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Simple and stability indicating RP-HPLC assay method development and validation of telmisartan in bulk and dosage form

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ABSTRACT

A new simple, accurate, precise, sensitive and validated by RP-HPLC method was developed for the estimation of Telmisartan in bulk and pharmaceutical dosage form. The Chromatographic conditions used for the separation was Zorabax SBC18(150x4.6 MM, 5µ) and the mobile phase comprised of Acetonitrile and (0.1ml Phosphoric acid and 0.2ml Try Ethyl Amine in100mlof Triple distilled Milli-Q-water) Buffer (35:65 v/v). The flow rate was 1.2 ml/ 1minute with the detection at 234 nm. The Assay method was validated as per ICH guidelines. The retention time was found to be 5.332 minutes. The linearity was found to be in the range of 0.1 – 0.6 mg/ml (25% to 150%) with correlation coefficient (r) 0.9996. The proposed method is accurate with 99.8576% - 99.988% recovery for Telmisartan and precise. %RSD of repeatability, intraday and inter day variations were 0.2125 - 0.98. The method can be successfully applied to pharmaceutical formulation.

Key words: Telmisartan, Phosphoric acid, RP-HPLC, Method development and validation.

INTRODUCTION

Telmisartan (TEL) is chemically known as 4[(1, 4-dimethyl-2-propyl (2, 6-bi-1H-benzimidazol]-1-yl) methyl] [1, 1-biphenyl]-2-carboxylic acid (Fig. 1). Telmisartan is a potent, long lasting, orally acting nonpeptide antagonist of angiotensin II Type 1receptor (AT1) used in the management of hypertension. It selectively inhibits stimulation of the AT1 receptor by angiotensin II without affecting other receptor systems involved in cardiovascular regulation [1]. The most recent clinical trials demonstrated that Telmisartan also has preventive roles against ischemic heart diseases in diabetic patients with a similar potency to angiotensin converting enzyme inhibitor. Several studies recently [2] suggest that the effects of Telmisartan are mediated via not only blockade of ARB but also activation of peroxisome proliferators-activated- γ receptor (PPAR -γ) a central regulator of insulin and glucose metabolism. It is believed that Telmisartan dual mode of action may provide protective benefits against the vascular and renal damage caused by diabetes and cardiovascular disease (CVD). Diabetes Mellitus, hypertension and obesity are the most common diseases of this era. The insulin resistance associated with obesity contributes to the development of cardiovascular risk factors such as dyslipidemia, hypertension, and type 2 diabetes. The risk of type 2 diabetes and hypertension are strongly related to obesity and central distribution of fat [3].The coexistence of hypertension and diabetes increases the risk for macrovascular and microvascular complications, thus predisposing patients to cardiac death, congestive heart failure, coronary heart disease, cerebral and peripheral vascular diseases, nephropathy, and retinopathy [4]. However, achieving and maintaining good glycemic control has always been a challenge, implying the need of an adjunct therapy. Antihypertensive treatment in diabetics decreases cardiovascular mortality and slows the decline in glomerular function. The structural similarity of Telmisartan to Pioglitazone is expected to be useful in the treatment of both hemodynamic and biochemical aspects of type 2 diabetes [5,6]. TEL is official in IP [7], BP [8], USP [9]. Some analytical methods for the quantitative determination of TEL in pharmaceutical formulations are

described in literature like Titrimetric [10], voltametry [11], Spectroflourimetric [12], UV spectrophotometric [13], HPLC [14-20], HPTLC [21], LC–MS/MS [22], method in human plasma have been reported.

Telmisartan has some published methods for estimation of assay and impurity profile by HPLC and UV/visible spectroscopy techniques. The objective of the research is to develop a simple RP-HPLC Assay method. Method validation has performed as per the ICH and regulatory guidelines and review articles were revealed for method development and validation.

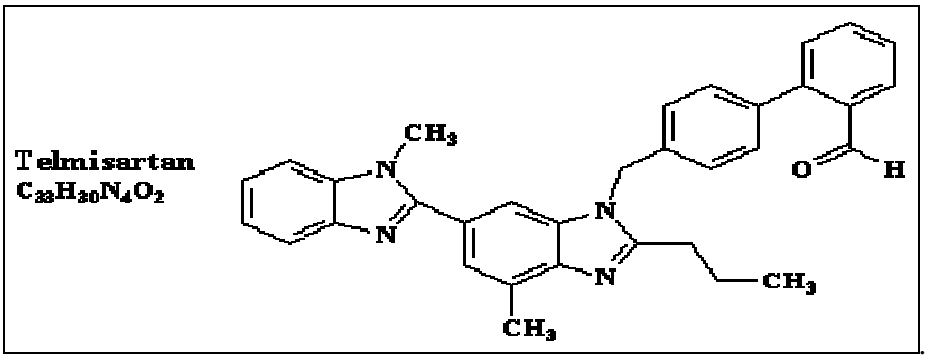


Figure-1: Structure of Telmisartan

MATERIALS AND METHODS

Reagents and Materials:
The reference sample of Telmisartan was supplied as a gift sample from Hetero labs limited, Hyderabad, Telangana. The commercially available Telmisartan (AXETEN-40mg, MSD) solid dosage forms were procured from the local market. Triple distilled Milli-Q-water was used throughout this research. HPLC grade Acetonitrile, Milli-Q-water, analytical grade Phosphoric acid and Try Ethyl Amine was used in this method.

Chromatograhic Parameters:
The chromatography was performed on a LC 10 AT vp HPLC instrument (Shimadzu Corporation, Japan) equipped with SPD-10A vp detector, SCL- HT A autosampler and CTO-AS vp column oven.The data was monitored with LC solutions software.Zorbax SB-C18(150x4.6 mm, 5µ,Agilent technologies,USA) was used as stationary phase. The flow rate was set at 1.2 ml/ 1min.An injection volume of 10µl was used for the analysis.The detector was monitored at 234nm.The column Temperaturewas maintained at 50^0C. Sartorius BT 224s analytical balance was used for this research experiments.

Selection of mobile phase:
Based on sample solubility, stability and suitability various mobile phase compositions were tried to get a good resolution and sharp peaks. The standard solution was run in different mobile phases. From the various mobile phases tried, the following compositions were given good response to the Drug solution.

Preparation of buffer solution:
0.5ml of Phosphoric acid and 1.0ml of Try Ethyl Amine were dissolved in 500 ml of mill-Q-water mixed thoroughly.

Preparation of Mobile phase:
Mobile phase was prepared by Mixing Phosphoric acid buffer and Acetonitrile in the ratio 35:65 v/v. The mixture was filtered and degassed through 0.45µm membrane filter paper.

Preparation of Diluent:
The same mobile phase was used as diluents for the preparation of standards and test samples.

Preparation of standard stock solutions:
25 mg of Telmisartan standard was accurately weighed and transferred into a 25 ml of volumetric flask and was initially dissolved in 15 ml of Methanol. The solution is then made up to a volume with 10ml of diluent so as to obtain a stock solution of 1 mg/1 ml. From the stock suitable dilutions were prepared.

Preparation of calibration curve:
Aliquots of 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 ml standard stock solution (1mg/ml) was transferred to the 10 ml of volumetric flasks and made up to the mark with mobile phase to get concentration of 10, 20, 30, 40, 50 and 60µg/ml.The fixed standard solution was prepared by transferring 3 ml of Telmisartan standard solution to 10 ml of volumetric flask and made up to the mark with mobile phase to get 30 µg/ml of Telmisartan.

Sample preparation (Tablet):
One tablet of Telmisartan (AXETEN-40mg) was taken and recorded the weight. Ground the tablet in a Agate mortar and weighed a quantity equivalent to 10mg (about 59.5mg) of Telmisartan. Transferred to10ml volumetric flask and added 5ml of diluent. The flask was shaken for 15 min and diluted to the mark with diluent. The solution was then filtered through 0.45 micron membrane filter and solicited. From this stock solution, 3ml is transferred to 10 ml volumetric flask dilute and made up to the mark with mobile phase.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT:
To develop simple and stability indicating RP-HPLC method for Telmisartan (AXETEN-40mg, MSD) determination, several research experiments were performed with different salt, acid buffers and mobile phase compositions. Finally, satisfactory separation with high peak symmetry were obtained with Zorbax SBC18 (150x4.6 mm, 5µ) and the mobile phase comprised of Acetonitrile and Phosphoric acid (pH adjusted 4.8) in the ratio of (35:65 v/v)at a flow rate of 1.2 ml/min to get better reproducibility and repeatability. Quantification was achieved with UV at 234 nm based on peak area. The retention time was found to be 5.33 min. The optimized method was validated as per ICH and other regulatory guidelines. System suitability, specificity, linearity, accuracy, robustness and ruggedness were performed.

Table 1: Optimized Chromatographic Conditions

Mobile phase	Acetonitrile and Phosphoric acid in the ratio of (35:65 v/v)
Stationary phase	Zorabax SBC18(150x4.6 MM, 5µ)
Wavelength	234nm
Run time	15 min
Flow rate	1.2 ml/min
Injection volume	10 µl
Temperature	50^0C
Mode of operation	Isocratic elution
PH	4.8

METHOD VALIDATION:
The optimized Chromatographic method has high accuracy, linearity and all method validation parameters were performed and reported below.

System suitability test:
10 µL of the standard solution (0.3 mg/ml) was injected under optimized chromatographic conditions to evaluate the suitability of system. The values of system suitability parameters were shown in Table 2.

Table 2: System Suitability Test Parameters

System suitability parameters	Result
Retention time	5.332
Area	13219908(0.3mg/ml)
Theoretical plate number	3561
Tailing factor	2.2

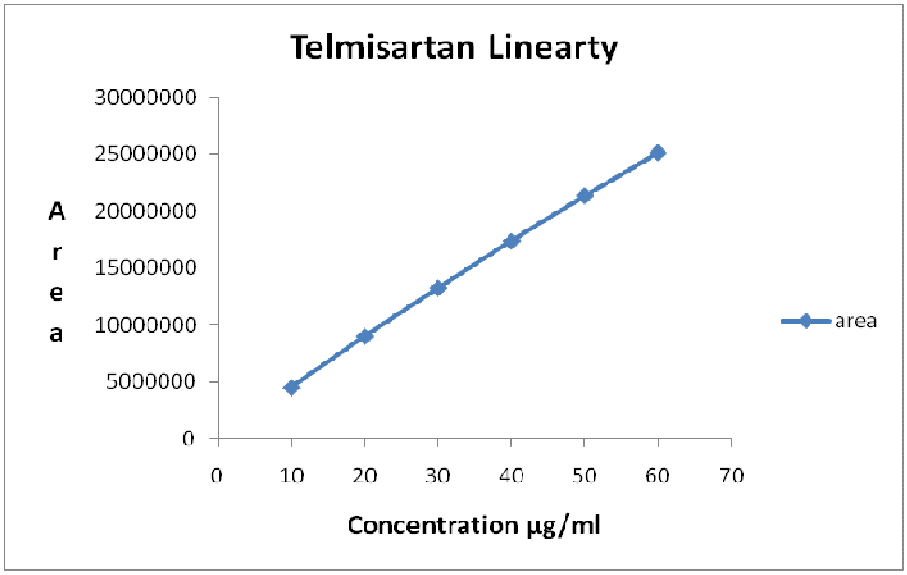
Specificity:
Specificity of the RP-HPLC method was demonstrated by the separation of the analytes from other potential components such as impurities and excipients. A volume of 10µl of working placebo sample solution was injected and the chromatogram was recorded. No peaks were found at retention time of 5.332 min. Hence, the proposed method was specific for Telmisartan.

Linearity
The linearity of calibration curve in pure solution was carried over the concentration range of 0.1-0.6 mg/ml through proposed RP-HPLC method. The data was represented in Table 3. The Correlation Coefficient is 0.9996 indicates that the method is Linear.

Table 3: Linearity Data

Linearity level	Concentration (mg/ml)	Peak area
1	0.1	4487500
2	0.2	8974808
3	0.3	13219908
4	0.4	17331575
5	0.5	21338611
6	0.6	25108183
Slope		41230426
Intercept		646115
Regression equation		Y= 41230426x+ 646115
Correlation Coefficient®		0.9996
Coefficient of determination(r^2)		0.9992

Figure-2: Linearity Graph



Precision:
The precision of the method was determined by injecting 0.3 mg/ml concentration in replicate (5times).

Repeatability:
The Repeatability of the proposed method was ascertained by injecting five replicates of fixed concentration within the Beer's range and finding out the peak area by the proposed method. The Precision was carried out by intraday and interday measurement. From this peak area % RSD was calculated. (Table 4) The calculated %RSD observed is well below 0.213% indicates that the method is Precise.

Table-4

Precision	
Repeatability (%RSD,n=5)	0.213
Intraday Precision(%RSD,n=5)	0.213-0.3088
Interday Precision(%RSD,n=5)	0.3088-0.98

Accuracy:
For the accuracy of proposed method, recovery studies were performed by standard addition method at five different levels (25%, 50%, 75 %, 125% and 150% of final concentration). A known amount of standard pure drug was added to pre-analyzed tablet powder and the sample was then analyzed by proposed method. Results of recovery studies were found to be satisfactory and reported in Table 5a and 5b.

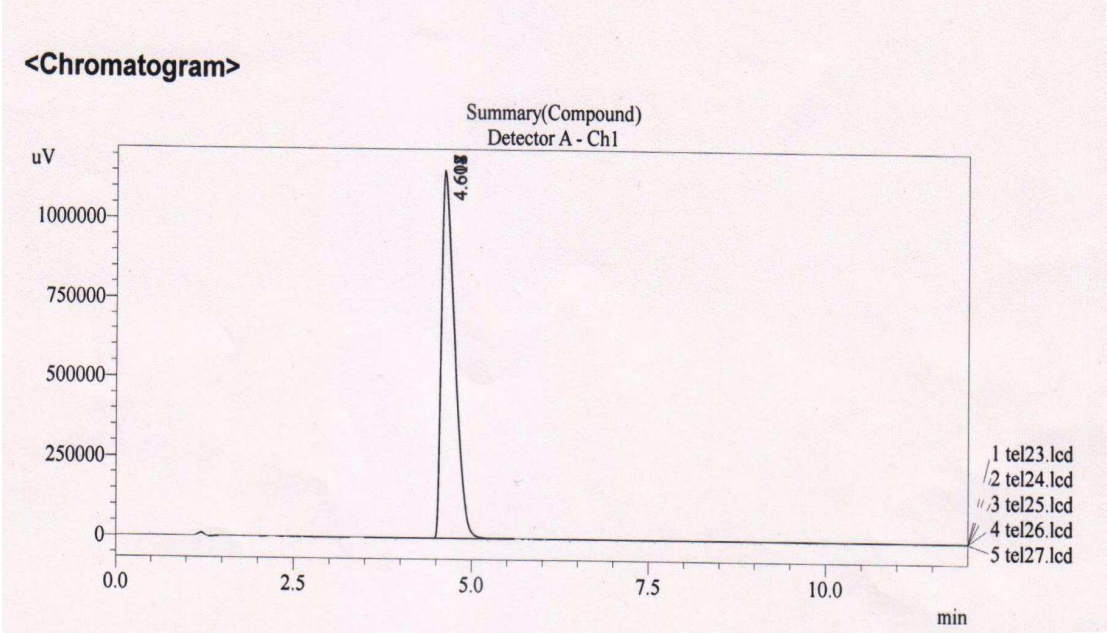


Figure-3: Telmisartan Standard Precision chromatogram overlay

Table-5a: Accuracy Results

Drug name	Levels	Amount added (mg/ml)	Telmisartan content (mg)	Percent recovery	Average of percentage recovery	Standard deviation	%RSD
Telmisartan	25%	0.1	25.89	99.858	99.93	0.058	0.058
	50%	0.2	51.789	99.93			
	75%	0.3	76.276	99.978			
	125%	0.5	123.12	99.988			
	150%	0.6	144.87	99.878			

Table-5b: Accuracy Results for Tablet

Brand name	Drug name	Amount labeled	Amount found	%Recovery	Average area	Standard deviation	%RSD
(AXETEN-40mg, MSD)	Telmisartan	40mg	39.9867	99.967	13215543	140827	1.056

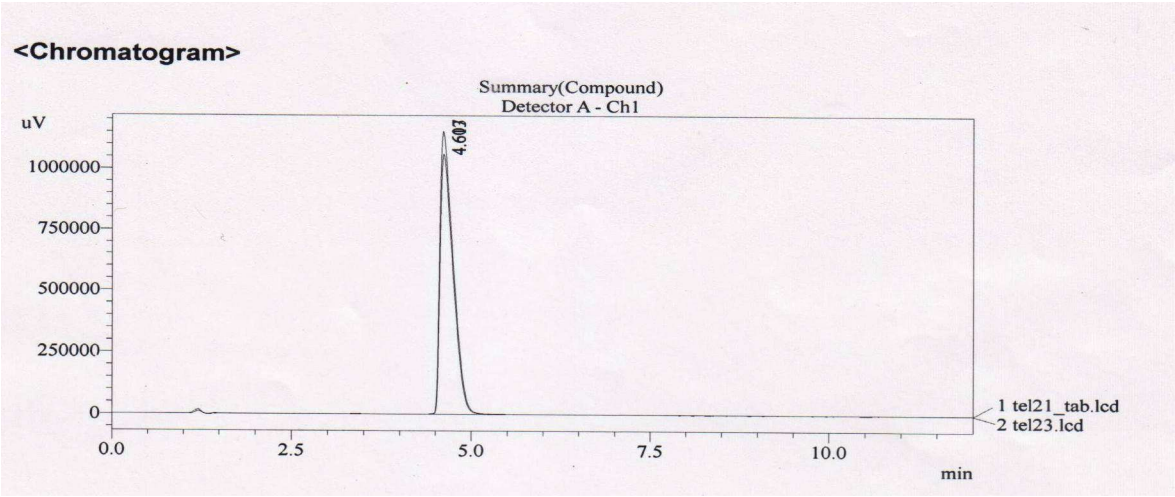


Figure -4: Telmisartan Standard and Tablet solution chromatogram overlay

Stability of the analytical solutions

The stability of the drug is determined by placing the sample solution for the short term stability by keeping at room temperature up to 24 hours and then comparing the obtained peak area with that of the similarly prepared fresh

sample. Further, auto-sampler stability for up to 24 hours was studied and established. The result indicates that the sample solution is stable upto 24 hours.

Stress degradation:
The following conditions were observed for study of the stability of the drug-
a. The drug is mixed with 10 ml of 0.05 N HCL and kept for 24 hours
b. The drug is mixed with 10 ml of 0.1N HCL and kept for 24 hours
c. The drug is mixed with 10 ml of 0.1 NaOH and kept for 24 hours
d. The drug is kept in oven at 80^0 C for 48 hours
e. The drug is placed in sunlight (UV) for 2 days.

The stress studies involving acid 0.05N, 0.1N HCl, Base 0.1 NaOH, Sun light (UV) and heat (80^oC) revealed that Telmisartan was stable under the stress conditions. For all stress conditions studied, the drug content was within 97 – 99 % indicating the stability and specificity of the analytical method to differentiate the degradation peaks.

Robustness:
Robustness was studied by deliberately changing the Flow rate and Temperature of the column. Analyzed the standard solution was changing the flow rate about ± 0.1 µl to the original flow rate 1.2 µl/ml and also recorded the analysis data for changing the column oven temperature about ± 2^0C to the original 50^0C temarature. Method precision verified with different Flow rates and Temperatures. The % RSD for 1.1µL, 1.3µL and 48^0C, 52^0C is within the limits.

CONCLUSION

A rapid and reliable isocratic RP-HPLC-UV method for the determination of Telmisartan has been developed and validated. This chromatographic assay fulfilled all the requirements to be identified as a reliable and feasible method. It is highly simple, accurate, precise, sensitive, validated and analytical procedure and its retention time 5.332 min allows the analysis of large number of samples in a short period of time. This method can be used for the routine quality control analysis.

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