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RESEARCH ARTICLE

VALIDATED RP-HPLC METHOD FOR THE ESTIMATION OF TELMISARTAN IN TABLET FORMULATION

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A simple, specific, accurate and precise reverse phase high performance liquid chromatographic method is developed and validated for the estimation of telmisartan in tablet dosage form. The expected separation and peak shapes were obtained on chromosil C18 (250 mm x 4.6 mm, 5 μm) column. To have an ideal separation of the drug under isocratic conditions, mixtures of solvents like methanol, water and acetonitrile with or without different buffers in different combinations were tested as mobile phases on a chromosil C18 column. A mixture of methanol : 0.1% orthophosphoric acid : acetonitrile in the ratio of 80:05:15 *v/v/v* was proved to be the most suitable of all the combinations since the chromatographic peak obtained was better defined and resolved and was almost free from tailing. The flow rate was 1.5 ml/min and effluents were monitored at 256 nm. The retention time for telmisartan was 2.7 min. The method was validated and found to be accurate, and precise. Recovery of telmisartan from tablet formulation was found to be 99.41%. The proposed method was successfully applied for the quantitative determination of telmisartan in tablet formulation.

Key words: Telmisartan, HPLC, UV detection, Validation, Methanol, Acetonitrile.

INTRODUCTION

Telmisartan is 2-(4-{[4-methyl-6-(1-methyl-1*H*-1,3-benzodiazol-2-yl)-2-propyl-1*H* -1,3-benzodiazol-1-yl] methyl} phenyl) benzoic acid, with molecular formula $C_{33}H_{30}N_4O_2$ (**Figure 1**). It is a member of a family of drugs called angiotensin receptor blockers (ARBs), which include losartan (Cozaar), valsartan (Diovan), irbesartan (Avapro), candesartan (Atacand) and is used alone or in combination with other medications to treat high blood pressure (hypertension). High blood pressure reduction helps to prevent strokes, heart attacks, and kidney problems. It has the longest half-life of any ARB (24 h) and the largest volume of distribution. In addition to blocking the RAs, telmisartan acts as a selective modulator of peroxisome proliferato-activated receptor gamma (PPAR-γ), a central regulator of insulin and glucose metabolism. Telmisartan's dual mode of action may provide protective benefits against the vascular and renal damage caused by diabetes and cardiovascular disease (Benson *et al* 2004; PubMed Health).

For the determination of telmisartan in pure and pharmaceutical dosage forms and in biological samples, the chromatographic conditions comprised of a reversed phase C8 column (4.6 × 150 mm, 3.5 m) with mobile phase composed of buffer and methanol (40 : 60 *v/v*, adjusted the pH to 3.0 with ortho phosphoric acid). Selected mobile phase was a combination of acetonitrile:buffer (0.01 M potassium dihydrogen phosphate) in ratio 65:35, pH 4.0 adjusted with

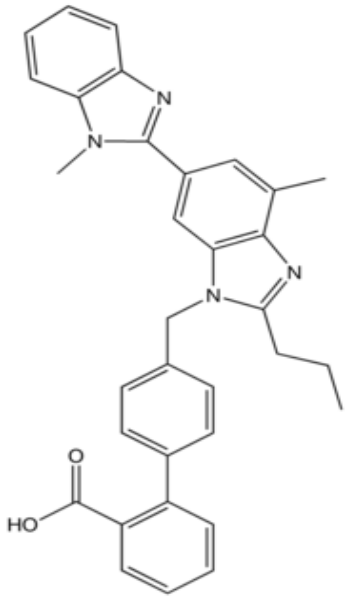

Fig. 1. Structure of telmisartan

orthophosphoric acid. The method was carried out in TLC precoated silica gel on aluminum plate 60 F 254, (10 cm ×10 cm, prewashed by methanol and activated at 60° C for 5 min prior to chromatography). A Genesis C18 column having dimensions of 4.6 × 250 mm and particle size of 5 μm in isocratic mode, with mobile phase containing a mixture of 0.01 M potassium dihydrogen phosphate buffer (adjusted to pH 3.4 using orthophosphoric acid) : methanol : acetonitrile (15:15:70 *v/v/v*) was used. Chromatography was performed on a ODS Hypersil C18 (25 cm × 4.6 mm ID) column from thermo in isocratic mode with mobile phase containing acetonitrile : 0.05 M KH_2PO_4 (pH 3.0) (60:40).

Literature is enriched with several scientific reports indicating availability of UV and RP-HPLC methods for determination of drugs (Kurade *et al* 2009; Sujana *et al* 2011; Kottai Muthu *et al* 2011; Prasanthi *et al* 2011; Bhimavarapu *et al* 2011) but yet no method is reported for the estimation of telmisartan in tablet formulation. So, in continuation of our work on developing new RP-HPLC methods for determination of drugs (Basaveswara Rao *et al* 2012), an attempt was made to develop and validate a new RP-HPLC method for the estimation of telmisartan in tablet formulation.

EXPERIMENTAL

Instrumentation

Peak HPLC containing LC 20AT pump and variable wavelength programmable UV-Visible detector and Rheodyne injector was employed for investigation. The chromatographic analysis was performed on a Chromosil C18 column (250 mm × 4.6 mm, 5 μm). Degassing of the mobile phase was done using a Loba ultrasonic bath sonicator. A Denwar analytical balance was used for weighing the materials.

Chemicals and solvents

The reference sample of Telmisartan (API) was obtained from Cipla, Mumbai. The Formulation CRESAR (Telmisartan) was procured from the local market. Methanol, acetonitrile used were of HPLC grade and purchased from Merck Specialities Private Limited, Mumbai, India. Orthophosphoric acid used was AR grade purchased from local market.

The mobile phase

A mixture of methanol : 0.1% orthophosphoric acid : acetonitrile in the ratio of 40:50:10 *v/v/v* was prepared and used as mobile phase.

The buffer and standard solution of the drug

About 1.0 ml of orthophosphoric acid was diluted to 1000 ml with water and filtered through 0.45 μ nylon filter. For analysis, 100 ppm standard solution was prepared and required concentrations were obtained from the 100 ppm solution by appropriate dilutions.

Sample (tablet) solution

The formulation tablets of telmisartan (CRESAR - 20 mg) were crushed to give finely powdered material. From the powder, 12 ppm solution was prepared in mobile phase and then filtered through Ultipor N_{66} Nylon 6, 6 membrane sample filter paper.

Method development

For developing the method (ICH Q2A, 1995; ICH Q2B, 1997; Shabir, 2003; USP 30, 2007), a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wave length and choosing stationary and mobile phases. The following studies were conducted for this purpose:

Detection of wavelength

The spectrum of 10 ppm solution of telmisartan in methanol was recorded separately on UV spectrophotometer. The peak of maximum absorbance wavelength was observed.

Choice of stationary phase and mobile phase
Finally the expected separation and peak shapes were obtained on chromosil C18 (250 mm × 4.6 mm, 5 μm) column.

Flow rate
Flow rates of the mobile phase were changed from 0.5-1.5 ml/min for optimum separation. It was found from experiments that 1.5 ml/min flow rate was ideal for elution of analyte.

Optimization of chromatographic conditions
Chromatographic conditions are required to be optimized. These optimized conditions were followed for the determination of telmisartan in bulk samples and in its formulations.

Validation of proposed method
The proposed method was validated as per ICH guidelines (Hokanson, 1994). The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification and solution stability.

Specificity
The specificity of method was performed by comparing chromatograms of blank, standard and sample (prepared from formulation).

Linearity
Linearity was performed by preparing mixed standard solutions of telmisartan at different concentration levels including working concentration mentioned in experimental condition *i.e.* 12 ppm. The response was read and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were calculated and linearity plots of concentration over the mean peak areas were constructed individually.

Intraday and interday precision
Precision of the method was performed as intraday precision and interday precision. To study the intraday precision, six replicate standard solution (12 ppm) of telmisartan was injected. The percent relative standard deviation (% RSD) was calculated.

Accuracy
The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. The standard addition method was performed at 50%, 100% and 150% level of 12 ppm. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and % RSD was calculated.

Robustness
The robustness study was performed by slight modification in flow rate of mobile phase, pH of the buffer and composition of the mobile phase. Telmisartan at 6 ppm concentration was analyzed under these changed experimental conditions.

System suitability
The system suitability was studied under each validation parameters by injecting the six replicates of the standard solution (2 ppm).

Limit of detection and Limit of quantification
Limit of detection (LOD) is the lowest concentration of analyte that gives a detectable response whereas limit of quantification (LOQ) is the lowest concentration that can be quantified reliably with a specified level of accuracy and precision. For this, sample was dissolved by using mobile phase and injected until peak was disappeared.

Formulation
The proposed method was applied to the assay of commercial tablets containing telmisartan. Sample was analyzed for five times after extracting the drug as mentioned in sample preparation for assay as described in the experimental section.

RESULTS AND DISCUSSION
To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was found in a mixture of acetonitrile : methanol : 0.1% orthophosphoric acid in the ratio of 80:15:05 *v/v* and 1.5 ml/min flow rate proved to be better than the other mixtures in terms of resolution and peak shape (**Table 1**). The chromatogram of standard (4 ppm) is shown in the **Figure 2**.
The optimum wavelength for detection was set at 256 nm at which much better detector responses for drug was obtained. The regressions of the plots were computed by least

Table 1. Optimized chromatographic conditions for estimation of telmisartan

Mobile phase	MeOH : 0.1 % OPA : ACN :: 80:05:15 *v/v/v*
Pump mode	Isocratic
Mobile phase pH	5.8
Diluent	Mobile phase
Column	Chromosil C18 column (250 mm × 4.6 mm, 5 *µ*)
Column temperature	Ambient
Wavelength	256 nm
Injection volume	20 *µ*l
Flow rate	1.5 ml/min
Run time	6 min
Retention time	2.7 min

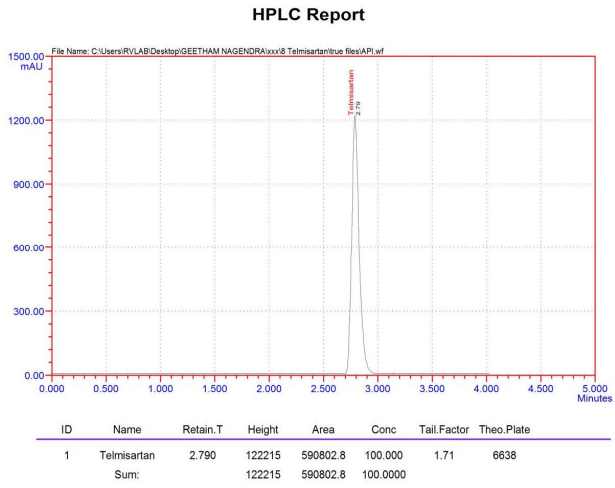

Fig. 2. Chromatogram of standard drug

square regression method. Linearity results are presented in **Figure 3**. The calibration curve was obtained for a series of concentrations in the range of 2-12 *µ*g/ml and it was found to be linear.

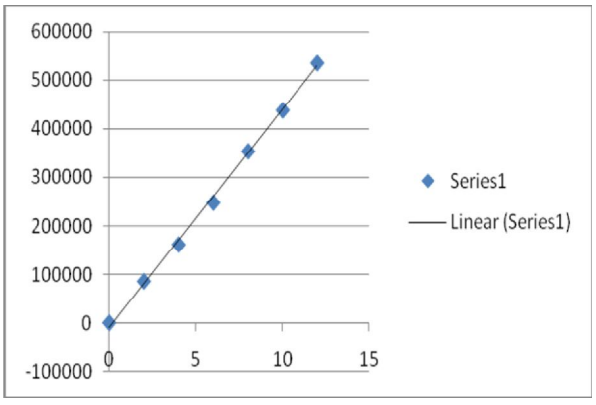

Fig. 3. Linearity studies

The standard deviation of the slope and intercept were low. Calibration curve found to be linear with r^2=0.999, intercept (-150323.5) and slope (45459.12). The results obtained were within acceptable limits where capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2000. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%.

The retention times were 2.7 min for telmisartan. The number of theoretical plates was found to be 6638.20, which indicated efficient performance of the column. It was found that there was no interference due to excipients in the tablet formulation and also showed good correlation between the retention times of standard and sample. The specificity results suggested no peak for blank and retention time 2.790 min for telmisartan. Results of system precision studies are shown in **Table 2** and **Table 3**.

The R.S.D. for intraday precision studies was found to be 0.039 and that of interday precision was 0.123, which were well within the acceptable criteria of not more than 2.0. Low values of standard deviation denoted very good repeatability of the measurement. For the interday precision, a study carried out on consecutive days indicated good method precision. Standard addition method at 50%, 100% and 150% to the proposed HPLC method was carried out to find the accuracy of the telmisartan. The results showed good recoveries ranging from 99.00 to 101.45%. The mean recovery data obtained for each level as well as for all levels combined were within 2.0% of the label claim for the active substance with an R.S.D. <2.0%, which satisfied the acceptance criteria set for the study.

Results of accuracy studies are presented in **Table 4**. Satisfactory recoveries ranging from 99.0 to 102.0 were obtained by the proposed method.

Table 2. Results of intraday precision studies

Sample	Concentration (ppm)	Injection number	Peaks area	R.S.D. (acceptance criteria ≤ 2.0%)
Telmisartan	12	1	588925.5	0.039
		2	588289.1	
		3	588470.8	
		4	588815.8	
		5	588548.2	
		6	588557.4	

Table 3. Results of interday precision studies

Sample	Concentration (ppm)	Injection number	Peaks area	R.S.D. (acceptance criteria ≤ 2.0%)
Telmisartan	12	1	586338.7	0.123
		2	585740.8	
		3	582157.1	
		4	582307.0	
		5	581967.1	
		6	586231.3	

This indicated that the proposed method was accurate. From robustness study, it was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature. The results obtained from system suitability studies were within acceptable limits (Tailing factor ≤ 2 and Theoretical plates ≥ 2000). The results are tabulated in the **Table 5** and **Table 6**.

Table 4. Percent recovery and % R.S.D. data

Level	Amount of telmisartan spiked (ppm)	Amount of telmisartan recovered (ppm)	% Recovery	% R.S.D.
50 %	6 6 6	5.98 5.95 5.95	99.66 99.16 99.16	0.289
100%	8 8 8	7.98 7.94 7.93	99.75 99.75 99.12	0.370
150%	10 10 10	9.98 9.91 9.92	99.8 99.1 99.2	0.380
			Mean % recovery = 99.41	Mean R.S.D. = 0.346

Table 5. Results of robustness studies

Condition	Mean area	% Assay	% Difference
Unaltered	590802.8	100.0	0.0
Flow rate at 1.4 ml/min Flow rate at 1.6 ml/min	585143.0 586252.8	99.04 99.22	0.96 0.72
Mobile phase MEOH: ACN: 0.1% OPA 75% 20% 05% 85% 10% 05%	 589314.9 596219.5	 99.74 100.9	 0.26 0.10
pH of mobile phase at 5.6	592326.2	100.25	0.25
pH of mobile phase at 6.0	593572.1	100.4	0.4

Table 6. Results of system suitability studies

Parameter	Tailing factor	Theoretical plates
Specificity study	1.71	6638
Linearity study	1.20	7694.97
Precision study	1.71	6705.46

CONCLUSION

The statistical evaluation of the proposed method revealed its good linearity, reproducibility and validation for different parameters and can be used for the rapid and reliable determination of telmisartan in tablet formulation. All these factors lead to the conclusion that proposed method is accurate, precise, sensitive and rapid and can be applied successfully for estimation of telmisartan in bulk and pharmaceutical formulations without interference and with good sensitivity.

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