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A Validated RP-HPLC Method for Estimation of Telmisartan and Metoprolol in its Bulk Form

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Abstract

This method is used for the estimation of Metoprolol and Telmisartan present in dosage forms. Chromatogram was performed on Xbridge C18 (5u,4.6x250mm), with a mobile phase composed of methanol: water adjusted pH 3.5 with (58:42% v/v) at flow rate of 1mL/min. the identification was carried out at 224 nm. Parameters studied and reported as in ICH. The linearity range (60-140%) coefficients (0.999) and % recovery was found to be (98-102%).Retention time was (2.091) & (5.089). The test sample in all formulations were within in their label claims and this technique is used for analysis.

Keywords: Telmisartan; Metoprolol; RP-HPLC; ICH; Validation.

Introduction

Analytical method validation

As per ICH Building up documented evidence, which gives degree of proof that a particular activity will deliver a desired procedure or item meeting its determined specs and quality characters [1,2].

Objectives of validation

The varying nature of the inequality between the analytical development laboratory and q.c lab is a good reason for validation program. This study includes

1. Linearity
2. Accuracy
3. Precision
4. LOD
5. LOQ
6. Robustness
7. System suitability
8. Stability criteria.

Accuracy

The values obtained by % mean recovery. The test results acquired by the method to the genuine value (concentration) of the analyte by recreate examination of tests containing known measure of analyte across its range.

Precision

It is an analytical method used to describe individual measures of an analyte test values of different injections expressed by, Harmonization (ICH) divides into three types:

1. Repeatability
2. Intermediate precision
3. Reproducibility

Linearity

Preparing the different concentrations from the given procedure & each conc. of analyte in sample is within the range. The curve is given for each analyte.

Limit of Detection (LOD)

The identification limit is the lower amount of specimen which can be identified but not really quantitated as a correct value.

Limit of Quantification (LOQ)

The LOQ is the lower measure of analyte in a specimen which is quantitatively decided with appropriate accuracy and precision.

Robustness

It is characterized as a measure of its capacity to stay unaffected by little but little variation in technique provide a sign of its indication during its usage.

System Suitability Testing

The parameters, includes, Resolution (Rs), Tailing factor, k and/or α, Plate number (N), and (%RSD) of peak height or peak area for continuous injections (Table 1)

Table 1: Acceptance criteria of validation for HPLC.

S.No	Characteristics	Acceptance criteria
1	Accuracy	98-102%
2	Precision	RSD<2
3	System Suitability Testing	-
4	Detection limit	S/N >3:1
5	Quantitation limit	S/N>10:1
6	Linearity	R^2=1 (or)=0.999

Selection of initial conditions for method development

Determination of solubility of drug solubility

Taken small amount of sample and dissolved it in various solvents and the solubility of drugs.

Selection of chromatographic methods

The proper selection of methods relies on the idea of the sample its mol wt and stability. The drugs selected are polar, ionic and hence Reversed phase chromatography was selected.

Drug Profile

Drug description of Metoprolol and Telmisartan is summarised in Tables 2 and 3.

Table 2: Drug Profile of Metoprolol.

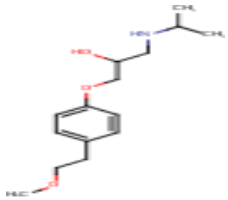
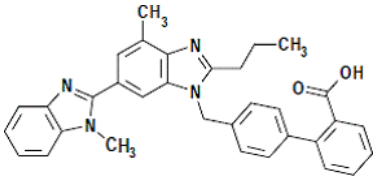
Drug Name	**Metoprolol**
Synonym	1-(isopropylamino)-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol
Brand name	Lopressor
Drug category	Antihypertensive Agents
Structure	
Chemical name / IUPAC Name	{2-hydroxy-3-[4-(2-methoxyethyl) phenoxy] propyl} (propan-2-yl) amine
Molecular Formula	$C_{15}H_{25}NO_3$
Molecular Weight	267.3639 gm/mole.
Description (Physical State):	Solid form (uncoated tablet)
Solubility	Soluble in methanol, acetonitrile, water
Half-life	3-7 h
Adverse effects/Side effects	Headache, pains, fever
Metabolism	Primarily hepatic
Storage Conditions	store at room temperature
Dosage	25 mg
Manufacture	Assure pharma

Table 3: Drug Profile of Telmisatan.

Drug Name	**Telmisartan**
Synonym	Telmax,Telsar beta
Brand name	Micardis Plus,Telma,Telmisartan
Drug category	Angiotensin II receptor antagonist
Structure	
Chemical name/ IUPAC Name	4'-((1,4'-Dimethyl-2'-propyl (2,6'-bi-1H- benzimidazol)-1'-yl) methyl) -(1, 1'-biphenyl)- 2-carboxylic acid.
Molecular Formula	$C_{33}H_{30}N_4O_2$
Molecular Weight:	514.6169 gm/mole.
Description (Physical State):	Solid form (uncoated tablet)
Solubility	Methanol, 1M sodium hydroxide, methylene chloride.
Half-life	24 h
Adverse effects/Side effects	Headache, dizziness, back pain and pains
Storage Conditions	room temperature
Dosage	40-80 mg/day
Manufacture	Assure pharrma

HPLC Method Development

Selection of initial conditions for method development

Determination of solubility of drug solubility

Taken small amount of sample and dissolved it in various solvents and the solubility of drugs.

Selection of chromatographic methods

The proper selection of methods relies on the idea of the sample its mol wt and stability. The drugs selected are polar, ionic and hence Reversed phase chromatography was selected (Table 4).

Analytical Method Validation

Validation

Building up documented evidence, which gives a high level of affirmation that a action will reliably create a desired outcome or product meeting its predetermined details and quality attributes [3-6].

Validation parameters

- Accuracy
- Precision
- Linearity
- LOD
- LOQ
- Robustness

Accuracy

Procedure

Inject the Three injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Record and measure the peak responses. Calculate the VOL found, and VOL added for Telmisartan & Metoprolol and calculate each recovery and mean recovery values.

Acceptance criteria

The % for each level should be B/W 98.1 to 102.1 %

Precision

The standard solution was infused for 5 times and measure the area for each of the 5 infusions in HPLC. The %RSD was observed to be within the limits.

Acceptance criteria

The %RSD for five std injection results should not be>2%

Intermediate precision

To evaluate the intermediate precision (Ruggedness) of the method, Precision was done on different days by maintaining same conditions.

Procedure

Day 1:

The standard solution was infused for 5 times and measure the area for each of the five infusions in HPLC. The %RSD for the area of five copy infusions was seen to be inside the limits.

Day 2:

The standard solution was infused for 5 times and measure the area for each of the five infusions in HPLC was in limit

Acceptance criteria

The % RSD of 5 different sample solutions should not >2%.

Table 4: Method development trials parameters and optimized parameters.

Parameters	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5 (Optimized)
Column	Hypersil C18 (4.6×250mm)' 5µ	Hypersil C18 (4.6×250mm)'5µ	ODS C18 (4.6×250mm) 5µ	Symmetry C18 (4.6×250mm) 5µ	X Bridge C18 (4.6×250mm) 5µ
Column temp	30ºC	30ºC	30ºC	30ºC	40ºC
Wavelength(nm)	224	224	244	244	224
Mobile phase ratio	Water (100% v/v)	Methanol: Water (15:85% V/V)	ACN: Water (50:50%) V/V	Methanol: ACN: Water (20:40:40%) V/V	Methanol: Water adjusted the pH 3.5 with OPA (58:42%)
Flow rate mL/min	0.4	0.6	1.1	0.9	1
Injection Volume (µL)	35	20	20	15	10
Run time (min)	10	20	8	10	10

Linearity

Procedure

Inject each preparation (10, 20, 30, 40, 50 ppm of drug) into the system and measure the peak area. Plot a graph of peak area vs concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient [7-12].

Acceptance criteria

Correlation coefficient should be not <0.999.

Limit of Quantification

LOQ is characteristic should be detected mainly impurities, forced degradation studies .it can be

expressed by S/N ratio (acceptable precision & accuracy).

Robustness

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

Effect of variation of flow

Sample was analysed at 0.9 mL/min and 1.1 mL/min instead of 1mL/min, remaining conditions are same. The 20 µL of the above sample was injected twice.

Effect of variation of mobile phase organic composition

Sample was analyzed by variation of mobile phase i.e. Methanol: Phosphate buffer 3.6 pH: ACN was taken in the ratio and 54:46, 63:37 instead of 58:42, remaining conditions are same. 20µl of the above sample was injected twice and chromatograms were recorded. [13-18].

Results and Discussion

Method development

The present investigation was to develop a new method and validation by RP-HPLC (Figures 1-6) (Tables 5-10).

Trail 1:

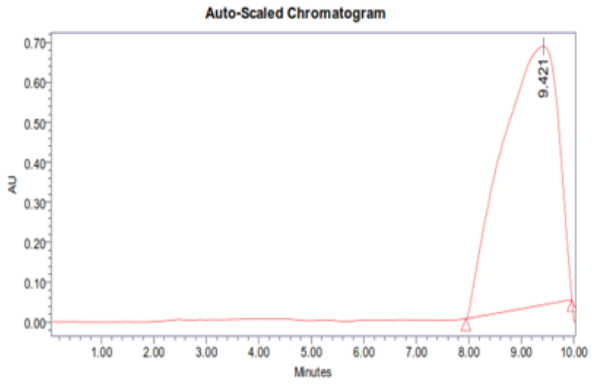


Figure 1: Chromatogram of Trail 1.

Table 5: Observations of Trail 1 Chromatogram.

Peak Name	Rt	Area	Height	USP Tailing	USP Plate count
Telmisartan & Metoprolol	9.421	48743060	646676	0.69	287

Observation

In this trial it shows less plate count and no good separation of two peaks in the chromatogram. So, it's required more trials to obtain good peaks.

Trial 2:

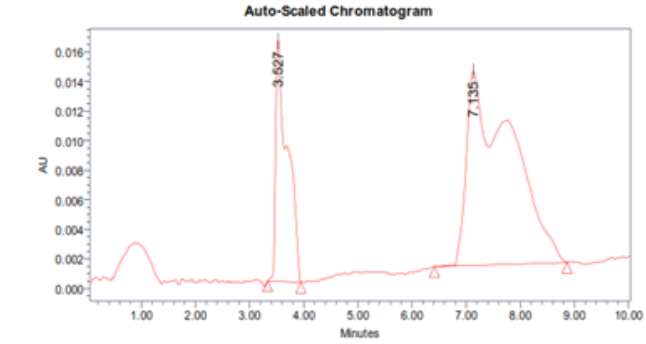


Figure 2: Chromatogram of Trail 2.

Table 6: Observations of Trail 2 Chromatogram.

Peak Name	Rt	Area	Height	USP Tailing	USP Plate count
Telmisartan & Metoprolol	3.527	247204	16277	2.70	1331
Telmisartan & Metoprolol	7.135	758138	13163	3.05	35

Observation

In this trial it shows improper baseline and more tailing. So, it's required more trials to obtain good peaks

Trail 3:

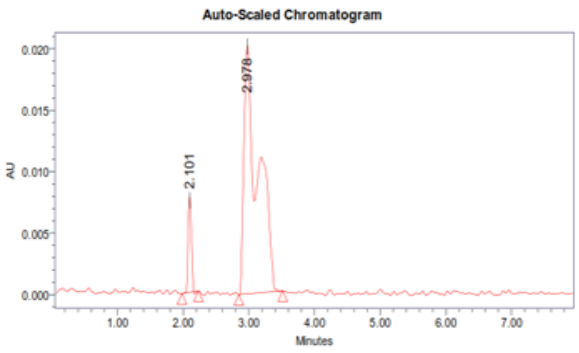


Figure 3: Chromatogram of Trail 3.

Table 7: Observations of Trail 3 Chromatogram.

Peak Name	Rt	Area	Height	USP Tailing	USP Plate count
Telmisartan & Metoprolol	2.101	27118	7668	1.07	7813
Telmisartan & Metoprolol	2.978	298974	20162	2.45	2847

Observation

In this trail the peak shape is improper and more plate count. So, it's required more trials to obtain good peaks.

Trail 4:

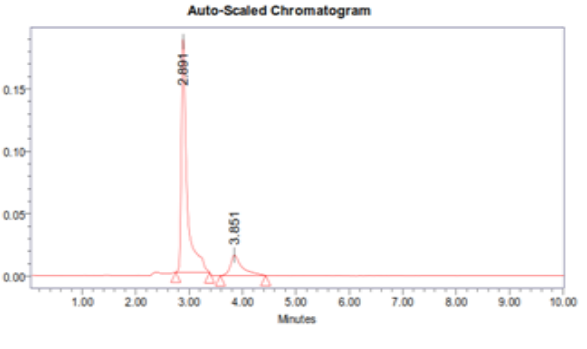


Figure 4: Chromatogram of Trail 4.

Table 8: Observations of Trail 4 Chromatogram.

Peak Name	Rt	Area	Height	USP Tailing	USP Plate count
Telmisartan & Metoprolol	3.527	247204	16277	2.70	1331
Telmisartan & Metoprolol	7.135	758138	13163	3.05	35

Observation

In this trail the peak doesn't shows more difference between two drugs. So, it's required more trials to obtain good peaks.

Trial 5:

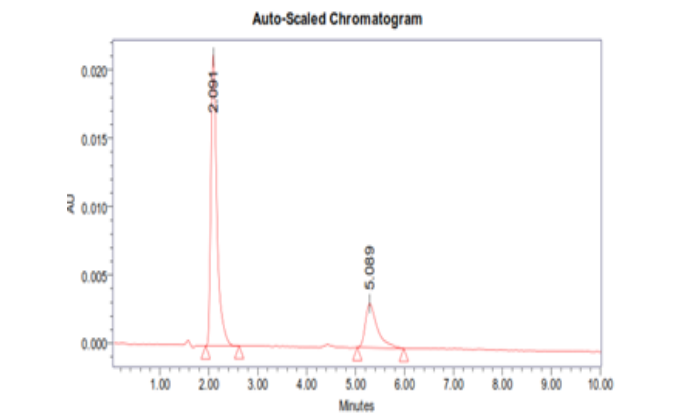


Figure 5: Optimized Chromatogram (Standard).

Table 9: Optimized Chromatogram (Standard)

Name	Rt	Area	Height	USP Tailing	USP Plate count
Telmisartan	2.091	182472	21370	1.67	5596
Metoprolol	5.089	54621	3234	1.35	7566

Observation

From the above trail we concluded that all the system suitability parameters are in limits. Hence the method was optimized.

Optimized Chromatogram (Sample)

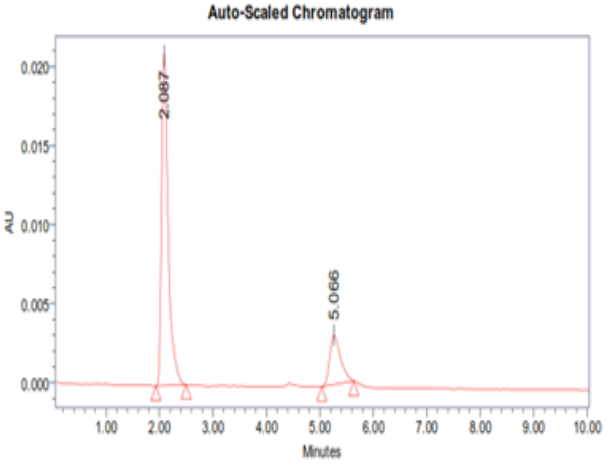


Figure 6: Optimized Chromatogram (Sample).

Table 10: Optimized Chromatogram (Sample).

Name	Rt	Area	Height	USP Tailing	USP Plate count
Telmisartan	2.087	180782	21054	1.69	5566
Metoprolol	5.066	43532	3116	1.38	6241

Acceptance criteria

- 1Theoretical plates must be not <2000
- Tailing factor must be not <2.

Method Validation

Accuracy

Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated.

The Accuracy studies were shown as % recovery for Telmisartan & Metoprolol at 50%, 100% & 150% the limits of % recovery should be in range of 98-102%. The results obtained for Telmisartan & Metoprolol were found to be within limits. Hence method was found to be accurate. The accuracy studies showed % recovery of the Telmisaratn & Metoprolol is 100% (Tables 11 and 12).

Table 11: The accuracy results for Telmisartan.

% Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	%Recovery	Mean Recovery
50%	91523.67	12	12	100	100
100%	185837.7	24	24	100	
150%	275572.7	36	36	100	

Table 12: The accuracy results for Metoprolol.

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	%Recovery	Mean Recovery
50%	20178.33	7.5	7.5	100	100
100%	42030	15	15	100	
150%	63890.67	22.5	22.5	100	

Precision

Repeatability

Obtained Five duplicates of 100% accuracy solution as per experimental conditions recorded the peak areas and calculated %RSD.

In the precision study %RSD was found to be less than 2%. For Telmisartan %RSD is 0.24 and Metoprolol % RSD is 0.81 which indicates that the system has good reproducibility. For precision studies 5 duplicate injections of Telmisartan and Metoprolol was performed. % RSD was determined for peak area of Telmisartan and Metoprolol. The acceptance limit should be not >2% and the results were found to be within the acceptance limits. Hence method is precise (Tables 13 and 14).

Intermediate precision

Day 1:

In the Day1 precision study for Telmisartan and Metoprolol's %RSD was 0.01817 & 0.758 respectively and found to be less than 2% which indicates that the system has good reproducibility. For intermediate precision studies 6 duplicate injections of Telmisartan and Metoprolol was performed and %RSD was determined for peak area of Telmisartan and Metoprolol. The limit should be not >2% and results were found to be within the limit.

Day 2:

In the Day 2 precision study %RSD was found to be less than 2%. For Telmisartan and Metoprolol % SD is 0.78 & 0.87 respectively which indicates that the system has good reproducibility. For intermediate precision studies 6 injections of Telmisartan and Metoprolol was performed and % RSD was determined for peak area of Telmisartan and Metoprolol. The acceptance limit should be not >2% and results were found to be within the limit (Tables 15 and 16)..

Table 13: Results of repeatability for Telmisartan.

S. No	Peak name	Retention time	Area (µV*s ec)	Height (µV)	USP Plate Count	USP Tailing
1	Telmisartan	2.083	182045	20760	1.79	5475
2	Telmisartan	2.081	182483	21046	1.79	5569
3	Telmisartan	2.086	181593	20990	1.75	5532
4	Telmisartan	2.084	182440	20747	1.80	5503
5	Telmisartan	2.081	182765	20964	1.82	5516
Mean			182265.2			
SD			455.009			
%RSD			0.249641			

Table 14: Results of repeatability for Metoprolol.

S. No	Peak name	Retention time	Area (µV*s ec)	Height (µV)	USP Plate Count	USP Tailing
1	Metaprolol	5.099	44969	3173	1.44	6251
2	Metaprolol	5.005	44769	3239	1.40	6456
3	Metaprolol	5.035	44067	3132	1.37	6428

4	Metaprolol	5.002	44332	3198	1.39	6346
5	Metaprolol	5.077	44698	3221	1.39	6374
Mean			44567			
SD			362.213			
%RSD			0.81273			

Table 15: Results of Intermediate precision Day 2 for Telmisartan.

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Telmisartan	2.084	182440	20747	5503	1.80
2	Telmisartan	2.066	186051	30734	5317	1.26
3	Telmisartan	2.081	182483	21046	5568	1.79
4	Telmisartan	2.089	182263	20929	5538	1.85
5	Telmisartan	2.082	183156	21058	5582	1.23
6	Telmisartan	2.080	182802	21103	5562	1.26
Mean			183199.16			
SD			1432.35			
%RSD			0.78185			

Table 16: Results of Intermediate precision Day 2 for Metoprolol.

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Metoprolol	5.002	42332	3198	6346	1.39
2	Metoprolol	5.022	42079	7763	6753	1.13
3	Metoprolol	5.077	42698	3221	6374	1.39
4	Metoprolol	5.031	42124	3249	6513	1.39
5	Metoprolol	5.052	42728	3260	6432	1.36
6	Metoprolol	5.010	42999	3313	6654	1.36
Mean			42493.3			

SD			370.611			
%RSD			0.87216			

Linearity

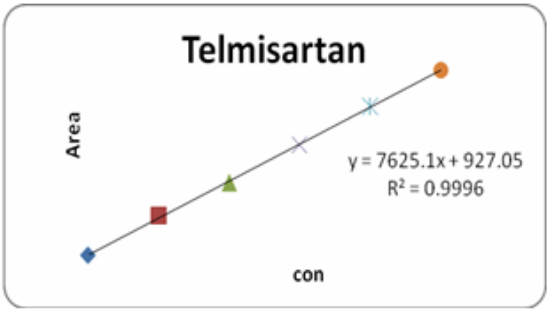


Figure 7: Calibration curve for Telmisartan.

Table 17: Linearity Observation of Telmisartan.

Concentration Level (%)	Concentration µg/ml	Average Peak Area
60	8	65676
80	16	119856
100	24	182758
120	32	246136
140	40	306150

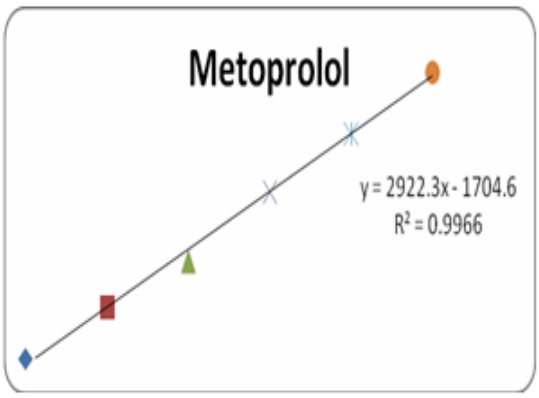


Figure 8: Calibration curve for Metoprolol.

Table 18: Linearity Observation of Metoprolol.

Concentration Level (%)	Concentration µg/ml	Average Peak Area
60	8	65676
80	16	119856
100	24	182758
120	32	246136
140	40	306150

The linearity range was found to be 10-50 µg/ml for Telmisartan & Metoprolol. Calibration curve was plotted and correlation co-efficient for the drug found to be 0.999 & 0.996 are within limit (Figures 7 and 8) (Tables 17 and 18).

Limit of detection (LOD)

Telmisartan

From the above, the LOD values of Telmisartan and Metoprolol were found to be 1.25 µg/ml & 1.69 µg/ml respectively. The LOQ values were found to be 3.817 µg/ml & 5.12 µg/ml respectively. Thus, the method developed was found to be sensitive.

$LOD = 3.3 \times \sigma / s$
Where,
σ=STD deviation of the response
S=Slope of the calibration curve
=1.25 µg/mL

Metoprolol

$LOD = 3.3 \times \sigma / s$
Where,
σ=STD deviation of the response
S=Slope of the calibration curve
=1.69 µg/mL

Limit of Quantitation (LOQ)

Telmisartan

$LOQ = 10 \times \sigma / S$

Where,
σ=Standard deviation of the response
S=Slope of the calibration curve
=3.817 µg/ml

Metoprolol

LOQ=10×σ/S
Where,
σ=STD deviation of the response
S=Slope of the calibration curve
=5.12 µg/ml

Robustness

The observations for more/high flow rate and less and more organic phase compositions was depicted in the Tables 19-24.

Table 19: Observations for Low flow rate chromatogram.

Parameter used for sample Analysis	Name	Peak Area	Rt	Theoretical plates	Tailing factor
Less Flow rate of 0.7 mL/min	Telmisartan	242504	2.736	5561	1.00
Less Flow rate of 0.7 mL/min	Metoprolol	64590	6.746	6735	1.07

Table 20: Observations for More flow rate chromatogram 0.9 mL/min.

Parameter used for sample Analysis	Name	Peak Area	Rt	Theoretical Plates	Tailing factor
More Flow rate of 0.9 mL/min	Telmisartan	147415	1.673	5387	1.03
More Flow rate of 0.9 mL/min	Metoprolol	39979	4.032	6905	1.30

Table 21: Observations for Less organic phase composition.

Parameter used for sample Analysis	Name	Peak Area	Rt	Theoretical plates	Tailing factor
Less organic phase (about 5% decrease in organic phase)	Telmisartan	11858838	3.637	7998	1.07
Less organic phase (about 5% decrease in organic phase)	Metoprolol	4345129	3.918	4202	1.43

Table 22: Observations for More Organic phase composition.

Parameter used for sample Analysis	Name	Peak Area	Rt	Theoretical plates	Tailing factor
More organic phase (about 5 % decrease in organic phase)	Telmisartan	178629	2.049	5020	1.46
More organic phase (about 5 % decrease in organic phase)	Metoprolol	52588	2.847	6362	1.53

Table 23: Results for Robustness (Telmisartan).

Parameter used for sample analysis	Peak Area	Rt	Theoretical plates	Tailing factor
Actual Flow rate of 0.8 mL/min	182472	2.091	5596	1.67
Less Flow rate of 0.7 mL/min	242504	2.736	5561	1.00
More Flow rate of 0.9 mL/min	147415	1.673	53807	1.03
Less organic phase (about 5 % decrease in organic phase)	11858838	3.637	7998	1.07
More organic phase (about 5 % Increase in organic phase)	178629	2.049	5020	1.46

Table 24: Results for Robustness (Metoprolol).

Parameter used for sample analysis	Peak Area	Rt	Theoretical plates	Tailing factor
Actual Flow rate of 0.8 mL/min	54621	5.089	7566	1.35
Less Flow rate of 0.7 mL/min	64590	6.746	6735	1.07
More Flow rate of 0.9 mL/min	39979	4.032	6905	1.30
Less organic phase (about 5 % decrease in organic phase)	4345129	3.918	4202	1.43
More organic phase (about 5 % Increase in organic phase)	52588	2.847	6362	1.53

Acceptance criteria

The tailing factor should be < 2.0 and the number of theoretical plates (N) should be >2000.

Summary and Conclusion

RP-HPLC technique was produced for estimation of Telmisartan and Metoprolol in its mass and pharmaceutical dosage form. Chromatographic separation was performed on X Bridge C18 (4.6×250mm) 5µ, with mobile phase including mixture Methanol: Water balanced the pH 3.5 with OPA (58:42% v/v), at the stream rate 1ml/min. The recognition was carried at 224 nm (Table 25).

The proposed RP-HPLC technique was observed to be exact, particular, precise, quick and economical for estimation of Telmisartan and Metoprolol in its bulk and pharmaceutical dosage form. The sample recoveries in all formulations were in great in terms with their Label Claims and this technique can be utilized for routine analysis. It can be connected for routine analysis in labs and is reasonable for the quality control of the crude materials, formulations, disintegration studies can be utilized for bioequivalence studies for the same formulation.

Table 25: Summary for RP-HPLC Method.

S.No	Parameter	Acceptance criteria	Results obtained	
			Telmisartan	Metoprolol
1	System suitability	Theoretical Plates-NLT 2000	5596	7566
		Tailing factor-NMT 2	1.67	1.35

		Retention time	2.091	5.089
2	Precision	%RSD- NLT 2	0.24	0.81
3	Linearity	Correlation Coefficient NLT 0.999	0.99	0.99
4	Accuracy	Percentage Recovery 98-102%	100	100
5	Limit of detection (LOD)		1.25	1.69
6	Limit of quantification (LOQ)		3.817	5.12
7	Robustness		Tailing factor< 2.0 and (N)>2000	Tailing factor< 2.0 and (N)>2000

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