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Research Article

METHOD DEVELOPMENT OF NOVEL RPHPLC METHOD AND FORCED DEGRADATION STUDIES OF NISOLDIPINE AND TELMISARTAN

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ABSTRACT

A new RP-HPLC method was developed and validated for the simultaneous estimation of Nisoldipine and Telmisartan in pharmaceutical formulations. Using an Inertsil ODS C18 column (4.6×250mm, 5µm) with a mobile phase of acetonitrile (ACN) and potassium dihydrogen phosphate (KH2PO4) (70:30, pH 3), the method achieved effective separation with detection at 225 nm. Retention times were 2.798 minutes for Nisoldipine and 3.587 minutes for Telmisartan. The % purity for Nisoldipine and Telmisartan was 99.87% and 100.27%, respectively. System suitability parameters and resolution were within acceptable limits. Validation according to ICH guidelines demonstrated high linearity (r^2 = 0.999), accuracy (% recovery: 98.56-99.96%), and precision (% RSD: 1.2-1.9%). Low LOD (3.72-0.0242 µg/mL) and LOQ (7.40-0.0202 µg/mL) values indicated method sensitivity. This method is suitable for routine analysis of Nisoldipine and Telmisartan, ensuring reliable quantification in pharmaceutical formulations.

Keywords: Medical devices, Regulation, Global harmonization, Healthcare industry, Patient safety.

INTRODUCTION

The accurate and simultaneous quantification of pharmaceutical compounds in combined dosage forms is critical for ensuring therapeutic efficacy and patient safety [1, 2]. Nisoldipine, a dihydropyridine calcium channel blocker marketed by Daiichi-Sankyo Pharmaceuticals, Inc. in Japan, is known for its gradual onset of action and sustained blood pressure reduction with minimal impact on heart rate. It is also being studied for its potential benefits in postischemic stroke management. Telmisartan, on the other hand, is an angiotensin II receptor antagonist (ARB) used extensively in the management of hypertension [3, 4]. ARBs like Telmisartan bind with high affinity to angiotensin II type 1 (AT1) receptors, inhibiting the action of angiotensin II on vascular smooth muscle and leading to a decrease in arterial blood pressure [5]. Additionally, recent studies suggest that Telmisartan may exhibit PPAR-gamma agonistic properties, potentially offering beneficial metabolic effects [6]. Given the therapeutic significance of these two drugs, there is a need for a reliable analytical method for their simultaneous estimation in combined

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pharmaceutical preparations [7]. This study aims to develop a novel, simple, rapid, accurate, efficient, and reproducible reversed-phase high-performance liquid chromatography (RP-HPLC) method coupled with a spectroscopic method for the concurrent analysis of Nisoldipine and Telmisartan. The proposed method will be validated in accordance with the International Council for Harmonisation (ICH) guidelines, specifically ICH Q2 (R1), to ensure its reliability and accuracy.

The development of this RP-HPLC method will involve optimizing the chromatographic conditions to achieve the best possible separation and quantification of the two drugs. The validation process will cover various parameters, including system suitability, specificity, accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ), robustness, and stability, as outlined in the ICH guidelines [8]. The successful development and validation of this analytical method will provide a valuable tool for the quality control and routine analysis of Nisoldipine and Telmisartan in pharmaceutical formulations.

METHODOLOGY

RPHPLC Method Development

Mobile Phase Optimization:

Initially the mobile phase tried was methanol: Ortho phosphoric acid buffer and Methanol: phosphate buffer ,Acetonitrile : methanol with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to Phosphate buffer (pH 3.0), Acetonitrile in proportion 70: 30 v/v respectively.

Wave length selection

UV spectrum of 10 µg/ml Telmisartan and 10 µg/ml Nisoldipine in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 225 nm. At this wavelength both the drugs show good absorbance.

Preparation of Buffer and Mobile Phase

Preparation of Phosphate buffer

3.4g of Potassium di hydrogen ortho phosphateis taken in 1000 ml of HPLC water pH was adjusted with 0.1M NAOH up to 3.0.final solution was filtered through 0.45 m Membrane filter and sonicate it for 10 mins.

Preparation of mobile phase

Accurately measured 700 ml (70%) of above buffer and 300 ml of Acetonitrile HPLC (30%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent

Preparation of the Telmisartan & Nisoldipine Standard & Sample Solution

Standard Solution Preparation

Accurately weigh and transfer 40 mg of Telmisartan and 5 mg of Nisoldipine working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.75 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. [9]

Sample Solution Preparation

Accurately weigh and transfer the equivalent weight of 40 mg of Telmisartan and 5 mg of Nisoldipine Tablet powder into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.75 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Telmisartan and Nisoldipine peaks and calculate the %Assay by using the formulae

System Suitability

Tailing factor for the peaks due to Telmisartan and Nisoldipine in Standard solution should not be more than 2.0. Theoretical plates for the Telmisartan and Nisoldipine peaks in Standard solution should not be less than 2000. Resolution for the Telmisartan and Nisoldipine peaks in standard solution should not be less than 2.

METHOD VALIDATION [10]

Precision

Preparation of stock solution

Accurately weigh and transfer 40 mg of Telmisartan and 5 mg of Nisoldipine working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.75 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure

The standard solution was injected for six times and measured the area for all six. Injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Intermediate Precision/Ruggedness

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day.

Preparation of stock solution

Accurately weigh and transfer 40 mg of Telmisartan and 5 mg of Nisoldipine workingstandard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.75 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Linearity

Preparation of stock solution:

Accurately weigh and transfer 40 mg of Telmisartan and 5 mg of Nisoldipine workingstandard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate todissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I: 0.25 ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluent.

Preparation of Level – II : 0.5 ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluent.

Preparation of Level – III : 0.75 ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluent.

Preparation of Level – IV : 1 ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluents.

Preparation of Level – V : 1.25 ml of above stock solutions has taken in 10ml of volumetric flask, dilute up to the mark with diluents.

Procedure: Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

LOD and LOQ

The LOD and LOQ solutions was prepared injected, for three times and measured the area for all three injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Degradation Studies

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Telmisartan and Nisoldipine using the proposed method.

Hydrolytic degradation under acidic condition

Pipette 0.75 ml of above solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.44 microns syringe filters and place in vials.

Hydrolytic degradation under alkaline condition

Pipette 0.75 ml of above solution into a 10ml volumetric and add 3ml of 0.1N NaOH was added in 10ml of volumetric flask. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent. Filter the solution with 0.44 microns syringe filters and place in vials.

Thermal induced degradation

Telmisartan and Nisoldipinee sample was taken in petridish and kept in Hot air oven at 110^0C fo 3 hours. Then the sample was taken and diluted with diluents and injected into UPLC and analysed.

Oxidative degradation

Pipette 0.75 ml above stock solution into a 10ml volumetric flask and 1ml of 30% w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials.

Photo degradation:

Pipette 0.75 ml above stock solution into a 10ml volumetric flask and expose to sunlight for 24hrs and the volume was made up to the mark with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

Procedure:

The standard solutions prepared in the precision was injected on the other day, for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

RESULTS

Optimized Chromatographic Conditions

Linearity

The developed RP-HPLC method demonstrated excellent linearity for both Nisoldipine and Telmisartan. For Nisoldipine, the linearity was evaluated over a concentration range of 100 to 500 µg/ml, with the calibration curve showing a strong linear relationship between the concentration and the peak area. The corresponding areas for concentrations of 100, 200, 300, 400, and 500 µg/ml were 65,787, 131,783, 194,311,

256,245, and 317,748, respectively. This linearity was confirmed by a correlation coefficient of 0.999, indicating the method's reliability for quantifying Nisoldipine. Similarly, the linearity for Telmisartan was evaluated over a concentration range of 20 to 100 µg/ml. The areas corresponding to concentrations of 20, 40, 60, 80, and 100 µg/ml were 32,441, 67,728, 100,630, 134,448, and 172,463, respectively. The calibration curve also showed a strong linear relationship, with a correlation coefficient of 0.999, confirming the method's reliability for quantifying Telmisartan.

Accuracy

The accuracy of the RP-HPLC method for quantifying Telmisartan and Nisoldipine was evaluated through recovery studies at different concentration levels. For Telmisartan, at specification levels of 50%, 100%, and 150%, the amounts added were 10 mg, 20 mg, and 30 mg, respectively. The corresponding amounts found were 9.97 mg, 19.97 mg, and 29.77 mg, resulting in percentage recoveries of 99.67%, 99.87%, and 99.25%, respectively. The mean recoveries for Telmisartan were calculated as 99.59%, indicating high accuracy across the specified concentration levels. Similarly, for Nisoldipine, accuracy was assessed at specification levels of 50%, 100%, and 150%, with added amounts of 4.05 mg, 8.1 mg, and 12.04 mg, respectively. The amounts found were 4.06 mg, 8.09 mg, and 12.04 mg, resulting in percentage recoveries of 100.23%, 99.90%, and 99.89%, respectively. The mean recoveries for Nisoldipine were determined to be 100.01%, demonstrating excellent accuracy and reliability of the method for quantifying both Telmisartan and Nisoldipine in pharmaceutical samples.

Table: 1 Forced degradation studies for Telmisartan and Nisoldipine

Stress condition	Telmisartan		Nisoldipine	
	Area	% Degraded	Area	% Degraded
Standard drug	191642		107223	
Acid	183252	4.38	98959	7.71
Base	183532	4.23	98921	7.74
Peroxide	183253	4.38	98978	7.69
Thermal degradation	187552	2.13	98851	7.81
Photolytic degradation	186452	2.71	98789	7.87

Figure: 1UV Spectrum

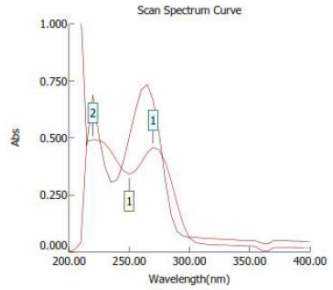


Figure: 2 Chromatogram of blank

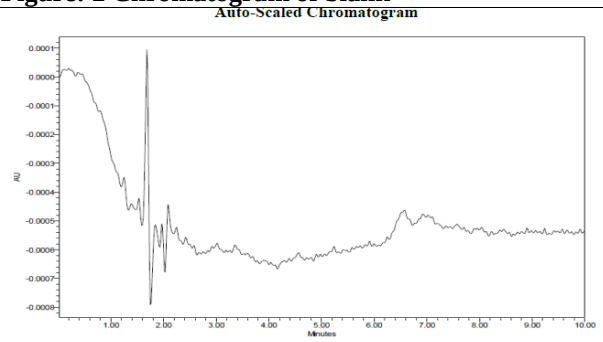


Figure: 3 Chromatogram of Telmisartan standard

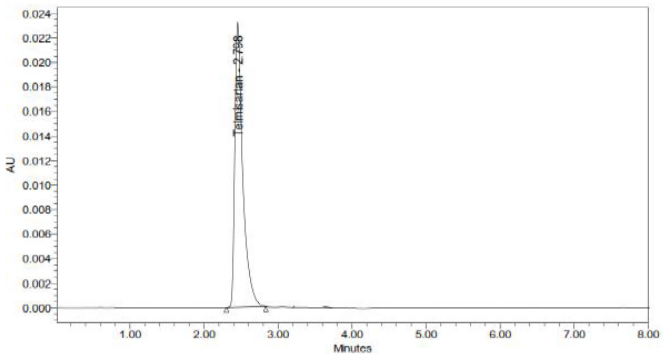


Figure: 4 Chromatogram of Nisoldipine standard

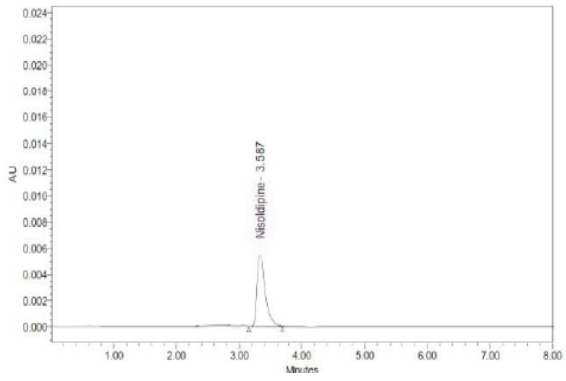


Figure: 5 Chromatogram of Standard chromatogram for Telmisartan and Nisoldipine

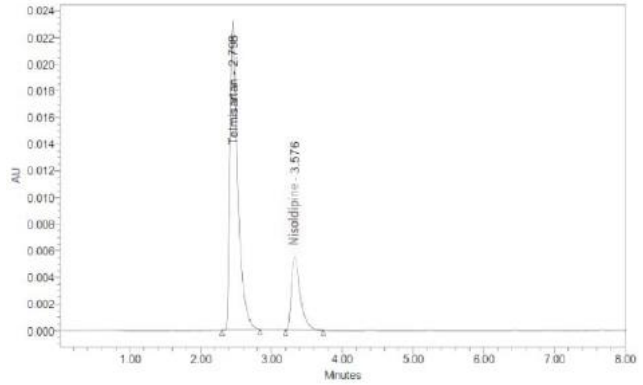


Figure: 6 Linearity results of Telmisartan

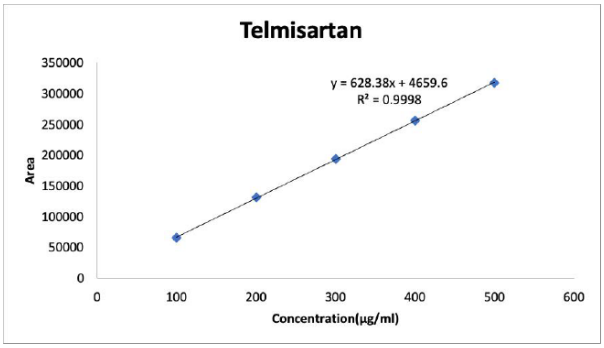


Figure: 7 Linearity results of Nisoldipine

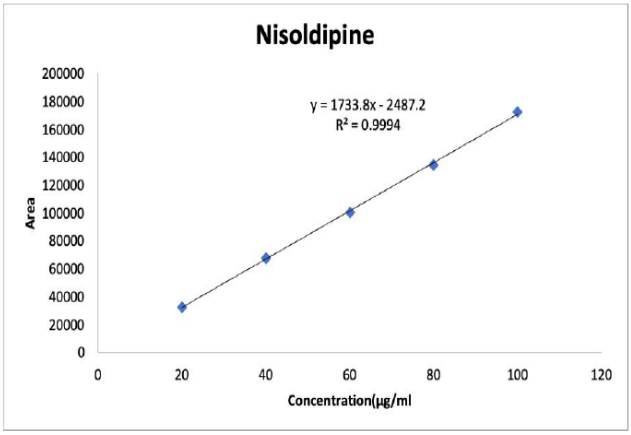


Figure: 8 LOD of Telmisartan and Nisoldipine

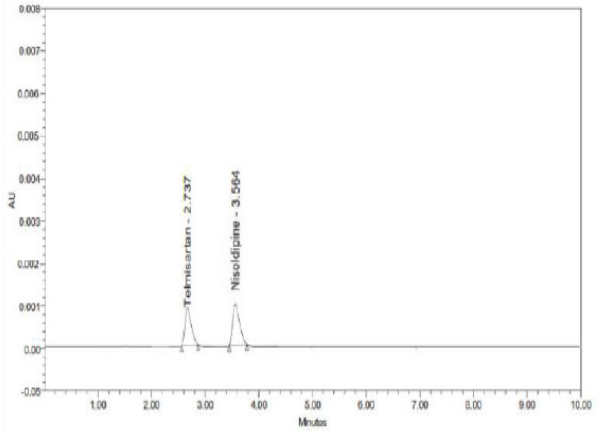
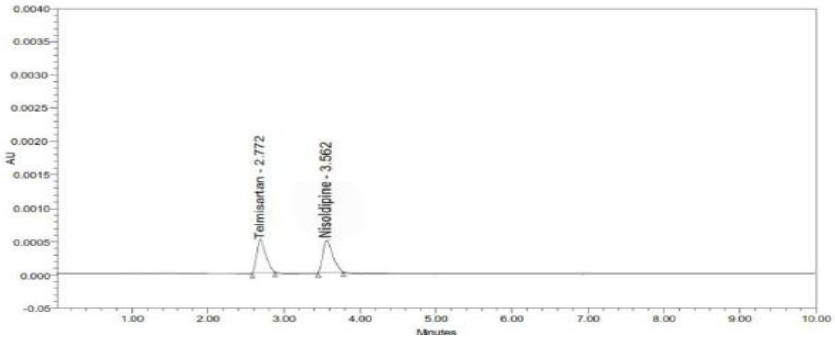


Figure: 9 LOQ of Telmisartan and Nisoldipine.



DISCUSSION

The developed RP-HPLC method for the simultaneous estimation of Nisoldipine and Telmisartan has demonstrated robust performance across various analytical parameters. The chromatographic conditions, including the use of an Inertsil ODS C18 column (4.6×250mm, 5µm), a mobile phase consisting of acetonitrile (ACN) and potassium dihydrogen phosphate (KH2PO4) at a ratio of 70:30 (v/v) adjusted to pH 3, and a detection wavelength of 225 nm, provided effective separation of the two drugs. The retention times of Nisoldipine and Telmisartan were determined to be 2.798 minutes and 3.587 minutes, respectively, indicating their efficient elution under the specified conditions. The % purity of Nisoldipine and Telmisartan was found to be 99.87% and 100.27%, respectively, affirming the method's ability to accurately quantify these compounds without interference from excipients or impurities.

System suitability parameters such as theoretical plates (>4000) and tailing factor (close to 1.2) for both analytes were within acceptable limits, ensuring adequate chromatographic performance. The resolution between Nisoldipine and Telmisartan was found to be 7.67, indicating good peak separation. Validation of the method according to ICH guidelines (ICH, Q2 (R1)) further substantiates its reliability. The linearity study across the concentration ranges of 50-250 µg/mL for Nisoldipine and 15-55 µg/mL for Telmisartan yielded correlation coefficients (r^2) of 0.999 for both drugs, demonstrating a strong linear relationship between concentration and peak area response. The % recovery values of 98.56% for Nisoldipine and 99.96% for Telmisartan indicate excellent accuracy of the method in quantifying the drugs in pharmaceutical formulations. Precision studies revealed low % RSD values of 1.2% for repeatability and 1.9% for intermediate precision, highlighting the method's reproducibility under different experimental conditions. The LOD and LOQ values were determined to be 3.72 and 7.40 µg/mL for Nisoldipine, and 0.0242 and 0.0202 µg/mL for Telmisartan, respectively, indicating the method's sensitivity to detect low concentrations of the analytes in samples.

CONCLUSIONS

The developed RP-HPLC method offers a reliable and efficient means for the routine analysis of Nisoldipine and Telmisartan, both as individual active pharmaceutical ingredients (APIs) and in combination formulations. Its ability to provide accurate quantification, coupled with robust analytical performance and compliance with regulatory guidelines, positions it as a valuable tool for quality control and pharmaceutical analysis laboratories.

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