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Research Article

Development and Validation of Analytical Method for Simultaneous Estimation of Bisoprolol Fumarate and Telmisartan by Using RP-HPLC Method

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ABSTRACT

A simple, rapid and selective HPLC method has been developed for quantitation of Bisoprolol fumarate and Telmisartan from bulk drug and pharmaceutical formulations using a mobile phase consisting mixture of methanol and water (75:25 v/v) at the flow rate of 1ml/min. An Waters X Bridge RP C18 (4.6 x 250 mm) column was used as stationary phase. The retention time of Bisoprolol fumarate and Telmisartan were 5.7 min. and 7.6 min. respectively. Linearity was observed in the concentration range of 5-25 µg/ml for Bisoprolol fumarate and 40-200 µg/ml for Telmisartan. Percent recoveries obtained for Bisoprolol fumarate and Telmisartan were 99-101 % and 99-100 % respectively. The proposed method is precise, accurate, selective and rapid for the simultaneous determination of Bisoprolol fumarate and Telmisartan.

Keywords: Bisoprolol fumarate, Telmisartan, RP-HPLC Method, Validation.

INTRODUCTION[1-8]

Bisoprolol fumarate chemical name is (±) - 1- (4-((2-(-methyl ethoxy) ethoxy) methyl) phenoxy)- 3-((1-methylethyl) amino)-2- propanol (e) -2-bartender and it is a cardio selective β- blocker. Bisoprolol fumarate was used for the treatment of heart attacks and kidney problems[1]. Telmisartan is chemically 2-(4-{[4-methyl-6-(1-methyl-1*H*-1,3-benzodiazol-2-yl)-2-propyl-1*H* 1,3-benzodiazol-1-yl]methyl}phenyl)benzoic acid. Telmisartan is an angiotensin II receptor antagonist (ARB) used in the management of hypertension. Literature surveys revealed that no RP-HPLC method have been reported for these combination. A combination of Bisoprolol fumarate and Telmisartan are available in combined tablet dosage form for treatment of hypertension. A successful attempt has been made to estimate the two drugs simultaneously by RP-HPLC method. This method describe simple, rapid, accurate, reproducible and economical methods for the simultaneous determination of Bisoprolol fumarate and Telmisartan in tablet formulations using RP-HPLC method

MATERIALS AND METHODS

Chemicals and Reagents

Analytical pure samples of Bisoprolol fumarate and Telmisartan were obtained as a gift sample from Supriya Lifescience Ltd. Mumbai and Lupin Ltd., were used in the study. The pharmaceutical dosage form used in this study was Besicor T 5 labeled to contain Bisoprolol Fumarate / Telmisartan 5/40 mg per tablet. The solvents used were of HPLC grade methanol and double distilled water used in preparation of mobile phase.

Apparatus and Chromatographic Conditions

Chromatographic separation was performed on a Thermo (USA) HPLC system consisting of Auto Sampler, P1000 pump, UV2000 Detector, ChromQuest 4.1 Data processor, Hemlet injection syringe with 20 µl loop volume, An Waters X Bridge RP C18 (4.6 x 250 mm) column was used for separation. The elution was carried out isocratically at flow rate of 1 ml/min using methanol: water (75:25 v/v) mobile phase.

Preparation of Standard Stock Solution

Standard stock solution was prepared by dissolving 5 mg of Bisoprolol fumarate and 40 mg of Telmisartan in 10 ml Methanol that give concentration 500 µg/ml and 4000 µg/ml for Bisoprolol fumarate and Telmisartan respectively.

Preparation of Sample Solution

Four tablets were weighed and the average weight was determined. Accurately weighed tablet powder equivalent to 5 mg Bisoprolol fumarate and 40 mg of Telmisartan (i.e.305 mg) was transferred in a 10 ml volumetric flask and methanol was added. It was sonicated for 10 to 15 minutes. Later the volume was made up to mark with methanol. The solution was filtered through 0-0.45 µm filter paper.

Method Validation[9-15]

The validation of method was carried out as per ICH guideline. The parameters assessed were linearity, precision, accuracy, LOD, LOQ, robustness.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are

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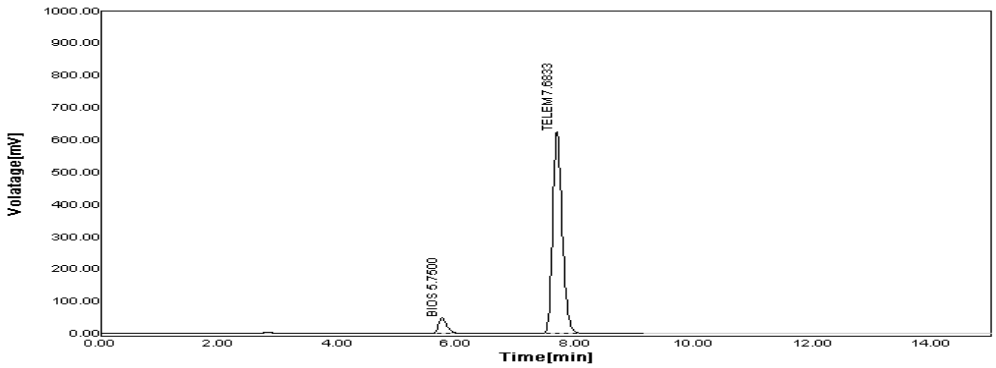

Figure 1: Chromatogram of Bisoprolol fumarate and Telmisartan with mobile phase Methanol:Water (75:25), wavelength-231 nm.

Table 1: Linearity of response.

Conc. µg/ml	Bisoprolol fumarate	Conc. µg/ml	Telmisartan
5	223.075	40	692.455
10	450.815	80	1391.57
15	664.05	120	2051.695
20	867.045	160	2701.475
25	1111.24	200	3417.65

Table 2: Optimized chromatographic condition.

Chromatographic mode	Chromatographic condition
HPLC system	Thermo (USA)
Pump	P1000
Detector	UV2000
Data processor	ChromQuest 4.1
Stationary phase	RP C_{18} (Waters X Bridge)
Mobile phase	Methanol:Water (75:25 v/v)
Wavelength	231 nm
Flow rate	1 ml/min
Sample size	20 ul

directly proportional to the concentration (amount) of analyte in the sample.

Precision

The intraday (repeatability precision) and interday precision studies (intermediate precision) were carried out by estimating the corresponding response three times on the same day and on three different days for two same concentrations and result was reported in terms of the relative standard deviation.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

Known amounts of Bisoprolol fumarate and Telmisartan were spiked to placebo at 80%, 100% and 120% of specification in duplicate and analyzed as per the proposed method to determine the accuracy of the method. Percentage recovery was calculated from the amount found and amount added. The percentage recovery is within the acceptance criterion, which indicates the accuracy of the method.

Limit of Detection and Limit of Quantitation

The LOD can be defined as the smallest level of analyte that gives a measurable responses and LOQ was determined as the lowest amount of the analyte that was reproducibly quantified. These two parameters were calculated using formula based on standard deviation of the response and slope. LOD and LOQ were calculated by the equation, LOD=3.3 x σ/s and LOQ= 10 x σ/s, where s = standard deviation, S = slope of calibration curve.

Robustness

The robustness of the method was evaluated by deliberately varying the chromatographic conditions viz variation in flow rate by ±1%, change in organic phase by ± 1% v/v,

change in wavelength of detection by ± 1 nm. At these changed condition the standard and test preparation were injected. The system suitability was evaluated in each varied condition. The amount of Bisoprolol fumarate and Telmisartan was calculated from test preparation in each varied condition. The results were compared with the

Table 3: Results of Bisoprolol fumarate and Telmisartan for system suitability parameter.

	Sr. No.	Peak Area		Tailing Factor		Theoretical Plate	
		Biso	Telmi	Biso	Telmi	Biso	Telmi
	1	222.0048	215.001	1.3571	1.3125	6664.1	7222.9
	2	225.1475	213.012	1.2500	1.2222	5397.9	8739.7
	3	223.0174	216.02	1.3650	1.1160	5647.3	7659.8
Mean	-	223.3899	214.6777	1.324033	1.2169	5903.1	7874.133
± S.D.	-	1.604122	1.24913	0.064236	0.080308	670.739	637.508
% R.S.D.	-	0.7180817	0.581862	1.8515407	1.5993918	11.362487	8.0962310

controlled data (Method Precision data). Results are

Table 4: Precision studies for Bisoprolol fumarate:

Sr. No.	Conc. µg/ml	Measured area (µg/ml) ± S.D, RSD (%)	
		Repeatability (n=2)	Intermediate Precision (n=2)
1	5	228.07 ± 0.76, 0.33	224.28 ± 2.76, 1.23
2	15	645.66 ± 2.27, 0.35	645.63 ± 6.54, 1.01
3	25	1110.89 ± 3.66, 0.33	1119.89 ± 3.16, 0.28

Table 5: Precision studies for Telmisartan.

Sr. No.	Conc. µg/ml	Measured area (µg/ml) ± S.D, RSD (%)	
		Repeatability (n=2)	Intermediate Precision (n=2)
1	40	682.95 ± 1.82, 0.27	684.83 ± 3.83, 0.56
2	120	2047.55 ± 5.78, 0.28	2044.63 ± 5.75, 0.28
3	200	3417.04 ± 12.98, 0.38	3431.89 ± 17.46, 0.51

Table 6: Recovery data for Bisoprolol fumarate and Telmisartan.

Drugs	Spiked level %	% Recovery	% R.S.D.
Bisoprolol fumarate	80	101.06	0.52
	100	99.45	0.78
	120	101.58	0.11
Telmisartan	80	100.07	0.47
	100	100.40	0.84
	120	99.54	0.45

Table 7: Results of LOD and LOQ.

Drugs	LOD (µg/ml)	LOQ (µg/ml)
Bisoprolol fumarate	0.27	0.83
Telmisartan	2.05	6.23

indicates that the method is robust under varied conditions.

Assay

20 µl of standard and sample solutions were injected into an injector of liquid chromatograph, from the peak area of Bisoprolol fumarate and Telmisartan amount of drug in samples were computed.

RESULTS AND DISCUSSION

The present work describes RP-HPLC method for simultaneous estimation of Bisoprolol fumarate and Telmisartan in tablet dosage form by using a mobile phase consisting mixture of methanol and water (75:25 v/v) at the flow rate of 1 ml/min. Waters X Bridge RP C18 (4.6 x 250 mm) column was used as stationary phase and the detection wavelength was 231 nm. The retention time for Bisoprolol fumarate and Telmisartan was found to be 5.7 and 7.6 min. respectively. Linearity response for both Bisoprolol fumarate and Telmisartan were found to be linear in concentration range of 5-25 µg/ml and 40-200 µg/ml respectively in the linearity study, regression equation and coefficient of correlation for Bisoprolol fumarate and Telmisartan were found to be ($y = 43.85x + 5.471$, $r2 = 0.999$) and ($y = 16.90x + 22.87$, $r2 = 0.999$). The results shows that an excellent correlation exists between peak area and concentration of drugs within the concentration range indicated above.

The % RSD in all two replicate was not more than 2.0% hence the method was found to be precise. Percentage recovery for both drugs Bisoprolol fumarate and Telmisartan was found in range of 99-101 % and 99-100 % indicating accuracy of the proposed work. The LOD value of Bisoprolol fumarate and Telmisartan was found to be 0.27 µg/ml and 2.05 µg/ml respectively. The LOQ value of Bisoprolol fumarate and Telmisartan was found to be 0.83 µg/ml and 6.23 µg/ml respectively. The results of the robustness study also indicated that the method is robust and is unaffected by deliberate variation in the chromatographic conditions. The % label claim of Bisoprolol fumarate and Telmisartan was found to be 98.57 % and 98.05 % respectively with % RSD not more than 2.

Hence, it can be concluded that the developed RP-HPLC method is accurate, precise, & selective and can be employed successfully for the estimation of Bisoprolol

Table 8: Robustness data of Bisoprolol fumarate and Telmisartan.

Table 8a: Effect of variation in Flow rate of mobile phase by ±1%.

Sr. No.	Flow rate	Conc. (µg/ml)		Mean		S.D.		% R.S.D.	
		Biso	Telmi	Biso	Telmi	Biso	Telmi	Biso	Telmi
1	0.9	10	80	494.87	15.41	3.01	3.22	0.61	0.21
2	1.1	10	80	386.33	1193.97	6.34	3.57	1.64	0.30

Table 8b: Effect of variation in mobile phase composition by±1 % v/v.

Sr. No.	Mobile Phase Composition	Conc. (µg/ml)		Mean		S.D.		% R.S.D.	
		Biso	Telmi	Biso	Telmi	Biso	Telmi	Biso	Telmi
1	M:W(74:26)	10	80	437.4	1383.5	2.13	13.51	0.49	0.98
2	M:W(76:24)	10	80	412.46	1293.01	11.69	13.13	1.83	1.02

Table 8c: Effect of variation in wavelengths.

Sr. No.	Wavelength Change(nm)	Conc. (µg/ml)		Mean		S.D.		% R.S.D.	
		Biso	Telmi	Biso	Telmi	Biso	Telmi	Biso	Telmi
1	230	10	80	409.6	1274.7	9.05	4.48	1.21	0.35
2	232	10	80	414.30	1268.31	4.21	7.45	1.02	0.59

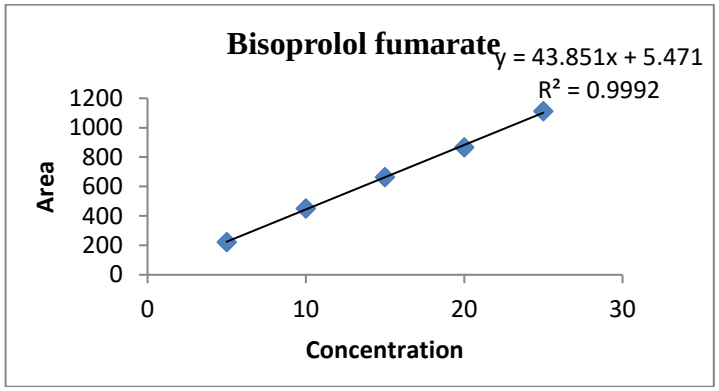

Figure 2: Plot of Linearity and Range Study of Bisoprolol fumarate.

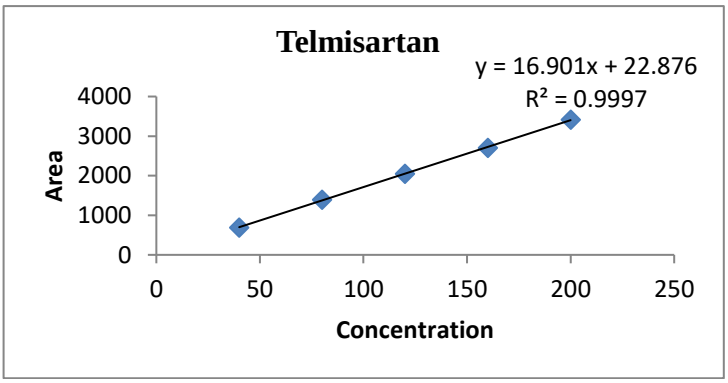

Figure 3: Plot of Linearity and Range Study of Telmisartan.

Table 9: Results for estimation of Bisoprolol fumarate and Telmisartan in marketed formulation.

Drugs	Conc. (µg/ml)	Amount found	% label claim	S.D.	% R.S.D.
Biso	15	14.78	98.57	0.33	0.27
Telmi	120	39.67	117.65	0.53	0.13

fumarate and Telmisartan in tablet dosage formulation.

CONCLUSION

The developed RP-HPLC methods are precise, specific, accurate. Statistical analysis proves that these methods are suitable for the analysis of Bisoprolol fumarate and Telmisartan in bulk and pharmaceutical formulation without any interference from the excipients. All these factors using these methods make easy quantification of drugs in bulk and pharmaceutical dosage form. It can therefore be concluded that use of these methods can save much time and money and hence can be used in small laboratories with very high accuracy over a wide linear range.

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