Original Article

A New Validated Analytical Method For Simultaneous Estimation Of Trimetazidine Dihydrochloride And Bisoprolol Fumarate From Dosage Forms Using HPLC

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Abstract

Developed and validated analytical method which simultaneously estimate both the drugs Trimetazidine dihydrochloride and Bisoprolol Fumarate in Bulk drug and formulations in single method. This method developed on HPLC and it is very simple, precise and accurate. Separation of both drugs achieved using a C18 column Gemini 4.6 x 50 mm, 3µ. Mixture of pH 6.8 Ammonium fumarate solution and Acetonitrile in the ratio 80:20 v/v used as Mobile phase. The flow rate was 0.8 mL/min and selected wavelength was 272 nm. Linearity covered for Bisoprolol fumarate and Trimetazidine dihydrochloride in the range of 0.25-0.75 mg/mL and 4-12 mg/mL respectively. Using this method analysed the Marketed samples and observed Assay are 98.8% and 99.0% for Trimetazidine dihydrochloride and Bisoprolol fumarate respectively. According to International Conference on Harmonization (ICH) guideline method was validated. The proposed method is very easy and cost effective.

KEYWORDS: Bisoprolol, Trimetazidine, validated assay method, Bisoprolol and trimetazidine, validation.

INTRODUCTION:

Number of researchers published their articles on Effects of bisoprolol fumarate when gives along with trimetazidine dihydrochloride in the treatment of heart failure and in chronic obstructive pulmonary disease. The overall study concludes the combination of these two drugs is found more effective and significant improvement in health condition[1-3] Currently there is no such combination Bisoprolol fumarate and trimetazidine dihydrochloride combined in single dose product is available in the market. However, doctors use individual table of bisoprolol fumarate and trimetazidine dihydrochloride are using for treatment. But One of innovator company is developing the combined dosage form of Bisoprolol fumarate and Trimetazidine dihydrochloride[15]. This is new drug abbreviation. European agency recently listed this drug in his report and this combination specified product waiver is agreed[12]

Based on above reference initiated the development of analytical method for such combination considering in upcoming day this combination drug i.e. Bisoprolol fumarate and trimetazidine dihydrochloride will be in the market. For pharmaceuticals who going to produce this drug and they definitely required the analytical testing method for these drugs. Till date there is no any single analytical method is available to determine the purity of

both bisoprolol fumarate and trimetazidine dihydrochloride. Individual analytical method for Bisoprolol fumarate and For Trimetazidine are available but there is no single method available till date[4-11,19]. Hence developed new analytical method which simultaneous estimate both the drugs within single analytical method. This method will be helpful to determine the potency, purity of Trimetazidine dihydrochloride and bisoprolol fumarate both drug in single method.

Bisoprolol fumarate (BISO) is a beta-1 adrenergic blocking agent. Bisoprolol is used to prevent heart failure, myocardial infarction and to treat mild to moderate hypertension. Bisoprolol is a cardio selective β1-adrenergic blocking agent used to treat high blood pressure. Chemically, Bisoprolol Fumarate is E)-but-2-enedioic acid;1-(propan-2-ylamino)-3-[4-(2-propan-2-yloxyethoxymethyl) phenoxy]propan-2-ol.[17,18] Trimetazidine hydrochloride (TMZ) is an aromatic amine. A vasodilator used in ischemic heart disease. Trimetazidine is a piperazine derivative indicated as an adjunct therapy in symptomatic treatment of stable angina pectoris. IUPAC name is 1-[(2,3,4-trimethoxyphenyl)methyl]piperazine dihydrochloride[14,16]

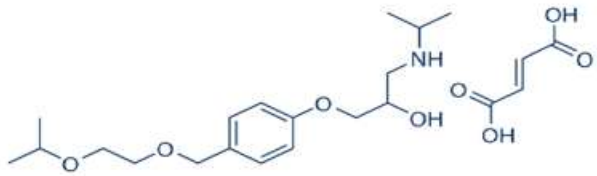

Figure-1. Structure of Bisoprolol fumarate[20]: $C_{18}H_{31}NO_4$ (BISO)

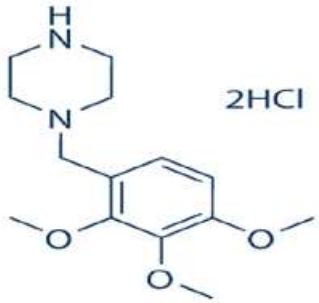

Figure-2.Trimetazidine dihydrochloride[21]: $C_{14}H_{24}Cl_2N_2O_3$ (TMZ)

MATERIALS AND METHODS:

Materials and Methods

Following instrument and equipment's were used for activity. Shimadzu (2010 CHT) and Waters two liquid chromatographic system having UV/PDA detector were used for separation, Column was used Gemini 50 x 4.6 mm, 3µ, having flow rate 0.8 mL/min. Balance was used Mettler Toledo AB265-S/FACT make and pH meter make was Venus.

Reagents and Chemicals:

Active drugs Bisoprolol Fumarate and trimetazidine dihydrochloride received through Scientia Qualitek®. Navi Mumbai and tablet formulations (Concor 5) with of 5mg of Bisoprolol Fumarate and 35mg of trimetazidine dihydrochloride Manufactured by Sardia Pharmaceutical Limited were purchase from local medical shop. HPLC grade Solvents such as Acetonitrile and water were manufactured by Merck Limited and same was used for analysis.

Chromatographic Conditions

Gemini 4.6 x 50 mm, 3µ reverse phase HPLC column was used for resolution of TMZ and BISO peaks, UV-wavelength was fixed at 272nm. Mobile phase was prepared by mixing buffer pH 6.8 and Acetonitrile in 80:20 v/v ratio and mixture of water and methanol in ratio 80:20 v/v was used as diluent for solution preparation such as standard solution, sample solutions. Mobile phase flow rate was selected 0.8 mL/min and 10 µL was used as injection volume.

Preparation of Standard solution

Dissolved 50 mg of Bisoprolol fumarate in 25 mL of diluent and mixed well. (Bisoprolol fumarate stock solution). Weighed and transferred 160mg of trimetazidine dihydrochloride in 20 mL of volumetric flask, added 5 mL of bisoprolol stock solution, sonicated to dissolve and diluted to volume with diluents. Final concentration were 0.5mg/ml bisoprolol fumarate and 8 mg/ml trimetazidine dihydrochloride.

Preparation of Sample solution

Weighed each 20 Tablets of Bisoprolol fumarate and Trimetazidine dihydrochloride and crushed individually. Weighed powdered sample equivalent to 10mg of Bisoprolol Fumarate and 160mg of trimetazidine dihydrochloride and transferred in to a 20mL volumetric flask. Added 4 mL of methanol, stirred and followed by sonicated for 15 minutes to dissolve the drug content, added 10 mL of water and repeated same procedure as above and final volume make up with water and mixed well. Filtered the solution through 0.45µ membrane filter. Final solution contains 0.5mg/mL of Bisoprolol Fumarate and 8mg/mL of Trimetazidine dihydrochloride.

Enhancement of RP-HPLC method

The Reverse phase HPLC method was optimized with the goal of development of a single chromatographic method which can easily estimate the content of Trimetazidine di-HCl and Bisoprolol Fumarate. Different type of chromatographic solvents, different pH, buffers were used but desirable retention time, USP plates count and separation/resolution were achieved with Gemini 4.6 x 50 mm, 3µ column and mobile phase mixture of Ammonium formate buffer pH 6.8 and Acetonitrile in 80:20 v/v ratio.

Validation of the RP-HPLC Method

The developed analytical method has been validated according to ICH Q2 (B) guidelines.

a) System suitability

Injected five replicate injection of standard solution with concentration 500µg/mL and 8000 µg/mL of Bisoprolol fumarate and Trimetazidine dihydrochloride respectively in to the reverse phase HPLC system. Plates count (N), tailing factors (T) were reported in Table 1

b) Specificity

Injected individual placebo and blank solution. Checked for their interference with respect to sample solution and such way proved the Specificity of a method. At the Retention time of TMZ and BISO, no peak observed due to blank and placebo. Placebo prepared using excipients[23,24]. The Forced degradation study perform to prove the specificity of the method. Based on result can be concluded that the method is found specific. The chromatogram and spectra of Bisoprolol Fumarate and Trimetazidine dihydrochloride were shown in Figure 3. Chromatogram of blank, placebo and sample shown in Figure 3(a), 3(b) and 3(c) respectively.

c) Linearity

Determined the linearity of Bisoprolol fumarate and trimetazidine dihydrochloride in the range of 0.25-0.75 mg/mL and 4-12 mg/mL respectively. There were much difference in concentration of both the drug and was selected based on their individual label claims.[3,22] Still the method found linear within provided range. Prepared the linearity solution from series of dilutions using higher concentrated solutions of both bisoprolol fumarate and trimetazidine dihydrochloride. Three replicates of Each solution were injected under linearity study. Plotted graph concentration against area and determined the regression coefficient. The Linearity Plot for Bisoprolol Fumarate

and trimetazidine dihydrochloride indicted in Figure 4 and 5. Their related parameters were given in Table 2 and 3 for Bisoprolol fumarate and Trimetazidine diHCl respectively.

d) Precision

As part of Precision study, the target concentration solution i.e 100% concentration solution was injected six times under the chromatographic system. Precision and intermediate precision performed on two different day and calculated the %RSD of precision and intermediate study. Based on result it can be concluded that the method is precise. For result refer Table 4, 4(a) and 4(b).

e) Accuracy

Spiking of standard method was followed to prove the accuracy of the method. Spiked exact amount of drug into the placebo and prepared the solution as per defined procedure under sample solution preparation and analyzed by the developed chromatographic method. Reported the obtained results in Table 5 and 6 for Trimetazidine dihydrochloride and Bisoprolol fumarate respectively.

f) Robustness

To prove the method is Robust altered many parameters such as mobile phase composition, UV-wavelength detection, mobile phase flow rate, etc. and the % difference with respect to unaltered condition were reported. Altered the wavelength ±2nm in and flow rate ±0.2mL/min, and Buffer pH ±0.5 and performed the analysis. A solution of 100% drug sample was injected under the above reverse phase chromatographic system. Refer Table-7.

g) Forced degradation study

Forced degradation study performed using Placebo, API and tablet formulation exposed to different conditions such as acid (Hydrochloric acid), base (Sodium hydroxide), peroxide (hydrogen peroxide), thermal and Ultraviolet. And results are summarized under Table 8 and 9 for Trimetazidine diHCl and Bisoprolol fumarate respectively.

h) Assay of Marketed Formulations

Purchased the individual samples from market local Pharmacy shop and prepared sample solution using sample procedure given under the sample preparation. Prepared solution contains 0.5mg/mL of bisoprolol fumarate and 8mg/mL of trimetazidine dihydrochloride. Injected 10µL of this solution under the reverse phase HPLC chromatographic system. Performed analysis in triplicate. Calculated the amount of both drugs. Refer Chromatogram given in Figure-6. And for results refer Table 10

RESULT:

System Suitability

Table 1. System suitability of Trimetazidine dil.HCl (TMZ) and Bisoprolol fumarate (BISO)

Parameter	TMZ	BISO
Tailing factor (T)	1.3	1.6
Theoretical Plate (N)	1662	5528
%RSD of Area	0.42	0.30
%RSD of RT	0.12	0.12

Specificity

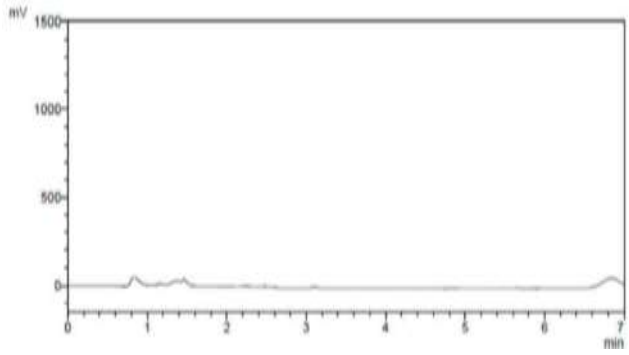

Figure 3a. Chromatogram of Blank

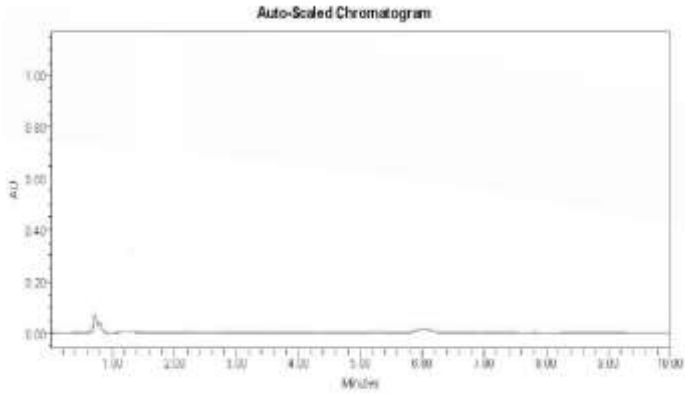

Figure 3b. Chromatogram of Placebo

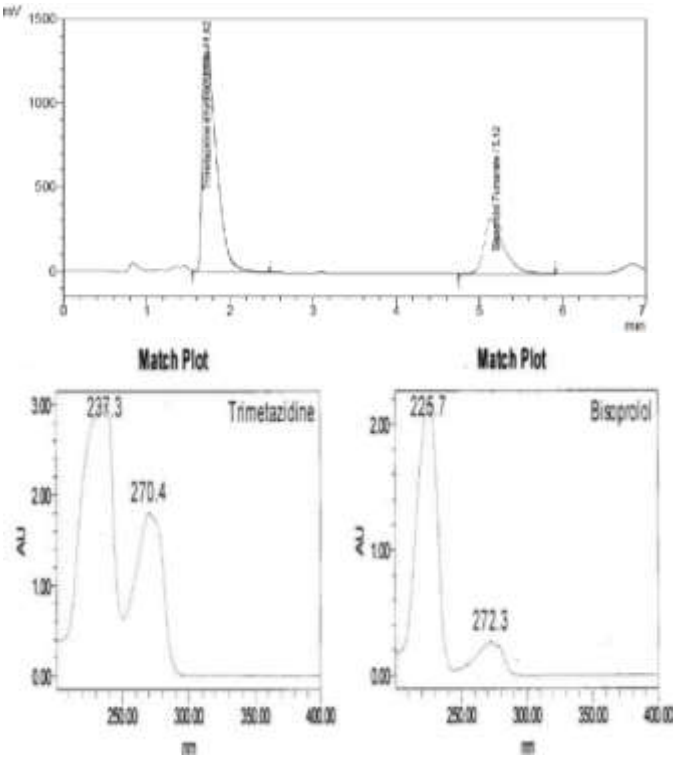

Figure-3c. Chromatogram of Sample with spectra

Linearity

Table 2. Linearity results for Bisoprolol fumarate (BISO)

Slope:	2134.188
intercept:	-1790.97332
Correlation coefficient(r):	0.999
(r)2	0.999
y-intercept	-0.17

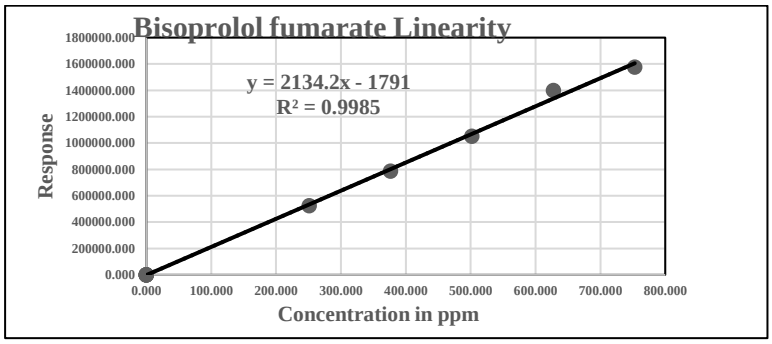

Figure 4. Calibration Plot for Bisoprolol fumarate

Table 3 Linearity results for Trimetazidine dihydrochloride (TMZ)

Slope:	1688.120
intercept:	-1820.67038
Correlation coefficient(r):	1.000
(r)2	1.000
y-intercept	-0.01

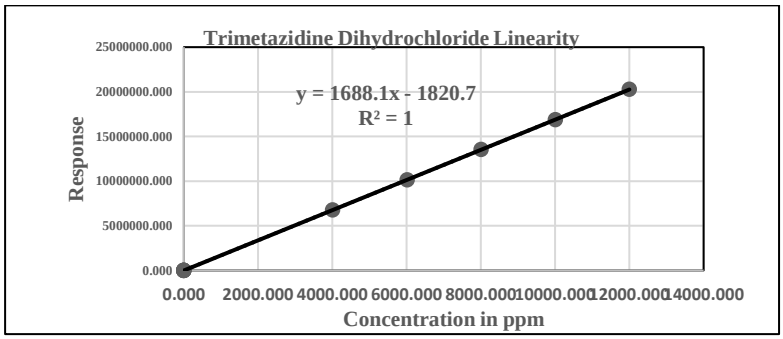

Figure 5. Calibration Plot for Trimetazidine Dihydrochloride (TMZ)

Precision

Table-4. Result of Precision and intermediate precision study

S.No	Parameter	TMZ	BISO
1.	Precision (%RSD)	0.40	0.13
2.	Intermediate Precision (IP) (%RSD)	0.24	0.42
3.	Overall %RSD of Precision and IP	0.33	0.36

Table 4(a). Result for Trimetazidine dil.HCl

Sample. No	%Assay	Sample No.	%Assay
Precision		**Intermediate Precision**	
1	100.3	1	100.3
2	99.5	2	99.8
3	100.1	3	100.2

4	100.0	4	100.4
5	100.0	5	99.9
6	100.8	6	100.3
Mean of Precision and IP			100.1
S.D. of			0.3312
%RSD			0.33

Table 4(b). Result for Bisoprolol fumarate

Sample. No	**%Assay**	**Sample No.**	**%Assay**
Precision		**Intermediate Precision**	
1	100.1	1	99.9
2	99.9	2	101
3	100.1	3	100.6
4	100.2	4	100.8
5	100.2	5	100.1
6	100.3	6	100.7
Mean of Precision and IP			100.3
S.D. of			0.3621
%RSD			0.36

Accuracy

Table 5. Result for Trimetazidine dihydrochloride (TMZ)

S.NO	**Level**	**Trimetazidine dihydrochloride**		
		Amount added in mg	**Amount found in mg**	**%Recovery**
1.	50% Preparation -1	80.10	80.15	100.1
2.	50% Preparation-2	80.50	79.50	98.8
3.	50% Preparation-3	80.20	79.60	99.3
4.	100% Preparation-1	160.50	159.60	99.4
5.	100% Preparation-2	160.80	159.10	98.9
6.	100% Preparation-3	160.10	159.0	99.3
7.	150% Preparation-1	240.15	239.20	99.6
8.	150% Preparation-2	240.14	239.50	99.7
9.	150% Preparation-3	240.12	238.61	99.4

Table 6. Result for Bisoprolol fumarate (BISO)

S.NO	**Level**	**Bisoprolol fumarate**		
		Amount added in mg	**Amount found in mg**	**%Recovery**
1.	50% Preparation -1	5.02	5.02	100.0
2.	50% Preparation-2	5.01	5.02	99.8
3.	50% Preparation-3	5.00	5.01	99.8
4.	100% Preparation-1	10.01	10.00	100.1
5.	100% Preparation-2	10.01	10.02	100.1
6.	100% Preparation-3	10.00	10.02	99.8
7.	150% Preparation-1	15.00	15.01	99.8
8.	50% Preparation -1	5.02	5.02	100.0
9.	50% Preparation-2	5.01	5.02	99.8

Robustness

Table 7. Robustness Results for Trimetazidine diHCl (TMZ) and Bisoprolol fumarate (BISO)

S.NO	Parameter	Change	%Assay Difference w.r.t control sample	
			TMZ	BISO
1.	Wavelength	270nm	0.2	0.1
		274nm	0.2	0.1
2.	Flow	0.6 mL/min	0.3	0.2
		1.0 mL/min	0.4	0.2
3.	pH	pH- 6.3	0.5	0.5
		pH- 7.5	0.6	0.4

Forced degradation

Table 8. Forced degradation Results for Trimetazidine diHCl (TMZ)

Condition	%Trimetazidine diHCl	Difference w.r.t control sample	Peak purity*
Control	98.2	NA	Pass
Acid 1N, 1 mL 60°C for 1 Hour	97.5	0.7	Pass
Base 1N, 1 mL 60°C for 1 Hour	92.8	5.4	Pass
Peroxide, 30%, 1mL 60°C for 1 hour	97.5	0.7	Pass
Thermal 80% for 24 hour	96.9	1.3	Pass
UV degradation	98.1	0.1	Pass

*Peak purity confirmed using PDA detector empower software i.e Purity threshold>Purity Angle.

Table 9. Forced degradation Results for Bisoprolol fumarate (BISO)

Condition	%Bisoprolol fumarate	Difference w.r.t control sample	Peak purity*
Control	99.1	NA	Pass
Acid 1N, 1 mL 60°C for 1 Hour	97.3	1.8	Pass
Base 1N, 1 mL 60°C for 1 Hour	95.2	3.9	Pass
Peroxide, 30%, 1mL 60°C for 1 hour	93.2	5.9	Pass
Thermal, 80°C for 24 hour	96.1	3.0	Pass
UV degradation	98.9	0.2	Pass

*Peak purity confirmed using PDA detector empower software i.e Purity threshold>Purity Angle.

Assay of Marketed Sample

Table 10. Result of Marketed Sample

Drug	Label claim	Amount recovered	% recovery
Bisoprolol fumarate	5	4.95	99.0
Trimetazidine dihydrochloride	35	158.1	98.8

DISCUSSION:

After extensive trials of different types of buffer, different mobile phase composition, different organic solvent and different pH, the Ammonia buffer pH 6.8 buffer and acetonitrile in the ratio 80:20v/v was found suitable for better separation/resolution, reproducibility and symmetric peaks. There was much difference in label claims[3] of the both the drugs. Hence Concentration of Trimetazidine dihydrochloride is much high as compared to Bisoprolol fumarate in the sample solution. But linearity and accuracy study results were proved that the method is capable to analyse both the drug within the specified range with accuracy and precision. Trimetazidine diHCl and

Bisoprolol Fumarate shows absorbance at 272nm when determined UV-spectra using photo diode detector and hence UV-wavelength 272nm was selected.

CONCLUSION:

The Developed and Validated method is very simple with short run time which simultaneously estimate both Trimetazidine dihydrochloride and bisoprolol fumarate from tablet formulation. Validation has been done as per ICH guideline[13]. The validated method is very simple. The method is specific and selective. The method is accurate and precise and finally method is robust as well. So, the above reverse phase developed chromatographic method can be utilize for the analysis of bulk drug and tablet formulations.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation**.**

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