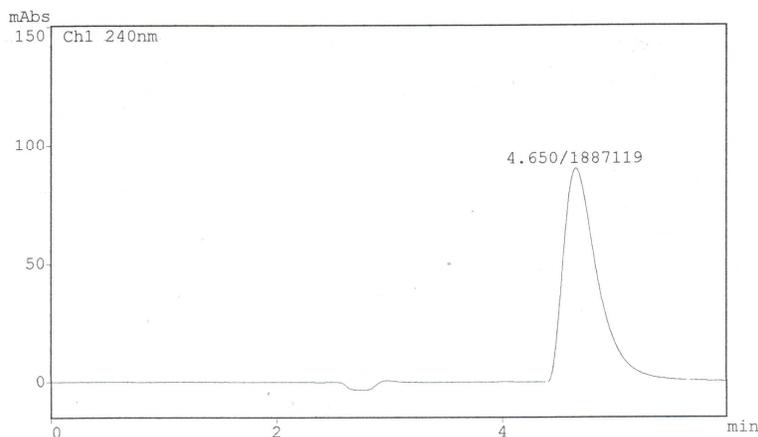


Figure 2: Chromatogram of TMZ

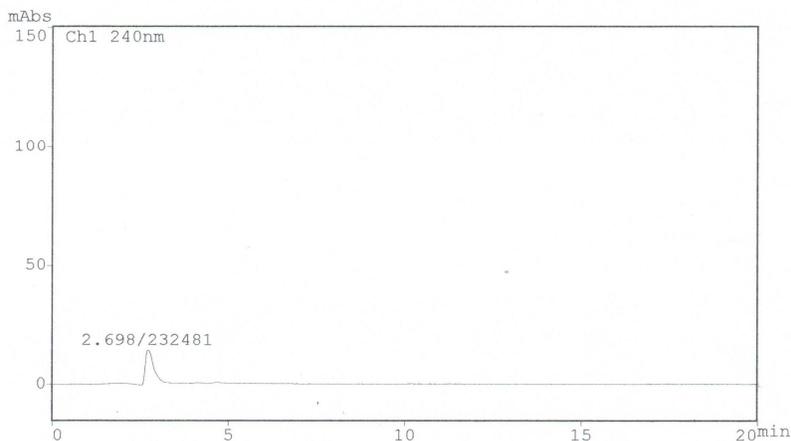


Figure 3: Chromatogram of Placebo

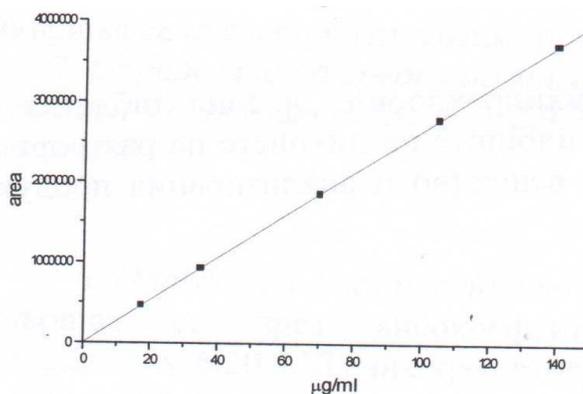


Figure 4: Linearity of TMZ

Table 1: Linearity Results, Limit of Detection (LOD) and Limit of Quantification (LOQ)

Compounds	r ²	Calibration curve equation	LOQ (ng)	LOD (ng)
Trimetazidine	0.9999	Y=26516.1X – 347.4	14	7

Table 2: Results of Precision

Drug	Concentration (µg/ml)	Intraday (n=3)		Interday (n=3)	
		Found Concentration µg/ml	% CV	Found Concentration (µg/ml)	% CV
TMZ	70	69.55±0.43	0.43	69.0±0.44	0.47

Table 3: Accuracy of TMZ

Parameters	% Taken	Mass taken (mg/l tabl.)	Mass found (mg/l tabl.)	% Found	% Recovery
		17.70	17.60	49.72	99.44
	50.00	17.70	17.87	50.48	101.0
		17.70	17.65	49.86	99.72
		35.40	35.27	99.64	99.64
	100.0	35.40	35.54	100.4	100.4
		35.40	35.34	99.83	99.83
		53.10	53.04	149.8	99.89
	150.0	53.10	52.86	149.3	99.55
		53.10	53.35	150.7	100.5
X					99.97
SD					±0.433
% RSD					0.433
%SE					0.332

CONCLUSION

An economic, accurate, sensitive and precise HPLC method with ultra violet detection was developed and fully validated for quality control analysis of Trimetazidine Dihydrochloride in tablets. The proposed method is very rapid, where the total analytical run time is less than 6 min.

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