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[Research article]

RP-HPLC Method Development and Validation for the Simultaneous Estimation of Metoprolol and Telmisartan in Tablet Dosage Form

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ABSTRACT

A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous determination of Metoprolol and Telmisartan in pharmaceutical tablet dosage form. Chromatographic analysis was performed on a Symmetry X-terra C8 (4.6 mm x 100 mm, 5 µm) column at ambient temperature with a mixture of ortho phosphoric acid buffer and Acetonitrile, Methanol in the ratio 45:10:45 v/v as mobile phase, at a flow rate of 0.7 mL min^{-1}. UV detection was performed at 226 nm. The retention times of Metoprolol and Telmisartan were 2.473 and 3.407 min, respectively. The correlation coefficient of Metoprolol and Telmisartan was found to be 0.999. Calibration plots were linear over the concentration ranges 12.5–62.5 µg mL^{-1} and 10-50 µg mL^{-1} for Metoprolol and Telmisartan, respectively. The Limit of detection was 0.667 and 0.846µg mL^{-1} and the quantification limit was 2.021 µg mL^{-1} and 2.565 µg mL^{-1} for Metoprolol and Telmisartan, respectively. The accuracy of the proposed method was determined by recovery studies and found to be 99.93% to 101.09%.The method was validated for accuracy, linearity, sensitivity, precision, robustness, system suitability Commercial tablet formulation was successfully analyzed using the developed method and the proposed method is applicable to routine analysis of determination of Metoprolol and Telmisartan in pharmaceutical tablet dosage form.

Keywords: Metoprolol, Telmisartan, RP-HPLC, Validation.

INTRODUCTION

Metoprolol is chemically described as: (RS)-1-(Isopropyl amino)-3-[4-(2-methoxyethyl) phenoxy] propan-2-ol. It is used mainly in treatment of several diseases of the cardiovascular system, especially hypertension it is a beta-adrenergic receptor blocking agent. Telmisartan is chemically described as: 2-(4-{[4-methyl-6-(1-methyl-1 H-1, 3-benzodiazol-2-yl)-2-propyl-1H-1, 3-benzodiazol-1-yl] methyl} phenyl) benzoic acid. It is used as antihypertensive and orally active nonpeptide angiotensin II antagonist that acts on the AT1 receptor subtype. Angiotensin II is the principal precursor agent of the renin-angiotensin system, with effects that include vasoconstriction, stimulation of synthesis and release of aldosterone, cardiac stimulation and renal reabsorption of sodium. Fix dosage combination containing Metoprolol (50 mg) and Telmisartan (40mg) available in market. A new combination formulation of metoprolol and Telmisartan seems to be beneficial in the treatment and management

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of essential hypertension in terms of its convenience and patient compliance. Literature survey revealed HPLC8-10, HPTLC13, and simultaneous UV spectrophotometric methods15 have been reported for the estimation of MET, TEL either alone or in combination with other drugs like Hydrochlorothiazide 10, Indapamide13, Ramipril 14, etc. The present research work describes the rapid, accurate, sensitive and reproducible RP-HPLC method for simultaneous estimation of Metoprolol and Telmisartan from the tablet formulation.

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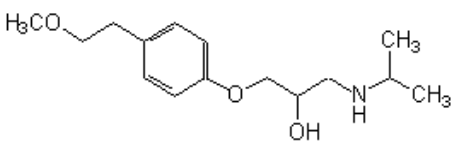
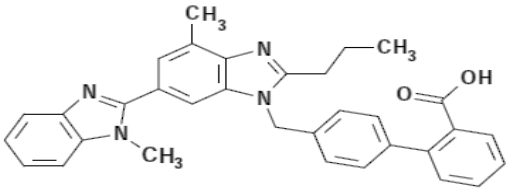
METOPROLOL



TELMISARTAN



MATERIALS AND METHODS

Chemicals/ Reagents and Solvents

Metoprolol -50mg and Telmisartan-40mg were obtained from, GLENMARK Pharmaceutical Ltd. Mumbai. Double Distilled Water (HPLC grade), Methanol (HPLC grade), Acetonitrile (HPLC grade), orthophosphoric acid and Potassium-dihydrogen phosphate were of reagent grade. The pharmaceutical preparations of combination of Metoprolol and Telmisartan that is TELMAXX 50 tablet (GLENMARK Pharmaceutical Ltd. Mumbai.).

Instrumentation and Equipments

The HPLC analysis was accomplished on WATERS high pressure liquid chromatography outfitted with 515 reciprocating dual column HPLC pump, a manually operating Rheodyne injector with 20 µL sample loop, X-terra C_8 4.6 mm x 150 mm analytical column reversed-phase material of 5µ size and a 2487 model UV-Visible detector. All the parameters of HPLC were controlled by N 2000 chromatographic system software. Other instruments used were TECHCOMP UV-Vis spectrophotometer of model 2310, Shimadzu electronic balance of model XEX-200, ADWA of model AD102U digital pH meter and ENERTECH of model SE60US ultrasonic bath sonicator.

ANALYTICAL METHOD DEVELOPMENT

Optimization of UV conditions

A symmetry X-terra C_8(4.6mm x150mm,5µm) was used for chromatographic separation. The mobile phase composed of pH 3 Buffer (Ortho phosphoric acid):Acetonitrile:Methanol (45:10:45) at flow rate 0.7 mL/min with run time 6 mins. Mobile phase and sample solution were filtered through a 0.45 µm membrane filter and degassed. The detection of both drugs was carried out at 226 nm.

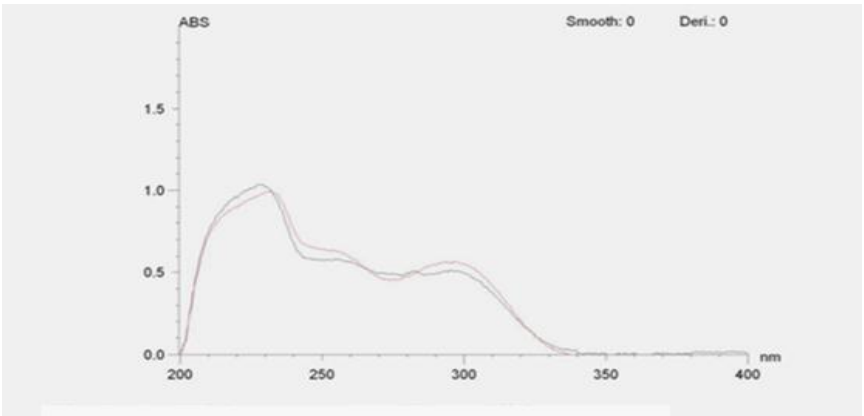


Figure-1. Isobestic point of Metoprolol and Telmisartan.

Optimized Method Parameters

MobilePhase : Phosphate buffer (3.0 pH): Acetonitrile: Methanol (45:10:45)
Column (Stationary Phase): X-terra (C_8) (4.6mm x 150mm, 5µm)
Flow rate (ml/min): 0.7
Column temperature (°C): Ambient
Volume of injection loop (µl): 20
Detection wavelength (nm):226
Drug RT (min**):** Metoprolol- 2.4, Telmisartan- 3.4

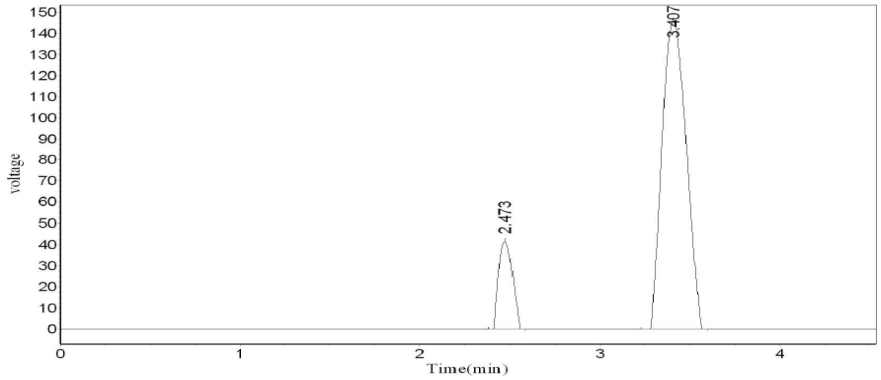


Fig: 1 Optimized chromatogram

PROCEDURE FOR PREPARATION OF SOLUTION

Preparation of buffer

Take 1000ml of HPLC grade water. Dissolve 2.72 grams of Potassium di hydrogen phosphate salt and Adjusted the pH to 3.0 with orthophosphoric acid.

Preparation of mobile phase

A mixture of above prepared buffer 450 ml (45%), 450 ml of HPLC grade Methanol and 100 mL of Acetonitrile (10%) were mixed and degassed in ultrasonic water bath for 5 minutes. The mobile phase was filterred through 0.45 µ filter under vacuum.

Diluent Preparation

Use Mobile phase Diluent Phase

ASSAY

Preparation of the Metoprolol And Telmisartan standard & sample solution

Preparation of Standard Solution

Accurately weighed and transferred 12.5 mg of Metoprolol and 10 mg of Telmisartan working standard into a 100 ml clean dry volumetric flask and added about 70 ml of diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluent. (Stock solution)

From this, 3 ml of the solution was pipetted into another 10 ml volumetric flask and diluted up to the mark with diluent.

Sample Solution Preparation

Accurately weighed and transferred tablet powder equivalent to 12.5 mg of Metoprolol and 10 mg of Telmisartan (136.3 mg) into a 100ml clean dry volumetric flask and added about 70ml of diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluent. (Stock solution)

From this, 3 ml of the solution was pipetted into another 10 ml volumetric flask and diluted up to the mark with diluent

Procedure

20 µL of the standard and sample solutions were injected into the chromatographic system and areas for the Metoprolol and Telmisartan peaks were measured. % Assay was calculated by using the formulae.

Calculation:

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.\ Wt}{Label\ Claim} \times 100$$

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 mg/ml.

ANALYTICAL METHOD VALIDATION

The HPLC method was validated in accordance with ICH guidelines.

Accuracy

Accuracy was carried out by % recovery studies at three different concentration levels. To the pre-analyzed sample solution of METO and TELM a known amount of standard drug powder of METO and TELM were added at 80, 100 and 120 % level.

Precision

The system precision of the method was verified by five replicate injections of standard solution containing METO and TELM. The method precision was carried out the analyte five times using the proposed method. Repeatability was measured by multiple injections of a homogenous sample of METO and TELM.

Linearity

The linearity was determined separately for METO and TELM Linearity of the method was studied by injecting 5 concentrations of both drugs prepared in methanol and calibration curves were constructed by plotting peak area against the respective concentrations.

Limit of detection and Limit of quantitation

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD = 3.3 x ASD/S and LOQ = 10 x ASD/S, Where, 'ASD' is the average standard deviation and 'S' is the slope of the line.

Robustness

Robustness was evaluated by making deliberate variations in method parameters such as variation of wave length; flow rate and change in mobile phase composition. The robustness of the method was studied for METO and TELM

RESULTS

Selection of Chromatographic Conditions and Optimization of Mobile Phase

Mobile phase was optimized to separate METO and TELM using Symmetry C8 column (150 mm x 4.6 mm i.d., 5µm). Initially, ACN and phosphate buffer and methanol in the Equal proportions were tried as mobile phase but the splitting of the peaks for both these drugs was observed. Therefore, after adjustment of pH of mixed phosphate buffer to 3.0 with ortho-phosphoric acid, and mobile phase composition (phosphate buffer, ACN and methanol in 45:10:45 % v/v*)* was tried for resolution of both drugs. Good resolution and symmetric peaks were obtained for both drugs when the pH of the mobile phase (buffer) was adjusted to 3.0. The flow rate of the mobile phase was 0.7 mL min-1. Under optimum chromatographic conditions, the retention time for METO and TELM was found to be 2.473 and 3.407 min, respectively when the detection was carried out at 226nm. A typical chromatogram of two drugs is shown in (Figure 1).

Table-1 : ACCURACY DATA

Drugs	Amount Added (mg)	Amount Found (mg)	% Recovery	% of mean recovery
Metoprolol	67.5	67.43	99.8	99.93
	75	76.3	102	
	82	81.55	98	
Telmisartan	54	53.86	99.42	101.09
	60	60.41	101.37	
	66	66.90	102.50	

Table-2 : PRECISION

S.NO	RT	METOPROLOL AREA	RT	TELMISARTAN AREA
1	2.472	1745269	3.397	6017053
2	2.543	1744223	3.477	6061793
3	2.498	1755032	3.440	6084698
4	2.473	1745274	3.398	6017163
5	2.548	1744271	3.482	6061885
Average	2.5068	1746614	3.4388	6048520
Standard Deviation	0.0368	4726.359	0.041	30151.48
% RSD	1.4	0.27	1.2	0.50

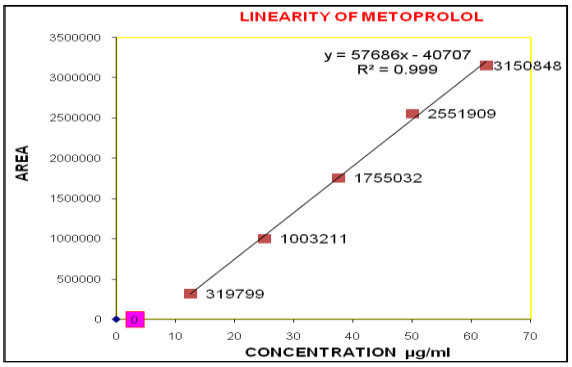
Table-3 METHOD PRECISION

S.NO	RT	AREA	RT	AREA
1	2.490	1723623	3.423	5925015
2	2.515	1709983	3.448	5864329
3	2.492	1720330	3.418	5891368
4	2.488	1723516	3.423	5924883
5	2.498	1720296	3.423	5909737
Average	2.4966	**1719549.6**	3.427	**5903066**
Standard Deviation	0.010	**5590.41**	0.011	**25688.72**
% RSD	**0.4**	**0.33**	**0.32**	**0.44**

Table-4: LINEARITY RESULTS OF METOPROLOL AND TELMISARTAN

METOPROLOL		**TELMISARTAN**	
Conc(mcg/ml)	**Mean Area**	**Conc(mcg/ml)**	**Mean Area**
12.5	319799	10	1677519
25	1003211	20	3739380
37.5	1755032	30	6084698
50	2551909	40	8174726
62.5	3150848	50	10315646

Fig 2: LINEARITY GRAPHS OF METOPROLOL AND TELMISARTAN



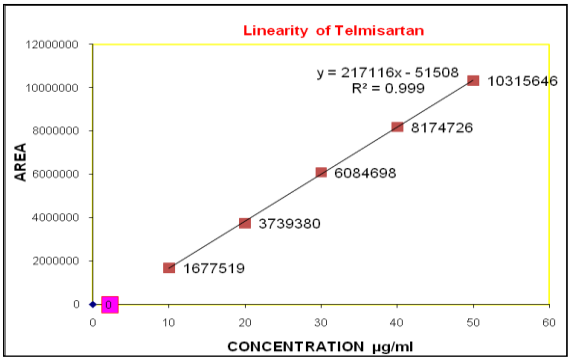


Table-5 LOD AND LOQ RESULTS

S.No	Drug name	Standard deviation	Slope	LOD	LOQ
1	Metoprolol	11659	57686	0.66	2.02
2	Telmisartan	55707	217116	0.84	2.56

Table-6: ROBUSTNESS RESULTS

S.No	Parameter	Metoprolol Theoretical plates per column	Tailing factor	Resolution	Telmisartan Theoretical plates per column	Tailing factor	Resolution
1	Less flow(0.63ml/min)	2122	1.287	-	4101	1.139	5.462
2	More flow(0.77ml/min)	2095	1.295	-	3952	1.149	5.326
3	%10 Less organic	2101	1.255	-	4028	1.134	5.404
4	%10 More organic	2042	1.245	-	3892	1.124	5.323

RESULTS AND DISCUSSION

Accuracy

The accuracy of the method studied at three different concentration levels i.e. 80%, 100 % and 120 % showed acceptable % recoveries in the range of 99.93% for METO and 101.09% for TELM. The results are shown in Table 1.

Precision

The precision study was evaluated on the basis of % RSD value was found to be in the range 0.27–1.4 and 0.32-0.4 %, respectively. As the RSD values were < 2% therefore developed method was precise. Results of precision study are shown in Table 2 & 3.

Linearity

The linearity was determined separately for METO and TELM. Linearity of the method was studied by injecting 5 concentrations of both drugs prepared in methanol and calibration curves were constructed by plotting peak area against the respective concentrations. The METO and TELM followed linearity in the concentration range of 12.5– 62.5 µg mL-1 and 10-50µg mL-1; respectively. The results are shown in Table 4.and Fig no 2.

Limit of detection and Limit of quantitation

The LOD for METO and TELM was found to be 0.667 and 0.846 µg, respectively. The LOQ for METO and TELM was found to be 2.021 and 2.565 µg, respectively. The low values of LOD and LOQ indicates high sensitivity of the method. The results are shown in Table 5.

Robustness study

Robustness of the method was studied by making deliberate changes in the chromatographic conditions and the effects on the results were examined. The low value changes of theoretical plates, tailing factor indicating robustness of the method. The results are shown in Table 6.

Analysis of marketed tablet formulation

3 replicates of the samples solutions (20 µL) were injected for quantitative analysis. The amounts of METO and TELM estimated were found to 99.09 % and 98.42 %, respectively. A good separation and resolution of both drugs indicates that there was no interference from the excipients commonly present in pharmaceutical formulations. The results are shown in Table 7.

System Suitability Test

The system suitability parameters such as resolution, number of theoretical plates and tailing factor were studied and were summarized in Table 8.

Table 7: ASSAY RESULTS

Assay Results Drug	Amount present/tablet	% of Assay
METOPROLOL	50mg	99.09
TELMISARTAN	40 mg	98.42

Table 8: SYSTEM SUITABILITY PARAMETERS

System suitability Parameters	METOPROLOL	TELMISARTAN
Tailing Factor	1.275	1.145
Theoritical plates	2076	3997
Resolution	--	5.381

CONCLUSION

The developed RP-HPLC method is simple, precise, accurate, selective and reproducible. The method has been found to be adequately in linearty and robustness can be used for simultaneous determination of Metoprolol and Telmisartan in tablet formulation. The method was validated as per ICH guidelines.

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