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A VALIDATED RP-HPLC METHOD FOR ESTIMATION OF TELMISARTAN AND METOPROLOL IN ITS BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Present work describes a simple, accurate, precise and reproducible reverse phase High Performance Liquid Chromatographic method for simultaneous estimation of Telmisartan and Metoprolol in bulk and tablet dosage form on C18 (4.6×250mm) 5µ, with mobile phase comprising of mixture of buffer Methanol: Water adjusted the pH3.5 with OPA (58:42% v/v), at the flow rate 1ml/min. The detection was carried out at 224nm.The retention time for Telmisartan and Metoprolol was found to be 2.09 and 5.08 min. respectively. Detection response for both Telmisartan and Metoprolol were found to be linear low in concentration range of 8-40 µg/ml and 5-25 µg/ml respectively in the linearity study, regression equation and coefficient of correlation for Metoprolol and Telmisartan were found to be (y = 7625x +927.0 , r^2 =0.9999) and (y = 2922x – 1704, r^2 = 0.9999). Proposed method was validated for accuracy, precision, linearity, range, ruggedness & robustness.

KEYWORDS: Metoprolol, Telmisartan, RP- HPLC method; PDA detection; Tablet dosage forms.

INTRODUCTION

Telmisartan[1] a non-peptide molecule, is chemically described as 4'-[(1,4'-dimethyl-2'-propyl[2,6'-bi-1Hbenzimidazol]-1'-yl)methyl]-[1,1'-biphenyl]-2-carboxylic acid. It is used

mainly in treatment of several diseases of the cardiovascular system,especially hypertension. Used as antihypertension and antidiabetic. Fixed dose combination containing Metoprolol (50mg) and Telmisartan (40mg) available in market as tablet.Its empirical formula is $C_{33}H_{30}N_4O_2$, its molecular weight is 514.63, and its structural formula is figure 1.

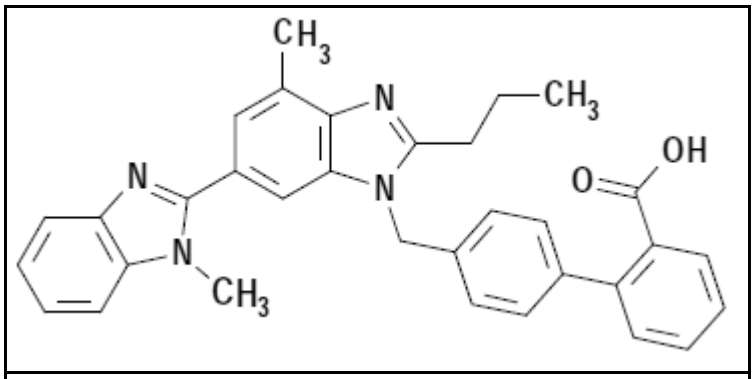

Fig 1 Chemmical Strcuture of Telmisartan

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.

Metoprolol Succinate[1]: is a beta-selective (cardioselective) adrenoceptor blocking agent, for oral administration, available as extended-release tablets. MPS has been formulated to provide a controlled and predictable release of metoprolol for once-daily administration. The tablets comprise a multiple unit system containing metoprolol succinate in a multitude of controlled release pellets. Each pellet acts as a separate drug delivery unit and is designed to deliver metoprolol continuously over the dosage interval. The tablets contain 23.75, 47.5, 95 and 190 mg of metoprolol succinate equivalent to 25, 50, 100 and 200 mg of metoprolol tartrate, USP, respectively. Its chemical name is (±)1(isopropylamino)-3-[p-(2-methoxyethyl) phenoxy]-2-propanol succinate (2:1) (salt). Its structural formula is figure 2.

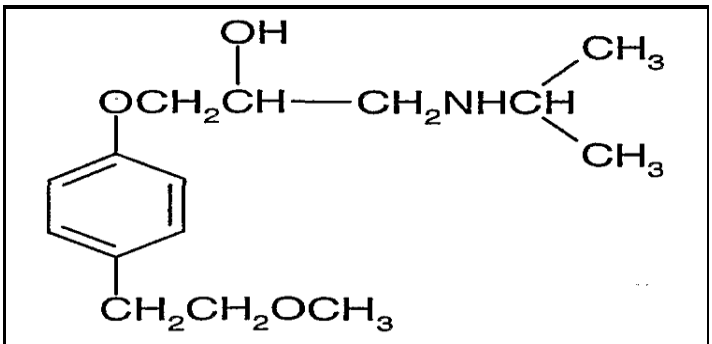

Fig 2 Chemical Structure of Metaprolol

Metoprolol succinate is a white crystalline powder with a molecular weight of 652.8. It is freely soluble in water; soluble in methanol; sparingly soluble in ethanol; slightly soluble in dichloromethane and 2-propanol; practically insoluble in ethyl-acetate, acetone, diethylether and heptane. Inactive ingredients: silicon dioxide, cellulose compounds, sodium stearyl fumarate, polyethylene glycol, titanium dioxide, paraffin.

Literature review show that there are developed methods including spectrophotometric, HPLC Method for the estimation of Metoprolol alone and in combination of other drugs Atorvastatin The developed method for Telmisartan includes spectrophotometric,HPLC, TLC densiometry, LC-MS, HPTLC detection alone and combination with other drugs Hydrochlorothiazide,Ramipril.[2-9] But till this date no simultaneous estimation of both drugs. So the aim of our study is to develop simple, fast, accurate and specific HPLC[9-11] with UV detection method for simultaneous estimation of Metaprolol and Telmisartan in tablet dosage form.

MATERIALS AND METHODS

Instrumentation: HPLC equipped with Quaternary pump, Autosampler, Photo-Diode Array Detector and Epower-2 software,make- WATERS.2695.

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 Metaprolol and Telmisartan was provided by Sura Labs Pvt Ltd Dilshuknagar, Hyderabad. Tablet formulation contaning Metoprolol (50mg),Telmisartan(40mg) was produce from Shreya Life Sciences Pvt Ltd. Research & Development Centre.

Chromatographic Conditions: When several mobile phases were tried, the mobile phase containing XBridge C18 (4.6×250mm) 5µ, with mobile phase comprising of mixture of buffer Methanol: Water adjusted the pH3.5 with OPA (58:42% v/v), at the flow rate 1ml/min. The detection was carried out at 224nm.

The mobile phases was filtered through a 0.45mcm membrane filter and then ultrasonicated for 15 minute flow rate was set to 1.0ml/min and uv detection was Carried out at 224nm.All determinations were performed at constant column temp.figure 3. (40°C).

Chromatographic Condition

Optimized Method Parameters

Mobile phase ratio	: Methanol: Water adjusted the pH3.5 with OPA (58:42% v/v)
Column	: XBridge C18 (4.6×250mm) 5µ
Column temperature	: 40ºC
Wavelength	: 224nm
Flow rate	: 1ml/min
Injection volume	: 10µl
Run time	: 10minutes

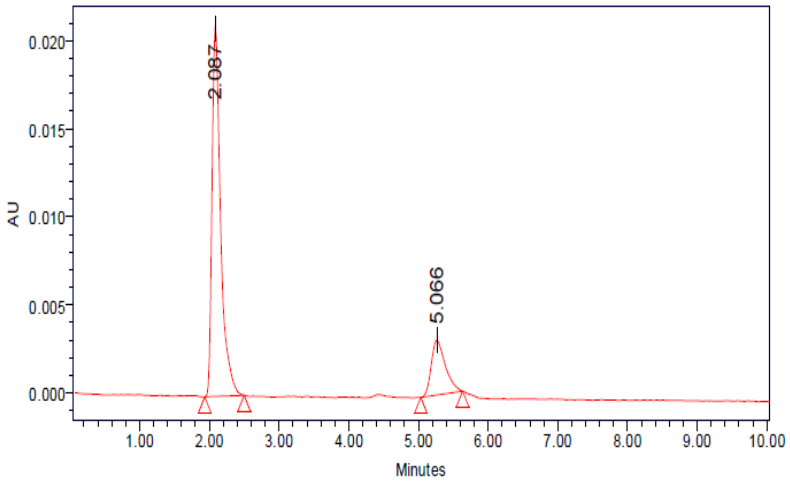

Fig 3 Typical Optimum chromatogram for Telmisartan and Metaprolol.

Calibration Curves: To prepare the calibration curve for Metoprolol and Telmisartan, 1.2, 1.6, 2.0, 2.4, 2.8ml of Standard stock solution of Metoprolol (7mcg/ml) and Telmisartan (40mcg/ml) was transferred to a series of five, 100ml of volumetric flasks. The volume of each flask was adjusted to 100ml of mobile phase. Detection response for both Telmisartan and Metaprolol were found to be linearlow in concentration range of 8-40 µg/ml in the linearity study, regression equation and Coefficient of correlation for Telmisartan and Metaprolol were found to be $r^2 = 0.999$ figure 4&5 (y = 7625x –927.0)

Table 1: Linearity Observation of Telmisartan

Concentration µg/ml	Average Peak Area
8	65676
16	119856
24	182758
32	246136
40	306150

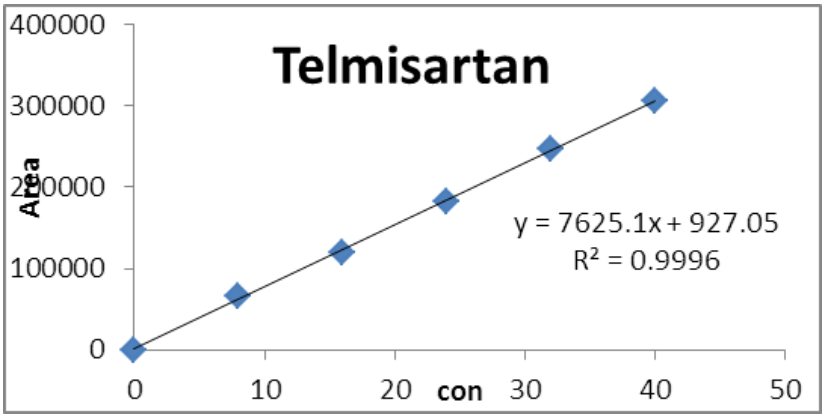

Figure: 4 Calibration curve for Telmisartan

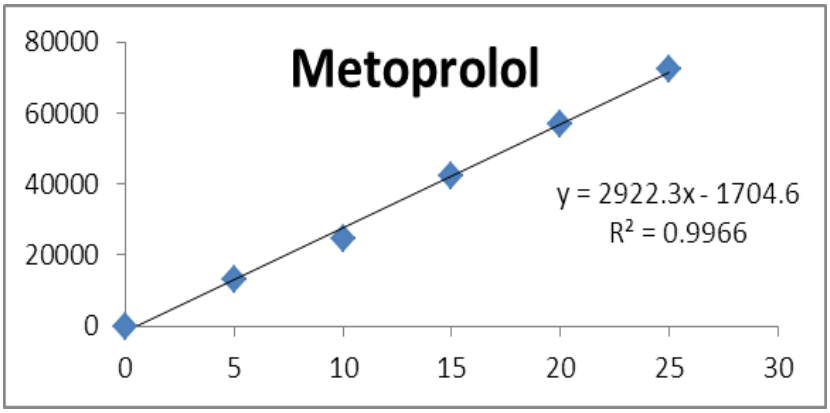

Figure 5: Calibration curve for Metoprolol

Table 2 Linearity Observation of Metoprolol

Concentration µg/ml	Average Peak Area
5	12995
10	24567
15	42033
20	56917
25	72435

The linearity range was found to be 5-25 and 8-40µg/ml for Telmisartan & Metoprolol. Calibration curve was plotted and correlation co-efficient for the drug found to be 0.999& 0.996. Hence the results obtained were within limits table 1& 2.

Limit of Detection (LOD) For Telmisartan

LOD= 3.3 × σ / s

Where
σ = Standard deviation of the response

S = Slope of the calibration curve
=1.25µg/ml

Limit of Detection (LOD) For Metoprolol

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

=1.69µg/m

Limit of Quantitationt (LOQ) For Telmisartan

LOQ=10×σ/S

where

σ = Standard deviation of the response

S = Slope of the calibration curve

=3.817µg/ml

Limit of Quantitationt (LOQ) For Metoprolol

LOQ=10×σ/S

where

σ = Standard deviation of the response

S = Slope of the calibration curve

LOQ=10×σ/S

=5.12µg/ml

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

2): Precision

a) System Precision

The system precision was evaluated by measuring the peak responses of Metoprolol and Telmisartan for six replicate injections of standard solution, prepared as the proposed method. The results shown in the **Table – 3** indicate that the precision of the system is with in the limit.

(Acceptance criteria: % RSD NMT 2.0%)

b) Method Precision

The method precision was determined by preparing a sample solution of single batch Metoprolol and Telmisartan Tablet six times and analyzing as per the proposed method. The results are

Table: 3 Results of Intermediate precision for Telmisartan

S.No	Peak Name	RT	Area (µV*sec)	USP Plate countt	USP Tailing
1	Telmisartan	2.080	182802	6562	1.86
2	Telmisartan	2.084	182594	5488	1.81
3	Telmisartan	2.078	182067	6643	1.86
4	Telmisartan	2.082	183156	6582	1.83
5	Telmisartan	2.089	182263	5538	1.85
6	Telmisartan	2.083	182826	5541	1.80
Mean			182618		
Std. Dev.			33.1906		
% RSD			0.01817		

Table 4: Results of Intermediate precision for Metoprolol

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate countt	USP Tailing
1	Metoprolol	5.010	42999	3313	5654	1.36
2	Metoprolol	5.003	42500	3218	6581	1.36
3	Metoprolol	5.076	42922	3331	6611	1.41
4	Metoprolol	5.052	42728	3260	5432	1.36
5	Metoprolol	5.031	42124	3249	6513	1.39
6	Metoprolol	5.028	42823	3157	3545	1.35
Mean			42682.6			
Std. Dev.			323.904			
% RSD			0.758			

In the Day1 precision study % RSD was found to be less than 2%. For Telmisartan and Metoprolol % RSD is 0.01 & 0.75 respectively which indicates that the system has good reproducibility.

For intermediate precision studies 6 replicate injections of Telmisartan and Metoprolol was performed and % RSD was determined for peak area of Telmisartan and Metoprolol. The acceptance limit should be not more than 2% and results were found to be within the acceptance limits.

ROBUSTNESS

The Robustness of the proposed method determined by analyzing the same batch of Metoprolol and Telmisartan Tablets by two different analysts using two different instruments,

different columns on different days. The overall mean standard deviation and % RSD of the assay values are shown in **Table-6**.

Table 6: 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 7: Results for Robustness Telmisartan

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual -1.0ml/min	182472	2.091	5596	1.67
Less -0.9mL/min	242504	2.736	5561	1.00
More -1.1mL/min	147415	1.673	5387	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 7: Results for Robustness: Metoprolol

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.8 mL/min	54621	5.089	7566	1.35
Less Flow rate of 0.7mL/min	64590	6.746	6735	1.07
More Flow rate of 0.9mL/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

Accuracy

Known amounts of Metoprolol and Telmisartan were spiked to placebo at 50%, 100% and 150% of specification in triplicate 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 results are shown in **Table-8**. The percentage recovery is within the acceptance criterion, which indicates the 80 of the method. (Acceptance criteria: % Recovery should be between (98 – 102)

Table 8 The accuracy results for Telmisartan

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

Table 9: 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	

The Accuracy studies were shown as % recovery for Telmisaratn & Metoprolol at 50%, 100% & 150% the limits of % recovery should be in range of 98-102%.

The results obtained for Telmisaratn & 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%.

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