Available online on 15.08.2022 at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Simultaneous Estimation of Metoprolol and Telmisartan in Combined Tablet Dosage Form by Using RP-HPLC and UV Spectrophotometry

Sravanthi Gandu[1], Parameshwari[1], Ravinder.M[3], Kumaraswamy Gandla[2*] Gade.Narmada[2]

[1]Trinity College of Pharmaceutical Sciences, Peddapalli, Karimnagar-Telangana, India.

[2]Department of Pharmacy, Chaitanya (Deemed to be) University), Hanamkonda, Telangana.

[3]Department of Chemistry, Chaitanya (Deemed to be) University), Hanamkonda, Telangana.

A B S T R A C T

An accurate, precise and reproducible RP-HPLC and UV Spectrophotometric method was developed and validated for the simultaneous estimation of Metoprolol and Telmisartan in tablet dosage form. The chromatographic separation was carried out on X-tera C_8 column (100mm*4.6mm*5µ), by using the mobile phase (0.05M Sodium phosphate buffer pH 2.8 and methanol) in the ratio 35:65, at a flow rate 1.2ml/min. The detection was carried out at a wave length of 226nm. The retention time for Metoprolol and Telmisartan was found to be 2.338 and 5.559 respectively. UV method involves solving simultaneous equations based on measurement of absorbance at two wavelengths 223nm and 296nm λmax of Metoprolol and Telmisartan respectively. Beer's law was obeyed in the concentration range of 1.25-6.25µg/ml and 2-10µg/ml for Metoprolol and Telmisartan respectively. The developed methods were validated according to ICH guidelines.

Key Words: Metoprolol, Telmisartan, RP-HPLC, UV-Visible Detector; ICH Validation.

A R T I C L E I N F O: Received; 20 March 2022; Review Complete; 27 June 2022 Accepted; 15 July 2022 Available online 15 August 2022

Cite this article as:

Gandu S , Parameshwari, Ravinder M, Gandla K, Narmada G, Simultaneous Estimation of Metoprolol and Telmisartan in Combined Tablet Dosage Form by Using RP-HPLC and UV Spectrophotometry, Asian Journal of Pharmaceutical Research and Development. 2022; 10(4):22-33. DOI: http://dx.doi.org/10.22270/ajprd.v10i4.1153

*Address for Correspondence:
Dr. Kumaraswamy. Gandla, Professor & HOD, Department of Pharmacy, Chaitanya (Deemed to be University), Hanamkonda, Telangana.

INTRODUCTION

Metoprolol is chemically[1-4] described as (RS)-1- (Isopropyl amino) -3-[4-(2-methoxyethyl) phenoxy] propan-2-ol. It is used mainly in the treatment of several diseases of the cardiovascular system, especially hypertension. It is a white crystalline powder, which is freely soluble in water, soluble in methanol, slightly soluble in 2-propanol, insoluble in acetone. The brand names of it are ToprolXL, Lopressor.

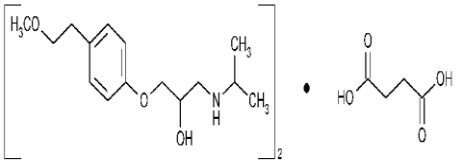

Figure 1: Chemical structure of Metoprolol

Telmisartan is chemically described[4-7] as 2-(4-{[4-methyl-6-(1-methyl-1H-1, 3-benzodiazol-2-yl)-2-propyl-1H-1, 3-benzodiazol-1-yl] methyl} phenyl) benzoic acid. It is used as antihypertensive and anti diabetic. It is a white to

slightly yellow solid, which is practically insoluble in water, sparingly soluble in strong acid (except insoluble in hydrochloric acid), and soluble in strong base. The brand names of it is Micardis. Its structure is

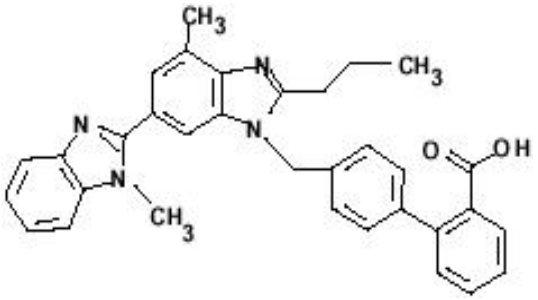

Figure: 02.Chemical structure of Telmisartan

Fixed dosage combination containing Metoprolol and Telmisartan available in market with the brand names of

TELSAR BETA (Telmisartan(40mg) +Metoprolol(25mg)

TELMAXX (Telmisartan(40mg) +Metoprolol(25mg)

Literature review[8-12] shows that there are developed methods such as spectrophotometric, HPLC method for the estimation of Metoprolol and Telmisartan alone and in combination. So the aim of our study is to develop fast, accurate and specific HPLC and AUC method for simultaneous estimation of Metoprolol and Telmisartan in tablet dosage form.

Experimental:

Instrumentation:

HPLC equipped with Quaternary pump, Auto sampler, UV Detector and Empower 2 software, make- WATERS. A double beam UV-visible spectrophotometer LAB INDIA-3000 series with UV –win software and 1cm quartz cell.

Chemicals and Reagents: The solvents used were of HPLC/AR grade. Double distilled water was used in preparation of mobile phase. Pure drug sample of Telmisartan was obtained as a gift sample from Sun Pharma pvt. Ltd. and Metoprolol from Cerex Pharmaceuticals Ltd. Tablet formulation containing Metoprolol (25mg), Telmisartan (40mg) was developed in Glenmark Pharmaceuticals Ltd.

Chromatographic conditions:

When several mobile phases were tried, the mobile phase containing Buffer (0.05M NaH_2PO_4, pH 2.8±0.02) and Methanol (35:65v/v) was considered appropriate. The Column X-Tera (C_8, 100mm x 4.6mm, 5µ,) was selected for the quantitative determination. The mobile phase was filtered through 0.45µm membrane filter and then ultrasonicated for 15 minutes. The flow rate was set to 1.2ml/min and UV detection was carried out at 226nm.

Preparation of Phosphate Buffer:

Weighed 7.8 grams of NaH_2PO_4 into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjusted the pH to 2.8 with Orthophosphoric acid.

Preparation of Mobile Phase:

Mix a mixture of above buffer 350 ml (35%) and 650 ml of Methanol HPLC grade (65%) and degas in ultrasonic waterbath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Diluent Preparation: Use the Mobile phase as Diluent.

Method Development: Method development by using HPLC and UV Spectrophotometry was intiated by taking λmax and isoabsorptive point.

Determination of λmax and isoabsorptive point:

Standard stock solutions were prepared by dissolving accurately weighed 10 mg of Metoprolol and Telmisartan in Methanol and the volume was made up to 10 ml with methanol in 10 ml volumetric flasks (Stock solution-I, 1000 mcg / ml). 1 ml of stock solution-I was diluted to 10ml with methanol (Stock solution-II, 100 mcg / ml). 1 ml of stock solution-II was taken in 10 ml standard flask diluted to 10 ml with methanol to get the concentration 10 mcg / ml. The absorbance of resulting solution was measured against respective blank solution in the UV region of 200-400 nm, which shows maximum absorbance at 223nm and 296nm for Metoprolol and Telmisartan respectively and isoabsorptive point was found to be at 226nm.

PART A: HPLC

Preparation of Metoprolol &Telmisartan standard and sample solution:

Standard solution preparation

Accurately weigh and transfer 10 mg of Metoprolol and 10mg of Telmisartan working standard into a 10ml clean dry volumetric flask add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.(Stock solution).

Further pipette 0.75ml of Metoprolol & 1.2ml of Telmisartan from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh 10 tablets and powdered. Transfer 456mg of Metoprolol and Telmisartan tablet powder into a 100ml clean dry volumetric flask and add about 70ml of Diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent.(Stock solution). Further pipette 0.3ml of Metoprolol & Telmisartan from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for the

Metoprolol and Telmisartan peaks and calculate the %Assay by using the formulae.

ASSAY % =

AT WS DT P Avg. Wt

---------- x ---------- x --------- x ---------- x---------- - X 100

AS DS WT 100 Label Claim

Where:

AT = average area counts of sample preparation.

As= average area counts of standard preparation.

WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC = LABEL CLAIM in mg/ml.

System Suitability:

Tailing factor for the peaks due to Metoprolol & Telmisartan in standard solution should not be more than 2.Theoretical plates for the Metoprolol & Telmisartan peaks in Standard solution should not be less than 2000.

Results:

Part A: RP-HPLC:

Assay results:

Metoprolol:

2934560 10 0.75 100 10 99.9 456

---------- x ----------x --------- x ----------x------- x --------- x -------- x 100 = 98.0%

2990516 10 10 456 3 100 25

Telmisartan:

1415093 10 1. 2 100 10 99.8 456

---------- x ----------x--------- x ----------x------- x -------- x --------- x 100 = 99.1%

1425433 10 10 456 3 100 40

System Suitability Results:

Metoprolol:

1). Tailing factor Obtained from the standard injection is 1.5

2). Theoretical Plates Obtained from the standard injection is 2986.4

Telmisartan:

1). Tailing factor Obtained from the standard injection is 1.3

2). Theoretical Plates Obtained from the standard injection is 2698.

Part B : Uv Spectrophotometry (Vierodt's Method)

For the simultaneous determination, suitable dilutions of the standard stock solutions (1000 µg/mL) of Metoprolol and Telmisartan were prepared separately in methanol and further diluted with methanol to make appropriate conc. and the absorbance and absorptivity values of the Metoprolol and Telmisartan were measured at λmax of Metoprolol (223nm) and Telmisartan(296nm). Concentration of Metoprolol and Telmisartan in the sample solution were calculated using the following equations

Cx =A2 ay1- A1 ay2 / ax2 ay1- ax1 ay2

Cy=A1 ax2 -A2 ax1 / ax2 ay1- ax1 ay2

Where,

ax1 and ax2 are the absorptivities of Metoprolol at 223nm and 296nm, respectively.

ay1 and ay2 are the absorptivities of Telmisartan at 223nm and 296nm, respectively.

A1 and A2 are absorbances of sample solution at 223nm and 296nm, respectively.

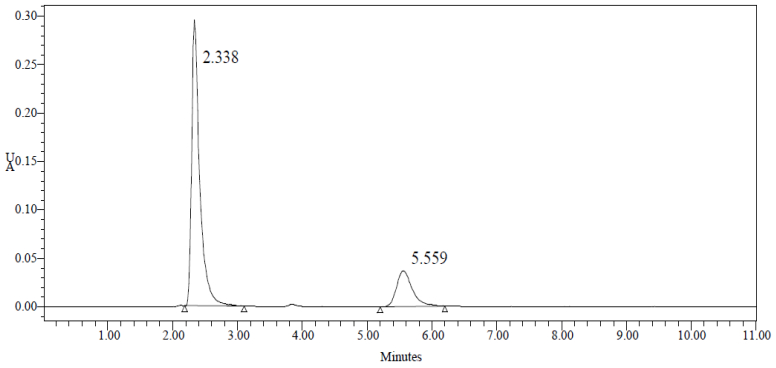

Figure: 03: Typical chromatogram of sample solution

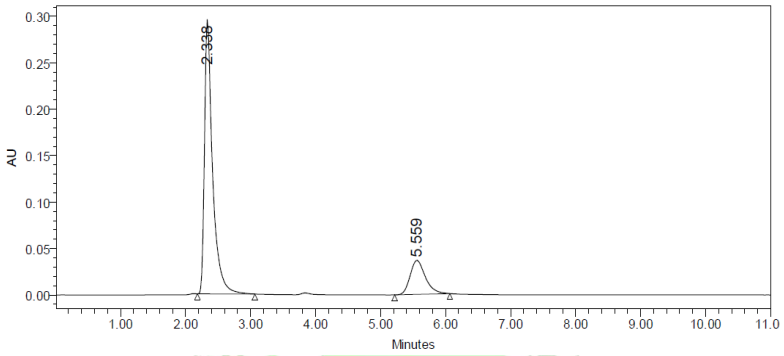

Figure 04: Typical chromatogram of standard solution

Validation parameters:

Precision:

Table 01: The Precision data of Metoprolol

Injection	Peak Area
Injection-1	2558981
Injection-2	2564477
Injection-3	2569726
Injection-4	2575001
Injection-5	2562204
Average	2566078
Standard Deviation	6341.4
%RSD	0.25

Table: 03: The results are summarized (Telmisartan)

Injection	Peak Area
Injection-1	617894
Injection-2	614922
Injection-3	613895
Injection-4	614875
Injection-5	615635
Average	615444
Standard Deviation	1502.8
%RSD	0.24

Acceptance Criteria:

The % RSD for the area of five standard injections results should not be more than 2%

Intermediate Precision:

Table: 02: The Data of Intermediate Results (Metoprolol)

Injection	Peak Area
Injection-1	2559635
Injection-2	2558586
Injection-3	2565586
Injection-4	2580623
Injection-5	2581026
Average	2569091
Standard Deviation	11039.7
% RSD	0.43

Table 03: The Intermediate Precission data of Telmisartan:

Injection	Peak area
Injection-1	603319
Injection-2	601602
Injection-3	602939
Injection-4	602159
Injection-5	601943
Average	602392
Standard Deviation	713.7
% RSD	0.12

Acceptance Criteria:

The % RSD for the area of five sample injections results should not be more than 2%

Accuracy:

Table: 04. The accuracy data of Metoprolol

%Concentration (at specification Level)	Area	Amount Added(mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	1644154	5.0	4.94	98.8%	99.3%
100%	3324514	10.0	9..99	99.9%	
150%	4965725	15.0	14.9	99.4%	

Table: 05: The accuracy results for Telmisartan:

%Concentration (at specification Level)	Area	Amount Added(mg	Amount Found(mg)	% Recovery	Mean Recovery
50%	397180	5.0	5.07	101.4%	100.4%
100%	782509	10.0	9.99	99.9%	
150%	1175881	15.0	15.0	100.0%	

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102.0%

Linearity:

Table: 06: Linearity data of Metoprolol

S. No	Linearity Level	concentration	Peak area
1	I	25ppm	809345
2	II	50ppm	1690982
3	III	75ppm	2538528
4	IV	100ppm	3285114
5	V	125ppm	4136334
Correlation Coefficient			0.999

Table 07: Linearity data of Telmisartan

S. No	Linearity Level	Concentration	Peak area
1	I	40ppm	206287
2	II	80ppm	393264
3	III	120ppm	598837
4	IV	160ppm	771735
5	V	200ppm	997889
Correlation Coefficient			0.999

Acceptance Criteria:

Correlation coefficient should be not less than 0.999

Linearity graph of Metoprolol

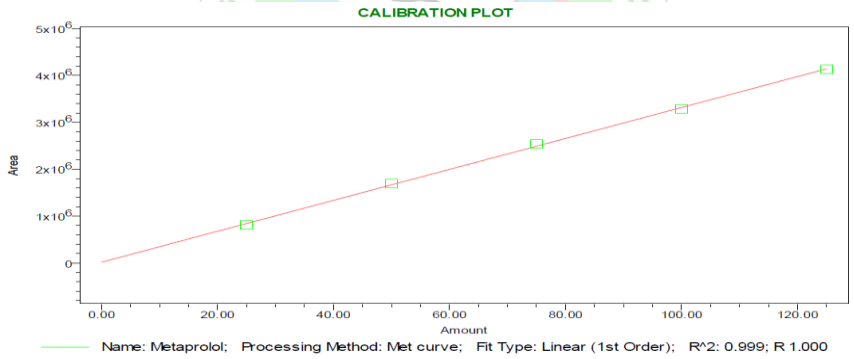

Figure 05: Calibartion curve of Metoprolol

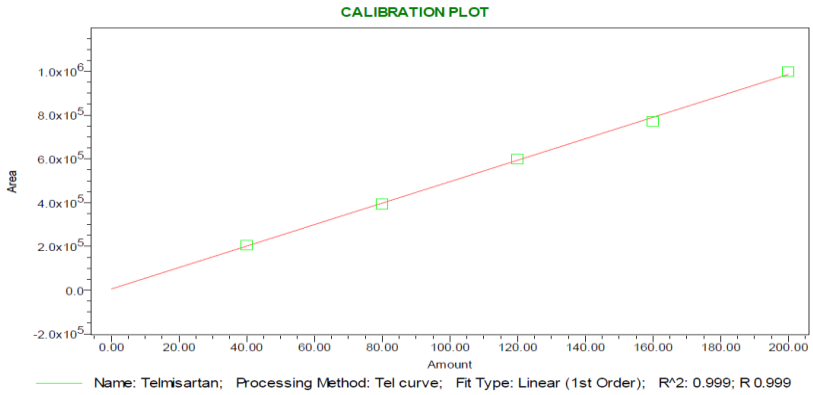

Figure: 06 Calibration curve of Telmisartan

Limit of Detection:

Metoprolol:

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank : 52 µV

Signal Obtained from LOD solution (0.6% of target assay concentration) : 154 µV

S/N = 154/52 : 2.96

Telmisartan:

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank : 52 µV

Signal Obtained from LOD solution (4% of target assay concentration) : 153 µV

S/N = 153/52 : 2.94

Acceptance Criteria:

S/N Ratio value shall be 3 for LOD solution.

Limit of Quantification:

Metoprolol:

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank : 52 µV

Signal Obtained from LOQ solution (0.18% of target assay concentration) : 519µV

S/N = 519/52 : 9.98

Telmisartan:

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank : 52 µ

Signal Obtained from LOQ solution (1.4% of target assay concentration) : 517µV

S/N = 517/52 : 9.94

Acceptance Criteria:

S/N Ratio value shall be 10 for LOQ solution

Robustness

The flow rate was varied at 1.0ml/min to 1.4 ml/min

The results are summarized : On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.The method is robust only in less flow condition.

Table 07: System suitability data of Metoprolol

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	1.0	2961.9	1.5
2	1.2	2986.4	1.5
3	1.4	2857.8	1.5

Table 08: System suitability data of Telmisartan

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	1.0	2912.6	1.1
2	1.2	2698.8	1.3
3	1.4	2677.4	1.2

***** Results for actual flow (1.2ml/min) have been considered from Assay standard

b) The Organic composition in the Mobile phase was varied from 60% to 70%.

The results are summarized

On evaluation of the above results, it can be concluded that the variation in 10% Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ±1

Part B: UV Spectrophotometry (Vierodt's Method)

Standard Spectrum of Metoprolol and Telmisartan:

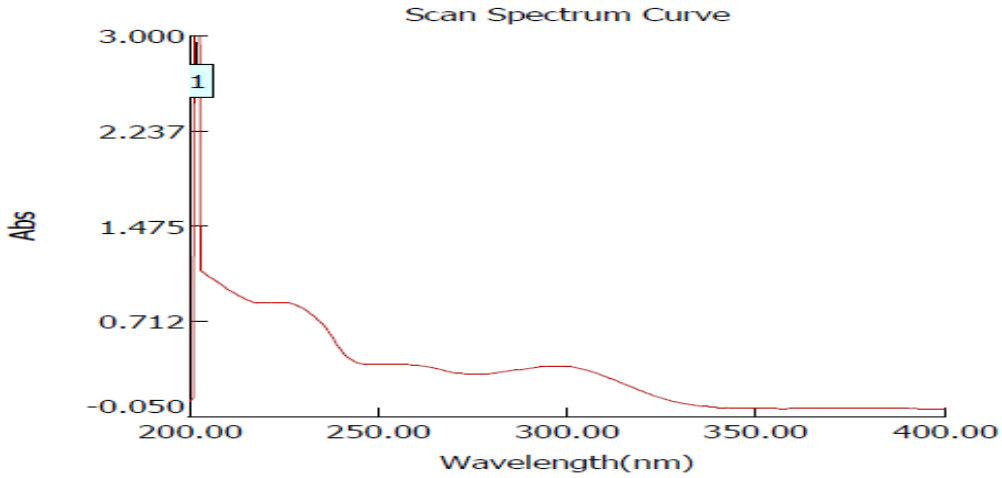

Figure. No.7: Standard Spectrum for Metoprolol and Telmisartan

Sample Spectrum of Metoprolol and Telmisartan:

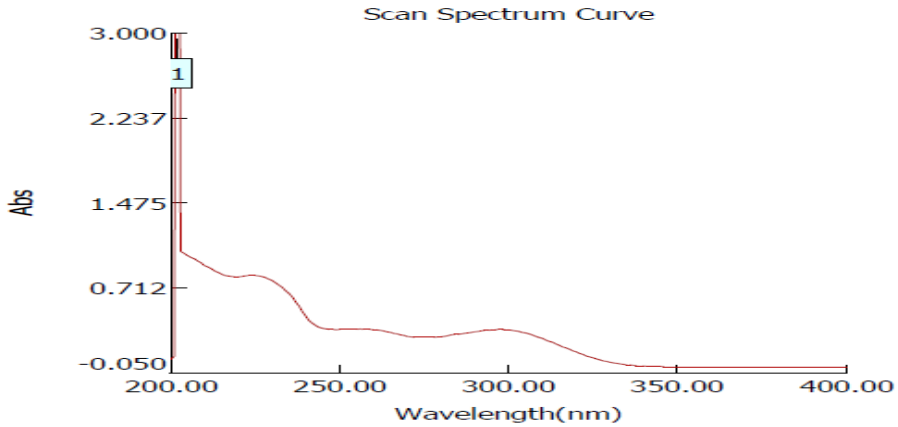

Figure .No.2: Sample Spectrum of Metoprolol and Telmisartan

Validation Parameters:

A) Accuracy:

Table: 09: Accuracy results for Metoprolol

S.NO	Amount of drug taken (µg/ml)	Amount of drug added (µg/ml)	Total amount of drug (µg/ml)	Total amount of drug found (µg/ml)	% Recovery
1.	3.75	1.875	5.625	5.575	99.12
2.	3.75	3.75	7.5	7.430	99.07
3.	3.75	5.625	9.375	9.370	99.94

Table:10 Accuracy results for Telmisartan

S.NO	Amount of drug taken (µg/ml)	Amount of drug added (µg/ml)	Total amount of drug (µg/ml)	Total amount of drug found(µg/ml)	% Recovery
1.	6	3	9	8.95	99.55
2.	6	6	12	11.92	99.37
3.	6	9	15	18.13	100.76

B) Precision:

Table: 11. Precision observation of Metoprolol

Solutions	Absorbance
Solution-1	0.152
Solution-2	0.153
Solution-3	0.152
Solution-4	0.151
Solution-5	0.151
Average	0.1518
Standard Deviation	0.0008
%RSD	0.55

Table: 12. Precision observation of Telmisartan

Solutions	Absorbance
Solution-1	0.344
Solution-2	0.344
Solution-3	0.344
Solution-4	0.347
Solution-5	0.346
Average	0.345
Standard Deviation	0.0014
%RSD	0.40

C) Intermediate Precision:

Table: 1 Intermediate Precision observation of Metoprolol

Solutions	Absorbance
Solution-1	0.154
Solution-2	0.153
Solution-3	0.153
Solution-4	0.153
Solution-5	0.153
Average	0.1532
Standard Deviation	0.0004
%RSD	0.29

Table: 2. Intermediate Precision observation of Telmisartan:

Solutions	Absorbance
Solution-1	0.347
Solution-2	0.346
Solution-3	0.350
Solution-4	0.348
Solution-5	0.348
Average	0.347
Standard Deviation	0.0014
%RSD	0.42

D) Linearity:

Table: 4. Linearity results for Metoprolol

S.NO	Concentration	Absorbance
1	1.25	0.058
2	2.5	0.097
3	3.75	0.144
4	5	0.195
5	6.25	0.235
	Correlation Coefficient	0.999

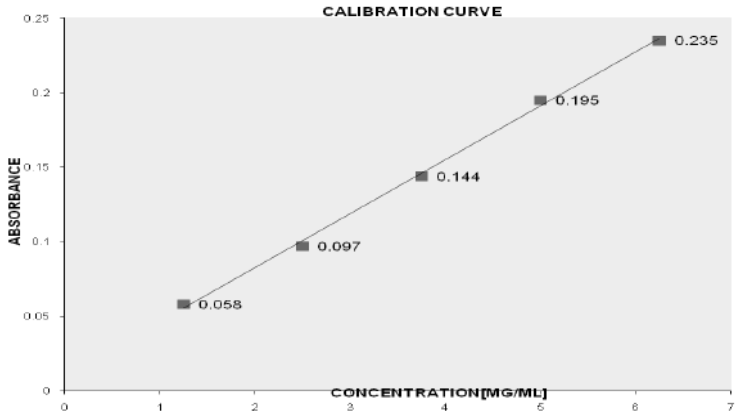

Figure.1 Calibration Curve for Metoprolol

Table: 5. Linearity results for Telmisartan

S. N	Concentration	Absorbance
1	2	0.115
2	4	0.230
3	6	0.346
4	8	0.470
5	10	0.582
	Correlation Coefficient	0.9999

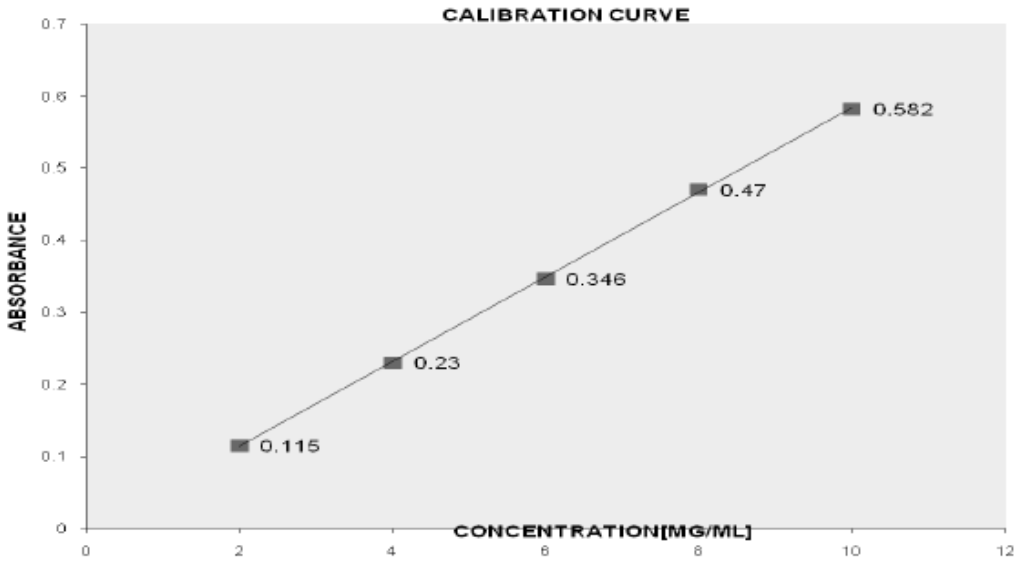

Figure: 2 Calibration Curve for Telmisartan

Table: Analysis of Marketed Tablets

S.NO	Formulation Brand Name	Drug	Vierodt's		
			Amount Present (mg)	Amount Found (mg)	% Labelled claim
1.	TELMAXX	Metoprolol	25	24.65	98.6
		Telmisartan	40	38.6	96.7

CONCLUSION:

The proposed HPLC and UV- Spectrophotometry method (Vierodt's method) was found to be specific, precise, accurate, rapid and economical for simultaneous estimation of Metoprolol and Telmisartan in Tablet dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness and results were validated statistically according to ICH and USP guidelines. The sample recoveries in all formulations were in good agreement with their respective Label Claims and this method can be used for routine Analysis.

Acknowledgment

Authors are very thank full to Management, Principal, Trinity College of Pharmaceutical Sciences, Peddapalli for providing necessary facilities to carried out Research work

REFERENCES:

1. Halama A, Kruliš R, Rymeš J. A convenient synthesis of rivaroxaban from (S)-epichlorohydrin. Organic Preparations and Procedures International. 2020; 52(3):201-211.
2. Perzborn E, Roehrig S, Straub A, Kubitza D, Mueck W, Laux V. Rivaroxaban: a new oral factor Xa inhibitor. Arteriosclerosis, thrombosis, and vascular biology. 2010; 30(3):376-381.
3. International Agency for Research on Cancer. Epichlorohydrin. IARC Summary & Evaluation, No. 71. http://www. inchem.

org/documents/iarc/vol71/020-epichlorohydrin. html. 1999; 603 Guideline, ICH Harmonized Tripartite.

4. Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic RISK M7. International conference on harmonization of technical requirements for registration of pharmaceuticals for human use (ICH): Geneva. 2014.
5. Snodin DJ, McCrossen SD. Guidelines and pharmacopoeial standards for pharmaceutical impurities: overview and critical assessment. Regulatory Toxicology and Pharmacology. 2012; 63(2):298-312.
6. Chan CC, Lee YC, Lam H, Zhang XM. Analytical method validation and instrument performance verification. John Wiley & Sons; 2004. p. 23.
7. Guideline ICH. Validation of analytical procedures: text and methodology Q2 (R1). International conference on harmonization, Geneva, Switzerland 2005 Nov (pp. 11-12).
8. Guguloth R, Madhukar A, Umadevi GT: Lalitha and Ravinder A: Analytical Method Development and Validation for the Determination of Metoprolol Succinate in tablet dosage form by RP-HPLC techniques. Journal of Scientific Research in Pharmacy 2016; 5(6):74-77.
9. Ashok P, Narenderan S, Meyyanathan S, Babu B and Vadivelan R: Development and Validation of a RP-HPLC Method for Estimation of Telmisartan in Human Plasma. International Journal of Applied Pharmaceutics 2019; 11(1):237-40.
10. Ruhina T and Mamatha T: A Novel RP HPLC Method for Developmentand Validation of Clinidipine in Bulk and Pharmaceutical Dosage Form. Asian Journal of Pharmaceutical Technology & Innovation 2017; 5(14):72 -81.
11. Suthakaran R, Bikshapathi D, Shankar C, Prem Kumar B and Ghouse S: A Validated RP-HPLC Method for Estimation of Telmisartan and Metoprolol in its Bulk Form. International Journal of Biomedical Investigation 2018; 1(2):1-13.
12. Prasad KC, Krishna MR, Babu DJ, Kumar NA and Babu GR: Stability indicating Simultaneous Estimation of Metoprolol and Clinidipine by using RP-HPLC. World Journal of Pharmacy and Pharmaceutical Sciences 2017; 6(1):1395-03.
13. Nagaraju K and Chowdary YA: Simultaneous Estimation of Telmisartan and Clinidipine in Combined Tablet Dosage Form by RP-HPLC. International Journal of Engineering Science Invention 2018; 18:39-42.