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ISOCRATIC RP-HPLC METHOD VALIDATION OF TELMISARTAN IN PHARMACEUTICAL FORMULATION WITH STRESS TEST STABILITY EVALUATION OF DRUG SUBSTANCE

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ABSTRACT

A simple, rapid and accurate and stability indicating RP-HPLC method was developed for the determination of Telmisartan in pharmaceutical dosage forms. An isocratic RP-HPLC was achieved on Waters 2695 using Xterra C18 (150mm × 4.6mm × 5µm) column with the mobile phase consisting of 20 mM Potassium dihydrogen phosphate, pH adjusted to 3.5 using *ortho*-phosphoric acid (solvent A), and Acetonitrile (solvent B) in the ratio of 40:60 % V/V. The method showed a linear response for concentrations in the range of 10-50 µg/mL using Potassium dihydrogen phosphate (pH 3.5) buffer: Acetonitrile [40:60] as the mobile phase with detection at 272 nm and a flow rate of 0.8 mL min^{-1} and retention time 3.533 min. The method was statistically validated for accuracy, precision, linearity, robustness, forced degradation and selectivity. The proposed method was validated as per ICH guidelines. The method was found to be suitable for the quality control of Telmisartan in bulk and pharmaceutical dosage forms as well as the stability-indicating studies.

KEY WORDS: Telmisartan, RP-HPLC, Degradation studies.

1.INTRODUCTION

Telmisartan is chemically 2-(4-{[4-methyl-6-(1-methyl-1*H*-1,3-benzodiazol-2-yl)-2-propyl-1*H*-1,3-benzodiazol-1-yl]methyl}phenyl)benzoic acid. Telmisartan is an angiotensin II receptor antagonist (ARB) used in the management of hypertension. Telmisartan is a new antihypertensive drug, which is non-peptide potent highly selective, orally active antagonist at the angiotensin II AT1-receptors. It is available as tablets for oral administration containing 40 mg of Telmisartan. The structure of the drug are shown in Fig.1.

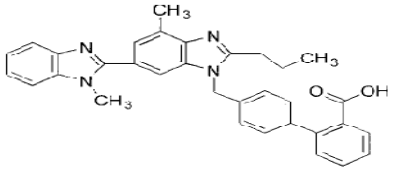

Figure 1.The chemical structures of Telmisartan

Many methods (Budavri,1996; ICH,2003; Phani Kishore,2010; Prem Anand,2011; Sujana,2011; Gangola,2011; Sunil,2010; Patel,2010; Bhatia,2010; Bankey,2009) have been reported on individual as well as simultaneous estimation on these drugs. The present method is relatively very simple, rapid and highly sensitive for the analysis of Telmisartan and Hydrochlorothiazide in bulk or in any other formulations. The present method developed is also validated as per ICH guidelines in the analysis of the multicomponents of interest and it can be used for routine Quality control analysis in laboratories.

The literature has demonstrated that a stability-indicating LC method for determination of Telmisartan related substances was developed (Patel,2010). Thus, the aim of our study was to develop a simple, selective, economic, specific stability indicating the LC method that can be used to determine the assay of Telmisartan.

2.EXPERIMENTAL

Materials and Reagents: Pharmaceutical grade working standards of Telmisartan (having potency 99.1% on as is basis) obtained as a gift sample from M/s Alkem laboratories Ltd., (Ahmedabad, India). Acetonitrile (HPLC grade) were obtained from Merck of analytical grade, Mumbai, India. All the other chemicals of analytical grade Potassium dihydrogen phosphate (AR grade) of HPLC grade from Spectrochemicals. All dilutions were performed in standard volumetric glassware. High pure water was prepared by using Millipore Milli Q plus purification system. Micardis ® commercial formulations were purchased from the local market.

Instrumentation: The instrument used was a Waters HPLC, Empower software, Separation module (2695), dual wavelength absorbance detector (2487) as Diode array detector processed by an auto sampler and a rheodyne variable injector fitted with 20 µL volume sample loop. The output signal was monitored and processed using Empower software (designed by Waters Technologies, USA). The samples were injected through a micro liter syringe. Chromatographic separation was performed on XTerra® C-18 column (150 × 4.6mm i.d, 5 µm) column coupled with a guard column of the same material. The mobile phase was composed of potassium dihydrogen phosphate buffer: Acetonitrile (40:60 % V/V) and pH of mobile phase was adjusted to 3.5 ± 0.1 and degassed by filtering it under vacuum through a 0.45µm nylon filter. The flow rate of mobile phase through analytical column was 0.8 mL min^{-1}. The column temperature was maintained at 23±1°C. The detection wavelength was set at 272 nm.

Chromatographic conditions: The chromatographic column used was Waters using XTerra® C18 (150mm × 4.6mm × 5µm). The mobile phase consists of a mixture of potassium dihydrogen phosphate, pH adjusted to 3.5 (solvent A), and acetonitrile (solvent B) in 40:60 ratio. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 0.8 mL/min for 6 min. The column temperature was maintained at 23±1°C. The eluate was monitored at 272 nm using the DAD. The injection volume was 20 µL. Mobile phase was used as diluent during the standard and test samples preparation.

Preparation of standard solutions: Stock solution of Telmisartan was prepared by dissolving 40 mg of Telmisartan in 50 mL of volumetric flask containing 20 mL mobile phase. The solution was sonicated for about 20 min and then made up to volume with mobile phase (Stock solution). The mobile phase standards containing Telmisartan was prepared by appropriately diluting the stocks to give final concentrations of 10-50µg mL^{-1}.

Preparation of sample solution for assay: Twenty tablets were weighed, finely powdered, and an accurately weighed sample of powdered tablets equivalent to 40mg of Telmisartan was treated with 20mL of mobile phase in a 50 mL volumetric flask using ultra sonicator. This solution was filtered through 0.45 µm filter paper and made upto the mark with mobile phase. Further dilute 1 mL of the sample from above stock solution into a 10 mL volumetric flask and diluted upto the mark with mobile phase and final concentration of 30 µg $mL^{-1}$was prepared and 20 µL of this solution was injected for HPLC analysis.

Quality Control Standards: The quality control (QC) standard for Telmisartan was prepared from stock solutions by dissolving 40 mg of Telmisartan in 50 mL of mobile phase. The working solutions of Telmisartan were prepared in the concentration ranges of low (10 µg mL^{-1}), medium (30 µg mL^{-1}), high (50 µg mL^{-1}) as target concentrations using mobile phase as a solvent.

Method Validation: The method was validated in terms of linearity, specificity, accuracy, and precision, limit of detection (LOD) and limit of quantitation (LOQ).

Linearity and Calibration standards: Five different concentrations of a mixture of all three drugs were prepared for linearity studies. The response was measured as peak area. The calibration curve obtained by plotting peak area against concentration showed linearity in the concentration range of 10-50 µg mL^{-1} of Telmisartan.

Precision: Intra-day precision was found out by carrying out the analysis of sample on five times on the same days. The standard drug solution containing 30 µg mL^{-1} of Telmisartan was injected into the chromatographic system, the peak area was noted and % RSD was calculated. Inter-day precision was found out by carrying out the analysis of sample on five different days. The standard drug solution containing 30 µg mL^{-1} of Telmisartan was injected into the chromatographic system, the peak area was noted and % RSD was calculated.

Recovery: The accuracy of the method was checked by spiking the sample with reference compound. 80%, 100% and 120% concentrations of Telmisartan were prepared with respect to target concentration.

Limit of Detection: The limit of detection is the lowest level of analyte such as Telmisartan of 0.46% concentration was prepared and that can be detected but not necessarily determined in a quantitative fashion, using a specific method under the required experimental conditions and it was calculated from signal-to-noise ratio method.

Limit of Quantification: The limit of quantification is the lowest concentration of analyte such as Telmisartan of 0.239% concentration was prepared and that can be determined with acceptable accuracy and precision when the required procedure is applied. It was calculated from signal-to-noise ratio method.

Forced Degradation Behaviour of Telmisartan in API: In forced degradation studies all solutions were prepared by dissolving API of 40 mg Telmisartan in small volume of Acetonitrile in 5 different 50mL volumetric flasks and later

add 5 mL of 3% aqueous hydrogen peroxide, 0.1M aqueous hydrochloric acid and 0.1M aqueous sodium hydroxide into each flask, it was subjected to forced degradation for about 12 hours. After the degradation these solutions were neutralized and diluted with mobile phase to obtain final concentration of 30 µg mL^{-1}. The no stress treatment sample (as control) has been evaluated relative to the standard concentration.

In case of acidic stress the solutions were prepared in Acetonitrile and 0.1M hydrochloric acid (20:80 % V/V), in case of alkaline stress the solutions were prepared in Acetonitrile and 0.1M sodium hydroxide (20:80 % V/V), in case of peroxide stress the solutions were prepared in Acetonitrile and 30% hydrogen peroxide (20:80 % V/V), in case of the thermal stress bulk powder of Telmisartan was exposed to 105^{o}C and the resultant samples was kept aside for about 12 hours then the samples that exposed to stress conditions were neutralized, except thermal stress sample and diluted with mobile phase to obtain final concentration of 30 µg mL^{-1} then the solutions were analyzed after five minutes of the preparation.

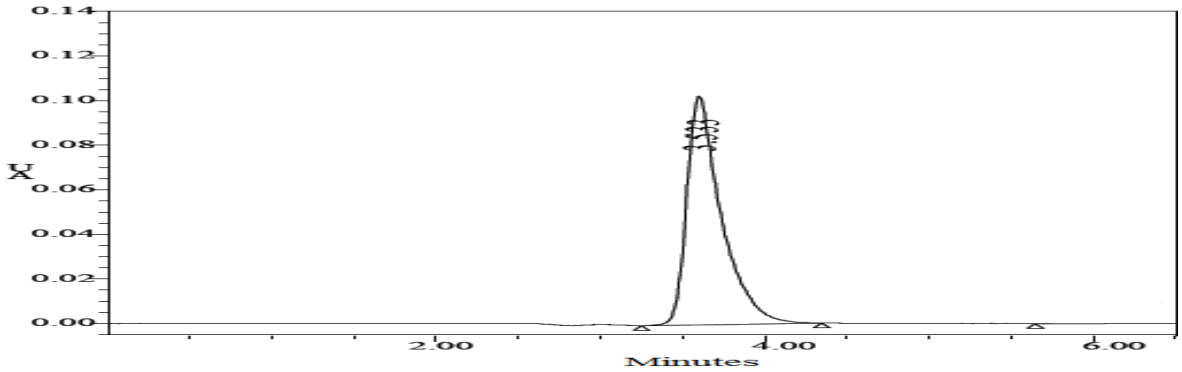

Figure 2. Telmisartan (30 µg mL^{-1}) pure drug

3.RESULTS

Optimized Chromatographic Conditions: Spectroscopic analysis of Telmisartan showed that maximum UV absorbance (λ_{max}) at 272.0 nm. Therefore the chromatographic detection was performed at 272 nm using a D A D detector. Chromatographic conditions were optimized by changing the mobile phase composition, by altering the pH of mobile phase a good separation was achieved. The optimized mobile phase was determined as a mixture of pH 3.5 buffer : Acetonitrile (40:60, V/V) at a flow rate of 0.8 mL min^{-1}. Under these conditions Telmisartan were eluted at a retention time of 3.533 minute respectively with a run time of 6 min. A typical chromatograms for Telmisartan obtained by using the afore mentioned mobile phase from 20 µL for the assay standard is illustrated in Fig. 2.

Method Validation

System suitability parameters: For system suitability parameters, three replicate injections of Telmisartan standard solution were injected and parameters such as the Tailing factor, Theoretical plate and Retention time of the peak were calculated. The results are shown in Table 1.

Linearity and Calibration standards: Five different concentrations of Telmisartan standards were prepared for linearity studies. The response was measured as peak area. The calibration curve obtained by plotting peak area against concentration showed linearity in the concentration range of 10-50 µg mL^{-1}. The best fit for the calibration curve could be achieved by a linear regression equation of Telmisartan found to be y = 10105.6 x + 15484 regression coefficient values (r^2) were found to be 0.9999 indicating a high degree of linearity for all drugs. Calibration results can be shown in Table 2.

Precision: Intra-day precision was ascertained by carrying out the analysis of the sample at a particular concentration five times on the same day. The sample was injected into the chromatographic system, peak areas were noted and the % relative standard deviation was 1.24% was found to be well within the limits indicating the sample repeatability of the method. Inter-day precision was found out by carrying out the analysis of sample on five different days. The sample was injected into the chromatographic system, the peak area was noted and % relative standard deviation was 1.45% was found to be well within the limits indicating the injection repeatability of the method.

Recovery: To check the recovery of the proposed method, recovery studies were carried out at 80%, 100% and 120% of the test concentration as per ICH guidelines. The recovery study was performed 3 times at each level; the method is accurate within the acceptance limit of 2%. The results of recovery study were found to be accurate and are given in Table 3.

Specificity: The specificity of the HPLC method was determined by complete separation of Telmisartan when it was

subjected to forced degradation as per ICH guidelines which were carried out with 0.1M Hydrochloric acid, 0.1M Sodium hydroxide, 3% Hydrogen peroxide and Heat degradation at 105°C. The method does not permit detection of degradation products for Telmisartan when it was subjected to stress conditions as per ICH guidelines. The drug degrades as observed by the decreased area in the peak of the drug when compared with peak area of the same concentration of the non degraded drug at 0 hr (control), without giving any additional degradation peaks. However it showed stability towards all these stress conditions. Percent degradation was calculated by comparing the areas of the degraded peaks in each degradation condition with the corresponding areas of the peaks of three drugs under non degradation condition. The results of specificity data for degradation study are given in (Table 4).

Robustness: The robustness of the method was determined as a measure of the analytical methods capability to be unaffected by small variations in method parameters. The effect of these variations on the content of Telmisartan was determined. The different variations are as given below.

a. Variation in flow rates: The flow rate of the mobile phase varied by ± 0.1 mL min^{-1}.

b. Variation in composition: The composition of the organic phase component of Mobile phase was varied by ±5%.

c. Variation in pH: The pH of the mobile phase was varied by ± 0.1.

The robustness study indicated that the selected factors remained unaffected by small variations of flow rate, these were 0.70 mL min^{-1} and 0.90 mL min^{-1} and composition of the mobile phase were (35:65 %V/V) and (45:55 %V/V). The results of the analysis of the samples under the conditions of the above variations indicated that the method was robust.

LOD and LOQ: The LOD and LOQ were separately determined based on the S/N Ratio. For LOD the S/N ratio is 3:1 and for LOQ the ratio is 10:1. The limit of detection for Telmisartan was found to be 0.3689 µg mL^{-1} and the limit of quantitation (LOQ) for Telmisartan was found to be 1.912 µg mL^{-1}.

Estimation of Telmisartan tablet formulation: The value of analysis of tablets obtained by the proposed method was 96.56% for Telmisartan which can shown in Table 5. This result showed that the estimation of dosage forms was accurate with the acceptance level of 90% to 110%.

Results of forced degradation studies: Intentional degradation was attempted to under different stress conditions to evaluate the ability of the proposed method to separate Telmisartan from its degradation products. Degradation was not observed in Telmisartan samples under stress condition like acid hydrolysis. However, mild degradation was observed when the drug was exposed to thermal exposure, oxidative and alkaline hydrolysis. The concentration of Telmisartan was more slightly decreasing with time in thermal and oxidative hydrolysis. This degradation is mainly observed in terms of loss of assay. Table 4 indicates the extent of degradation of Telmisartan under various stress conditions. Therefore, it may be concluded that Telmisartan is susceptible to degrade in oxidative and thermal conditions. Photodiode array detection was used as an evidence of the specificity of the method and to evaluate the homogeneity of the drug peak.

DISCUSSION

The calibration curves obtained for each drug were linear over a wide range of concentrations. Both precision and accuracy at the LOQ, a low, medium and high concentration of Telmisartan was within acceptable limits. However the present study we applied our method for stability indicating Telmisartan in bulk. The peaks due to Telmisartan was found to be symmetrical and well defined. The total run time is 10 min. The optimum wavelength for detection was found to be 272 nm. The linearity of the calibration curves indicated the suitability of the method over a wide range of concentration of 10-50 µg mL^{-1}. The method was robust and the recovery obtained by the proposed method was found to be 103.95% - 106.54% between within the acceptance level of 90% to 110%.

Table 1. Data for System suitability of the method

Parameters	Telmisartan
Tailing factor	1.1
Theoretical plates	4452.0
Resolution	---
%RSD	0.43
Limit of detection (LOD; µg mL^{-1})	0.3689
Limit of Quantification (LOQ; µg mL^{-1})	1.9120

Table 2. Data for Regression Analysis for Calibration Curves of Telmisartan

Parameters	Telmisartan
Linearity range(mg mL^{-1})	10-50
Correlation coefficient (r^2)	0.9999
Slope (m)	10105.6
Intercept (c)	15484

Table 3. Data for Recovery of Telmisartan

Compound	Conc.(%)	Amount added pure drug(mg)	Amont found ($\mu g\ mL^{-1}$)	% Recovery	Mean Recovery
3TC	80	32.0	34.09	106.54	105.35
	100	40.0	42.22	105.55	
	120	48.0	48.89	103.95	

*Average of three determinations

Table No 4. Data for forced degradation Study on Telmisartan

Drug Name	Stress behaviour	Time(hrs)	Rt(min)	% Degradation	% of Active drug present after degradation
Telmisartan	Control	12	3.533	---	---
	Acid hydrolysis	12	3.027	0.7207	99.49214
	Alkaline hydrolysis	12	3.008	3.9209	96.26487
	Thermal stress	12	3.175	0.3990	90.00002
	Oxidative stress	12	3.026	3.4559	92.67147

Tables 5. Data for Estimation of Telmisartan in tablet formulation

Drug	Qty.claimed(mg/tablet)	Qty.found(mg/tablet)	% Qty.found
Telmisartan	40	38.97	96.56

4.CONCLUSION

It can be concluded that the proposed method were developed and fully validated and it was found to be simple, accurate, precise, reproducible, and robust stability indicating RP - HPLC method to estimate the levels of Telmisartan considering the fact that the present method involves a shorter running time. The results of stress testing undertaken according to the ICH guidelines revealed that the method is selective and stability-indicating. In addition it can be applied to routine quality control analysis for assay of other drugs in this class and its similar formulation studies.

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