INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN:2320-2831

IJPAR |Vol.9 | Issue 2 | Apr - Jun - 2020
Journal Home page: www.ijpar.com

Research article *Open Access*

Development and validation of RP-HPLC method for the simultaneous estimation of bisoprolol fumarate and telmisartan from pharmaceutical formulations

Snehal Patil*[1], Ashpak Tamboli[2], Sunil More[3], Shubhada Pawar[1]

[1]*Student, Department of Pharmaceutical Chemistry, Sahyadri College of Pharmacy, Methwade, Sangola, 413307, Solapur, Maharashtra, India*

[2]*Associate Professor, Department of Pharmaceutical Chemistry, Sahyadri College of Pharmacy, Methwade, Sangola, 413307, Solapur, Maharashtra, India*

[3]*Research Associate, Department of Pharmaceutical Chemistry, Sahyadri College of Pharmacy, Methwade, Sangola, 413307, Solapur, Maharashtra, India*

***Corresponding Author: Snehal Patil**

Email: sp.snehalpatil97@gmail.com

ABSTRACT

A simple, sensitive, accurate, precise HPLC method developed for the simultaneous estimation of Bisoprolol fumarate and Telmisartan in pharmaceutical dosage forms. Chromatography is carried out at 40ºc temperature on inert sustain C18 Column (4.6 x 150mm, 5µm. particle size) column using a mobile phase of 0.1% of Tri fluroacetic acid in Water: Acetonitrile (80:20v/v) with flow rate 1ml/min (DAD scan at 227nm). Validation parameters such as system suitability, linearity, precision, accuracy are considered as reported in the International Conference on Harmonization guidelines. The retention times for Bisoprolol fumarate and Telmisartan are 2.79min and 3.52min. The linearity range for Bisoprolol fumarate and Telmisartan is 5-25µg/ml and 40-200µg/ml. The % RSD for intra-day and inter-day precision were found to be less than 2 %. The percentage recoveries obtained for Bisoprolol fumarate and Telmisartan ranges from 100.07-100.69% and 99.43-100.60% respectively. Hence the proposed method was found to be accurate, precise, reproducible and specific and can be used for simultaneous analysis of these drugs in tablet formulation.

Keywords: RP-HPLC, Bisoprolol fumarate (BSL), Telmisartan (TEL).

INTRODUCTION

Bisoprolol fumarate is chemically 2- propanol,-[4-[[2-(1-methylethoxy) ethoxy] methyl] phenoxy]-3-[(1-methylethyl) amino]-, (±)-, (E)-2-butenedioate (Fig.1) [1, 2]. Bisoprolol fumarate is a white crystalline powder, which is readily soluble in water, methanol, ethanol, and chloroform. Bisoprolol is a cardio-selective beta-blocker. Bisoprolol is given as the fumarate in the

management of hypertension and angina pectoris and chronic heart failure by blocking action epinephrine on heart and blood vessels [3, 4].

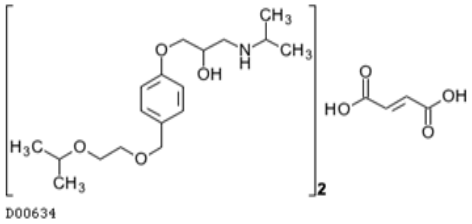

Fig.1: Structure of Bisoprolol fumarate
Molecular Formula: $(C_{18}H_{31}NO_4)_2.C_4H_4O_4$
Molecular Weight: 767.0 g/mol

Telmisartan is 4'- {[4-methyl-6- (1-methyl-1H-benzimidazol-2-yl) -2-propyl-1H-benzimidazol-1-yl] methyl}-2-biphenyl-carboxylic acid (Fig.2) [1, 3].Telmisartan is a white to slightly yellowish solid. It is practically insoluble in water and in the pH range of 3 to 9, sparingly soluble in strong acid (except insoluble in hydrochloric acid), and soluble in strong base. It is believed that telmisartan's dual mode of action may provide protective benefits against the vascular and renal damage caused by diabetes and cardiovascular diseases (CVD) [5, 6].

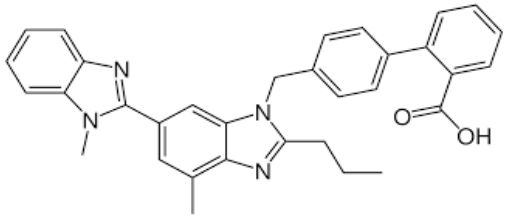

Fig. 2: Structure of Telmisartan
Molecular Formula: $C_{33}H_{30}N_4O_2$.
Molecular Weight: 514 g/mol.

EXPERIMENTAL

Instrumentation

Chromatography was performed with Agilent HPLC system provided with auto sampler and Diode Array HPLC Detector.

Reagents and Materials

Bisoprolol fumarate and Telmisartan reference standard were procured as a gift sample from Unichem laboratories Ltd, Pilerna Goa, India. The commercial fixed dose Besicor T was procured from local market. Purified water prepared using Milipore Milli-Q water purification system. Acetonitrile and Trifluroacetic acid (all AR grade) were purchased from Merck Limited (Mumbai,India).

Preparation of mobile phase

1000 ml of mobile phase was prepared by mixing 0.1% Trifluroacetic acid in 800ml water and 200ml acetonitrile.

Degassing of mobile phase

The prepared mobile phase was degassed by ultra-sonication for 15 min, so to avoid the disturbances caused by dissolved gases.

Filtration of mobile phase

The degassed mobile phase was filtered through 0.45µmfilters to avoid the column clogging due to smaller particles.

Preparation of standard stock solution

Standard stock solution of Bisoprolol fumarate and Telmisartan were prepared separately by dissolving 10 mg of drug in 10 ml of mobile phase

to get concentration of 1000 µg/ml. From the respective standard stock solution, working standard solution was prepared containing 5µg/ml of BSL and 40µg/ml of TEL separately in mobile phase (Fig. 3).

Selection of Detection Wavelength

From the standard stock solution of Bisoprolol fumarate and Telmisartan further dilutions were made using mobile phase and scanned over the range of 200 - 400 nm and the spectrum was obtained. It was observed that both the drug showed considerable absorbance at 227nm.

Loading of standard solution

10µl standard solution of Bisoprolol fumarate and Telmisartan were loaded into the HPLC vial stand placed in sample tray & were injected.

Table1. Optimized chromatographic conditions

System	Agilent
Mobile phase	0.1%TFA in Water: Acetonitrile
"Mobile phase ratio (%v/v)	80:20
Injection volume	10µl
Flow rate	1ml/min
Column (4.6 x 150mm)	Inert sustain C18
Column temperature	40°c
Detector	Diode Array HPLC
Scan wavelength	227 nm

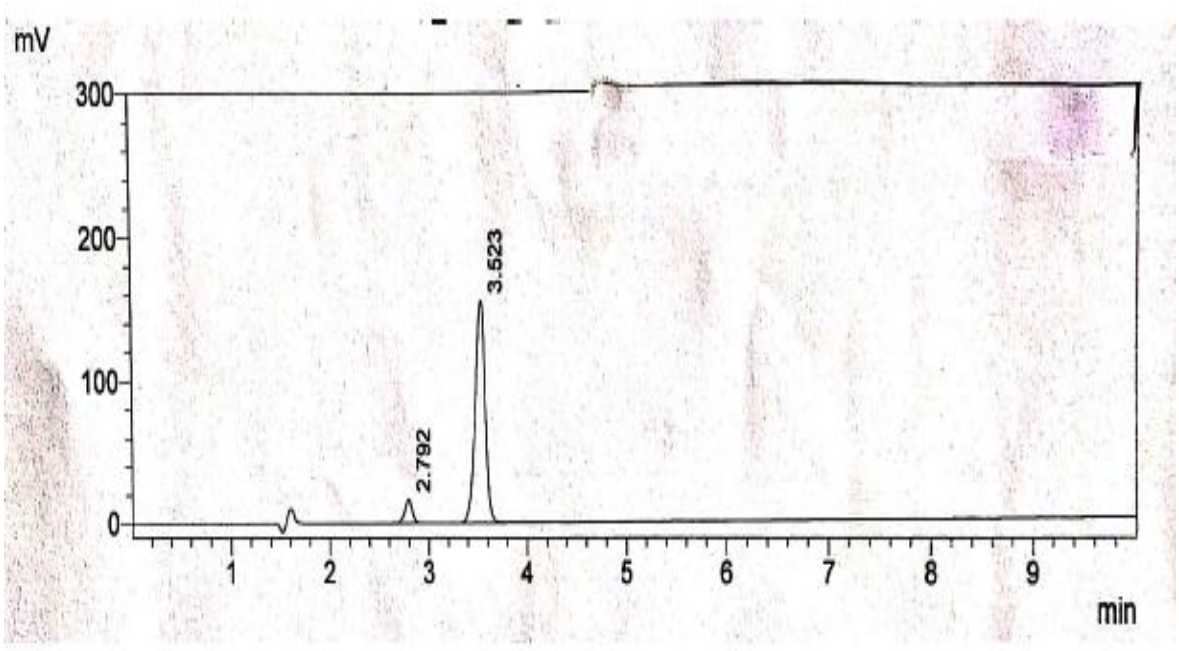

Fig. 3: Chromatogram of standard mixture of Bisoprolol fumarate and Telmisartan

Preparation of sample solution (Tablet formulation analysis)

Ten tablets each containing 5 mg of BSL and 40 mg of TEL and was weighed and powdered (Each uncoated tablet contains Bisoprolol fumarate IP 5 mg and Telmisartan IP 10 mg). Powder equivalent to 5 mg of BSL (40 mg of TEL) was transferred to 10 ml volumetric flask and was diluted with mobile phase then ultra-sonication was done and volume made to 10 ml (500 µg/ml of BSL and 4000 µg/ml of TEL) with mobile phase then further dilutions were made with mobile phase to get the final concentration of 5 µg/ml of BSL and 40 µg/ml of TEL .Solution was filtered by 0.45µm nylon membrane filters by using vacuum filter. Tablet formulation analysis was carried out as mentioned under section Tablet Formulation Analysis.

Procedure was repeated for six times. Sample solution was injected and area was recorded for each drug. Concentration and % purity was determined from linear equation shown in (Table 2)

Table 2: Assay results for BSL and TEL

Sr. no.	BSL			TEL		
	Peak area	Amount recovered (µg/ml)	% recovery	Peak area	Amount recovered (µg/ml)	% recovery
1	60637.3	5.065	101.3114	2211035	40.011	100.02
2	60518	5.055	101.1152	2212065	40.029	100.07
3	60421.2	5.047	100.9561	2212987	40.046	100.11
4	60345.1	5.041	100.831	2234581	40.439	101.09
5	60308.2	5.038	100.7703	2236547	40.474	101.18
6	60515.1	5.055	101.1105	2212458	40.037	100.09
Mean	60457.48	5.050	101.0158	2213279	40.173	100.43
%RSD	0.203318	0.200041	0.200041	0.550033	0.548748	0.548748

METHOD VALIDATION [7-10]

Linearity

A series of standard solutions 5-25µg/ml of Bisoprolol fumarate and 40-200µg/ml of Telmisartan were prepared. An aliquot of 10µl of each solution was injected 6 times for each standard solutions and peak area was observed. Plot of average peak area versus the concentration is plotted and from this the correlation coefficient and regression equation were generated. The results obtained are shown in (Table 3 and Fig.4) for BSL and in (Table 4 and Fig.5) for TEL

Table 3: Linearity study of BSL

Replicates	Concentrations of BSL(µg/ml)				
	5	10	15	20	25
	Peak Area				
1	60619	119290	181892	242545	303152
2	60656	119245	181902	242532	303168
3	60630	119264	181891	242515	303145
4	60671	119254	181890	242527	303132
5	60629	119299	181915	242513	303149
6	60632	119231	181906	242534	303169
Mean	60639.5	119236.8	181899.3	242527.7	303152.5
Std. dev.	19.705	26.271	10.073	12.1271	14.15274
%RSD	0.0324	0.0220	0.0055	0.005	0.0046

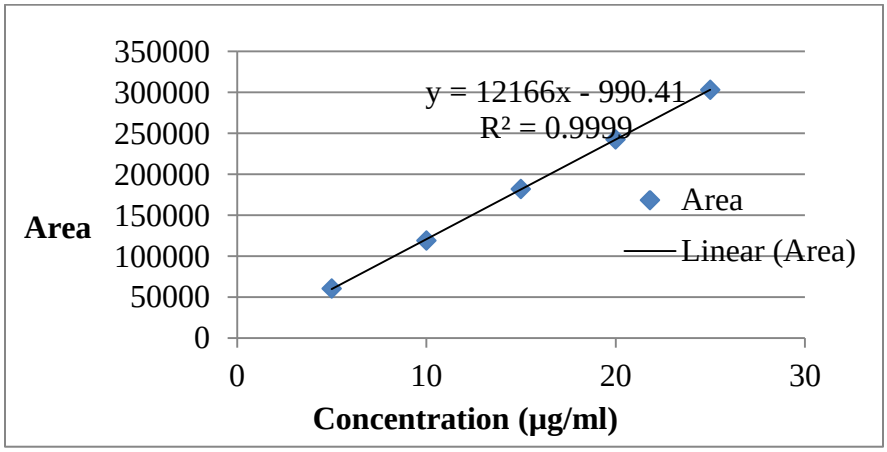

Fig. 4: Calibration curve for BSL

Table 4: Linearity study of TEL

Replicates	Concentrations of TEL(µg/ml)				
	40	80	120	160	200
	Peak Area				
1	2211063	4421059	6632092	8744110	11055175
2	2211042	4421095	6632108	8744163	11055165
3	2211052	4421038	6632089	8744159	11055199
4	2211023	4421123	6632125	8744102	11055147
5	2211012	4421075	6632117	8744132	11055182
6	2211016	4421046	6632075	8744143	11055168
Mean	2211035	4421073	6632101	8744135	11055173
Std. dev.	20.76215	32.05412	18.87856	25.08718	17.46616
%RSD	0.000939	0.000725	0.000285	0.000287	0.000158

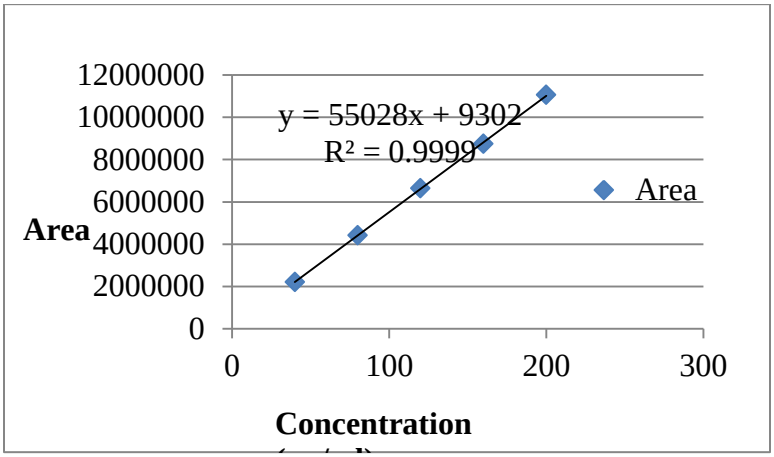

Fig. 5: Calibration curve for TEL

Precision

The precision of this method was determined by intraday and interday precision. The % RSD was found less than 2 this indicate that the method is precise. The intraday precision for Bisoprolol fumarate and Telmisartan is shown in table 5 & 6. The interday precision for Bisoprolol fumarate and Telmisartan is shown in table 7 & 8.

Table 5: Intra-day precision study of BSL

Concentration (µg/ml)	Area	% Recovery	Avg % Recovery ± SD	Mean % Recovery ± % RSD
	60639	101.314		
5	60642	101.319	101.312±0.008	
	60632	101.302		
	119264	98.844		100.130±1.061
10	119290	98.866	98.861±0.014	
	119299	98.873		
	181898	100.218		
15	181896	100.217	100.218±0.0013	
	181901	100.220		

Table 6: Intra-day precision study of TEL

Concentration (µg/ml)	Area	% Recovery	Avg % Recovery ± SD	Mean % Recovery ± % RSD
	2211063	100.02		
40	2231035	100.93	100.33±0.524	
	2211049	100.21		
	4421086	100.21		
80	4411075	99.98	100.14±0.132	100.22±0.295
	4421199	100.21		
	6612165	99.99		
120	6632099	100.29	100.19±0.174	
	6632075	100.29		

Table 7: Inter-day precision of BSL

Concentration (µg/ml)	Area	% Recovery	Avg % Recovery ± SD	Mean % Recovery ± % RSD
	60715	101.439		
5	60695	101.406	101.422±0.016	
	60704	101.421		
	120212	99.623		
10	121424	100.620	99.994±0.544	100.60±0.685
	120354	99.740		
	182198	100.382		
15	182645	100.627	100.604±0.211	
	182965	100.803		

Table 8: Inter-day precision of TEL

Concentration (µg/ml)	Area	% Recovery	Avg % Recovery ± SD	Mean % Recovery ± % RSD
	2212096	100.076		
40	2231197	100.943	100.671±0.516	
	2232315	100.994		
	4422023	100.238		
80	4421198	100.219	100.24±0.021	
	4423098	100.262		100.308±0.386
	6613654	100.015		
120	6613994	100.020	100.014±0.005	
	6613264	100.009		

Accuracy

The accuracy of the methods was determined by calculating recovery values of Bisoprolol fumarate and Telmisartan by the standard addition method. The accuracy of the method was determined by preparing solutions of different concentrations in which the amount of marketed formulation(BesicorT5) was kept constant (10mg of Bisoprolol fumarate and 80mg of Telmisartan) and the amount of pure drug was varied that is 50%, 100% and 150% respectively. The solutions were prepared in triplicates and the accuracy was indicated by % recovery was shown in table 9 &10.

Table 9: Recovery studies of BSL

Level	Conc. (µg/ml)		Area	% Recovery	Mean % Recovery ± RSD
	Sample	Std.			
			182856	100.743	
50 %	10	5	181899	100.218	100.690±0.444
			183525	101.109	
			242227	99.957	
100 %	10	10	242598	100.110	100.071±0.100
			242689	100.147	
			303189	100.009	
150 %	10	15	303526	100.120	100.025±0.087
			302999	99.947	

Table10: Recovery studies of TEL

Level	Conc. (µg/ml)		Area	% Recovery	Mean % Recovery ± RSD
	Sample	Std.			
			6643254	100.463	
50 %	80	40	6635487	100.345	100.499±0.174
			6658254	100.690	
			8745698	99.226	
100 %	80	80	8749589	99.270	99.434±0.323
			8796587	99.804	
			11055173	100.365	
150 %	80	120	11098475	100.759	100.601±0.206
			11089654	100.679	

Table 11: System suitability parameters

Parameters	BSL	TEL
Retention time (min)	2.79	3.52
Area	181899	2211035
Plates	5511	5433
Resolution	--	4.280
Tailing factor (T)	0.989	0.956

Limit of detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ for Bisoprolol fumarate and Telmisartan were calculated as suggested by ICH guidelines using equations LOD = 3.3 σ/s and LOQ = 10 σ/s, respectively. Where, σ is the SD of the response and S is the slope of the calibration curve. The results were shown in Table 12.

Table No.12: LOD & LOQ studies of Bisoprolol fumarate & Telmisartan.

Drugs	LOD(µg/ml)	LOQ(µg/ml)
Bisoprolol fumarate	0.2627	0.7962
Telmisartan	2.8846	8.7414

Range

The range shown by BSL and TEL is given as follow
BSL: 5-25µg/ml
TEL: 40-200µg/ml

RESULT AND DISCUSSION

The present work comprised of development of analytical method for the simultaneous estimation of Bisoprolol fumarate and Telmisartan by reverse phase of high performance liquid chromatography as well as validation of the developed method. The commercially available tablet dosage forms selected for the estimation of Besicor -T containing

Bisoprlol fumarate5mg and Telmisartan 40mg. The calibration curve plotted between concentration and AUC measured at the selected wavelength of 227nm. The instruments used for method development was Agilent HPLC System, Diode Array HPLC detector, 40ºc temperature on Inert sustain C18 Column (4.6 x 150mm, 5µm. particle size) and mobile phase comprising of 0.1%TFA in Water: Acetonitrile:(80:20v/v). The retention time for Bisoprolol fumarate and Telmisartan were 2.79and 3.52 respectively flow rate kept at 1ml/min.

According to ICH guidelines method was validated linearity for detector which was observed in 5-25µg/ml for Bisoprolol fumarate and 40-200µg/ml for Telmisartan respectively. Percentage recovery of both drugs was found in range 99-102 indicating accuracy of proposed method. The intra-day and interday coefficient for Bisoprolol fumarate and Telmisartan were found to be 1.061-0.295% and 0.685-0.386%. The percentage RSD for both the tablet analysis and recovery studies is less than 2% indicating high degree of precision. The current developed HPLC method developed was found to be rapid, simple, precise, accurate and economic for routine estimation of Bisoprolol fumarate and Telmisartan in market able dosage forms.

CONCLUSION

All these factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive, rugged and rapid and can be applied successfully for the estimation of BSL and TEL in pharmaceutical formulations without interference and with good sensitivity; hence it can be used for the routine analysis in quality control department.

ACKNOWLEDGEMENT

I am very much thankful to Unichem laboratories Ltd, Pharmaceutical Company in Goa Industrial Estate, Goa, India. for providing standard sample of drugs and Sahyadri college of Pharmacy, Methwade (Sangola), Maharashtra, India for giving permission to carry out my work.

REFERENCE

[1]. Indian Pharmacopoeia "Government of India ministry of health and family welfare, published by Indian Pharmacopoeia commission", Government of India Ghaziabad, 3, 2018, 1392-1393, 3319-3320.

[2]. Barge V. U. Development and Validation of Analytical Method for Simultaneous Estimation of Bisoprolol Fumarate and Telmisartan by Using RP-HPLC Method. International Journal of Pharmaceutical and Clinical Research, 10(8), 2018, 219-223.

[3]. Snehal Patil, Simultaneous Determination of Bisoprolol fumarate and Telmisartan by Area under Curve UV Spectrophotometric Method, International Journal of Pharmacy and Pharmaceutical Research, 18(2), 2020, 147-156.

[4]. Snehal Patil, Development and Validation of UV Spectrophotometric Method for the Simultaneous Estimation of Bisoprolol fumarate and Telmisartan in Tablet Dosage Form, International Journal of Scientific Development and Research, 5(6), 2020, 58-63.

[5]. Sunil Jawla, Development and Validation of Simultaneous HPLC Method for Estimation of Telmisartan and Ramipril in Pharmaceutical Formulations International Journal of Pharm Tech Research, 2(2), 2020, 1625-1633.

[6]. Amit Tapkir, Development and validation of RP-HPLC method for simultaneous estimation of Telmisartan and Clorthalidone in bulk and tablet dosage form, Scholars Research Library, 5(1), 2013, 149-154.

[7]. More Sunil, Development and validation of RP-HPLC method for the simultaneous estimation of Pregabalin and Amitriptyline Hydrochloride from pharmaceutical formulations. Int. J. Res. Analytical Reviews, 6(2), 2019, 667-678.

[8]. Sathish Kumar Konidala, Development and validation of RP-HPLC method for the simultaneous determination of Amlodipine Besylate and Telmisartan in bulk and Pharmaceutical dosage forms, World Journal of Pharmacy and Pharmaceutical Sciences, 3(3), 2014, 1962-1972.

[9]. ICH Q2A Text on validation of analytical procedure, International conference on harmonization. Tripartite guideline. 1994, 1-5.

[10]. ICH Q2B Validation of analytical procedure: methodology, International conference on harmonization. Tripartite guideline. 1994, 1-10.