Development and validation of HPLC method for determination of Trimetazidine Hydrochloride in bulk and Tablet dosage form

Devendra Kr. Chaudhary[1*], Meenakshi Kukshal[2], Dr. Shivanand Patil[3], Bhatt Nisha[4]
DepartmentofPharmaceutics,Shree Dev Bhoomi Institute of Science and Technology,
Uttarakhand Technical University, Dehradun-248007,India
*Addressforcorrespondence:
E-mail:Key2devendra@gmail.com
Addressforcorresponding Author[2]:
E-mail: meenu.kandwal23@gmail.com

ABSTRACT:

A simple and reproducible method has been developed for Trimetazidine Hydrochloride by Reverse phase high performance liquid chromatography (RP-HPLC). The separationswere performed in C18 column at ambient temperature with a mobile phase of Methanol and Hexane-1Sulfonic Acid Sodium Salt, pH 3.0 adjusted with Phosphoric acid solution and flow rate of 1.2 mL/min. The wavelength for maximum absorbance was selected as 232 nm by the spectral scan of the Trimetazidine Hydrochloride standard solution in UV-VIS Spectrophotometer. The detection was done by using UV detector at 232 nm.

The developed method was validated in complete compliance with the current regulatory guidelines by using well developed analytical method validation techniques and tools that comprises of the analytical method validation parameters like linearity, accuracy, method precision, specificity, system suitability and robustness. The proposed method's results were found to be satisfactory and are suitable for the determination of Trimetazidine Hydrochloride for routine quality control of drugs in bulk drug and formulation.

The method was found to be linear with a regression coefficient value of 0.999 at a concentration of 80.0% – 120.0% of the Labelled amount of Trimetazidine Hydrochloride. The range was determined at 80%, 90%, 100%, 110% and 120% of the nominal test concentration for assay with acceptable accuracy (% Recovery: 99.51) and precision study (% RSD: 0.10) in five replicate analysis standard of Trimetazidine sample solution. The method showed good precision with a %RSD value of 0.10 for repeatability or intraday precision study and a mean %RSD value of 0.46 for intermediate precision or interday precision study. Accuracy was checked for replicate analysis of three concentration levels by the standard addition method. % recovery value obtained was 99.21-100.27 % with a % RSD of 0.73-1.02. Specificity was checked by comparing the peaks of standard and sample with excipients for the presence of any interference. The developed method was found to be robust with flow rate variation of 1 ml- 1.4 ml/min and column variation of 20 °C – 30 °C. System suitability parameters were checked for acceptance limit in terms of asymmetry, theoretical plates, tailing factor and relative standard deviation (%) of the five replicate injections in 20 µL value of the standardsolution at a specific concentration. which is useful for the routine determination of Trimetazidine in bulk drugs and its pharmaceutical dosage form. Further study on the degradation pathway of the compound will provide an idea about the stability indicating the nature of the method to discriminate the active constituent, Trimetazidine Hydrochloride from its related impurities and degradants.

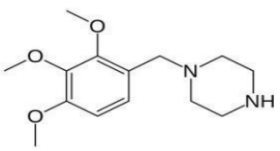
Keywords: *Trimetazidine Hydrochloride, Myocardial metabolism modifier, HPLC, Method validation*.

DOI Number: 10.14704/nq.2022.20.10.NQ55495 **NeuroQuantology 2022; 20(10): 5172-5177**

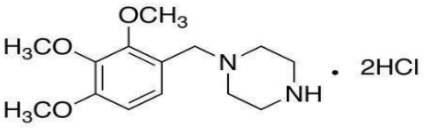
eISSN 1303-5150

INTRODUCTION

Trimetazidine (TMZ) is an effective and well tolerated cytoprotective anti-ischemic agent, which improves myocardial glucose utilization through inhibition of fatty acid metabolism. The drug is suitable for use as monotherapy in patients with angina pectoris and as adjunctive therapy in those with symptoms not sufficiently controlled by nitrates, beta-blockers or calcium antagonists. It is chemically 1-(2,3,4-trimethoxybenzyl)-piperazine hydrochloride.



Structure of Trimetazidine



Structure of TrimetazidineDihydrochloride

A literature survey revealed that various UV and HPLC methods have been reported for the estimation of Trimetazidine Hydrochloride. The objective of this project is to develop simple, accurate and economical and reproducible spectrophotometric methods for the estimation of Trimetazidine Hydrochloride in bulk and Pharmaceutical dosage forms.

EXPERIMENTAL

Standard solution Preparation

Weigh 50 mg of Trimetazidine Hydrochloride WS was weighed and transferred into a 100 ml volumetric flask. 50-60 ml of mobile phase was added and was shaken well to dissolve. Volume was maintainedupto the mark with the same and was mixed well. 5 ml of that solutionwas transferred into another 50 ml volumetric flask and the volume was maintained upto the mark with the same and was mixed well. The standard solution was transferred into a vial by passing through 0.45µm syringe filter. (0.05 mg/ml)

Assay of tablets

20 tablets were weighedand finely powdered in a mortar pestle. Accuratelyweighed portion of the powder equivalent to 50 mg of Trimetazidine Hydrochloride (about 278.5 mg of powder) and transferred into a dry 100 ml volumetric flask. 60-65 ml of mobile phase was then heated at about 50°C in the water bath for 20 minutes; again itwas sonicated for about 30 minutes. Cooled and made up the volume up tothemark with the mobile phase. Then each of the samples was stirred in a mechanical stirrer for about 30 minutes. The sample solution was filtered by the Whatmann filter paper and was transferred 5 ml of the solution into 50 ml volumetric flask. Volume was made up to the mark with the mobile phase and was mixed well. The sample solution was through 0.45 µm syringe filter. (0.05 mg/ml)

eISSN 1303-5150

Result of assay of tablet formulation

S.No	Sample	Label claim (mg)	Amount (mg/Tab)	Assay (%)
1.	Sample 1	35	34.64	98.71
2.	Sample 2		34.69	99.11
Average			34.67	98.91
% RSD			0.072	

Method Validation:

The proposed method was validated according to ICHQ2 (R1) guidelines.

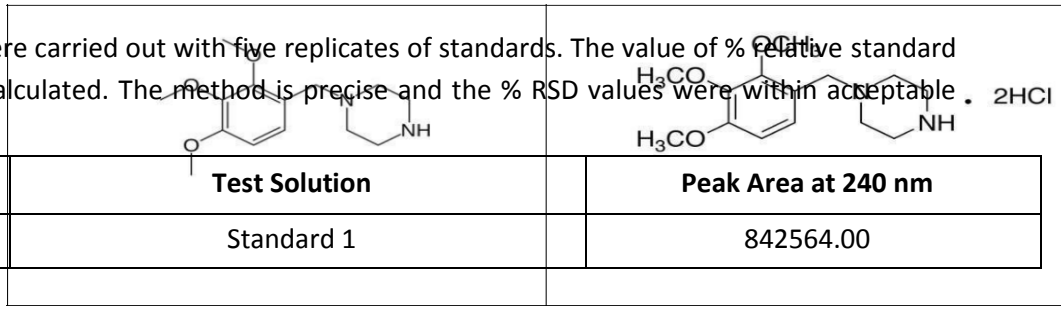
Accuracy:

The accuracy of the method was determined by the per cent recovery of the drug. Per cent recovery of TMZ was determined by three different levels 80%, 100% and 120% of the target concentration in triplets. The result of the accuracy study is shown below in the table.

Percentage	Amount present (mg)	% Recovered	Mean recovery (%)	% RSD
80	39.589	100.56	100.27	0.73
	39.689	100.82		
	39.145	99.44		
100	49.069	99.72	99.21	1.02
	49.150	99.88		
	48.250	98.05		
120	59.118	100.11	99.40	0.80
	58.787	99.55		
	58.188	98.54		
Mean recovery (%)	**99.63**			
Mean % RSD	**0.85**			

Precision:

The precision studies were carried out with five replicates of standards. The value of % relative standard deviation (%RSD) was calculated. The method is precise and the % RSD values were within acceptable limit.



S. No.	Test Solution	Peak Area at 240 nm
1	Standard 1	842564.00

eISSN 1303-5150

2	Standard 2	844369.00
3	Standard 3	842372.00
4	Standard 4	842328.00
5	Standard 5	842929.00
	% RSD	**0.10**

Linearity:

The value of the correlation coefficient for TMZ demonstrated a good relationship between absorbance and concentrations. Therefore, the developed method was linear.

S. No.	***Concentration of Analyte***	***Peak area at 240 nm***
1	80%	662733
2	90%	760046
3	100%	845561
4	110%	919930
5	120%	1006910
	Correlation Co-efficient (r^2)	**0.9990**

Range:

The range was carried out for the concentration of 80% to 120% and mean recovery, correlation coefficient and % RSD were calculated. The values were within the acceptable limit.

S. No.	**Concentration of Analyte**	**Peak Area at 240 nm**	**% Recovery**
1	80%	662733.00	98.28
2	90%	760046.00	100.19
3	100%	845561.00	100.31
4	110%	919930.00	99.22
5	120%	1006910.00	99.55
	Mean % Recovery	**99.51**	
	Correlation Co-efficient (r^2)	**0.9990**	
	% RSD	**0.83**	

eISSN 1303-5150

System Suitability:

System suitability was performed for the confirmation of chromatographic conditions by using a standard solution. The system suitability is within the acceptable limit.

Standard	**Observation**
Number of theoretical plates: NLT 2000	8895.349
% RSD: NMT 2%	0.10
Tailing Factor: NMT 2	1.174

Summary:

Validation of an analytical method for assay determination of Trimetazidine Hydrochloride Modified Release Tablet was performed.The parameters listed below show the status of compliance:

Sr. No	**Validation Parameters**	**Result Obtained**	**Acceptance criteria**	**Status of compliance**
1.	Accuracy	99.63 0.85	% Recovery 98 -102 RSD NMT 2%	Complies
2.	Precision: System Precision	0.10	% RSD NMT 2.0	Complies
3.	Linearity	0.9990	$r^2 \geq 0.98$	Complies
4.	Range	0.9990 99.51 0.83	$r^2 \geq 0.98$ % Recovery 98-102 % RSD NMT 2.0	Complies
5.	System suitability Theoretical plate Tailing Factor % RSD	 8895.349 1.174 0.10	 NLT 2000 NMT 2 NMT 2%	Complies

Conclusion:

It can be concluded that the developed methods are simple, rapid, accurate and economical for the estimation of Trimetazidine Hydrochloride. These methods were validated as per ICH guidelines. Thus, the developed methods can be used for routine estimation of Trimetazidine Hydrochloride in bulk and tablet dosage forms.

REFERENCES:

1. http://www.drugbank.ca/drugs/DB09069.
2. McClellan KJ, Plosker GL. Drugs. Trimetazidine: A review of its use in stable angina pectoris and other coronary conditions. 58(1); 1999: 143-57.
3. Government of India. Ministry of health and family welfare. Indian Pharmacopoeia Vol.

eISSN 1303-5150

III. The Controller of Publication, New Delhi; 2010: 2263-2264.

4. Prasanna NY, Archana S, Poornima Y, Lalitha S. Development and validation of a spectrophotometric method for determination of Trimetazidine in pharmaceutical dosage forms. International Journal of Pharmaceutical Science and Research. 4(3); 2013: 1133-1134.
5. Mani G, Pushparaj H, Arthanari SK, Peng MM. Spectrophotometric method for determination of Trimetazidine in formulation using chloranil as a chromogenic agent. Eurasian Journal of Analytical Chemistry. 6(1); 2011: 22-30.
6. Osama HA. Simple spectrophotometric and spectrofluorimetric methods for determination of trimetazidine dihydrochloride. Bulletin of Pharma Science. 27(2); 2004: 315-323.
7. Rajani B, Mukkanti K. RP-HPLC method development and validation for the estimation of Trimetazidine in the tablet dosage form. Indo-American Journal of Pharmaceutical Research. 4(2); 2014: 1657-1661.
8. Madhuri A, Hinge N, Rajvi J, Sojitra S. Development and validation of an RP-HPLC method for simultaneous determination of trimetazidine hydrochloride and metoprolol succinate. International Journal of Chemical and Pharmaceutical Analysis. 2(2); 2015: 77-83.
9. Mirjana BM, Darko PI, Igor BP, Andjelija MM, and Biljana SJ. Computer–Assisted optimization and validation of analysis of trimetazidine dihydrochloride and its impurities. Journal of Chromatographic Science. 46; 2008: 430-435.
10. Simmy OT, Amin PD. Trimetazidine: stability indicating RPLC assay method. Journal of Pharmaceutical and Biomedical Analysis. 25; 2001: 191-195.
11. Jain HK, Agrawal RA. Spectrophotometric methods for simultaneous estimation of amlodipine besylate and lisinopril in tablets. Indian Drugs. 37(4); 2000: 196-199.
12. Jain HK, Agrawal RK. Simultaneous estimation of gliclazide and metformin hydrochloride in combined dosage forms. Indian Journal of Pharmaceutical Sciences. 64(1); 2002: 88-91.
13. Jain HK, Patel KH. Development and validation of UV spectrophotometric area under curve method for estimation of loratadine in bulk and tablet dosage form. American Journal of Pharmtech Research. 3(4); 2013: 492-499.
14. ICH Guidelines on Validation of Analytical procedure: Text and Methodology Q 2 (R1), (2011).

eISSN 1303-5150