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Research Article

VALIDATED RP-HPLC METHOD FOR THE ESTIMATION OF TELMISARTAN IN SERUM SAMPLES

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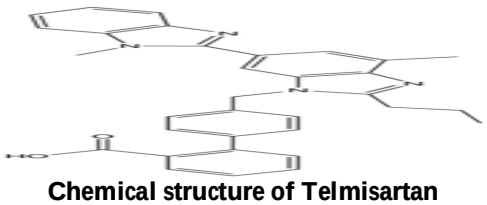
ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of telmisartan in serum samples. Isocratic elution at a flow rate of 1.0ml/min was employed on a Equsil, 250 X 4.6mm, 5µ at ambient temperature. The mobile phase consisted of buffer: Acetonitrile (35:65) (V/V). The UV detection wavelength was 282nm and 20µl sample was injected. The retention time for telmisartan was 3.32 min. The percentage RSD for precision and accuracy of the method was found to be less than 2. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of telmisartan in tablet dosage form and in serum.

Keywords: Telmisartan, RP-HPLC, UV detection, serum, recovery, precise.

INTRODUCTION

Telmisartan is an angiotensin receptor blocker that shows high affinity for the angiotensin II type 1 receptors, has a long duration of action, and has the longest half-life of an ARB. In addition to blocking the Renin-Angiotensin System (RAS), telmisartan acts as a selective modulator of Peroxisome proliferators - activated receptor gamma (PPAR-γ), a central regulator of insulin and glucose metabolism[1].Telmisartan molecular formula is $C_{33}H_{30}N_4O_2$ .Mol. mass is 514.617 g/mol and **IUPAC** Name is 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[2]



Chemical structure of Telmisartan

Literature survey reveals that various LC-MS/MS [3-4], RP-HPLC [5-6], HPTLC[7] methods have been reported for the estimation of telmisartan in plasma samples. Main aim of present work is to develop a method which is precise, accurate and economical to estimate telmisartan in rabbit plasma.

EXPERIMENTAL

Chemicals and reagents

HPLC grade acetonitrile, methanol and orthophosphoric acid was purchased from Merck Specialties Pvt.Ltd.

Instrumentation and analytical conditions

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 LC-20AT prominence liquid chromatography, a 20µl injection loop and SPD-20A prominence UV/VIS detector and running on Spinchrom CFR software. Isocratic elution with Buffer:Acetonitrile (35:65) (V/V)

(PH-4.5) was used at a flow rate of 1.0ml/min. The mobile phase was prepared freshly and degassed by sonicating for 5 min before use.

Stock and Working standard solutions

Accurately weigh and transfer 10mg of telmisartan working standard into a 10ml volumetric flask methanol and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Preparation of serum samples for calibration curve

0.2ml of spiked standard rabbit serum samples (calibration curve samples) were vortexed (extracted) with acetonitrile using 1ml v/v up to 2min and centrifuged up to 5min after centrifugation separated supernant layer up to 0.9ml and evaporated under nitrogen pressure. Finally dry residue was reconstituted with mobile phase.

Calibration curve range:-1, 10, 20,30,50,60 and 70 µg / ml in rabbit serum

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, stability and system suitability. Standard plots were constructed with five concentrations in the range of 1.0µg/ml to 70.0µg/ml prepared in triplicates to test linearity. The peak area of telmisartan was plotted against the concentration to obtain the calibration graph. The linearity was evaluated by linear regression analysis that was calculated by the least square regression method. The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from five replicate injections of freshly prepared telmisartan test solution in the same equipment at a concentration value of 100% (100µg/ml) of the intended test concentration value on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak area of the telmisartan was determined and precision was reported as %RSD. Method accuracy was tested (% recovery and %RSD of individual measurements) by analyzing sample of telmisartan at three different levels in pure solutions using three preparations for each level. The results were expressed as the percentage of telmisartan recovered in the samples. Sample solution short term stability was tested at ambient temperature (20±100C) for three days. In order to confirm the stability of both standard solutions at 100% level and tablet sample solutions, both solutions protected from light were reinjected after 24 and 48 hours at ambient temperature and compared with freshly prepared solutions.

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions

Proper selection of the stationary phase depends up on 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 orthophosphoric acid, buffer and acetonitrile was selected as mobile phase and the effect of composition of mobile phase on the retention time of telmisartan was thoroughly investigated. The concentration of the orthophosphoric acid, methanol and acetonitrile were optimized to give symmetric peak with short run time (Fig.1).

Analysis of Telmisartan in Serum

0.2ml of rabbit serum subject samples (subject samples) were vortexed (extracted) with acetonitrile using 1ml v/v up to 2min and centrifuged at 3000 rpm up to 5min. From this centrifuged solution supernatant layer up to 0.9ml was separated and evaporated to dryness nitrogen pressure. Finally dry residue was reconstituted with Mobile phase and mixed for 5 min by vortex mixer and evaporated the organic layer and finally the remaining sample was injected into HPLC and chromatogram was recorded. The amount of drug present in the blood sample was calculated from linearity graph (Fig. 2).

VALIDATION OF METHOD

Linearity

Five points graphs was constructed covering a concentration range 1.0-70.0µg/ml (Three

independent determinations were performed at each concentration). Linear relationships between the peak area signal of telmisartan the corresponding drug concentration was observed. The standard deviation of the slope and intercept were low. The statistical analysis of calibration is shown in Table 1.

Precision

Method precision and precision of the system was found to be within the limits of acceptance criteria. For the analytical method and system precision the relative standard deviation was found to be 1.4% and 0.4% respectively.

System suitability

The system suitability parameter like capacity factor, asymmetry factor, tailing factor and number of theoretical plates were also calculated. It was observed that all the values are within the limits. The statistical evaluation of the proposed method was revealed its good linearity, reproducibility and its validation for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of telmisartan in tablet formulation.

CONCLUSION

A validated RP-HPLC method has been developed for the determination of telmisartan in serum samples. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic runtime of 10 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of telmisartan in pharmaceutical dosage form.

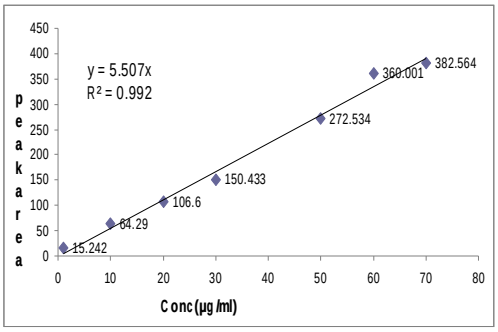


Fig. 2: Calibration curve for Telmisartan

Table 1: Linearity of Telmisartan

Concn. (µg/ml)	Drug Retention time (min)	Drug peak area
1	3.19	15.242
10	3.203	64.29
20	3.193	106.6
30	3.187	150.433
50	3.253	272.534
60	3.253	360.001
70	3.253	382.564

Table 2: System suitability parameters

Parameters	Values
λ max (nm)	282
Retention time (min)	3.25
Theoretical plates	5045
Tailing factor	1.82
Limit of detection (ng/ml)	30
Limit of quantification (ng/ml)	60

Table 3: Chromatographic condition

Buffer	10mM KH2PO4 + 0.3ML for 300ml of buffer TEA & finally adjusted pH-4.5 with OPA
Mobile phase	buffer:Acetonrile (35:65)
Wavelength	282nm
Flow rate	1ml/min
Inj vol	20µL
Detector	SPD-20A prominence UV/VIS detector

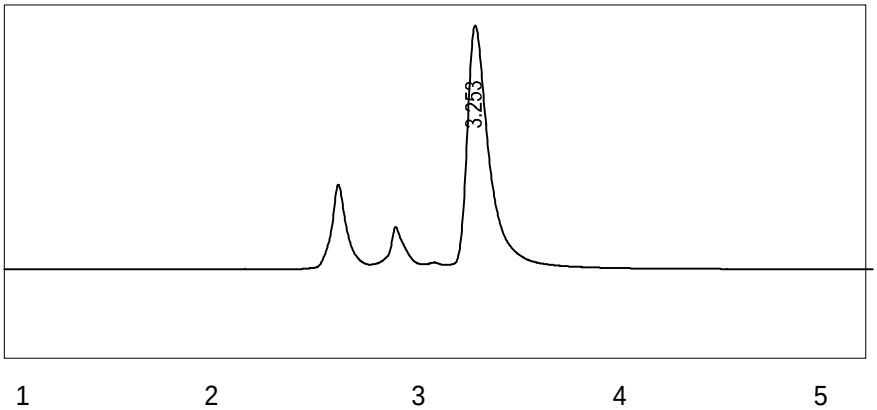


Fig. 1: Typical chromatogram of Telmisartan

REFERENCES

1. Pratik kumar A. Patel et al. Commercial Telmisartan Tablets: A Comparative Evaluation with Innovator Brand Micardis. International Journal of Pharma Sciences and Research. 2010;1(8):282-292.
2. Reynolds JEF. In; Martindale, The Extra Pharmacopoeia, The Pharmaceutical Press, London. 1996;31st Edn:334.
3. Ben-mei Chen,Yi-zeng Liang et al. Development and validation of liquid chromatography–mass spectrometry method for the determination of Telmisartan in human plasma. Analytica Chimica Acta. 2005;540(2):367-373.
4. Simultaneous determination of Ramipril, Ramiprilat and Telmisartan in human plasma using liquid chromatography tandem mass spectrometry. Talanta. p 709-16.
5. Sujana K, Gowri sankar D et al. Stability indicating RP-HPLC method for the determination of Telmisartan in Pure and pharmaceutical formulation. International Journal of Pharmacy and Pharmaceutical Sciences. 2011;3(2).
6. Phani Kishore CH, Bhanuprakash Reddy V et al. Development and validation of stability indicating HPLC method for the estimation of Telmisartan related substances in tablets formulation. Int J Res Pharm Sci. 2010;1(4):493-501.
7. Patel VA et al. Development and Validation of HPTLC Method for the Simultaneous Estimation of Telmisartan and Ramipril in Combined Dosage Form. International Journal on Pharmaceutical and Biological Research. 2010;1(1):18-24.