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BIOANALYTICAL ESTTIMATION OF ANTI-HYPERTENSIVE DRUG BY USING HPLC IN HUMAN PLASMA

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ABSTRACT:

An accurate, rapid and simple reversed-phase high performance liquid chromatography (RP-HPLC) bioanalytical method was developed for estimation Bisoprolol Fumarate and Telmisartan in human plasma. Chromatographic separation was accomplished using BDS Hypersil C_{18},(250 mm X 4.6 mm, 5µ) analytical column. The mobile phase consisted of Methanol:Buffer, pH adjusted with ortho-phosphoric acid in the ratio 85:15 with flow rate of 1ml/min. Detection was carried out at 288 nm. The retention times were found to be 8 min. All the analytical validation parameter were determined as per ICH guidelines.The bioanalytical method developed was selective, robust and reliable as accuracy, precision , recovery and other validation parameter were within the limits as specified by the guidelines The linearity range was found to be 5 to 15 µg /ml and 40 to 120 µg /ml for Bisoprolol Fumarate and Telmisartan respectively.The method was validated and was found to be suitable for analysis in biological fluid.

Keywords: Bisoprolol Fumarate, Telmisartan, RP-HPLC, Bioanalytical method, Human plasma..

INTRODUCTION

High blood pressure is a common condition that affects the body's arteries. It's hypertension. If you have high blood pressure, the force of the bloed pushing gaine artery walls is consistently too high. The heart has to work harder to pump blood Blood pressure is measured in millimeters of mercury (mm Hg). In general, hypertension blood pressure reading of 130/80 millimeters of mercury (mm Hg) or higher. The American College of Cardiology and the American Heart Association divide blood pressure into four general categories. Ideal blood pressure is categorized as normal.[1]

Bisoprolol Fumarate (Fig. 1) is a cardioselective β1-adrenergic blocking agent used to treat high blood pressure. It is considered a potent drug with a long-half life that can be used once daily to reduce the need

for multiple doses of antihypertensive drugs. Bisoprolol Fumarate is generally well tolerated, likely due to its β1-adrenergic receptor selectivity and is a useful alternative to non-selective β-blocker drugs in the treatment of hypertension such as Carvedilol and Labetalol. It may be used alone or in combination with other drugs to manage hypertension and can be useful in patients with chronic obstructive pulmonary disease (COPD) due to its receptor selectivity.[2-4]

Telmisartan (Fig.2) is an angiotensin II receptor antagonist (ARB) used in the management of hypertension. Generally, angiotensin II receptor blockers (ARBs) such as telmisartan bind to the angiotensin II type 1 (AT1) receptors with high affinity, causing inhibition of the action of angiotensin II on vascular smooth muscle, ultimately leading to a reduction in arterial blood pressure. Recent studies suggest that telmisartan may also have PPAR-gamma agonistic properties that could potentially confer beneficial metabolic effects. [5-7]

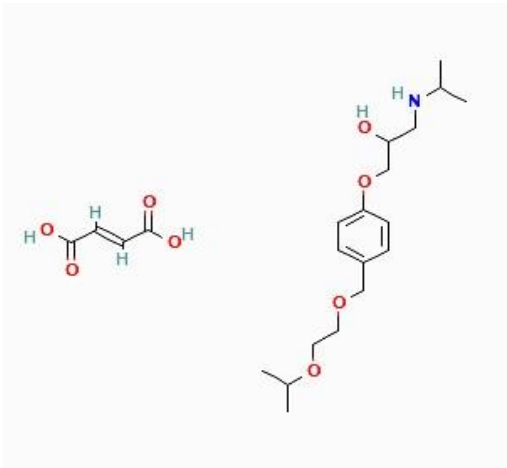

Fig 1: Structure of Bisoprolol Fumarate

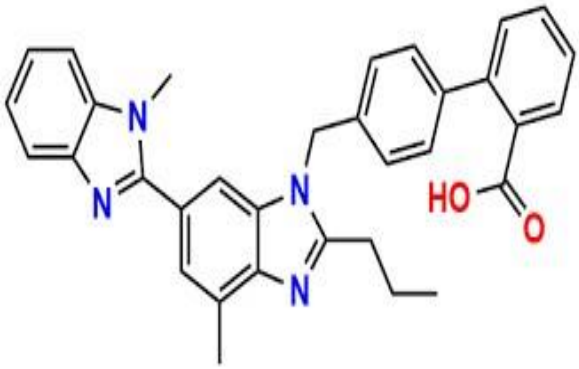

Fig.2: Structure of Telmisartan

MATERIALS AND METHOD

Chemicals and solutions

Bisoprolol Fumarate and Telmisartan were purchase from an Yarrow Chem Products, Mumbai. Human Plasma was obtained from a blood bank . All chemicals and reagents used in the study were of gradient grade, water is of HPLC grade and obtained from peaks analytical mondha Nagpur, Pvt Ltd.

Instrumentation:

HPLC was performed on Younglin-HPLC System, detector used was UV detector (730D) and UV spectrophotometer was shimadzuUV1800 spectrophotometer Japan corporation. A reverse phase enable C18 analytical column was used. Weighing was done on Shimadzu Model- ATX224 and software used is Autochrom3000.

Chromatographic condition:

Preliminary studies were conducted and trails are made for the bioanalytical method development. Seperation and analysis was carrid out on C_{18} column. The optimized mobile phase consists of MeOH:Buffer (85:15). The flow rate was maintained at 1.0 ml/min with run time of 8min. The detection wavelength was measured at 288nm.

Preparation Of Standard Stock Solution:

Weighed accurately 10 mg of Bisoprolol Fumarate standard and 80 mg of Telmisartan transfer to 100 ml of glass volumetric flask,shake well, sonicate this solution for about 2 min toget completely dissolved, make up volume to 100 ml with diluent 100 µg/ml.

Preparation Of Standard Solution:

Pipette out 2 ml from stock solution, transfer to 20 ml volumetric flask, diluted up to 20ml with diluent shake well, sonicate for about 2 min, filter through 0.2 µm syringe filter.

Selection of Wavelength:

An UV spectrum of 10 ug/ml Bisoprolol Fumarate and Telmisartan in methanol was recorded by scanning in the range of 200 nm to 400 nm. A wavelength which gaves good response for the drugs to be selected. From the UV spectrum a wavelength of 288 nm was selected. Both drugs showed optimal absorbance at this wavelength.

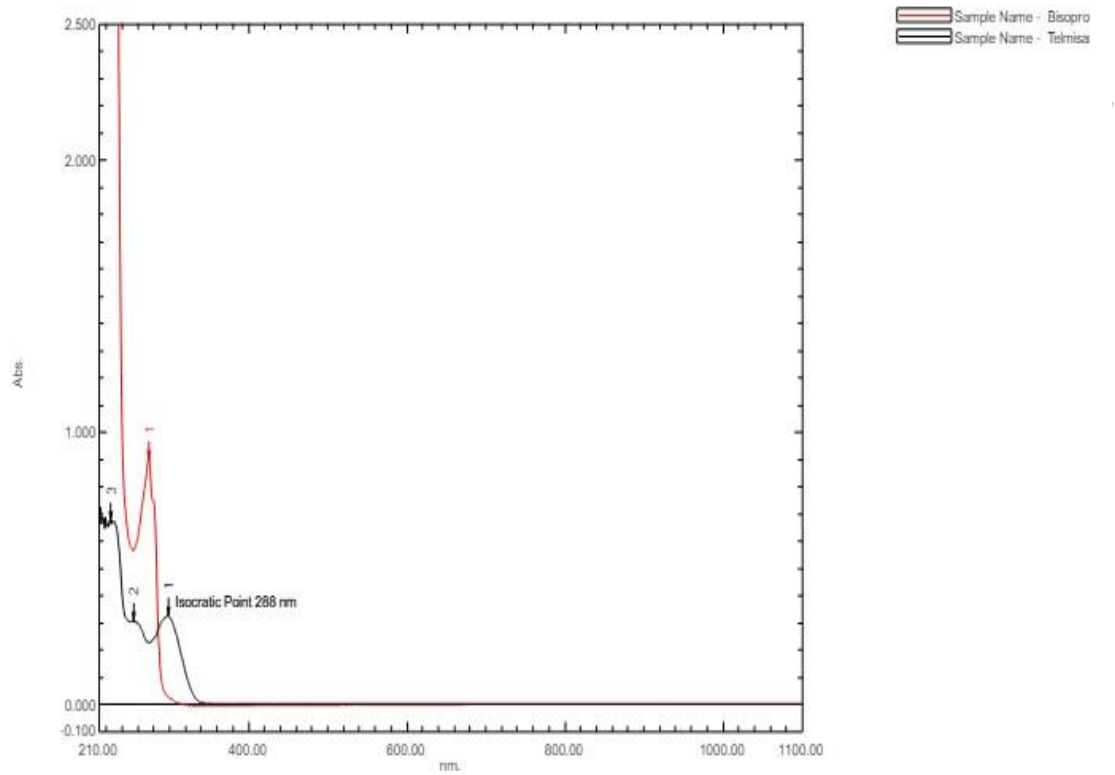

Fig 3: Overlay spectrum of Bisoprolol Fumarate and Telmisartan

Selection of Mobile Phase

The pure drug of Bisoprolol fumarate and Telmisartan was injected into the HPLC system and run in different solvent system. Each mobile phase was allowed to equilibrate with stationary phase until steady base line was obtained. Different mobile phase like Methanol: Buffer, ACN: Water, various proportions were tried to get a stable peak each mobile phase and sonicate on ultrasonic bath and then filter through 0.45 µm filter paper. After Trials the final combination of the solvent selected was Methanol, Buffer in the ratio 85:15 that gives sharp peak and good resolution.

Validation of Developed Method:

After developing a method its validation is necessary to prove the suitability of the method for the intended purpose.

Here the procedure followed for the validation of the developed method is described.

ACCURACY:

For accuracy studies, samples were prepared at three concentration levels: Low (LQC), Medium (MQC) and High (HQC) Quality Controls. Concentration of each injection was calculated and the standard deviation between the readings is calculated shown in table 1 and 2.Peaks are shown in Fig 6, 7 and 8.

Table 1 : Accuracy Data of Bisoprolol Fumarate By RP-HPLC

Accuracy Levels	MEAN % recovery	SD	%RSD (NMT 2)
Accuracy at 80 %	100.71	0.4676	**0.46**
Accuracy at 100 %	100.16	0.8414	**0.84**
Accuracy at 120 %	100.10	0.1116	**1.11**

Table 2 : Accuracy Data of Telmisartan By RP-HPLC

Accuracy Levels	MEAN % recovery	SD	%RSD (NMT 2)
Accuracy at 80 %	98.86	1.1347	**1.15**
Accuracy at 100 %	98.66	0.9034	**0.92**
Accuracy at 120 %	100.52	0.6797	**0.68**

PRECISION:

Precision can be determined by two types:

1.Intraday precision

2.Interday precision

The plasma concentration preparation were injected into HPLC four times and mean peak area was calculated separately for each concentration and from that precision percentage RSD values were calculated and shown in tables 3 and 4.

Table 3: Intra-Day Precision Data of Bisoprolol Fumarate By RP-HPLC Method

Name	Preparation	% ASSAY
Set-1	prep-01	**99.28**
	prep-02	**100.02**
Set-2	prep-01	**99.58**
	prep-02	**98.67**
Mean		99.39
SD		**0.5667**
% RSD (NMT 2.0)		**0.57**

Table 4: Intra-Day Precision Data of Telmisartan By RP-HPLC Method

Name	Preparation	% ASSAY
Set-1	prep-01	**99.90**
	prep-02	**99.45**
Set-2	prep-01	**101.21**
	prep-02	**99.44**
Mean		100
SD		**0.8347**
% RSD (NMT 2.0)		**0.83**

LINEARITY AND RANGE:

Linearity And Range for Bisoprolol Fumarate

The linearity graph of average peak area at each level against the concentration in µg/ml is plotted and found to be straight line graph. This method proved to be linear between µg/ml of Bisoprolol Fumarate, with a typical calibration curve of correlation equation.

$y = 25.702x - 13.151$

Correlation coefficient > 0.999 shown in table 5.

Table 5: Calibration Standards Peak Area

Level	Con. (ppm or µg/ml)	Area
1	5.00	116.7329
2	7.50	178.4900
3	10.00	242.8151
4	12.50	308.0748
5	15.00	373.2098
	Correlation coefficient (r)	0.9999
	(NLT 0.995)	
	Intercept	-13.151
	Slope	25.702

The chromatograms of calibration standards with concentrations 5.00, 7.50, 10.00, 12.50 and 15.00 µg/ml were recorded and shown in figures and their peak areas of drug was noted. The calibration curve for Bisoprolol Fumarate was plotted as peak Area vs. concentration of the Bisoprolol Fumarate calibration standards in plasma.

Chromatogram of Bisoprolol Fumarate calibration standards was shown in Fig .4.

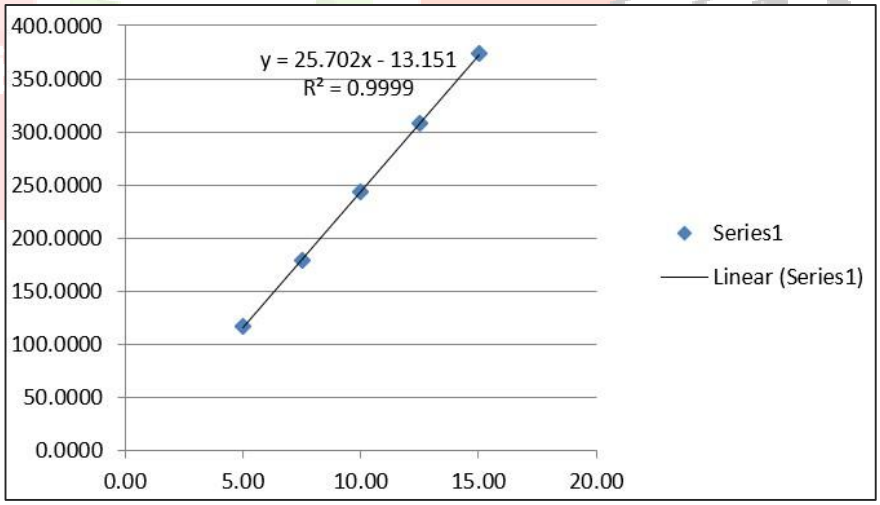

Fig.4 Calibration curve for Bisoprolol Fumarate

Linearity And Range for Telmisartan

The linearity graph of average peak area at each level against the concentration in µg/ml is plotted and found to be straight line graph. This method proved to be linear between µg/ml of Telmisartan, with a typical calibration curve of correlation equation. y = 10.671x - 20.12

Correlation coefficient > 0.9998 shown in table 6.

Table 6: Calibration Standards Peak Area For Telmisartan

Con. (ppm or ug/ml)	Area
40.00	404.5174
60.00	618.1824
80.00	835.8741
100.00	1056.8053
120.00	1252.2683
Correlation coiefficient (r)	0.9998
(NLT 0.995)	
Intercept	-20.1204
Slope	10.6706

The chromatograms of calibration standards with concentrations 40.00, 60.00, 80.00, 100.00 and 120.00 µg/ml were recorded and shown in figures and their peak areas of drug was noted. The calibration curve for Telmisartan was plotted as peak Area vs. concentration of the Telmisartan calibration standards in plasma. Chromatogram of Telmisartan calibration standards was shown in Fig. 5.

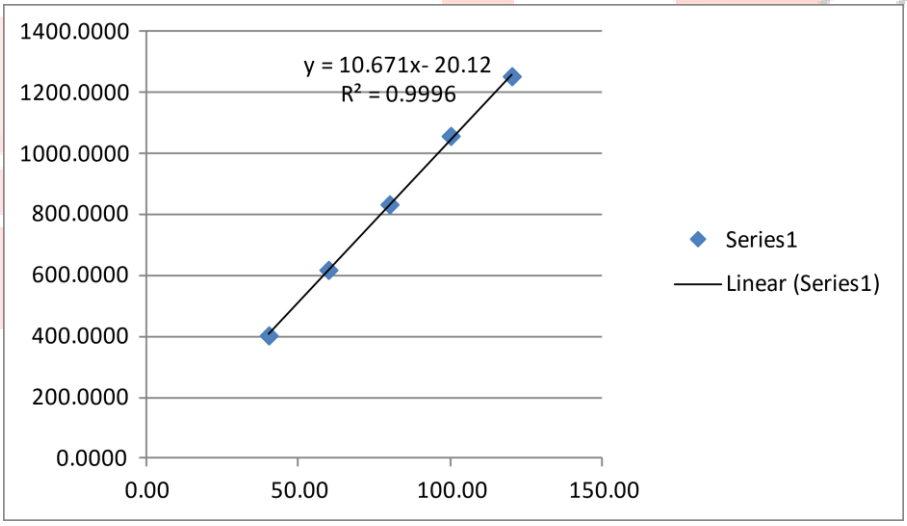

Fig.5: Calibration Curve for Telmisartan

The correlation coefficient of Telmisartan shown was 0.999 which was within limits. This calibration curve plotted was linear and showed that the method had adequate sensitivity to the concentration (40 -120µg/ml) of the drug. Finally, the data obtained, in this was within limits. Coefficient of correlation of Telmisartan was found to be 0.9998.

ROBUSTNESS:

Robustness was attempted by deliberately changing the chromatographic conditions to evaluate the difference in resolution, capacity factor, peak height and peak width (tailing factor).The parameters studied were flow rate and mobile phase composition.

LOWER LIMIT OF QUANTIFICATION AND LIMIT OF DETECTION:

The LOD is the smallest concentration of the analyte which shows a measurable response. The LLOQ is the smallest concentration of the analyte, which shows response that can be accurately quantified and LLOQ = 10 × D/S and LOD = 3.3× D/S where, D is the standard deviation of y – intercepts of regression line. S is the slope of the calibration curve. This signal to noise ratio were performed by comparing measured signal of known low concentration of drug with those of blank plasma sample.

The Limit of Detection (LOD) and Lower limit of quantification (LLOQ) for Bisoprolol Fumarate were determined and reported, based on the calibration curve was found to be 0.16 µg/ml and 0.50 µg/ml respectively. The Limit of Detection (LOD) and Lower limit of quantification (LLOQ) for Telmisartan were determined and reported, based on the calibration curve was found to be 2.37 µg/ml and 7.19 µg/ml respectively. Shown in table 7 and 8 respectively.

Table 7: LOD and LOQ data of Bisoprolol Fumarate

Parameters	Result
SLOPE	25.702
LOD (µg/ml)	0.16
LOQ (µg/ml)	0.50

Table 8: LOD and LOQ data of Telmisartan

Parameters	Result
SLOPE	10.6706
LOD (µg/ml)	2.37
LOQ (µg/ml)	7.19

SPECIFICITY:

Specificity was evaluated by compairing the chromatograms of mobile phase blank, placebo solution, standard solution of Bisoprolol Fumarate and Telmisartan and its sample solution shown in fig 9 and 10.

SYSTEM SUITABILITY:

These parameters were shown to be within specified limits. Column efficiency (theoretical plates), resolution factor and peak asymmetry factor, tailing factor, LLOQ are the system suitability parameters. These parameters of the optimized methods were found satisfactory. The results of the system suitability studies were shown in table 9 and 10. These parameters were shown to be within specified limits.

Table 9: System Suitability Studies for Bisoprolol Fumarate

Name	Area	RT(min)	TP (NLT 2000)	TF (NMT 2.0)	Resolution (NLT 2.0)
Standard _Inj_01	239.3732	3.42	13618	0.99	4.83
Standard_Inj_02	232.2733	3.43	13698	0.91	4.56

Standard_Inj_03	237.8633	3.40	11359	1.37	5.11
Standard_Inj_04	231.5037	3.40	10265	1.28	4.96
Standard_Inj_05	240.7749	3.48	14106	0.94	4.31
Mean	236.3577	3.43			
SD	4.2165	0.0329			
%RSD (NMT 2)	**1.78**	**0.96**			

Table 10: System Suitability Studies for Telmisartan

Name	**Area**	**RT(min)**	**TP (NLT 2000)**	**TF (NMT 2.0)**	**Resolution (NLT 2.0)**
Standard _Inj_01	829.5359	3.98	18384	1.03	4.83
Standard_Inj_02	820.3126	3.98	16556	1.09	4.56
Standard_Inj_03	849.8510	3.98	24945	1.07	5.11
Standard_Inj_04	846.1826	3.98	24903	1.05	4.96
Standard_Inj_05	826.5422	4.03	13644	1.10	4.31
Mean	834.4849	3.99			
SD	12.8587	0.0224			
%RSD (NMT 2)	**1.54**	**0.56**			

RESULT AND DISCUSSION

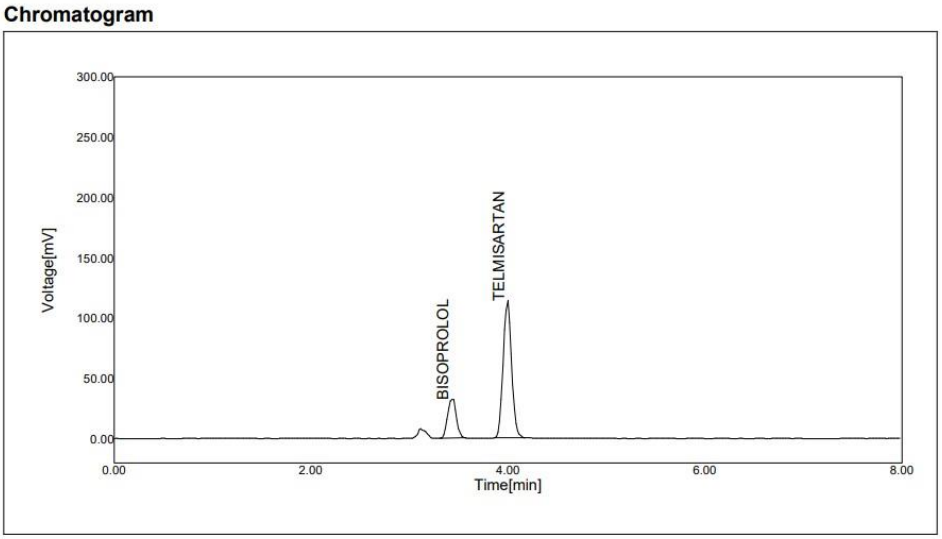

Fig.6: Chromatogram of Bisoprolol Fumarate and Telmisartan Accuracy at 80%Conc.

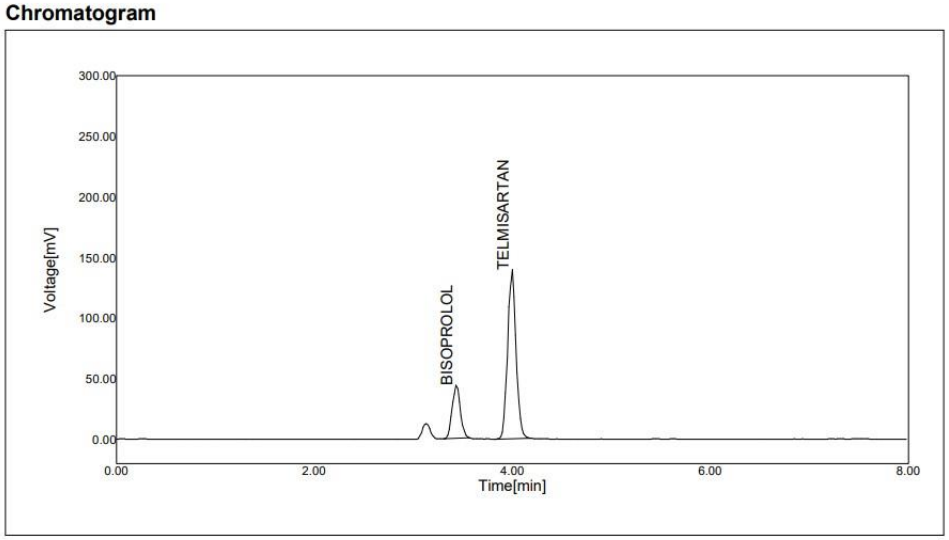

Fig.7: Chromatogram of Bisoprolol Fumarate and Telmisartan Accuracy at 100%Conc.

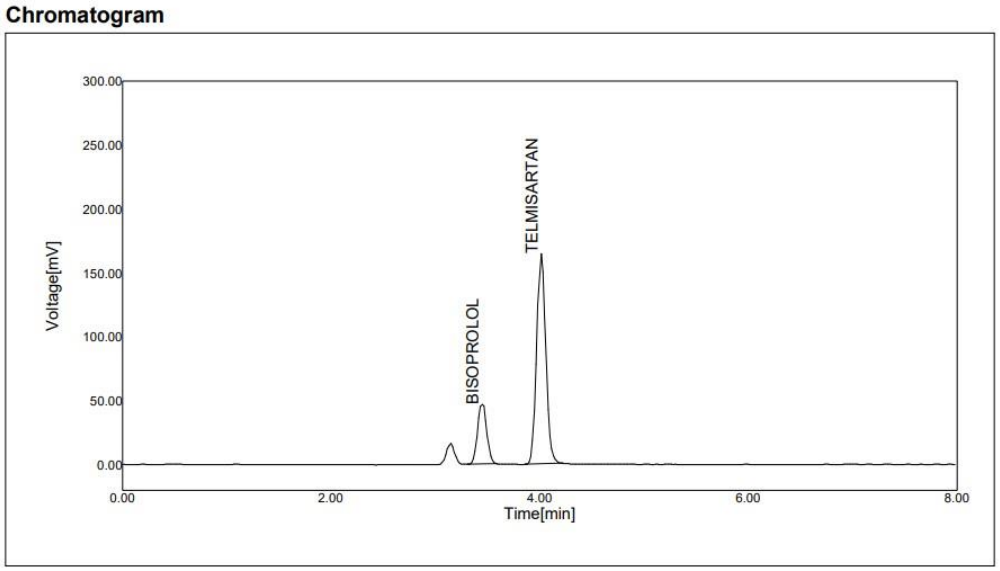

Fig.8: Chromatogram of Bisoprolol Fumarate and Telmisartan Accuracy at 120%Conc.

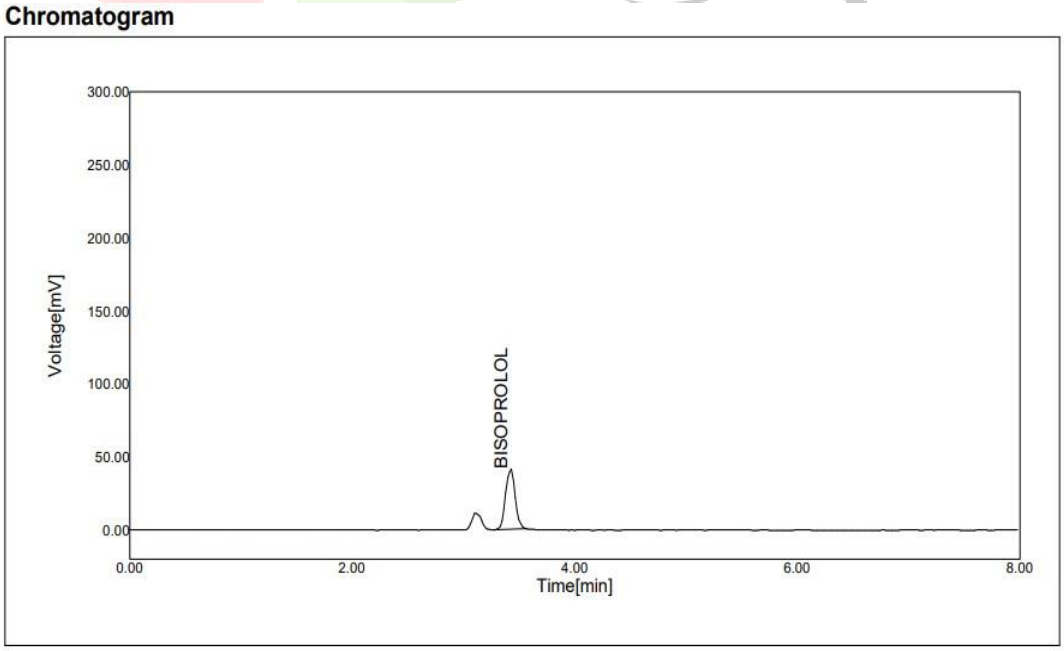

Fig.9: Chromatogram of Standard Bisoprolol Fumarate

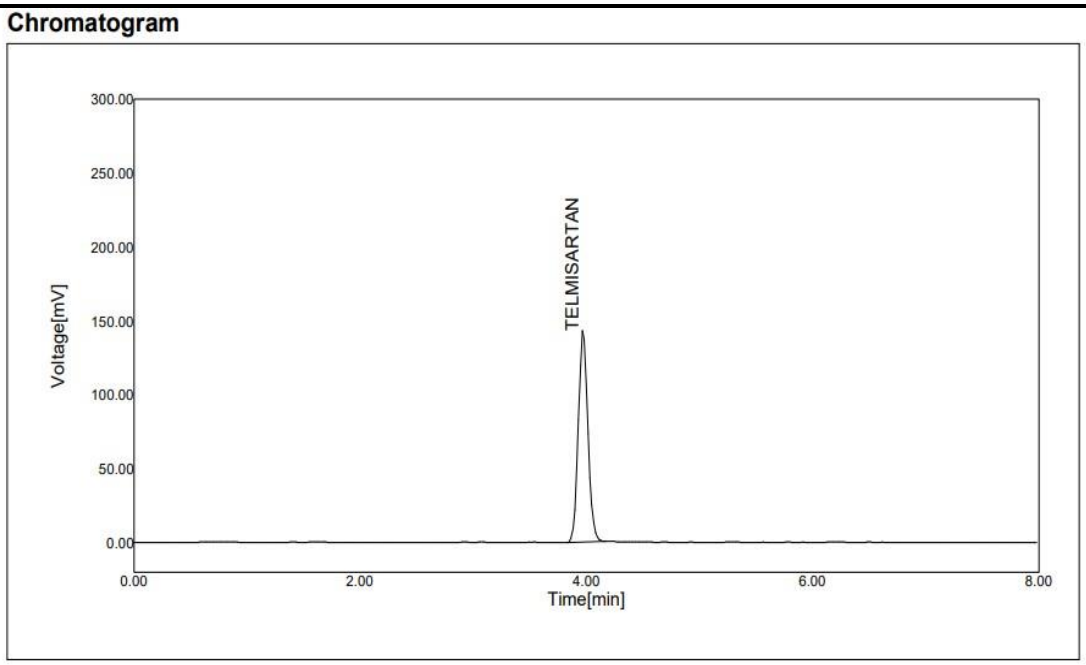

Fig10: Chromatogram of Standard Telmisartan

CONCLUSION

All the analytical validation parameters were determined and found in the limit as per ICH guidelines, which indicates the validity of the method. The method was validated with respect to specificity, linearity, accuracy, precision, ruggedness and robustness. The results of linearity, intraday and interday precision study and capability of the extraction method were within the limits of bioanalytical method development. The method was linear with a correlation coefficient within a acceptable range, which is suitable for the estimation of Bisoprolol Fumarate and Telmisartan in human plasma and other biological fluids.

From the studies it can be concluded that RP-HPLC technique can be succesfully used for the estimation of the Bisoprolol Fumarate and Telmisartan.The method shows good reproducibility Compared to UV-spectrophotometric methods, the RP-HPLC method is accurate, precise, specific, reproducible and sensitive.

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