

Research Article



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

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ABSTRACT

A simple, rapid, and precise reversed-phase High performance liquid chromatographic (RP-HPLC) method has been developed and validated for the determination of telmisartan in bulk and pharmaceutical dosage form. Chromatographic separation of telmisartan was achieved on a reverse phase C18 column using a mobile phase consisting of acetonitrile: phosphate potassium buffer (pH= 3): methanol in the ratio of 40:20:40 v/v. The mobile phase was pumped at a flow rate of 1 mL/min and the eluents were monitored at 295 nm. The method was successfully validated in accordance to ICH guidelines acceptance criteria for (linearity, accuracy, precision, selectivity, limit of detection, limit of quantification) all validation parameters were within the acceptance range. The proposed method was found to be suitable and accurate for quantitative determination of telmisartan in bulk samples and pharmaceutical preparation and it can be used for the quality control of formulation products.

Keywords: Telmisartan, Estimation, RP-HPLC, Validation, Tablets.

INTRODUCTION

Telmisartan (TELM), 4-((2-*n*-propyl-4-methyl-6-(1-methylbenzimidazol-2-yl)-benzimidazol-1-yl)methyl)-biphenyl-2-carboxylic acid (Fig.1), is a new highly selective, non-peptide angiotensin II type 1 (AT1)-receptor antagonist that lowers blood pressure through blockade of the rennin angiotensin – aldosterone system (RAAS).¹ It is widely used in treatment of hypertension. It can selectively block the angiotensin II type 1 (AT1) receptors with high affinity², causing inhibition of the action of angiotensin II on vascular smooth muscle, ultimately leading to a reduction in arterial blood pressure. It is useful in treatment of mild to moderate hypertension and well tolerated with lower incidence of cough than ACE inhibitors with a long terminal elimination half-life of 24h.³⁻⁵ Recent studies suggest that telmisartan may also have PPAR-gamma agonistic properties that could potentially confer beneficial metabolic effects.⁶⁻⁷ Telmisartan has become one of the most important advances in the treatment of hypertension.

A few spectroscopic⁸, HPTLC⁹, High performance liquid chromatographic (HPLC) methods coupled with fluorescence detection¹⁰ and LC-MS^{11, 12} methods were reported earlier for the determination of telmisartan in bulk and pharmaceutical dosage forms. But the HPLC methods using the most commonly available columns and detectors like UV were few.

The aim of this study is to develop a rapid, sensitive, accurate and precise reverse phase HPLC method for the estimation of telmisartan in bulk samples and in tablet dosage forms.

The results of analysis were treated statically, as per International Conference on Harmonization (ICH) guidelines¹³, for validation of analytical procedure.

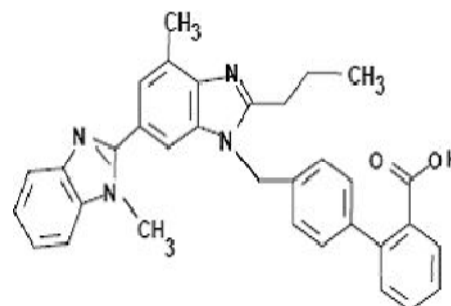


Figure 1: Chemical structure of telmisartan

MATERIALS AND METHODS

Instrumentation

The analysis of drug was carried out on a SHIMADZU HPLC system equipped with a reverse phase C18 column (250x4.6mm, 5µm in particle size), a 20µl injection loop and SPD-20A prominence UV/VIS detector, Degasser DGU-20A3.

Reagents and solutions

The reference sample of telmisartan was supplied by Sigma-Aldrich Chemical Co. (Hamburg, Germany).

HPLC grade water, methanol and acetonitrile were purchased from Merck Ltd., Mumbai (India).

Potassium dihydrogen phosphate and ortho phosphoric acid were obtained from BDH Laboratory Supplies (Poole, UK).



Chromatographic conditions

Chromatographic separation of telmisartan was achieved on a reverse phase C18 column in a mobile phase consisting of acetonitrile: phosphate potassium buffer (pH=3): methanol in the ratio of 40:20:40 v/v. The mobile phase was pumped at a flow rate of 1ml/min and the eluents were monitored at 295nm.

Stock solution

Accurately weighed 10 mg of telmisartan (TELM) was transferred to a 100 ml volumetric flask, 50 ml of methanol was added and allowed to sonicate for 15min and finally volume was made up to the mark by methanol. Standard stock solution of TELM (100µg/ml) was prepared.

Working solutions were prepared daily by appropriate dilution of this stock solution.

Preparation of phosphate buffer (pH 3.0)

1.3609 g of KH₂PO₄ was weighed into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC water. pH adjusted to 3.0 with orthophosphoric acid.

Preparation of mobile phase

200 ml of the phosphate buffer was mixed with 400 ml of acetonitrile and 400 ml of Methanol. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45µm filter under vacuum.

Optimization of the chromatographic Conditions

Proper selection of the stationary phase depends upon the nature of the sample molecular weight and solubility. The drug telmisartan is non-polar. Non-polar compounds preferably analyzed by reverse phase columns. Among C8 and C18, C18 column was selected. Non-polar compound is very attractive with reverse phase columns. So the elution of the compound from the column was influenced by polar mobile phase. Mixture of buffer, acetonitrile and Methanol was selected as mobile phase and the effect of composition of mobile phase on the retention time of telmisartan was thoroughly investigated.

The chromatographic conditions were optimized through several trials to achieve good resolution and symmetric peak shapes, as well as a short run time. It was found that a mixture of acetonitrile – phosphate buffer – methanol (40:20:40 v/v) was appropriate.

Procedure

A mixture of buffer, acetonitrile and Methanol in the ratio of 40:20:40 v/v was found to be the most suitable mobile phase for ideal separation of telmisartan. The solvent mixture was filtered through a 0.45 µm membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1ml/min. The column was equilibrated by pumping the mobile phase through the column for at least 30 min prior to the injection of the drug solution. The detection of the drug was monitored at 295nm. The run time was set at 10min.

Under these optimized chromatographic conditions the retention time obtained for the drug was 6.6min. A typical chromatogram showing the separation of the drug is given in (Fig. 2).

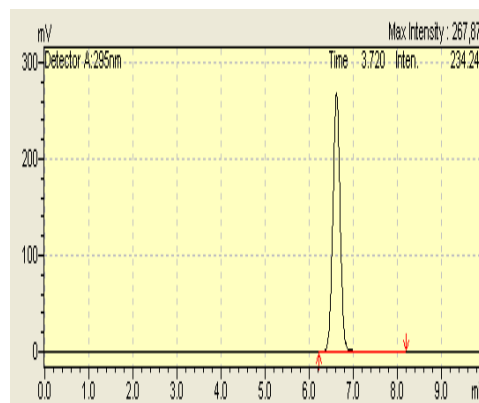


Figure 2: Typical chromatogram of Telmisartan

Validation procedure

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision (repeatability and intermediate precision), accuracy, specificity, and system suitability. Standard plots were constructed with five concentrations in the range of 40µg/ml to 60µg/ml to test linearity. The peak area of telmisartan was plotted against the concentration to obtain the calibration graph (Fig.3). To check the degree of accuracy of the method, recovery studies were performed in triplet by standard addition method at 80%, 100% and 120% concentration levels. Results of recovery studies are shown in Table 1. The precision of the assay was studied with respect to both repeatability and intermediate precision. The intra- and inter-day variability or precision data are summarized in Table 2.

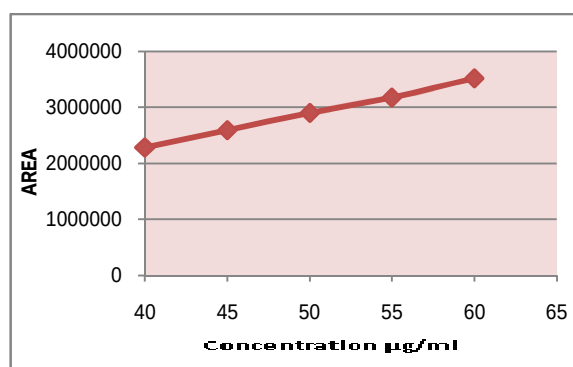


Figure 3: Calibration curve for telmisartan

Table 1: Recovery studies

Concentrations	Recovery %	Mean recovery %
80 %	98.87	99.55
100 %	99.95	
120 %	99.84	

Table 2: Precision of the proposed HPLC method

Telmisartan	Intra -day	Inter-day
%RSD	0.37	0.8

Specificity of the method was found out through non-interference of the blank, excipients peaks and standard peak.

The system suitability parameter like tailing factor and number of theoretical plates were also calculated. The system suitability parameters are given in Table 3.

Table 3: System suitability parameters

Parameter	Result
Correlation coefficient	0.999
Retention time (min)	6.6
Theoretical plates (N)	7367
Tailing factor	1.027
LOD ($\mu\text{g/ml}$)	0.07
LOQ ($\mu\text{g/ml}$)	0.25

Estimation of telmisartan in tablet dosage forms

Two commercial brands of tablets were chosen for testing the suitability of the proposed method to estimate telmisartan in tablet formulations.

Twenty tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 40mg of telmisartan was transferred into a 50ml volumetric flask and dissolved in 25ml methanol. The contents of the flask were sonicated for 15 min and the volume made up to 50ml and mix well, the solution was filtered through a 0.45 μ membrane filter. This solution was injected into the column and the amount of the drug present in the tablet dosage form was calculated. The relevant results are furnished in Table 4.

Table 4: Assay and recovery studies

Formulation	Label Claim (mg)	Amount found (mg) *	% Label claim
Formulation 1	40	38.6	96.5
Formulation 2	40	40.6	101.5

* Mean of three determinations

RESULTS AND DISCUSSION

In the proposed method, the retention time of telmisartan was found to be 6.6min. The number of theoretical plates calculated was 7367, which indicates efficient performance of the column. The limit of detection and limit of quantification were found to be 0.07 $\mu\text{g/ml}$ and 0.25 $\mu\text{g/ml}$ respectively, which indicate the sensitivity of the method.

The high percentage of recovery indicates that the proposed method is highly accurate.

The precision (measurements of intraday and interday) results showed good reproducibility with percent relative standard deviation (RSD %) are below 2.0. This indicated that the method is highly precised.

The results of assay indicate that the amount of each drug in the tablets is within the requirements of 90–110% of the label claim.

No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drug by the proposed HPLC method. The results were found to be accurate, reproducible and free from interference and better than the earlier reported methods.

CONCLUSION

A simple, accurate, sensitive and precise HPLC method has been developed for the determination of telmisartan in bulk and tablet dosage form. The proposed method was found to be suitable and accurate for quantitative determination of telmisartan in bulk samples and pharmaceutical preparation and it can be used for the quality control of formulation products.

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