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RESEARCH ARTICLE

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF TELMISARTAN IN BULK AND FORMULATION USING FLUORESCENCE DETECTOR.

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ABSTRACT

Asensitive, accurate and precise RP-HPLC method using Fluorescence detector has been developed and validated for the estimation of TELMISARTAN from bulk drug and Pharmaceutical Dosage form. The separation was achieved by a Prontonsil ODS analytical C18 column (250mm X 4.6mm, 5µm) in isocratic mode, with mobile phase comprises of Acetonitrile : Buffer in proportion of 90:10v/v, buffer was Phosphate Buffer (pH 2.4 adjusted with Ortho Phosphoric Acid). The flow rate of mobile phase was 1.0ml/min and employing fluorescence detection with 259 nm excitation and 399 nm emission wavelengths. The retention time of TELMISARTAN was 2.6min. The calibration curve was found to be linear within the concentration range of 10ng/ml to 90ng/ml. The regression data for calibration curve shows good linear relationship with r^2 = 0.9981. The method was validated in accordance with the requirements of ICH guidelines. Moreover, the proposed analytical method was applied to monitor the formulation commercially available.

Key words: Telmisartan (TEL), Validation, RP-HPLC, Fluorescence.

INTRODUCTION:

Telmisartan is an angiotensin II receptor antagonist (ARB) used in the management of hypertension. Generally, angiotensin II receptor blockers (ARBs) such as Telmisartan bind to the angiotensin II type 1 (AT1) receptors with high affinity, causing inhibition of the action of angiotensin II on vascular smooth muscle, ultimately leading to a reduction in arterial blood pressure. Recent studies suggest that telmisartan may also have PPAR-gamma agonistic properties that could potentially confer beneficial metabolic effects. Chemically it is 2-(4-{[4-methyl-6-(1-methyl-1H-1,3-benzodiazol-2-yl)-2-propyl-1H-1,3-benzodiazol-1-yl]methyl}phenyl)benzoic acid Figure 1.

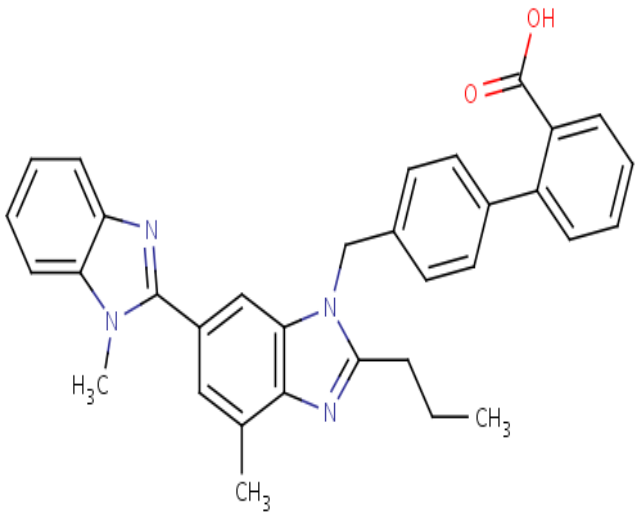

Figure 1: Structure of Telmisartan

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Several analytical techniques are available for estimation of Telmisartan in bulk dosage form by HPLC, HPTLC and UV Spectrophotometric method[4-14]. Keeping this objective in mind an attempt has been made to develop and validate the HPLC-fluorescence method for the analysis of Telmisartan which would be highly sensitive, having good resolution and reproducible. Various validation aspects of the analysis such as accuracy, precision, and the limits of detection and quantification etc. have been measured as per ICH guidelines[15].

MATERIALS & METHODS:

Material:

The HPLC system consisted of following components: Perkin-Elmer-Model Series 200 and software –Turbo chrome. Rhenodyne valve with 20µl fixed loop, quaternary gradient system pump, Chromatographic analysis was performed on Brownlee Analytical C18 column 250×4.6 mm, 5µm particle size.

Analytically pure Telmisartan was procured as gift samples from Torrent research centre, Ahmedabad, Gujarat, India. All other chemicals and reagents used were analytical grade and purchased from Merck Chemicals, India. Tablets were procured from the local market.

Methods:

Preparation of standard stock solution and solutions for calibration curve stock solutions of Telmisartan was prepared by dissolving 10 mg of Telmisartan in 10 ml of volumetric flask with methanol. Aliquot of 0.1ml of the standard stock solution of Telmisartan were transferred using A-grade bulb pipette into 100 ml volumetric flask and from that appropriate aliquots were taken to give concentration range of 10-70ng/ml for calibration curve.

Determination of Telmisartan in tablet dosage form:

Twenty tablets were weighed, finely powdered, and an accurately weighed sample of powdered tablets equivalent to 10 mg of Telmisartan was treated with mobile phase in a 10mL volumetric flask using ultra sonicator. This solution was filtered through 0.45 µm filter paper. Suitable aliquot of the filtered solution was added to a volumetric flask and make up to volume with mobile phase to get appropriate concentration in range

Chromatographic conditions:

Chromatographic estimation was performed using an equilibrated Prontonsil ODS C18 column (250mm×4.6mm, 5µ), mobile phase consisting Acetonitrile: Buffer in proportion of 90:10v/v, buffer was 5mM Phosphate buffer (pH 2.5 adjusted with Ortho Phosphoric Acid). Detection was done at excitation wavelength of 259 nm and 399 nm emission wavelengths. The sample was injected using a 20µl fixed loop, flow rate 1ml/min and the total run time was 10 minutes.

Validation:

The method was validated as per the ICH guideline.

Regression analysis- Regression of analytical method is expressed in terms of correlation co-efficient of the regression analysis. Accuracy- For determination of Accuracy, recovery study was carried out. That was performed by standard addition method at three different levels (50%, 100%, and 150%), to the pre-analyzed samples and the subsequent solutions were re-analyzed. At each level, three determinations were performed.

Precision- The precision of analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogeneous samples. Intraday precision- Intraday variance for the Telmisartan was done at the interval of 3 hrs. Interday precision- Interday variance for the Telmisartan was done at the interval of one day.

Limit Of Detection (LOD)- LOD was found out based on the standard deviation of the response and the slope method. Limit Of Quantification (LOQ)- LOQ was found out based on the standard deviation of the response and the slope method. Specificity- Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present.

RESULTS AND DISCUSSION:

Several mobile phase compositions were tried to resolve the peak of TEL. The mobile phase containing Acetonitrile : Buffer in proportion of 90:10v/v, buffer was 5mM Phosphate buffer (pH 2.5 adjusted with Ortho Phosphoric Acid)was found ideal to resolve the peak of TEL. Retention time of TEL was 2.65min [Figure 2]. Quantification was achieved by fluorescence detector with 259nm excitation and 399nm emission wavelengths.

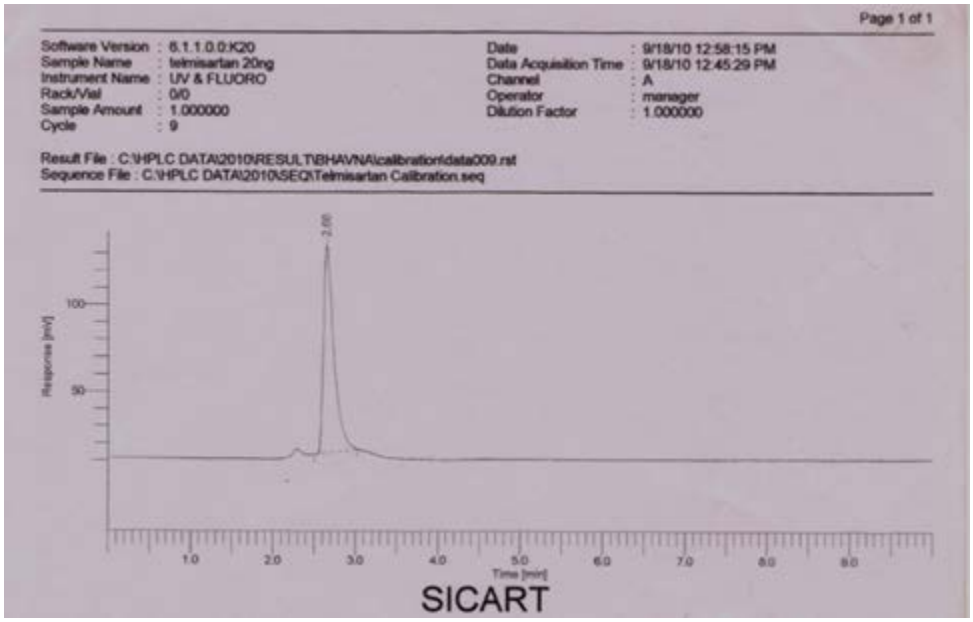

Figure 2: Chromatogram of standard Telmisartan solution (20 ng/ml)

The system suitability parameters are shown in [Table 1].

Table 1: System suitability parameters

Parameter	TEL
Retention time	2.65±0.02 min
No. of theoretical plate	2045.6
Tailing factor	1.5
Capacity Factor	0.76

Linear regression data showed a good liner relationship over a concentration range of 10-90ng/ml for TEL. The correlation coefficients (r^2) were 0.9981 [Figure 3].

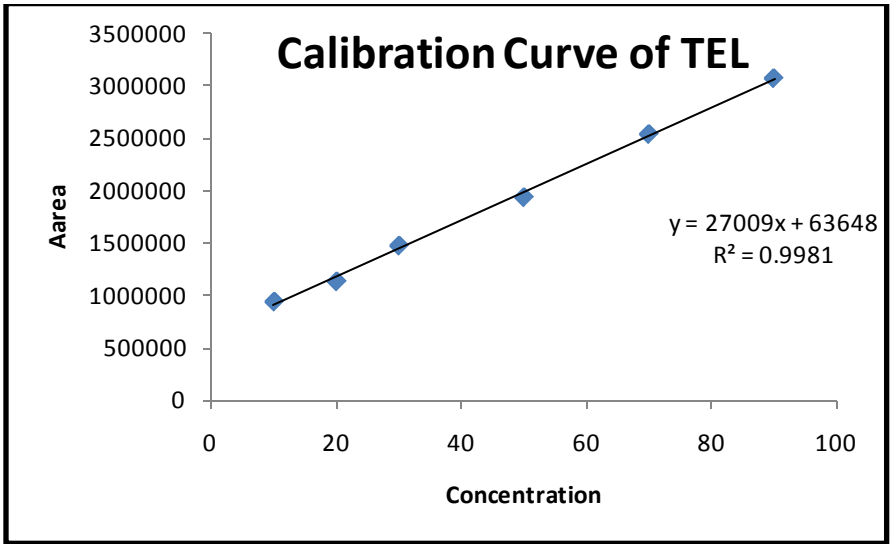

Figure 3: Calibration Curve of Telmisartan

The accuracy of the method was evaluated by carrying out recovery studies, were performed by standard addition method at three different levels I, II and III (50%, 100%, and 150%), to the pre-analyzed samples and the subsequent solutions were re-analyzed. At each level, three determinations were performed [Table 2].

Table 2: Determination of Accuracy for TEL

Level	Labelled amount (mg per tablet)	Amount obtained (mg per tablet)	Average assay Recovery (%)
Level I 50%	40	40.2	100.5
Level II 100%	40	40.4	101.0
Level III 150%	40	39.8	99.5

The limit of detection and limit of quantification were found to be 3.36 ng/ml and 9.16 ng/ml respectively. The intra-day and inter day precision was determined by analyzing standard solution of 20, 30 and 50ng/ml and the results are reported in terms of relative standard deviation shown in Table 3.

Table 3: Intra-Day and Inter-Day study of TEL

Conc. (ng/ml)	Intra-Day Area Mean (n=3) ± SD	%RSD	Inter-Day Area Mean (n=3) ± SD	%RSD
20	1156276 ±14578.67	1.2	1117514 ± 21033.91	1.8
30	1458540±15849.17	1.0	1459463± 16789.63	1.1
50	1939130± 21754.14	1.1	1915265 ± 26119.95	1.3

The assay result was repeated for three times and average was found to be 99.6% of labeled claim [Table 4].

Table 4: Assay Result of Marketed formulation

Formulation	Labelled claim	Amt. Recovered TEL	% Assay
TABLET	10mg per tablet	9.96 mg	99.6

CONCLUSIONS:

A method of quantitative determination of Telmisartan using HPLC with fluorescence detector has been developed. The validation results have demonstrated that this method is accurate, precise, linear, specific and sensitive. The method can also be applied for drug content in pharmaceutical preparations.

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