WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 3, 561-567. Research Article ISSN 2277–7105

ESTIMATION OF RELATED SUBSTANCES IN TRIMETAZIDINE DIHYDROCHLORIDE DRUG SUBSTANCE BY RP-HPLC

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Article Received on
06 December 2024,

Revised on 27 Dec. 2024,
Accepted on 16 Jan. 2025

DOI: 10.20959/wjpr20253-35200

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ABSTRACT

A simple, specific, sensitive, accurate, robust, precise, and stability indicating RP-HPLC method was developed and validated for the stability indicating analytical method development and validation for the determination of related substances of the Trimetazidine dihydrochloride drug substance. The method was developed by using potassium hydrogen phosphate buffer pH 4.0, acetonitrile as mobile phase by gradient pump mode. The column used for separation was Develosil ODS MG-5, (250 X 4.6 mm), 5µm, as it helps to achieve good separation between the related substances in the drug product with 1.2ml/min flow rate and PDA detection at 240nm. The retention time of Trimetazidine dihydrochloride is about 16.13 min and for related substances i.e., impurity-E, impurity-A, impurity-I, impurity-J, impurity-F, impurity-H, impurity-D, impurity-B, impurity-C were found to be 14.50 min, 19.40min, 21.60 min, 22.47 min, 22.78 min,28.38 min,29.46 min, 30.47 min, 31.11 min respectively. The validation was carried out on optimised method and obtained results were within the limits of acceptance criteria for all the parameters like System suitability, Specificity, Linearity, Accuracy, Precision, LOD, LOQ, Robustness and stability of the method has been checked by the forced degradation studies. Based on the above information the method can be used for the daily routine analysis.

KEYWORDS: RP-HPLC, Trimetazidine dihydrochloride, Related Substances, Forced degradation studies.

1. INTRODUCTION

Active Pharmaceutical Ingredient (API) is a substance intended to be used in the manufacture of a drug (medicinal) product and is responsible for eliciting the desired pharmacological activity.[1] Such substances are generally called drug substances and used to formulate the drug product which are consumed by the patients. These drug products furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment or prevention of disease or to affect the structure and function of the body. Thus, pharmaceutical sector has two demarcated sections, one is manufacturing of API's and other is formulating them in the tablets, capsules, injectables, syrup, patches, etc. International Conference of Harmonization has adopted stringent rules to maintain quality of the API used in pharmaceutical product. Quality of the drug is nothing but compliance of the substance (API) with respect to set of quality parameters such as chemical purity, chemical assay, impurity profile, content of known and unknown, and genotoxic impurities, inorganic contents, residual solvents, physical attributes such as polymorph, particle size, bulk density etc. All these set of analytical examination is formatted together and formatted to release the material to end users is known as Certificate of Analysis (COA). The role of analytical chemist in the pharmaceutical industry plays an extremely important role in developing analytical methods that ensure the safety, efficacy, purity, stability and overall quality of the API and formulated drug products.[2] Method validation of analytical methods is another milestone, which is critical to ensure that impurity levels are accurately and consistently reported throughout drug development.

Nandini R Pai *et.al.,* **A Novel RP-HPLC Method for Quantitative etermination of an Angina Pectoris Drug and Related Substances. 2013.**[3] 2,3,4- Trimetazidine Dihydrochloride is a cytoprotective anti-ischemic agent used for the treatment of angina pectoris. In the present work, a simple, sensitive, reproducible reverse phase High Performance Liquid Chromatography (RP-HPLC) method for separation and determination of related substances in Trimetazidine dihydrochloride was developed and validated.

Mirjana B *et.al.,* **Computer—Assisted Optimization and Validation of LC Analysis of Trimetazidine Dihydrochloride and Its Impurities, 2008.**[4] Trimetazidine dihydrochloride is an anti-anginal drug, which possesses protective properties against ischemia inducing heart damage. In this work, a new procedure for liquid chromatographic analysis was successfully developed, optimized, and applied in assessment of Trimetazidine dihydrochloride content

and its impurities, Y-145, Y-235, Y-234 at most 1.0%, 0.2% and 0.2%, respectively, in commercially available pharmaceutical preparation containing 35 mg of Trimetazidine dihydrochloride.

Description of Trimetazidine dihydrochloride drug substance.[5]

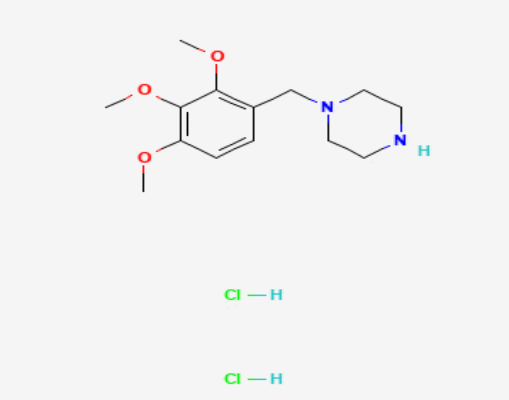

Figure 1: Chemical structure.

Diluents used: Water and methanol in the ratio of 90:10.

Preparation of Mobile phase

Mobile phase A: pH 2.80 buffer (100%) Mobile phase B: Methanol (100%).

Preparation of Blank

A portion of Diluent was filtered through 0.2µm membrane filter and used as blank.

Preparation of Standard solutions Working standard solution

Transfer about 40mg of the working standard to 100ml volumetric flask, dissolve and dilute to volume with diluent.

Standard Dilution

Dilute 1.0ml of the working standard solution to 100ml with diluent. From this solution 10 ml is taken and diluted to 100ml with diluent.

Preparation of Test solution

Weigh accurately about 40mg of the test sample in to a 50ml volumetric flask, dissolve and dilute to volume with diluent.

Impurity-A Stock solution

Transfer about 2mg of Trimetazidine impurity-A in to a 10ml volumetric flask, dissolve and dilute to volume with diluent.

System suitability solution

40mg of Trimetazidine Dihydrochloride working standard is accurately weighed and transferred to a 10ml volumetric flask and to this 2ml of impurity-A stock solution is added and make up the volume with diluent.

2. MATERIALS AND METHODOLOGY

RP-HPLC Method Development for Trimetazidine Dihydrochloride

Preparation of solutions Buffer Preparations

- 2.27gms of Potassium dihydrogen phosphate and 4.7gms of disodium hydrogen phosphate was weighed accurately and dissolved in 1000ml of Milli-Q water and mixed.
- The pH of the solution is adjusted to 2.8 with Orthophosphoric acid.
- The solution was filtered through 0.22µ membrane filter and degassed.

3. RESULTS AND DISCUSSIONS

OPTIMIZED METHOD

Chromatographic Conditions

Column	: Develosil ODS MG-5, 250×4.6 mm, 5µm or equivalent
Injection Volume	: 10µl
Flow rate	: 1.2ml/min Detection
Wavelength	: 240nm
Oven Temperature	: 30˚C
Run time	: 45 min
Pump mode	: Gradient

Trimetazidine dihydrochloride forced degradation study was performed and based on the above study it is concluded that Trimetazidine dihydrochloride is stable in acid, base, thermal, hydrolytic and photolytic conditions. The degradation is observed in peroxide degradation conditions.

The purity angle of Trimetazidine dihydrochloride peak is less than the purity threshold in all

degraded conditions samples. In acid, base, hydrolytic and peroxide degradation conditions, the assay (% w/w, on as is basis) decreased to 2.07%, 2.14%, 3.29% and 12.04% respectively.

In thermal and photolytic degradation conditions no significant change observed. The mass balance of the acid, base, peroxide, thermal, hydrolytic and photolytic degradation samples were found to be 100.7%, 100.9%, 102.1%, 99.8%, 97.3%, 100.1%.

Table 1: Summary of validation report.

S.No	Parameter		IMP-E	IMP-A	IMP-I	IMP-J	IMP-F
1.	Specificity		Specific	Specific	Specific	Specific	Specific
2.	Method precision % RSD		0.89	0.00	0.93	0.40	0.00
3.	Accuracy % Recovery		84.5	85.6	117.0	99.1	115.6
4.	Precision at LOQ % RSD		3.77	2.95	2.81	2.13	4.59
5.	Accuracy at LOQ % Recovery		75.7	83.5	105.1	97.3	75.1
6.	Intermed iate precision % RSD	n=6	0.50	0.00	0.80	1.45	0.50
		n=12	6.02	4.30	8.57	3.45	6.02
7.	Linearity Range (µg/ml)		0.50-5.88	0.19-5.76	0.40-4.72	0.32-6.05	0.50-5.88
8.	Regression equation (y=mx+c)		y = 17576331. 2980x 2989.916	y = 17695040. 4403x 341.1390	y = 9105212.3 279x 38.3776	y = 22679907. 5622x +37.9490	y = 17576331. 2980x 2989.916
9.	R2		0.9984	0.9999	1.0000	0.9984	0.9984
10.	Robustness (%RD)	pH-	0.00	11.76	-6.38	0.00	-5.83
		pH+	-14.42	-7.95	6.16	-12.12	-4.42
		CT+	2.04	6.86	0.00	1.98	-2.88
		CT-	2.04	4.90	-3.47	0.99	-3.85
		FR+	4.71	-4.55	2.74	9.09	7.96
		FR-	-5.88	-3.41	0.68	-5.05	-5.31
11.	LOD (S/N)		3.25	3.25	5.00	3.25	4.25
12.	LOQ (S/N)		10.0	10.0	15.25	10.0	12.50

S. No	Parameter		IMP-H	IMP-D	IMP-B	IMP-C	Limits
1.	Specificity		Specific	Specific	Specific	Specific	No interference of any peak
2.	Method precision % RSD		0.40	0.00	0.33	0.33	NMT 2.0%
3.	Accuracy % Recovery		105.2	85.1	116.1	111.7	80-120%
4.	Precision at LOQ % RSD		1.04	1.28	2.26	5.80	≤15%
5.	Accuracy at LOQ Recovery		97.2	110.8	116.0	97.5	70-130%
6.	Intermed-iate precision % RSD	n=6	0.00	0.80	1.45	0.00	≤30%
		n=12	4.30	8.57	3.45	0.60	

7.	**Linearity Range (µg/ml)**		0.19-5.76	0.40-4.72	0.32-6.05	0.10-6.07	
8.	**Regression equation (y=mx+c)**		y = 17695040.4403x 341.1390	y = 9105212.3279x 38.3776	y = 22679907.5622x +37.9490	y = 29938385.2389x +923.7074	
9.	**R2**		0.9999	1.0000	0.9984	0.9997	NLT 0.99
10.	**Robustness (%RD)**	**pH-**	0.87	-4.81	5.79	5.47	≤2.0%
		pH+	-14.85	-7.69	-4.24	-6.67	
		CT+	0.87	-7.69	9.09	3.91	
		CT-	-0.87	-8.65	1.65	3.91	
		FR+	4.95	-17.95	1.69	-2.50	
		FR-	0.99	-17.95	1.69	-2.50	
11.	**LOD (S/N)**		1.50	2.00	2.75	0.75	≥3
12.	**LOQ (S/N)**		4.75	5.75	8.00	2.50	≥10

4. CONCLUSION

A specific, selective, accurate, precise, robust and stability indicating analytical method was developed for the determination of related substances in Trimetazidine dihydrochloride drug substance by using RP-HPLC.

The method was optimized after many trails because in these all-related substances present in the drug substance were well separated. For the optimised method the column used was Develosil ODS MG-5, 250×4.6 mm, 5µm or equivalent in gradient pump mode with flow rate 1.2ml/min and injection volume 20µL with data acquisition time 45 min. The retention time of Trimetazidine dihydrochloride is about 16.13 min which was confirmed by comparing with the standard drug and the retention times of the impurity-E, impurity-A, impurity-I, impurity-J, impurity-F, impurity-H, impurity-D, impurity-B, impurity-C were found to be 14.50 min, 19.40min, 21.60 min, 22.47 min, 22.78 min,28.38 min,29.46 min, 30.47 min, 31.11 min respectively confirmed by injecting all the individual impurities separately.

The method was validated by for all the parameters like System suitability, Specificity, Linearity, Precision, Accuracy, LOD, LOQ, Robustness and degradation studies. The results obtained in all parameters were within the acceptance criteria. This method was Specific, as no interference peaks at the retention times of the related substances were found.

The method was Linear for the determination of the related substances R^2 values were in the range 0.9984-1.000. The LOD and LOQ values of impurity-E, impurity-A, impurity-I, impurity-J, impurity-F, impurity-H, impurity-D, impurity-B, impurity-C were found to be 3.25 & 10.00, 3.25 & 10.00, 5.00 & 15.25, 3.25 &10.00, 4.25 & 12.50, 1.50 &4.75, 2.00 &5.75,

2.75 &8.00, 0.75 & 2.50 (µg/ml) respectively.

The method was Accurate because the mean % recovery of related substances impurity- E, impurity-A, impurity-I, impurity-J, impurity-F, impurity-H, impurity-D, impurity-B, impurity-C were found to be 84.5%, 85.6%,117.0%, 99.1%, 115.6%, 105.2%, 85.1%, 116.0%, 97.5% which is in between 80% and 120% according to specification.

The method was Precise for the determination of related substances because for all the precise conditions the % RSD was less than 2%. The Robustness was carried out by varying the conditions like pH variation, column temperature variation and flow rate variation. No effect on method was observed. Hence, it's a robust method.

The method was Stable, it was confirmed by forced degradation conditions under various stress conditions like Acid, Base, Photolytic, Thermal, hydrolytic degradations. In all these conditions there were no interference of degraded peaks at the retention times of all the related substances. The drug substance was degraded with 10% hydrogen peroxide at 60°C for 1 hour in peroxide degradation condition.

Based on the above information, it was concluded that this method can be used for routine analysis for the determination of related substances in the Trimetazidine dihydrochloride drug substance.

5. REFERENCES

1. International conference on Harmonization (ICH) Q7A, Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients, 2005.
2. S. Gorog, J. Pharm. Biomed. Anal, 2008; 48; 2, 247-253.
3. Nandini R. Pai, Swapnali Suhas Patil. A Novel RP-HPLC Method for Quantitative Determination of an Angina Pectoris Drug and Related Substance. Research J. Pharm. and Tech, September 2013; 6(9): 1014-1018.
4. Mirjana B. Medenica, Darko P. Ivanovic, Igor B. Popovic, Andjelija M. Malenovic, Biljana S. Jancic, Computer—Assisted Optimization and Validation of LC Analysis of Trimetazidine Dihydrochloride and Its Impurities, Journal of Chromatographic Science, May-June 46(5): 430–435.
5. Trimetazidine Impurities and its Related Products, 2024, https://clearsynth.com/ product-category/Impurities/Trimetazidine-Impurities.