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A Validated RP-HPLC Method for the Determination of Telmisartan In Bulk and Pharmaceutical Dosage Form

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Abstract

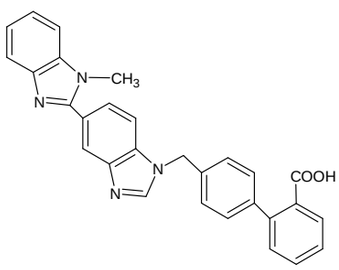
A RP-HPLC method has been developed and validated for the estimation of telmisartan in bulk and pharmaceutical dosage form. A RP-HPLC isocratic separation was achieved on C18 column (250×4.6 mm i.d., 5µm) utilizing a mobile phase comprising of methanol and acetonitrile in the ratio of 90: 10(v/v) and the eluents from the column were detected using a variable wavelength detector at 237nm. The proposed method has permitted the quantification of telmisartan in the linearity range of 20-100µg/ml and the flow rate was maintained at 1ml/min. The column was maintained at ambient temperature and the complete separation was achieved for telmisartan in an overall analytical run time of approximately 10 minutes. The retention time of telmisartan was found to be 3.3 minutes. The limit of detection and limit of quantification were found to be 2.82 and 8.54 µg/ml, respectively. The percentage recovery was found to be in between 87.3 to 103.18%. The method was found to be suitable for the routine quality control analysis of telmisartan in bulk drug and formulation. The method was validated as per ICH guidelines.

Keywords: Telmisartan, RP-HPLC, ICH guidelines, Validation

1. Introduction

Telmisartan is an angiotension receptor blocker that shows high affinity for the angiotension II, type 1 receptors, has a long duration of action, and has the longest half-life of an ARB. In addition to blocking the Renin Angiotensin System (RAS), telmisartan acts as a selective modulator of Peroxisome proliferators' activated receptor gamma (PPAR-γ), a central regulator of insulin and glucose metabolism. Telmisartan is chemically described as 4'- [(1, 4'-dimethyl-2'-propyl[2,6'-bi-1H-benzimidazol]-1'-yl) methyl]-[1,1'-biphenyl]-2-carboxylic acid. Its empirical formula is $C_{33}H_{30}N_4O_2$, As shown in Figure.1, its molecular weight is 514.63. Literature survey reveals that a few analytical methods like HPTLC, HPLC and some spectroscopic methods have been reported for the estimation of telmisartan alone and in combination with other drugs. The objective of this study was to develop the method with less economical, precise, simple and sensitive.

Figure 1: Chemical Structure of Telmisartan



2. Materials and Methods

2.1 Materials

Telmisartan was obtained as a gift sample from Unichem laboratories Ltd, Raigad. The commercially available tablet, "TELMA" 20 mg (Pfizer Ltd., Mumbai) containing 20 mg of telmisartan was procured from the local market and used for analysis. Acetonitrile, methanol and water used were of HPLC grade and purchased from Merck specialties private limited, Mumbai, India. All the glassware employed in the study was cleaned with hot water, followed by acetone and dried in hot air oven whenever required.

2.2 Instrumentation

Analysis of samples was performed by using JASCO PU 2080 HPLC system, a variable wavelength programmable UV/VIS detector with precision loop injector (Rhenodyne, 20 µl). The data was processed by using BORWIN 6.0 software. All samples were filtered through 0.45 µm membrane, Millipore filtration apparatus with vacuum pump.

2.3 Chromatographic conditions

The isocratic method was employed with the mobile phase consisting of 90 volumes of methanol and 10 volumes of acetonitrile. The chromatographic column used was a HiQ Sil C-18 Column with dimensions of 250×4.6 mm with 5 µm particle size. The column was maintained at ambient temperature and detection was performed at a wavelength of 237 nm. Prior to injection of analyte, the column was equilibrated for 30-40 minutes with mobile phase. The injection volume was 20 µL. Methanol was used as diluent for preparation of solutions.

2.4 Preparation of mobile phase

The HPLC grade solvents of methanol and acetonitrile were used for the preparation of mobile phase in the ratio of 90:10 (v/v). The contents of the mobile phase were filtered before use through a 0.45µm membrane filter, degassed by sonication and pumped from the solvent reservoir to the column at a flow rate of 1ml/min throughout the analysis.

2.5 Preparation of standard stock solution

Standard stock solution was prepared by dissolving the 10 mg of telmisartan in 10 ml methanol to obtain primary stock solution of 1000 µg/ml. From the primary stock solution; 5ml of the solution was pipette out and diluted up to 10 ml with methanol to get final concentration of 500 µg/ml.

2.6 Analysis of tablet formulation

Twenty tablets were accurately weighed and triturate thoroughly to get fine powder. The powder equivalent to 20 mg of telmisartan was weighed and transferred into 25ml volumetric flask. The contents of the flask were dissolved in the 10 ml of the methanol with the aid of ultrasonication for 10 minutes. The solution was filtered through whatmann filter paper no. 41 and volume was made up to 25ml with methanol. From the resultant solution, further dilutions were prepared with methanol to get final concentration of telmisartan. The absorbance's were measured at selected wavelengths and the concentration of the analyte was determined with the equation obtained from calibration curve. The results of assay of tablets are shown in Table 3.

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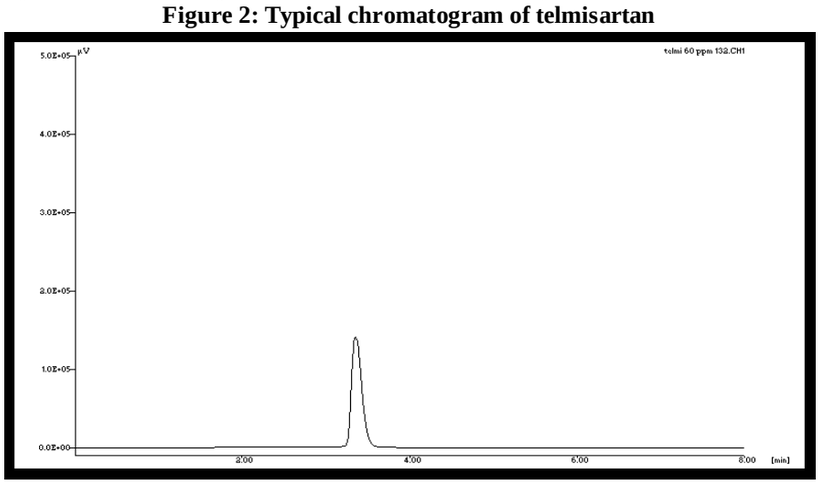
2.7 Procedure

A mixture of methanol and acetonitrile in the ratio of 90:10 v/v was prepared. The solvent mixture was filtered through a 0.45µm membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1 ml/min. The column was maintained at ambient temperature. The column was equilibrated by pumping the mobile phase through the column for at least 30 minute prior to the injection of the drug solution. The detection of the drug was monitored at 237 nm. The run time was set at 10 minute. Under these optimized chromatographic conditions the retention time obtained for the drug was 3.3 minute. A typical chromatogram showing the separation of the drug is given in Figure 2.

Figure 2: Typical chromatogram of telmisartan



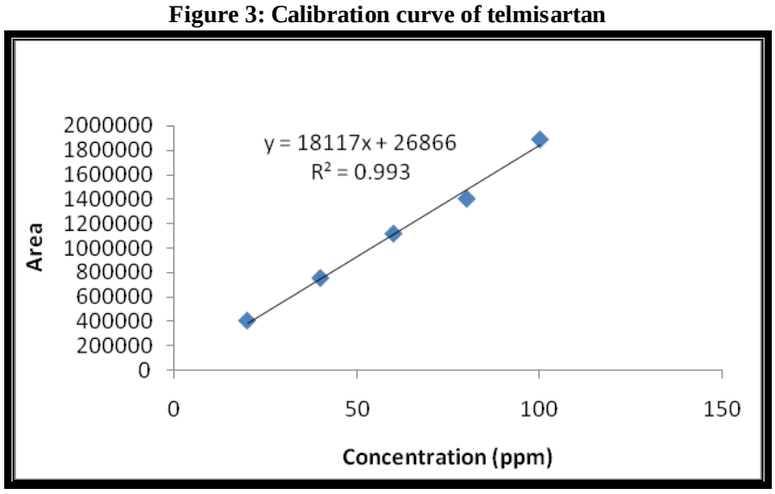
2.7.1 Preparation of calibration graph

Preparation of Level – I (20, 40, 60,80,100 µg/ml): 0.4, 0.8, 1.2, 1.6 and 2 ml of stock solution has taken in 10 ml of volumetric flask diluted up to the mark with diluent.

Procedure:

Injected each level into the chromatographic system and measured the peak area. Plotted a graph of peak area versus concentration and calculated found to be linear in the concentration range of 20-100 µg/ml of the drug (Figure 3).

Figure 3: Calibration curve of telmisartan



The relevant data are furnished in Table 2. The regression equation of this curve was computed. The regression equation was later used to estimate the amount of telmisartan in tablet dosage forms.

2.7.2 Validation of the proposed method

The specificity, linearity, precision, accuracy, limit of detection, limit of quantification and robustness parameters were studied systematically to validate the proposed HPLC method for the determination of telmisartan.

2.7.3 LOD and LOQ

In this study, LOD and LOQ were based on the standard deviation of the response (σ) and the slope of the corresponding curve (S) using the following equation:

$$LOD = 3.3\,\sigma/S, \quad LOQ = 10\,\sigma/S$$

Where, σ is the standard deviation of the response of blank

S is the slope of calibration curve

The LOD and LOQ of telmisartan were found to be 2.82 µg/ml and 8.54 µg/ml respectively.

2.7.4 Precision

Precision is the measure of how close the data values to each other for a number of measurements under the same analytical conditions. Precision of the method was determined by performing inter day variation, intraday variation and repeatability studies. Three replicate injections of the specific standard (3 different concentrations) at various time intervals on the same day were injected into the chromatograph and the value of % RSD was found to be within the limits. In inter day precision, same standard concentrations were injected on different days and % RSD was also found to be within the limits for telmisartan. In repeatability study, six determinations of the fixed concentration of telmisartan were analyzed separately. The results of precision data are given in Table 4.

2.7.5 Robustness

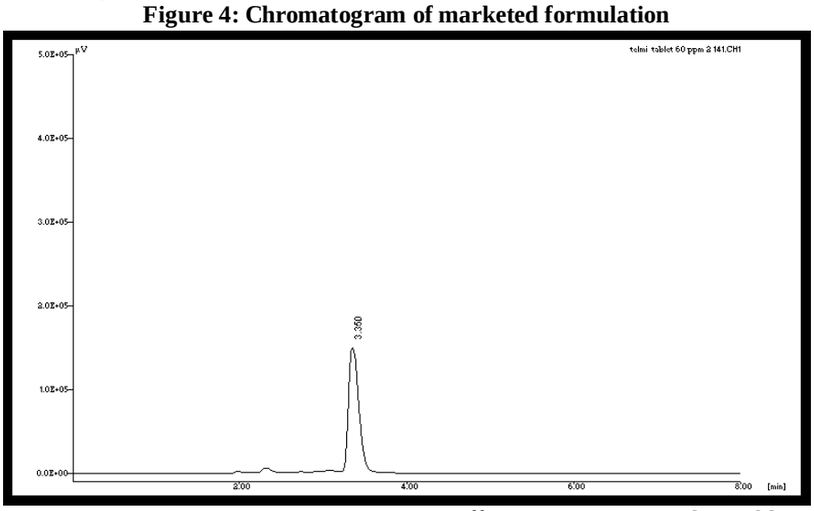
To determine the robustness of the developed method, experimental conditions were purposely altered and the resolution was evaluated. The flow rate of the mobile phase was 1 ml/min. To study the effect of flow rate on resolution, it was changed by 0.1 units from 0.9 to 1.1 ml/min. The effect of percent organic strength on resolution was studied by varying composition of mobile phase by 2 units from

methanol: acetonitrile (88:12 v/v) to methanol: acetonitrile (92:08 v/v). The results are given in Table 5.

2.7.6 Specificity

Marketed formulation were analyzed to determine the specificity of the optimized method in the presence of excipients and it was observed that single peak for telmisartan (Rt 3.3 min) were obtained under optimized conditions (Figure 4), showing no interference from excipients and impurities. Also the peak area were compared with the standard and % purity calculated was found to be within the limits.

Figure 4: Chromatogram of marketed formulation



2.7.7 Accuracy (Recovery studies)

The accuracy of the proposed method was determined by calculating the recoveries of telmisartan by the standard addition method. It was determined by preparing solutions of different concentrations at 80%, 100% and 120% of 60µg/ml in which the amount of marketed formulation was kept constant (30 µg/ml) and the amount of pure drug was varied that is 10µg/ml, 30µg/ml and 50µg/ml for 80%, 100% and 120% respectively. The amount of telmisartan recovered was estimated by applying obtained values to the regression line equation. The results of % recovery are given in Table 6.

3. Results and Discussions

A simple RP-HPLC method has been developed for determination of telmisartan. The method was optimized to provide a good separation of the component (acceptable theoretical plates) with a sufficient sensitivity and suitable peak symmetry in a short run. For this purpose, the analytical column, solvent selection, mobile phase composition, flow rate, and detector wavelength were studied. The chromatographic separation was achieved using an RP C18 column. Our experiments using methanol along with acetonitrile (HPLC grade) as mobile phase was eluted the telmisartan in a significant shorter retention time of 3.3 minutes. Therefore, we selected methanol and acetonitrile in the ratio of 90:10 (v/v) as a mobile phase. The method has many advantages, e.g., simplicity, isocratic conditions, and absence of buffers in the mobile phase that could damage the chromatographic column and equipment. Under these conditions, the retention time of telmisartan was about 3.3 minute, with a good peak shape (peak symmetry). The optimized chromatographic conditions are given in Table 1.

Table 1: Optimized chromatographic conditions

Parameters	Optimized conditions
Chromatograph	HPLC (Jasco with UV detector)
Column	HiQ sil C-18 HS (5 µm; 250 × 4.6 mm)
Mobile phase	Methanol : acetonitrile (90 : 10v/v)
Flow rate	1 ml/min
Detection wavelength	237 nm
Injection volume	20 µl

Table 2: Statistical data for linearity and calibration range

Parameters	Telmisartan
λmax (nm)	237nm (methanol)
Linearity range	20-100µg/ml
Correlation coefficient (r^2)	0.993
Regression equation (Y = mx + c)	y = 18117x + 26866
Slope (m)	18117
Intercept (c)	26866
LOD	2.82µg/ml
LOQ	8.54µg/ml

Table 3: Analysis of tablet formulation

Formulation	Concentration (ppm)	Percentage of drug estimated	Statistical analysis
Tab. Telma	60	58.42	Mean-58.94 %RSD-0.89
	60	59.47	
	60	58.94	

Table 4: Precision data

Days	Fortified amount (ppm)	Amount found (ppm)	% RSD
Intraday (n = 3)	40	39.39	1.48
	60	58.94	0.86
	100	101.13	1.36
Interday (n = 3)	40	37.79	1.29
	60	58.57	0.98
	100	100.86	1.46
Repeatability (n = 6)	60	57.99	0.51

Table 5: Results of robustness study

Chromatographic conditions	Normal	Variation	Drug amount	%RSD
Change in composition Mobile phase (methanol : acetonitrile)	90 : 10	88 : 12	54.44	0.86
		92 : 08	49.72	0.54
Flow rate(ml/min)	1.0	0.9	55.31	0.53
		1.1	60.93	0.76

Table 6: Results of % recovery in tablet formulation

Concentration % of spiked level	Amount of drug added (ppm)		Amount of pure drug found (ppm)	% Recovery	Statistical analysis of % recovery
	Pure drug	Formulation			
80% sample 1	18	30	48.50	101.68	Mean-103.18 %RSD-1.53
80% sample 2	18	30	49.45	104.83	
80% sample 3	18	30	48.91	103.03	
100% sample 1	30	30	60.28	100.96	Mean- 100.87 %RSD-1.82
100% sample 2	30	30	59.69	98.99	
100% sample 3	30	30	60.80	102.67	
120% sample 1	42	30	68.26	87.55	Mean- 87.3 %RSD-1.87
120% sample 2	42	30	67.65	85.55	
120% sample 3	42	30	68.42	88.80	

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4. Conclusion

A validated RP-HPLC analytical method has been developed for the determination of telmisartan in bulk and dosage form. The proposed method was simple, accurate, precise, specific and suitable to use for the routine analysis of telmisartan in either bulk API powder or in pharmaceutical dosage forms. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments such as LC–MS and GC–MS. These methods are complicated, costly and time consuming rather than a simple HPLC-UV method.

References

1. Sethi PD. Quantitative analysis of drugs in pharmaceutical formulations, Third edition, CBS Publishers 1997; 5-60.
2. ICH Q2B, Harmonized Triplicate Guideline, Validation of analytical Procedure Methodology. IFPMA, Proceeding of the International Conference on Harmonization. Geneva, March 1996.
3. ICH, Q2A, Harmonized Tripartite Guideline, Validation of analytical Procedure Methodology. IFPMA, Proceedings of the International Conference on Harmonization. Geneva. March 1994.
4. Telmisartan, Information from Drugs. com., http://www.drugs.com Monograph/ Telmisartan. html (Accessed on June 2012)
5. Gowri SD, Sujana K. ‘Stability indicating RP-HPLC method for determination of telmisartan in pure and pharmaceutical formulation’, *International Journal of Pharmacy and Pharmaceutical Sciences* 2011; 231-240.
6. Kale DM. ‘Stability indicating RP-HPLC method for estimation of telmisartan related substances in tablet formulation’, *International Journal of Research Pharm. Sciences*.2010; 1: 100-109.
7. Mahdik KR, Agrawal H. ‘Stability indicating RP-HPLC method for analysis of telmisartan in the bulk drug and formulation’, *Acta Chromatographica*.