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Development and Validation of RP - HPLC method for the estimation of Telmisartan in bulk and tablet dosage Form

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Abstract

A simple, precise, rapid, and reproducible RP -HPLC method was developed and validated for the determination of Telmisartan in Pharmaceutical dosage form. Separation was achieved under optimized chromatographic condition on a Zorbax-SB-18 ;(ODS) column (150 X 4.6 mm i.d., particle size 3.5µ).The mobile phase consisted of Pentane sulphonic acid sodium salt mono hydrate, add 1ml of Perchloric acid and adjust the pH-2.7±0.05 with Triethyl amine: Methanol in the ratio 40: 60 v/v. An isocratic elution at a flow rate of 1.2 ml/ min at ambient temperature. The detection was carried out at 230 nm using waters UV-Visible detector. The calibration curve was linear in the concentration range of 4–20µg/ ml (r2= 0.9999).The limit of detection and the limit of quantification were found to be 0.2515 µg/ml and 0.6623 µg/ml respectively. The amount of Telmisartan present in the formulation was found to be 99.95. The method was validated statistically using the SD, %RSD and SE and the values are found to be within the limits and the recovery studies were performed and the percentage recoveries was found to be 99.55± 0.7211 %. So, the proposed method was found to be simple, specific, linear, and rugged. Hence it can be used for applied for routine analysis of Telmisartan in the Pharmaceutical formulations.

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Key words:
Telmisartan, RP-HPLC, UV detection, Development and validation of method; Tablet dosage form.

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INTRODUCTION

Telmisartan is chemically 2-(4-{[4-methyl-6-(1-benzodiazol-1-yl] methyl} phenyl) benzoic acid. It is an angiotensin receptor blocker (ARB) that shows high affinity for the angiotensin II type 1 (AT1) receptors, has a long duration of action, and has the

longest half-life of any ARB [1]. In addition to blocking the renin-angiotensin system (RAS), telmisartan acts as a selective modulator of peroxisome proliferatoractivated receptor gamma (PPAR-γ), a central regulator of insulin and glucose metabolism. It is believed that telmisartan's dual mode of action may provide protective benefits against the vascular and renal damage caused by diabetes and cardiovascular disease (CVD). Telmisartan has binding affinity 3000 times with AT-2 receptor than AT-1 receptor. Telmisartan is also having maximum half-life in sartans - 24 hrs. (Fig.1). TST is not official in any pharmacopoeia.

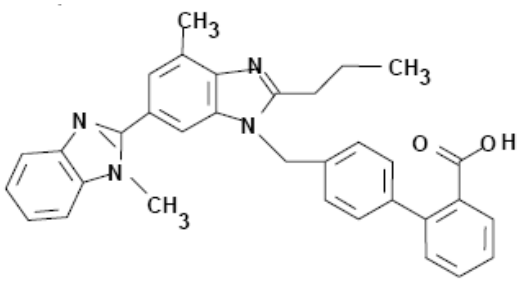

Fig.1: Chemical Structure of Telmisartan

The aim of the present work was to develop and validate a simple, fast and reliable isocratic RP-HPLC[7-13] method with UV detection for the determination of TST in bulk and in tablet dosage forms. The important features and novelty of the proposed method included simple sample treatment with sonication of small amount of powder sample at ambient temperature, short elution time (less than 5 min) TST, good precision (R.S.D.less than 2%) and high recovery (greater than 98%). Confirmation of the applicability of the developed method validated according to the International Conference on Harmonization (ICH)[14-15] for the determination of TST in bulk and in tablet dosage form.

Experimental

Instrumentation:

Quantitative HPLC was performed on a gradient High Pressure Liquid Chromatography (waters separation module) with variable wave length programmable Diode array Detector 2487, and Zorbax-SB-18 Column. The HPLC system was equipped with the software "Empower-2 series (waters)".

Preparation of standard drug solution:

Stock solutions (0.48 mg/ml) of TST (Telmisartan) was prepared by dissolving 48mg of Telmisartan in 100 ml volumetric flasks add about 75ml of diluent, add 2ml of 1N NaOH solution and sonicated for about 15 min. and then made up to volume with diluent. Dilute 5ml of this solution to 100ml with diluent. Filter the solution through 0.45µm nylon filter. The stock contains 24µg/ml.

Preparation of sample drug solution for pharmaceutical formulations:

Ten tablets were taken and pulverized. The sample of the powdered tablets were transerferd into a 1000ml volumetric flask and add about 750ml of diluent, add 20 ml of 1N NaOH solution and sonicate for about 45 min with intermittent shaking to disperse the tablets completely and makeup with diluent. Dilute 3ml of this solution to 100ml with diluent and filter through 0.45µm nylon filter; finally we get the concentration obtained was 24µg/ml

Reagents used:

- Water HPLC grade (Qualigens)
- Methanol HPLC grade (Qualigens)
- Triethyl amine ExcelaR grade (Qualigens)
- Sodium hydroxide ExcelaR grade (Qualigens)
- Pentane sulphonic acid sodium salt mono hydrate ExcelaR grade (Qualigens)
- Perchloric acid 70% w/w ExcelaR grade (Qualigens)

After systematic and detailed study of the various parameters involved, as described under results and discussion in this chapter, the following procedure was recommended for the determination of TST in bulk samples and pharmaceutical formulations.

Preparation of buffer:

Weigh and dissolve about 1g of Pentane sulphonic acid sodium salt mono hydrate in 1000ml water, add

1ml of Perchloric acid and adjust the pH-2.7±0.05 with Triethyl amine. Filter the solution through 0.45 µm membrane filter.

Preparation of mobile phase:

The mobile phase was prepared by mixing the buffer and methanol in the ratio of 40:60 v/v and the solution was filtered and degassed.

Preparation of diluent:

The diluent was prepared by mixing water and methanol in the ratio of 60:40 v/v and the solution was filtered and degassed.

Chromatographic conditions:

Column : Zorbax-SB-18, 150 mm x 4.6 mm; 3.5µ

Flow rate: 1.2 ml/min

Run time: 10 min

Column temperature: 40±2 °c

Injection volume: 10 µl

Detection wavelength: 230nm

3. Result and Discussion

3.1 Method Development

The method utilizing Methanol: Water as mobile phase yielded broad peak, whereas with MeOH: Water tailing was observed with methanol as diluent. Procedure utilizing Methanol: Water as mobile phase with water as diluents also yielded tailing where as with MeOH: Water mobile phase and acetonitrile as diluent sharp peak was obtained. During method development, a number of variations were tested like MeOH concentration and flow rate to give a symmetric peak. With a mobile phase MeOH: Water (75:25) at flow rate 1 ml min-1 and wavelength is 230 nm, symmetric peak was obtained [Fig. 2].

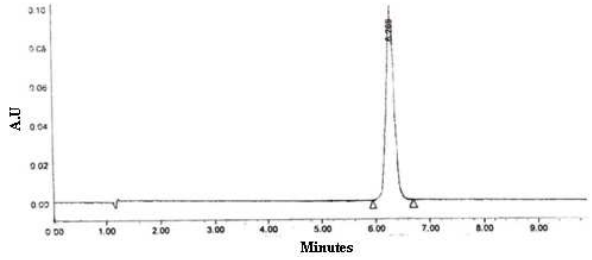

Fig. 2: Model Chromatogram for Telmisartan.

3.2 Validation

3.2.1 Linearity

Six serial dilutions were prepared in concentration range from 4 to 20 µg/ml. A volume of 20 µl from each concentration of the solution was injected and chromatograms were recorded; three independent determinations were performed at each concentration. A linear calibration graph (y = 9523x + 4253; where y and x are peak area and concentration, respectively) was obtained over six concentrations 4, 8, 12, 16, 20 µg/ml. Correlation coefficient was found to be 0.999.

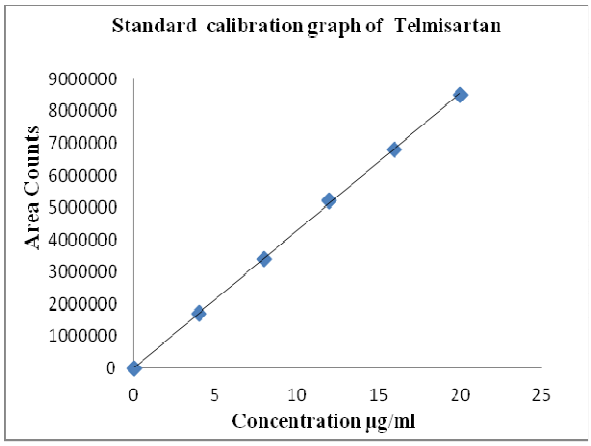

Fig 3: Standard calibration graph of Telmisartan

Table 1: Optical characteristic of Telmisartan

Parameter	TST
Retention time (t) (min)	6.269
Theoretical plates (n)	8375
Plates per meter (N)	55833
(HETP)(mts)	1.70×10^{-4}
Tailing factor (T)	1.10
Linearity range (µg ml^{-1})	4-20
Regression equation (Y = a + bC)	
Slope (b)	42653
Intercept (a)	9523
Standard error of estimation (S_e)	1.45×10^{-2}
Correlation coefficient (r)	0.999
Relative standard deviation (%)*	0.127
% Range of error (Confidence limits)*	
0.05 level	0.329
0.01 level	0.658
% Error in bulk samples**	0.037

3.2.2 Accuracy

To ensure the accuracy of the analytical method, the recovery studies were carried out. Known amount of Telmisartan was added to a pre quantified sample solution of its dosage form and the amounts of Telmisartan were estimated by measuring the peak area ratios and by fitting these values to the straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range of Telmisartan. Accuracy was evaluated at three different concentrations equivalent to 50% to 150% (50%, 100% & 150%) of the active ingredient by calculating the recovery of Telmisartan with %RSD.

Table 2: Accuracy of Telmisartan

Sample No.	Spike level	µg/ml added	µg/ml found	% Recovery	Mean% Recovery
1.	50	40	39.5	99.75	99.17
2.	50	40	39.2	98	
3.	50	40	39.9	99.75	
1.	100	80	79.5	99.35	99.16
2.	100	80	79.2	99	
3.	100	80	79.3	99.12	
1.	150	120	119.5	99.58	99.44
2.	150	120	119.1	99.25	
3.	150	120	119.4	99.5	

3.2.3 Precision

Intra-day precision of the method was determined by repeat analysis (three identical injections) at three concentration levels. Inter-day precision was established by performing the analysis next day on a freshly prepared solution. The low RSD values of Table 3 indicate the ruggedness of the method. The low RSD values indicate the ruggedness of the method.

Table 3: Precision study

No. of Injection	% Assay*
1	99.48
2	99.23
3	100.35
4	99.68
5	99.85
6	99.11
Mean	99.79 ± 0.8075
SD	0.8075
%RSD	8077

*n=6

3.2.4 Repeatability

The peak area of 16 ppm drug solution was analyzed six times on the same day. The %RSD was calculated for the resultant peak area.

Table 4: Repeatability of Telmisartan

S. No	%Assay
1.	100.1
2.	100.5
3.	100.8
4.	100.1
5.	100.5
6.	99.8
Mean	100.3
%RSD (Limit NMT 2.0%)	0.36

3.2.5 Robustness

The robustness was assessed by altering the following experimental conditions such as, by changing the flow rate from 0.8 to 1.2 ml/min, the mobile phase composition with Methanol: Water (60:43, 60:45) and analyzed in triplicate. In all varied Chromatographic conditions, there was no significant change in chromatographic parameters. There was

no effect of mobile phase composition on retention time as seen in Table 5.

System Suitability Parameters	Observed value with Flow rate			Acceptance Criteria
	0.8 ml/min	1.0 ml/min	1.2 ml/min	
Tailing factor for Tel-misartan peak	1.043	1.042	1.041	NMT 2.0
Relative standard devia-tion of Telmisartan pea k area for five injections of standard.	0.08%	0.05%	0.03%	NMT 2.0%

3.2.6. Ruggedness

The % assay and RSD for samples prepared by second analyst was calculated and found within limit. Then RSD of analyst 1 and analyst 2 was calculated and found within limit. This proved that the method is rugged, as depicted in Table 6.

Table No 6: Ruggedness Analysis (*n=6)

Analyst 1 Sample	% Assay	Analyst 2 Sample	% Assay
1	100.04	1	99.85
2	100.22	2	98.91
3	99.84	3	100.45
4	100.35	4	100.03
5	100.67	5	99.47
6	100.41	6	99.49
*Mean	100.25	Mean	99.75
SD	0.283	SD	0.193
RSD	0.271	RSD	0.159

CONCLUSION:

A RP-HPLC method has been developed for the determination of Telmisartan. The proposed method is simple, rapid, accurate and precise. Its chromatographic run time of 10 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of Telmisartan. The results of the study reveal that the proposed RP-HPLC method for the estimation of Telmisartan is simple and accurate in bulk and pharmaceutical dosage forms.

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