RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF TELMISARTAN IN BULK AND TABLET DOSAGE FORM

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RESEARCH ARTICLE

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ABSTRACT:

A simple, sensitive, precise and specific reverse phase high performance liquid chromatographic method was developed and validated for the determination of Telmisartan in bulk and tablet dosage forms. It was found that the excipient in the tablet dosage forms does not interfere in the quantification of active drug by proposed method. The HPLC separation was carried out by reverse phase chromatography on RP_{18}, column (250×4.6mm) with a mobile phase composed of 0.025M potassium dihydrogen phosphate : acetonitrile : methanol (45:50:5) at a flow rate of 1ml/min. The detection was monitored at 216 nm. The calibration curve for Telmisartan was linear from 100 to 500 ng/ml. The interday and intraday precision was found to be within limits. LOD and LOQ for Telmisartan were found to be 27 ng/ml and 83 ng/ml. Accuracy and reproducibility were also found within range. The proposed method has adequate sensitivity, reproducibility and specificity for the determination of Telmisartan in bulk and its tablet dosage forms.

Key words: Telmisartan, RP-HPLC, Validation, Tablet.

Introduction:

Telmisartan is 4'-[1, 4'-dimethyl-2-propyl [2, 6'- bi-benzimidazole]-1'-yl] methyl 1, 1'-biphenyl 2-carboxylic acid. Telmiartan is practically insoluble in water; sparingly soluble in strong acid; soluble in strong bases[1,2]. It blocks the vasoconstrictor and aldosterone secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in many tissues, such as vascular smooth muscle and the adrenal gland. As the analysis is an important component in the formulation development of any drug molecule. It becomes essential to develop a simple, sensitive, accurate, precise, reproducible method for the estimation of drug samples. The proposed method was optimized and validated as per the ICH guidelines[3,4] and was found to be simple, precise, sensitive and accurate for the determination of Telmisartan in bulk and tablet dosage form.

Experimental Work

Apparatus and Chromatographic conditions

Chromatographic separation was performed on a Shimadzu chromatographic system equipped with a UV/Visible detector SPD-20A and Redone 20µL fixed loop. Separation was carried out by reverse phase chromatography on RP_{18}, column (250×4.6mm) with a mobile phase composed of 0.025M potassium dihydrogen phosphate : acetonitrile : methanol (45:50:5) at a flow rate of 1ml/min. The detection was monitored at 216 nm. The mobile phase was filtered through a 0.45 µ membrane filter and sonicated for 15min. Analysis was performed at ambient temperature.

Reagents and Solutions

Telmisartan drug sample was supplied as gift sample by Oasis Test Laboratory Jaipur. Commercial tablets of Telmisartan were procured from the local market. All other

chemicals used were of analytical grade. Optimized chromatographic conditions are listed in **Table: 1**.

Standard solution

100 µg/ml of Telmisartan was prepared in methanol. This solution was further diluted with methanol to get a solution of appropriate concentrations.

Sample solution

Twenty tablets of Telmisartan were weighed, average weight was calculated and quantity equivalent to 20 mg of Telmisartan was weighed accurately and transferred to a 100 ml standard flask, extracted with methanol and volume was made up to the mark with methanol and then diluted appropriately.

Method Validation[5,9]

Once the HPLC method development was over, the method was validated in terms of parameters like precision, accuracy, linearity and range, LOD, LOQ etc. For all the parameters percentage relative standard deviation values were calculated. The proposed HPLC method was validated as per ICH guidelines.

Linearity and Range

The linearity of measurement was evaluated by analyzing different concentrations of the standard solutions of the Telmisartan. The Beer lamberts concentration was found to be 100-500 ng/ml. Calibration curve was constructed by plotting average peak area against concentration and regression equation was computed. The results were shown in **Table: 2**.The correlation coefficient value was found to be 0.99887. The results show that an excellent correlation exists between peak area and concentration.

Precision

Precision was evaluated by carrying out three independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in the sample preparation. Percentage relative standard deviation (% RSD) was found to be less than 1% for within a day and day to day variations, which proves that that method is precise (**Table 2)**.

Accuracy

To study the reliability, suitability and accuracy of the method recovery experiments were carried out. A known quantity of the pure drug was added to the pre-analyzed sample formulation at the level of 50% and 100%, dissolved in methanol and made up to 100 ml with same solvent and further dilutions were made. The contents were determined from the respective chromatograms. The % RSD was calculated **(Table 2)**.

Repeatability of solution

A standard solution of drug substance was injected ten times and corresponding peak areas were recorded. The % RSD was found to be less than 1%.

Specificity

Conditions of HPLC method like percentages of organic solvent in mobile phase, ionic strength, pH of buffer flow rate etc were changed. In spite of above changes no additional peaks were found, although there were shift retention times or little changes in peak shapes.

Limit of Detection and Limit of Quantification

The limit of Detection (LOD) and limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for Telmisartan was found to be 27 ng/ml. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ was 83 ng /ml for Telmisartan. It was concluded that the developed method is sensitive.

Results and Discussion

Flow rate of 1ml/min was selected for mobile phase 0.025M potassium Dihydrogen phosphate: Acetonitrile: Methanol (45:50:5). Telmisartan shows linearity in the range of 100-500 ng/ml, and the co-efficient was found to be 0.99887. Recovery studies were performed at different levels and were found to be within the limits mentioned as per ICH Guidelines. The low standard deviation and good percentage recovery indicates the reproducibility and accuracy of the method.

Regression analysis of the calibration curve for Telmisartan showed a linear relationship between the concentration and peak area with correlation coefficients higher than 0.99. The intraday precision was found to be within 1% RSD for different concentration and %RSD for interday precision was found to be within 2%. Repeatability of injection was performed for10 times and corresponding peak areas were recorded. The %RSD peak was reported. The sensitivity of method LOD and LOQ is shown in **Table 2** which were found accurately in the ratio of 1:3 for each other. Hence the proposed method was found to be simple, accurate, and rapid and can be employed for routine analysis of Telmisartan in bulk and tablet dosage form.

Conclusion

A convenient and rapid RP- HPLC method has been developed for estimation of Telmisartan in tablet dosage form. The assay provides a linear response across a wide range of concentrations. Low intra-day and inter-day % RSD coupled with excellent recoveries. The proposed method is simple, fast, accurate and precise for routine quality control analysis of Telmisartan in bulk and dosage form.

Table 1: Optimized chromatographic conditions

Parameter	Optimized condition
Instrument	Shimadzu with PDA
Column	RP-18, (250 × 4.6 mm)
Mobile Phase	0.025M potassium dihydrogen phosphate: acetonitrile: methanol (45: 50: 5 %)
Flow rate	1 ml/min
Injection volume	20µl
Column Temperature	Ambient

Table 2: Validation parameters

Parameters		Values
Linearity range		100 – 500 ng/ml
Intraday Precession (% RSD)		0.2274 – 0.2058
Interday Precision (% RSD)		1.58 – 1.79
Recovery studies (%Recovery)		99.7 - 101.31
Repeatability (%RSD)		0.5597
System suitability	Theoretical plates	7686
	Tailing factor	1.27
	LOD	27 ng/ml
	LOQ	83 ng/ml

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