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A NEW RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF METOPROLOL TARTRATE AND TELMISARTAN IN PHARMACEUTICAL TABLET DOSAGE FORM

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ABSTRACT

The Present work was to develop a simple, fast, accurate, precise, reproducible, reverse phase high performance liquid chromatographic method for simultaneous estimation of metoprolol tartrate and telmisartan in pharmaceutical tablet dosage form marketed as telsar beta. Chromatographic separation was done using Agilent – Zorbax SB-CN RP C18 column having dimension of 4.6×150mm having particle size of 3.5µm, with mobile phase consisting of phosphate buffer pH 3 ±0.02 pH adjusted with ortho phosphoric acid and methanol (30:70 %v/v), flow rate was adjusted to 0.8 ml/min and detection wavelength at 226nm. The retention times of metoprolol tartrate and telmisartan was found to be 2.3 and 4.2mins. The Proposed method has been validated for accuracy, precision, linearity; range and robustness were within the acceptance limit according to ICH guidelines. Linearity for metoprolol tartrate and telmisartan was found in range of 25µg-125µg & 20µg-100µg and correlation coefficient was found to be 0.999 and 0.999, %RSD for intermediate precision was found to be 0.11 and for repeatability was 0.40 and 0.23, % mean recovery for metoprolol tartrate and telmisartan was found to be 99.8% to 100.5%. The method was found to be robust even by change in the mobile phase ±5% and in less flow condition. The developed method can be successfully employed for the routine analysis of metoprolol tartrate and telmisartan in API and Pharmaceutical dosage forms.

Keywords: Metoprolol tartrate and Telmisartan, RP-HPLC, Method development, Method validation.

INTRODUCTION

Metaprolol tartrate and Telmisartan in pharmaceutical tablet dosage form marketed as telsar beta inthe ratio of 5:4. Chemically [1] 1-(Isopropylamino)-3-(p-(2-methoxyethyl)phenoxy)- 2-propanol (2:1) Dextro-tartrate salt has activity of anti hypertensive as β –blocking agent. Telmisartan [2] is 4'-[(1, 4'-dimethyl-2'-propyl-[2,6'-Bi-1H-benzimidazol]-1‘- yl) methyl]-[1,1'- biphenyl] -2- carboxylic acid, has antihypertensive activity as angiotensin II antagonist acting on the AT_1 receptor sub type. It is official in B.P and Merck index. Literature survey [3-7] reveals, UV, HPLC, MS/MS methos for analysis Metaprolol tartrate of single and combined dosage forms with other drugs.

MATERIAL AND METHODS

Instrument

The HPLC system used was Shimadzu with model no SPD-20A equipped with UV detector source of deuterium lamp. The chromatogram was recorded at and peaks quantified by means of PC based Spinchrome software.

Solvents used

Methanol and water for HPLC grade in the study and obtained from MERCK Company.

Preparation of solutions

Preparation of phosphate buffer

7.0 grams of KH_2PO_4 was weighed and was taken

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Fig 1. Chemical structure of Metaprolol tartrate

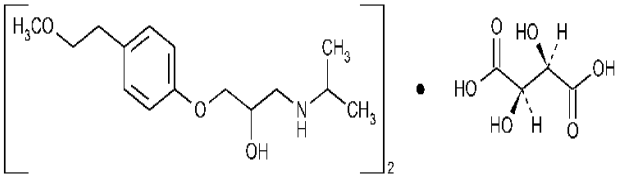
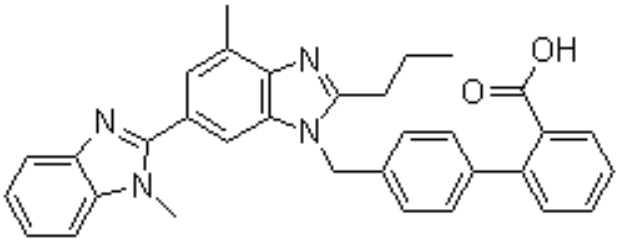


Fig 2. Chemical structure of Telmisartan



into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water and pH was adjusted to 3 with ortho phosphoric acid. The resulting solution was sonicate and filtered.

Preparation of mobile phase

Mix a mixture of above buffer 300 ml (30%) and 700 ml of methanol (HPLC grade-70%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

Diluent preparation

Mobile phase was used as the diluent.

Preparation of the individual metoprolol tartrate standard preparation

10mg of metoprolol tartrate working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and 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 out 0.75ml of metoprolol tartrate from the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Preparation of the individual telmisartan standard preparation

8mg of telmisartan working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and 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 out 0.75ml of telmisartan from the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Preparation of the metoprolol tartrate & telmisartan standard & sample solution

Sample solution preparation

441.3mg of metoprolol tartrate and telmisartan tablet powder was accurately weighed and transferred into a 100ml clean dry volumetric flask add about 70ml of diluent and sonic ate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 1.5ml of metoprolol tartrate & telmisartan the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Standard solution preparation

10mg of metoprolol tartrate and 8mg of telmisartan working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and 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 out 0.75ml of metoprolol tartrate & telmisartan from the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Procedure

20µL of the blank, standard and sample was injec ted into the chromatographic system and areas for the metoprolol tartrate and telmisartan from the peaks were used for calculating the % assay by using the formulae.

RESULTS AND DISCUSSION

Method Development

After the number of trials of we arrived at the optimized parameter for the simultaneous estimation for chosen drugs as shown table no 1.

Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank. The chromatograms are shown in Fig. No.3

Linearity

Each level was injected into the chromatographic system and peak area was measured. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and the correlation coefficient was calculated. The results are tabulated in Table.No.2-3. Calibration graph for MET and TEL are shown in Fig.No.4-5. Acceptance criteria correlation coefficient should be not less than 0.999.

Accuracy

The standard solutions of accuracy-80%, 100% and 120% was injected into chromatographic system. Calculate

Table 1. Showing optimised chromatographic condition

S.No	Parameter	Condition
1	Column	Agilent-Zorbax SB-CN RP C18 4.5×150 mm 3.5µm
2	Column temperature	Ambient
3	Wavelength	226nm
4	Mobile phase ratios	70:30 % v/v methanol : phosphate buffer pH was adjusted to 3 with ortho phosphoric acid
5	Flow rate	0.8 ml/min
6	Auto sampler temperature	Ambient
7	Injection volume	20µL
8	Run time	7 minutes

Table 2. Showing linearity results for metoprolol tartrate level -1to level-5

S.No	Linearity Level	Concentration	Peak Name	R_t	Area	Height
1	I	25ppm	Metoprolol tartrate	2.384	1660868	247080
2	II	50ppm	Metoprolol tartrate	2.396	2256788	330465
3	III	75ppm	Metoprolol tartrate	2.403	2806434	407746
4	IV	100ppm	Metoprolol tartrate	2.404	580920	83708
5	V	125ppm	Metoprolol tartrate	2.408	1069774	156758
Correlation Coefficient					0.999	

Table 3. Showing linearity results for telmisartan level -1to level-5

S.No	Linearity Level	Concentration	Peak Name	R_t	Area	Height
1	I	20ppm	Telmisartan	4.181	5020392	436746
2	II	40ppm	Telmisartan	4.211	6734083	568847
3	III	60ppm	Telmisartan	4.241	1672987	144097
4	IV	80ppm	Telmisartan	4.246	8405968	711441
5	V	100ppm	Telmisartan	4.248	3180475	272612
Correlation Coefficient					0.999	

Table 4. Showing accuracy results for metoprolol tartrate

% Concentration (at specification level)	Average Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
80%	1355837	8.0	8.0	100.5%	100.3%
100%	1690347	10.0	10	100.2%	
120%	2025922	12.0	12.0	100.1%	

Table 5. Showing accuracy results for telmisartan

%Concentration (at specification level)	Average Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
80%	4044874	6.4	6.4	100.2%	100.0%
100%	5041056	8.0	8.0	100.0%	
120%	6040236	9.60	9.58	99.8%	

Table 6. Showing results for precision of metoprolol tartrate

S.No	Injection	Peak Name	R_t	Area	Height
1	Injection-1	Metoprolol tartrate	2.405	1688138	236651
2	Injection-2	Metoprolol tartrate	2.406	1692826	236460
3	Injection-3	Metoprolol tartrate	2.409	1688757	236063
4	Injection-4	Metoprolol tartrate	2.410	1689699	237099
5	Injection-5	Metoprolol tartrate	2.415	1691189	237641
Average				1690122	
Standard Deviation				1899.244	
% RSD				0.11	

Table 7. Showing results for precision of telmisartan

S.No	Injection	Peak Name	R_t	Area	Height
1	Injection-1	Telmisartan	4.260	5041710	405414
2	Injection-2	Telmisartan	4.266	5032589	402727
3	Injection-3	Telmisartan	4.270	5047130	404890
4	Injection-4	Telmisartan	4.270	5044410	404494
5	Injection-5	Telmisartan	4.273	5037372	404188
Average				5040642	
Standard Deviation				5765.7	
% RSD				0.11	

Table 8. Showing results for Limit of Detection

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Metoprolol tartrate	41	121	2.95
Telmisartan	41	122	2.97

Table 9. Showing results for Limit of Quantification

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Metoprolol tartrate	41	409	9.7
Telmisartan	41	412	10

Table 10. Showing system suitability results for metoprolol tartrate

S.No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.6	2562.4	1.1
2	**0.8**	**2526.0**	**1.1**
3	1.0	2442.3	1.2

Table 11. Showing system suitability results for telmisartan

S.No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.6	3054.9	1.0
2	0.8	2933.6	1.1
3	1.0	2664.7	1.1

Fig 3. Chromatogram showing standard

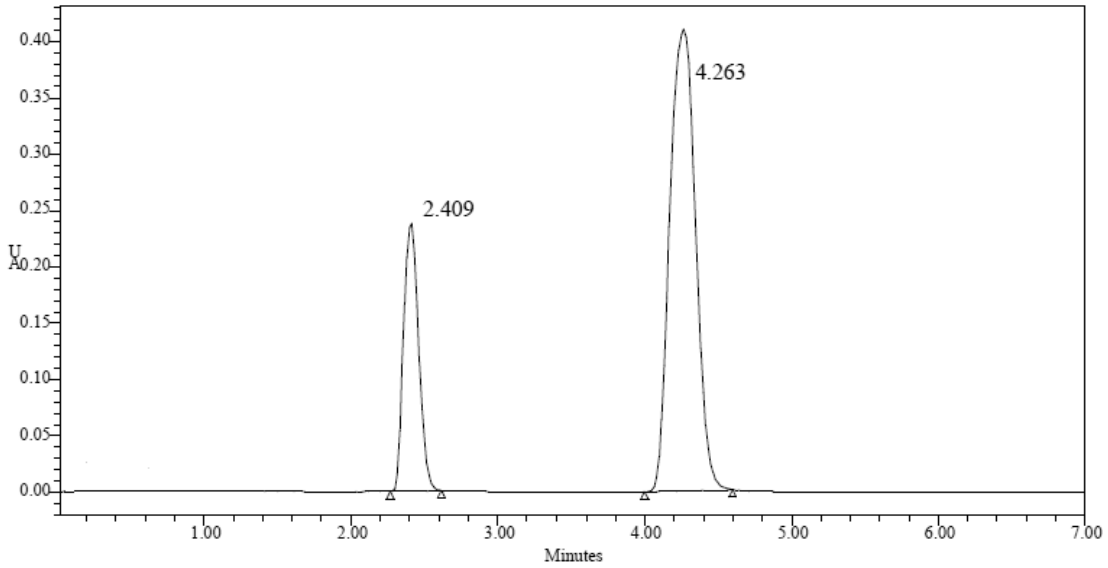
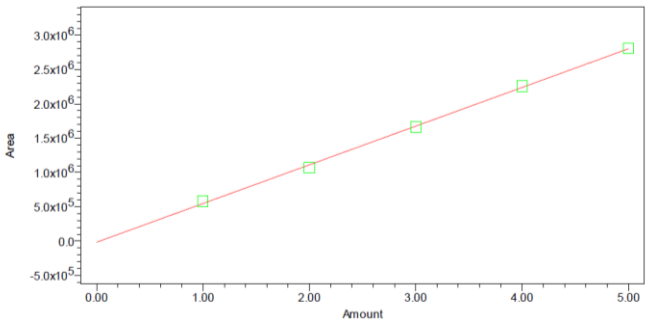
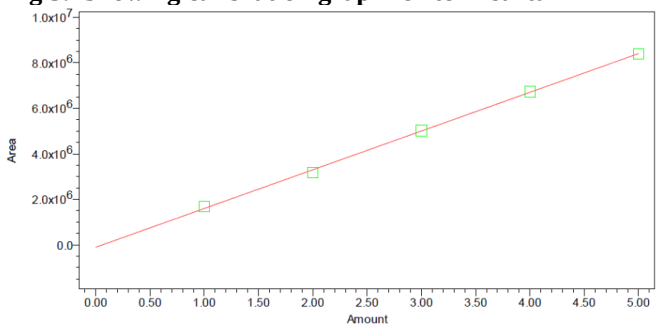


Fig 4. Showing calibration graph for metoprolol tartrate



Metoprolol tartrate $r^2 = 0.999$

Fig 5. Showing calibration graph for telmisartan



Telmisartan $r^2 = 0.999$

the amount found and amount added for metoprolol tartrate & telmisartan and calculate the individual % recovery and mean % recovery values. The results are tabulated in Table. No. 4-5.Acceptance criteria is % recovery for each level should be between 98.0 to 102.0%.

Precision

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. The results are tabulated in Table.No.6-7. Acceptance criteria % RSD for the area of five standard injections results should not be more than 2.

Robustness

The flow rate was varied at 0.6ml/min to 1.0 ml/min. Standard solution 75ppm of metoprolol tartrate & 60ppm of telmisartan was prepared and analysed using the varied flow rates along with method flow rate.

Detection limit

The LOD was performed for the concentration level of 0.03µg/ml and 0.016µg/ml for metoprolol tartrate and telmisartan. The S/N ratio was used for the calculation of LOD. The results are tabulated in Table. No.8.

Quantitation limit

The LOQ was performed for the concentration level of 0.12µg/ml and 0.05 µg/ml for metoprolol tartrate and telmisartan. The S/N ratio was used for the calculation of LOD. The results are tabulated in Table. No.9.

System suitability

10mg of metoprolol tartrate and 8mg of telmisartan working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and 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 out 0.75ml of metoprolol tartrate & telmisartan from the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent. The results are tabulated in Table.No.10-11.

CONCLUSION

A new method was established for simultaneous estimation of metoprolol tartrate and telmisartan by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of metoprolol tartrate and telmisartan by using AGILENT –ZORBAX SB-CN RP C18 column 4.5×150 mm 3.5µm , flow rate was 0.8ml/min, mobile phase ratio was (70:30 v/v) methanol : buffer pH 3 (pH was adjusted with ortho phosphoric acid), detection wave length was 226nm. The instrument used was WATER HPLC auto Sampler, Separation module 2695, UV Detector 2487, Empower-software version-2. The retention times were found to be 2.3mins and 4.2mins. The % purity was found to be 101% respectively. The system suitability parameters for metoprolol tartrate and telmisartan such as theortical plates and tailing factor were found to be 2528, 1.1 and 2924.8 & 1.2, the resolution was found to be 7. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for metoprolol tartrate and telmisartan was found in concentration range of 25µg-125µg & 20µg-100µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.8% to 100.5%, %RSD for precision was 0.40, 0.23, % RSD for intermediate precision was 0.11 respectively. The precision study was precise, robust, repeatable. LOD value was 2.95 and 2.97, and LOQ value was 9.7 and 10. Hence the suggested RP-HPLC method can be used for routine analysis of metoprolol tartrate and telmisartan in API and Pharmaceutical dosage form.

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