Research Article

Development and Validation of RP-HPLC Method for the Estimation of Telmisartan in Bulk

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ABSTRACT

The aim of the present work was to develop a RP-HPLC (Reversed-Phase High-Performance Liquid Chromatography) method for the estimation of Telmisartan in bulk. Chromatographic separation of Telmisartan was achieved by using a C18 column. A Mobile phase containing of methanol:water (90:10) was pumped at the flow rate of 1 mL/min. Detection was performed at 291 nm. Validation parameters were evaluated according to the International Conference on Harmonization (ICH) Q2R1 guidelines. The calibration curve was linear in the concentration range 5-40 µg/mL for Telmisartan with regression coefficient 0.999. RSD values were found to be 0.142 % in the case of intra-day precision studies, whereas 0.333% in the case of inter-day precision. The limits of detection and quantification were found to be 0.052, 0.16 µg/mL, for Telmisartan respectively. This method was found to be good as the percentage recovery for Telmisartan were found to be 100.145%, which indicates that the proposed method is highly accurate.

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INTRODUCTION

Analytical Chemistry deals with two major types of analysis such as qualitative analysis and quantitative analysis. Qualitative signifies identification or detection of analytes and quantitative analysis signifies the determination of the numerical concentration of the analytes. The main motto of the present study is to find out the latest innovation gap, the experimental design and new analytical tools for the analysis of drug substances. The HPLC plays an important role for analysis of various pharmaceutical dosage forms, since the method is accurate, specific, robust, linear and the limit of detection is low.[1-10]

IUPAC name for telmisartan is 4′-{[4-methyl-6-(1-methyl -1H-benzimidazol-2-yl)-2-propyl-1H-benzimidazol-1-yl] methyl}-2-biphenylcarboxylic acid. The molecular formula is C33H30N4O2 and molecular weight is 514.617.

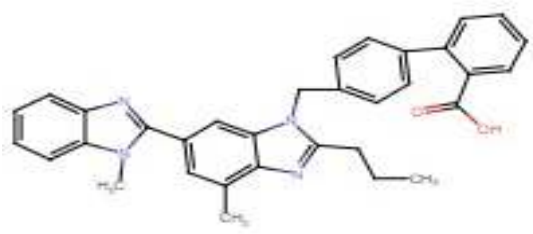

Figure 1: Telmisartan (It goes under category of antihypertensive.)

It describes as a White to off-white crystalline powder. It is practically insoluble in water, sparingly soluble in strong acid (except insoluble in hydrochloric acid), soluble in strong base. It stores protected from light and moisture [11-13]

MATERIALS AND METHODS

Instruments

The present method used HPLC (Shimadzu, Japan, Pump- LC-20AT, Detector- SPD20A), Syringe (Hamilton, Rheodyne-25µl), Syringe filter (Himedia Syringe-driven Filters, 0.22µ), Digital electric balance (Shimadzu, Japan, model-XP205), UV-Vis Spectrophotometer (Shimadzu, Japan, model- UV-1700), Ultra sonic bath sonicator (BandelinSonorex, Berlin, Germany, model- RK 102 CH Liter 3,0), Hot air oven (York Scientific Industry Pvt. Ltd., India, model- Universal).

Chemicals

Methanol, Water, Hydrogen peroxide, Hydrochloric acid, Chloroform, Sodium hydroxide, Ethanol, Acetonitrile chemicals are used for this estimation of Telmisartan.

Preparation of mobile phase

The mobile phase was prepared by mixing methanol: water (90:10). The mobile phase was sonicated and degassed.

Preparation of the standard solution

Accurately weighed 10 mg of each of the powdered drug Telmisartan was taken in a 10 ml volumetric flask and the prepared solvent HPLC grade methanol was added up to the mark which gives the

concentration of 1000 ppm. From the stock solution 1 ml of the solution was taken in a 10 ml volumetric flask and then it was made up to the mark with the same solvent to prepare the concentration of 100 ppm. From the above solution different aliquots of solution was prepared by taking 0.5,1,1.5,2,2.5,3,3.5,4 ml was taken in each 10 ml of volumetric flask separately and it was made up to mark with the same solvent to produce 5,10,15,20,25,30,35,40 ppm respectively.

Results and Discussion

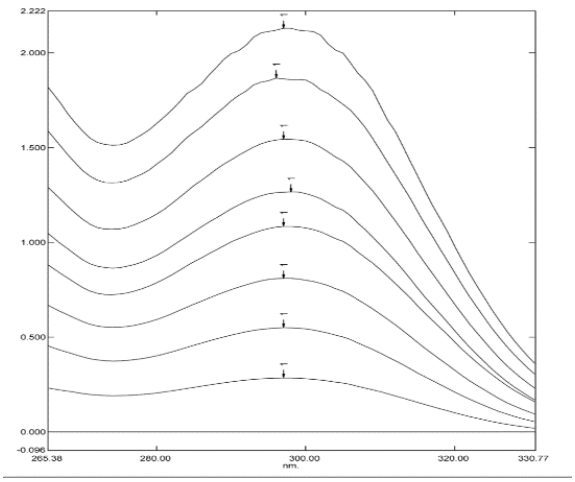

Figure 2: UV-Vis Spectra of Telmisartan

Selection of Working Wave Length:

The drug solutions of Telmisartan were scanned using UV-VIS spectrophotometer within the wavelength region of 200 – 400nm against methanol and water (90:10) as blank. The wavelength of maximum absorption (λ_{max}) of Telmisartan was found to be 291 nm in methanol :water (90:10).The resulting overlay spectra were shown in Figure 2.

Optimized Chromatographic Condition of Telmisartan for RP-HPLC

PARAMETER	CONDITION
Stationary phase(column)	ODS C-18 (250 x 4.6 mm, packed with 5 micron)
Mobile phase	Methanol : Water (90:10)
Flow rate(ml/min)	1 ml/min
Run time (min)	10 min
Column temperature (° C)	Ambient
Volume of injection (µl)	20
Detection wavelength (nm)	291 nm
Drug Rt (min)	2.803
SYSTEM SUITABILITY STUDIES	
Theoretical plate (n)	3611.275
Height equivalent to theoretical plate (HETP) (mm)	1.396 x 10^{-6}
Asymmetric factor	1.12
Efficiency/ No. of theoretical plates (N)	17, 898. 25/ Metre

Method development

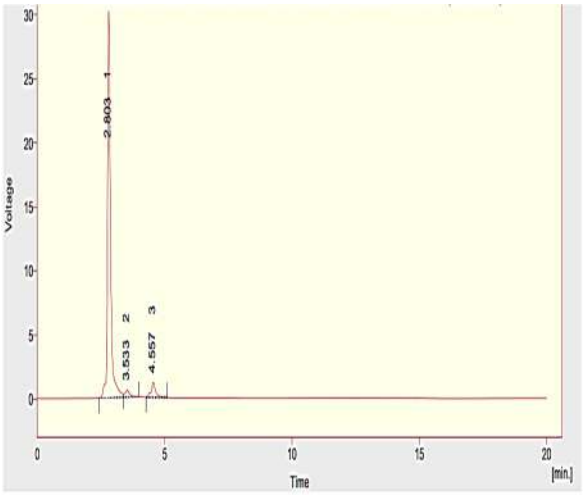

Figure 3: Overlay chromatogram of Telmisartan by HPLC

Due to the sharp peak and Rt (2.8min), which is the drug, this strength of solvent composition is used to study the HPLC analysis.

Accuracy

Accuracy of the proposed method was determined in five different sample solution of same concentration by analysing % recovery of Telmisartan and Amlodipine by standard recovery method. The result of recovery studies demonstrates accuracy of proposed method. The mean, standard deviation and % recovery were calculated and reported.

Table 1: Accuracy Data of RP-HPLC Method for Telmisartan

Sl. No.	No. of Preparation	Formulation	Pure Drug	% Recovery	Statistical Parameter
1	60%	10	6	99.320	MEAN= 99.760 SD= 0.622 %RSD= 0.623
2	60%	10	6	100.200	
3	80%	10	8	98.930	MEAN=99.515 SD= 0.827 %RSD= 0.831
4	80%	10	8	100.100	
5	120%	10	12	99.990	MEAN=100.145 SD= 0.219 % RSD= 0.218
6	120%	10	12	100.300	

Precision

The intraday and inter day precision studies of the drugs were carried out by estimating the corresponding responses on the same day and consecutive six days respectively. The results were reported in terms of standard deviation and %RSD.

Table 2: Inter Day Precession Data of the RP-HPLC for Telmisartan

Sl. No	Conc. (PPM)	Peak Area	Calculated Concentration	Statistical Parameter
1	20	497.321	20.401	Mean= 20.414
2	20	498.576	20.455	
3	20	496.258	20.356	SD= 0.068
4	20	499.491	20.494	
5	20	498.695	20.460	%RSD= 0.333
6	20	495.327	20.316	

Table 3: Intra Day Precession Data of the RP-HPLC method for Telmisartan

Sl. No	Conc. (PPM)	Peak area	Calculated Concentration	Statistical Parameter
1	20	498.364	20.446	MEAN= 20.437
2	20	497.293	20.400	
3	20	497.374	20.404	SD= 0.029
4	20	498.295	20.443	
5	20	499.127	20.478	%RSD= 0.142
6	20	498.471	20.450	

Linearity and Range

A calibration curve was plotted using the concentration on X-axis and peak area on Y-axis. The correlation coefficient R^2 was determined and the linearity was found to be 0.999. (Table 4; Figure 3).

Table 4: Calibration Table of the RP-HPLC Method for Telmisartan

Concentration (PPM)	Peak area
5	141.325
10	246.766
15	374.514
20	498.377
25	594.333
30	716.208
35	832.154
40	968.285

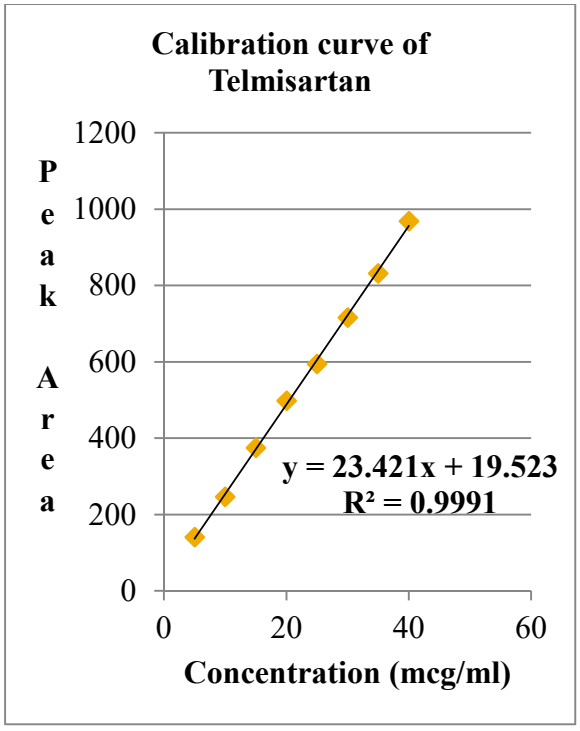

Figure 3: Calibration Curve of Telmisartan by RP-HPLC Method

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Calibration curves were plotted by using concentration in the expected detection limit range (0.1-0.5 µg/ml) of the drug. The Standard deviation of y-intercept of regression line was determined and substituted in the following equation for the determination of detection limit and quantification limit.

$$LOD = 3.3\ \sigma/S$$

$$LOQ = 10\ \sigma / S$$

Where "σ" is the standard deviation of the regression line and "S" is the slope of calibration curve.

Table 5: LOD AND LOQ data of the RP-HPLC method for Telmisartan

Conc. (ppm)	Peak area (reading 1)	Peak area (reading 2)	Peak area (reading 3)	Standard deviation
1	131.280	131.560	130.990	0.285
2	135.370	135.830	135.120	0.360
3	137.460	138.930	137.510	0.835
4	139.970	139.730	139.850	0.120
5	142.410	141.960	142.070	0.234
				Mean= 0.367

Limit of detection (LOD) = 3.3σ/S =

3.3 × 0.367/23.42 = 3.3×0.016 = 0.052

Limit of quantification (LOQ) = 10 σ/S = 10 × 0.367/23.42 = 0.16

Robustness

To verify the robustness of the method, three vital experimental variables such as composition of mobile phase, detection wavelength and flow rate were slightly varied. The analysis was performed by changing the flow rate. The data was then subjected to statistical analysis and the results are expressed in mean, standard deviation and %RSD.

Table 6: Robustness Data of the RP-HPLC method for Telmisartan for 0.9 ml/min flowrate

Sl. No.	Conc.	Flow rate	Peak area	Calculated concentration	Statistical parameter
1	20	0.9 ml/min	496.625	20.372	MEAN = 20.365
2	20	0.9 ml/min	489.792	20.079	
3	20	0.9 ml/min	499.836	20.509	SD= 0.154
4	20	0.9 ml/min	497.913	20.427	
5	20	0.9 ml/min	495.677	20.331	%RSD = 0.756
6	20	0.9 ml/min	498.964	20.471	

Table 7: Robustness Data of the RP-HPLC method for Telmisartan for 1.1 ml/min flow rate

Sl. No.	Conc.	Flow rate	Peak area	Calculated concentration	Statistical parameter
1	20	1.1 ml/min	501.283	20.570	MEAN= 20.402
2	20	1.1 ml/min	499.957	20.514	
3	20	1.1 ml/min	492.638	20.201	SD= 0.383
4	20	1.1 ml/min	498.927	20.469	
5	20	1.1 ml/min	482.387	19.764	%RSD= 1.878
6	20	1.1 ml/min	508.822	20.892	

CONCLUSION

The development RP-HPLC method was found to be suitable for the analysis of Telmisartan, in pure drug. The method was found to be fast, simple, reliable, sensitive, economical, accurate and precise. In RP-HPLC method the drug follows linearity within the range of 5-40 µg/ml. The method successfully validated in the optimized conditions. The validation parameter was within the limit.

For Telmisartan, the optimized chromatographic conditions were a reverse phase C-18 column, mobile phase methanol: water (90:10), flow rate was maintained at 1ml/min and eluents were monitored at 291 nm. Though method was found to be accurate with 0.219 standard deviation and 0.218 % relative standard deviation. The method was found to be precise, according to the repeatability data, intraday precision data and inter day precision data with the standard deviation and %RSD less than 2. The method was robust with the standard deviation and %RSD less than 2 in different flow rate. The limit of detection and limit of quantification was found to be 0.052 mcg/ml and 0.16 mcg/ml respectively.

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