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HPLC Determination of Telmisartan in Bulk and Pharmaceutical Formulations

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A rapid and sensitive high performance liquid chromatographic method was developed for the estimation of telmisartan in pharmaceutical dosage forms. Telmisartan was chromatographed on a reverse phase C_{18} column in a mobile phase consisting of methanol : acetonitrile : buffer (pH 2.8) in the ratio of 20 : 70 : 10 v/v. The mobile phase was pumped at a flow rate of 1 mL/min and the eluents were monitored at 290 nm. The calibration curve was linear in the range of 10–1000 ng/mL. The intra- and inter-day variation was found to be less than 1% showing high precision of the assay method. The mean recovery of the drug from the solution containing 50 ng/mL was 96.6 ± 1.13% indicating high accuracy of the proposed HPLC method. Due to its simplicity, rapidness, high precision and accuracy the proposed HPLC method may be used for determining telmisartan in bulk drug samples and pharmaceutical dosage forms.

Key Words: Telmisartan, Reverse phase HPLC, Dosage forms.

INTRODUCTION

Telmisartan (TMS) is nonpeptide angiotensin II receptor antagonist, used in hypertension[1]. TMS is 4′-[(1,4′-dimethyl-2′-propyl[2,6′-bi-1H-benzimidazole]-1′-yl)methyl]-[1,1′-biphenyl]-2-carboxylic acid. A few analytical methods like polarography[2], electrophoresis[3,4] and HPLC[5] have been reported for the estimation of telmisartan. All the methods considered were tedious. The HPLC methods using the most commonly available columns and detectors like UV were preferred. The present study describes the determination of telmisartan in bulk drug samples and pharmaceutical dosage forms by using RP-C_{18} column with UV detector. Owing to the widespread use of HPLC in routine analysis, it is important that well validated HPLC methods are to be developed for estimating telmisartan. The aim of this study is to develop a simple, precise, rapid and accurate reverse phase HPLC method for the determination of telmisartan in bulk drug samples or in pharmaceutical dosage forms.

In the present study, a simple, economical, accurate and reproducible analytical

method with better detection range for estimation of telmisartan in pure form and in its solid dosage forms was developed. The results of analysis were treated statistically, as per International Conference on Harmonization (ICH) guidelines, for validation of analytical procedures and by recovery studies. The results were found to be accurate, reproducible and free from interference and better than the earlier reported methods.

This method describes a reverse phase high performance liquid chromatographic method (LC method) using RP-C_{18} column in methanol : acetonitrile : buffer, pH 2.8 (20 : 70 : 10) with UV detection at 290 nm. Liquid chromatography was attempted to demonstrate the utility of UV detection for the estimation of telmisartan coupled with simple and economical mobile phase and reasonable analysis time with high precision. The method was also assessed for suitability as stability indicating assay. In this proposed method there is no need to extract the drug from the formulation excipients matrix thereby decreasing the error in quantities. Formulation sample can be directly used after dissolving and filtration. The developed method was used to estimate the total drug content in commercially available capsules of telmisartan. The results of the analysis were validated by statistical methods[6, 7] and recovery studies.

EXPERIMENTAL

Telmisartan was obtained as a gift sample from Aristo pharmaceuticals, Bhopal, India. HPLC grade acetonitrile and water was purchased from Qualigens. Commercially available capsules of telmisartan were selected from the local market on a random basis. These capsules normally contain common additives like diluents (lactose, aerosil, etc.), glidants and lubricants (magnesium stearate, etc.).

For LC estimation an isocratic HPLC (Waters) with Waters 510 HPLC pump, equipped with a 20 µL sample loop, and Waters 486 tunable absorbance detector. The output signal was monitored and integrated using Millenium NT workstation software.

Chromatographic conditions: The chromatographic column used was a reverse phase 4.6 × 250 mm Bondapak C_{18} HPLC column (Waters) with 5 µm particles. The column and the HPLC system was kept in ambient conditions. The mobile phase was methanol : acetonitrile : buffer, pH 2.8 (20 : 70 : 10) delivered at a flow rate of 1.0 mL/min. The injection volume was 20 µL. The eluate was analyzed at a wavelength of 290 nm.

Method development: To develop a rugged and suitable LC method for the analysis of telmisartan in formulations, different solvent systems were used. The criteria employed for assessing the suitability of a particular solvent system for the drug was cost, time required for analysis, sensitivity of the assay, solvent noise, preparatory steps involved and use of the same solvent system for extraction of the drug from the formulation excipients matrix for estimation of the drug content.

Preparation of standard curve for LC method

A stock solution (1000 µg/mL) of pure drug was prepared by dissolving 100 mg of telmisartan and 100 mg of ethamsylate (internal standard) separately in 100 mL volumetric flasks containing 70 mL of methanol, sonicated for at least 15 min and then made up to volume with methanol. Daily working standard solutions of telmisartan and internal standard were prepared by suitable dilution of the stock solution with appropriate mobile phase.

Composition and flow rate of the mobile phase was programmed from mother pump and the mobile phase methanol : acetonitrile : buffer, pH 2.8 (20 : 70 : 10) was passed through the 0.45 µm membrane filter using Millipore HPLC solvent filtration assembly and delivered at 1.0 mL/min for column stabilization. During this period, the baseline was continuously monitored. The wavelength of detection was selected at 290 nm. The prepared dilutions were injected serially. The obtained peaks were integrated and the area under the peak was calculated. The stability of the solution of telmisartan during analysis was determined by repeated analysis of samples during the course of the experiment on the same day and also on different days after storing at laboratory bench conditions and under refrigeration. The results are listed in Table-2. Chromatogram parameters, retention time and asymmetry factor were standardized.

(a) Accuracy and precision: Five separate solutions of telmisartan (50 ng/mL) standard and test solution were prepared in duplicate from freshly prepared stock solution and analyzed as per the procedure given above.

(b) Linearity: Five separate series of solutions of the drug 10–1000 ng/mL were prepared from the stock solution and analyzed.

(c) Specificity: Series of five solutions of the drug 20 ng/mL were prepared from the stock solution meant for method validation and analyzed.

(d) Limit of detection (LOD) and quantitation (LOQ): LOQ and LOD were calculated on the basis of signal to noise ratio. Experiments were performed to analyze the actual concentration that can be accurately quantified or detected by the method.

(e) Ruggedness: It was determined for the method by varying the analyst, instrument and different columns of it for LC method.

(f) Robustness: The robustness of LC method the % of acetonitrile was varied (40, 50, 60%) and the effect on retention time and peak parameters studied.

Estimation of telmisartan from the commercial capsules by the proposed method

Commercially available capsules of telmisartan (brands A and B) were taken randomly from the Indian market for estimation of total drug content per capsule by the proposed method. 20 capsules were weighed and the contents were thoroughly mixed and accurately weighed. Aliquot amount (equivalent to 100 mg telmisartan) was dissolved in 20 mL of methanol. The weighed amount 100 mg of active ingredient was extracted with methanol and made to get a stock solution

of 1 mg/mL. This solution was filtered through a 0.45 µm membrane filter. This solution was further diluted stepwise with mobile phase as under preparation of standard solution to get the different concentrations required. The area under the curve (LC method), the drug content per capsule (on an average weight basis) were calculated (Table-3).

RESULTS AND DISCUSSION

Method development

Again in case LC method for the determination of telmisartan different mobile phases was employed. Initially a mobile phase consisting of methanol : acetonitrile : buffer in the ratio of 20 : 60 : 20 was tried. Symmetry RP-C18 column 250 mm was used. Early elution with tailing of peaks was observed in the above condition. Then the composition of mobile phase was changed to 30 : 60 : 10; under these conditions broad peaks shape and pronounced tailing was observed. For the same mobile phase, if the ratio was changed to 20 : 70 : 10, telmisartan was eluted at around 4.0 min with symmetric peak shape.

LC method

A typical chromatogram for telmisartan using C_{18} RP HPLC column with mobile phase, composed of methanol : acetonitrile : buffer (20 : 70 : 10) at 1.0 mL/min flow rate is shown in Fig. 1. The λ_{max} of detection was fixed at 290 nm.

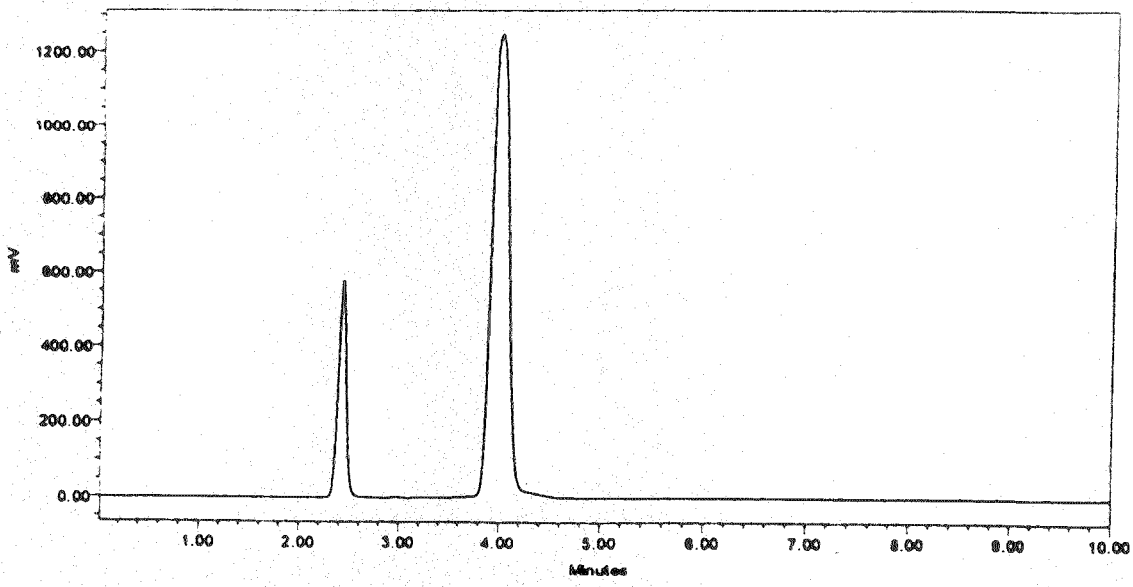

Fig. 1. Typical chromatogram of telmisartan

The calibration curve, area *vs.* concentration (ng/mL), was found to be linear. The values obtained for the calibration curve points and their standard deviation, coefficient of variance and standard error are presented in Table-1. Statistical calculations were done at 5% level of significance. The low values of standard deviation, standard error and coefficient of variation (Table-1) established the

precision of the proposed method. The drug was stable during analysis and for a period of 48 h stored at room temperature and under refrigerated condition in methanol : acetonitrile : buffer (20 : 70 : 10) mixture.

CHROMATOGRAPHIC CONDITIONS

Parameters	Method
Stationary phase (column)	Bondapak C-18 (250 × 4.6 mm, packed with 5 micron)
Mobile Phase	Methanol : acetonitrile : buffer (20 : 70 : 10) (pH 2.8)
Flow rate (mL/min)	1.0
Column back pressure (psi)	1540
Run time (min)	15
Column temperature (°C)	Ambient
Volume of injection loop (µL)	20
Detection wavelength (nm)	290
Internal standard	Ethamsylate
Drug RT (min)	4.0
Internal standard RT (min)	2.5

The correlation coefficient value was highly significant (Table-1). The reported slope value without intercept on ordinate, at 95% confidence limits, suggested that the calibration curve of telmisartan solution did not deviate from the origin and the above values were within the confidence limits (Table-1). The retention time was found to be 4.01 ± 0.04 min.

TABLE-1
OPTICAL AND REGRESSION CHARACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED HPLC METHODS FOR TELMISARTAN

Parameter	Method
Detection wavelength (nm)	290
Linearity range (ng/mL)	10–1000
Detection limits (ng/mL)	0.00128
Regression equation (Y = a + bC)	
Slope (b)	0.7560
Standard deviation of slope (S_b)	0.00196
Intercept (a)	0.00084
Standard deviation of intercept (S_a)	0.00324
Standard error of estimation (S_e)	0.00309
Correlation coefficient	0.9999
Relative standard deviation (%)*	0.0571
% Range of error (Confidence limits)*	
0.05 level	0.0477
0.01 level	0.0706
% error in bulk sample†	0.0691

*Average of eight determinations †Average of three determinations.

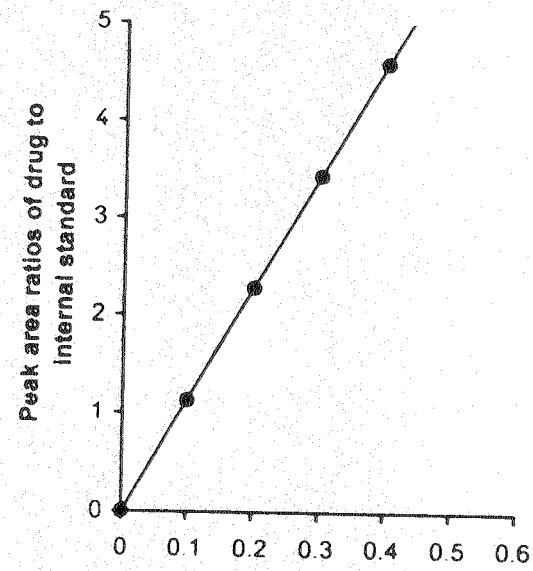

Fig. 2. Standard calibration graph of telmisartan

Intra-day, Inter-day precision

In order to assess the within-day precision of the assay procedure in standard solutions, eight replicate measurements of three solutions at different concentration levels were performed on the same day. The intra-day precision of the assay was assessed by the repeated analysis of telmisartan standard solutions over a period of eight consecutive days. Each day's representable peak area ratio, for the three concentration levels tested, was the mean value of six replicate injections. The results are shown in Table-2.

TABLE-2
INTER- AND INTRA-DAY PRECISION FOR TELMISARTAN ASSAY IN PHARMACEUTICAL DOSAGE FORMS BY THE PROPOSED HELC MPTHODS

Concentration of telmisartan (ng/mL)	Observed concentration of telmisartan (ng/mL)			
	Intra-day		Inter-day	
	Mean (n = 5)	% CV	Mean (n = 5)	% CV
50	49.70	0.55	47.80	0.62
70	70.01	0.19	68.25	0.62

Validation of the developed methods

The developed methods were validated according to the standard procedures[6, 7] and the results obtained are tabulated in Table-1. The linearity range of telmisartan solutions was found to be 10–1000 ng/mL. Since the reported slope values without intercept fell within 95% confidence limits for the proposed method, the linearity characteristics of the proposed method could be practically considered as 1–1000 ng/mL. The limit of detection (LOD) is (0.18 ng/mL) while

the limit of quantitation is (LOQ) (0.58 ng/mL). The lowest quantity accurately quantified was 0.58 ng/mL and the lowest quantity detected was 0.18 μg/mL (but it was omitted from the calibration curve due to constraint of signal-to-noise ratio requirement). The proposed method was found to be rugged when analyst or equipment of column was varied.

Recovery studies

The method was evaluated by estimation of telmisartan in pharmaceutical formulations by the proposed method and analysis of pure drug solution as reference. The results are presented in Table-3. The estimated drug content with low values of standard deviation established the precision of the proposed method. The accuracy of results of estimation was further tested by recovery experiments by adding known amount of pure drug to pre-analyzed samples of the formulations. The average accuracy was found to be 98.65%. Common formulation excipients in the concentration normally used had no effect. Recovery experiments using the developed assay procedures further indicated the absorbance of interference from commonly encountered pharmaceutical excipients used in the selected formulations.

TABLE-3
ESTIMATION OF TELMISARTAN IN CAPSULES

Pharmaceutical formulation	Labeled amount (mg)	Amount obtained by proposed method	% Recovery of proposed method
Capsules	40	39.87	99.6
Capsules	40	39.68	99.2

Conclusions

The proposed methods were found to be simple, precise, accurate and rapid for determination of telmisartan in pure form pure and its dosage form. As the LOQ of the proposed method is very low (0.58 ng/mL), the method can be adopted for sensitive quality testing and dissolution studies. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence, this can be easily and conveniently adopted for routine analysis of telmisartan in pure form and its dosage forms and can also be used for dissolution or similar studies.

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