FORM 2
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COMPLETE SPECIFICATION
(See section 10 and rule13)

TITLE OF THE INVENTION
METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ANALYSIS OF ATENOLOL-AMLODIPINE BY RP-HPLC

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the following specification particularly describes the invention and the manner in which it is to be performed.

FIELD OF INVENTION

The present invention relates to to pharmaceutical analysis, specifically to the development and validation of a robust and reliable reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous quantitative analysis of atenolol and amlodipine in pharmaceutical formulations.

BACKGROUND OF THE INVENTION

The accurate and simultaneous quantification of atenolol, a beta-blocker, and amlodipine, a calcium channel blocker, is critical for ensuring the efficacy and safety of combination therapies used in the management of hypertension and related cardiovascular disorders. These drugs, when formulated together, often require precise analytical methods to monitor their stability, dosage accuracy, and compliance with pharmacopoeial standards. However, the complex matrices of pharmaceutical formulations and potential interference from excipients pose significant challenges to achieving reliable quantification. Traditional methods often fall short in sensitivity, specificity, or speed, making routine quality control cumbersome and less efficient.

Existing analytical techniques such as UV-spectrophotometry or single-component HPLC methods are limited in their ability to simultaneously resolve and quantify atenolol and amlodipine in a single run. The lack of a validated, cost-effective, and efficient method often necessitates the use of separate analyses, leading to increased time, cost, and complexity in quality control processes. Furthermore, variations in retention times, poor resolution, or co-elution of peaks compromise the accuracy and reproducibility of the results, necessitating a more advanced approach.

To address these issues, there is a pressing need for a robust and validated reverse-phase high-performance liquid chromatography (RP-HPLC) method capable of simultaneous analysis of atenolol and amlodipine. This method must overcome matrix interference, achieve sharp peak resolution, and ensure high sensitivity and reproducibility. Such an advancement would streamline quality control processes, reduce operational costs, and improve compliance with regulatory requirements,

offering a practical solution for the pharmaceutical industry.

OBJECTS OF THE INVENTION

Some of the objects of the present disclosure, which at least one embodiment herein satisfies, are as follows.

It is an object of the present disclosure to ameliorate one or more problems of the prior art or to at least provide a useful alternative

An object of the present disclosure is to detects and quantifies low concentrations of atenolol and amlodipine with excellent precision.

Another object of the present disclosure is to provides reliable results with recovery rates between 98–102%.

Still another object of the present disclosure is to distinguishes atenolol and amlodipine from excipients and degradation products effectively.

Another object of the present disclosure is to elutes atenolol and amlodipine at 2.8 and 4.6 minutes, ensuring rapid analysis.

Still another object of the present disclosure is to suitable for routine quality control and stability testing of pharmaceutical formulations.

Still another object of the present disclosure is to consistent results under small deliberate changes in chromatographic conditions.

Yet another object of the present disclosure is to delivers reproducible results across different laboratories, analysts, and instruments.

Yet another object of the present disclosure is to utilizes a simple mobile phase and standard instrumentation, reducing operational costs.

Other objects and advantages of the present disclosure will be more apparent from the following description, which is not intended to limit the scope of the present disclosure.

SUMMARY OF THE INVENTION

The following presents a simplified summary of the invention in order to provide a basic understanding of some aspects of the invention. This summary is not an

extensive overview of the present invention. It is not intended to identify the key/critical elements of the invention or to delineate the scope of the invention. Its sole purpose is to present some concept of the invention in a simplified form as a prelude to a more detailed description of the invention presented later.

The present invention is generally provides a reliable RP-HPLC method for the simultaneous analysis of atenolol and amlodipine in pharmaceutical formulations, ensuring accurate quantification of both drugs.

An embodiment of the present invention the method utilizes a C18 column (250 mm × 4.6 mm, 5 µm), a methanol-phosphate buffer mobile phase (60:40 v/v), and a detection wavelength of 235 nm.

Another embodiment of the invention atenolol and amlodipine are eluted at retention times of approximately 2.8 minutes and 4.6 minutes, respectively, ensuring clear separation and accurate measurement.

Yet another embodiment of the invention is the method is validated for linearity, accuracy, precision, robustness, specificity, and sensitivity in compliance with ICH guidelines, ensuring reliability.

Yet another embodiment of the invention is the method exhibits high sensitivity, with LOD values of 0.2 µg/mL for atenolol and 0.1 µg/mL for amlodipine, and LOQ values of 0.6 µg/mL and 0.3 µg/mL, respectively.

Yet another embodiment of the invention is recovery studies show 98–102% accuracy, and precision tests yield %RSD values below 2%, confirming the method's reproducibility and accuracy.

Yet another embodiment of the invention is the method is robust under small variations in chromatographic conditions and rugged across different analysts, instruments, and laboratories.

DETAILED DESCRIPTION OF THE INVENTION

The following description is of exemplary embodiments only and is not intended to limit the scope, applicability or configuration of the invention in any way. Rather, the following description provides a convenient illustration for implementing exemplary embodiments of the invention. Various changes to the described

embodiments may be made in the function and arrangement of the elements described without departing from the scope of the invention.

The present invention relates to reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous analysis of atenolol and amlodipine in pharmaceutical formulations. The method utilizes a C18 column (250 mm × 4.6 mm, 5 µm), a mobile phase comprising methanol and phosphate buffer (60:40 v/v, pH adjusted to 3.0), and UV detection at 235 nm. With retention times of 2.8 minutes for atenolol and 4.6 minutes for amlodipine, the method ensures clear separation and accurate quantification of both drugs. Designed with simplicity and efficiency in mind, the method offers fast analysis and minimal solvent consumption, making it suitable for routine quality control and stability testing of combination drug formulations.

The method has been rigorously validated in compliance with ICH guidelines, demonstrating excellent linearity ($R^2 > 0.999$) over a concentration range of 5–50 µg/mL, high sensitivity with low LOD and LOQ values, and high precision (%RSD < 2%). It is robust under small variations in chromatographic conditions and specific enough to detect the drugs even in the presence of degradation products. Recovery rates between 98–102% confirm its accuracy, while its ruggedness ensures reproducibility across different laboratories and instruments. This method is a cost-effective, reliable, and practical solution for analyzing atenolol and amlodipine in pharmaceutical quality control settings.

EXAMPLE 1: Methodology

The RP-HPLC method was developed using a C18 reverse-phase column (250 mm × 4.6 mm, 5 µm particle size) with a mobile phase consisting of methanol and phosphate buffer (50 mM, pH adjusted to 3.0 with orthophosphoric acid) in a 60:40 v/v ratio. The flow rate was set at 1.0 mL/min, and the detection wavelength was 235 nm. The column temperature was maintained at 30°C to optimize analyte

separation. Standard and sample solutions were prepared in methanol, filtered through a 0.45 µm membrane filter, and diluted with the mobile phase to achieve working concentrations. A 20 µL volume of the prepared solutions was injected into the HPLC system, and atenolol and amlodipine were eluted at retention times of approximately 2.8 minutes and 4.6 minutes, respectively. Each sample was analyzed in triplicate, and the detector response was recorded for quantitative analysis. System suitability was verified before analysis to ensure reliable performance.

EXAMPLE 2: Linearity

The linearity of the developed method was evaluated by preparing calibration curves for atenolol and amlodipine across a concentration range of 5–50 µg/mL. Each concentration was injected into the HPLC system in triplicate, and the peak areas were plotted against the corresponding concentrations. The linear regression analysis for both drugs produced a correlation coefficient (R^2) greater than 0.999, demonstrating excellent linearity. This indicates that the method is capable of accurately quantifying atenolol and amlodipine within the specified range.

The method demonstrated excellent linearity for both atenolol and amlodipine across the tested concentration range of 5–50 µg/mL. The calibration curves for both drugs were highly linear, with correlation coefficients (R^2) of 0.9998 for atenolol and 0.9997 for amlodipine. The regression equations obtained were Y=10345X+22134Y = 10345X + 22134Y=10345X+22134 for atenolol and Y=15478X+10564Y = 15478X + 10564Y=15478X+10564 for amlodipine, where YYY represents the peak area and XXX the concentration in µg/mL.

EXAMPLE 3: Accuracy

The accuracy of the method was assessed by recovery studies conducted at three concentration levels: 80%, 100%, and 120% of the nominal drug concentration. Known amounts of atenolol and amlodipine were added to pre-analyzed samples, and the mixtures were analyzed using the developed method. The percentage

recoveries for atenolol and amlodipine were found to be within the range of 98% to 102%, confirming that the method is accurate and reliable for quantitative analysis of these drugs in pharmaceutical formulations.

The accuracy of the method was confirmed by recovery studies at three levels: 80%, 100%, and 120% of the nominal concentration. The recovery for atenolol was found to range from 98.5% to 101.2%, while that for amlodipine ranged from 98.7% to 100.8%. For instance, at the 100% level, the recoveries were 99.8% for atenolol and 99.5% for amlodipine.

EXAMPLE 4: Precision

The precision of the method was evaluated through intra-day and inter-day variability studies. Intra-day precision was determined by analyzing six replicates of atenolol and amlodipine at the same concentration within a single day, while inter-day precision involved analyzing the same samples over three consecutive days. The percentage relative standard deviation (%RSD) for both drugs was consistently below 2%, indicating that the method is highly precise and reproducible under the tested conditions.

The intra-day precision study showed %RSD values of 1.2% for atenolol and 1.1% for amlodipine, while the inter-day precision study yielded %RSD values of 1.5% for atenolol and 1.4% for amlodipine. These low %RSD values confirm the method's high precision and reproducibility under both intra-day and inter-day conditions, making it suitable for routine quality control.

EXAMPLE 5: Limit of Detection (LOD) and Limit of Quantification (LOQ)

The sensitivity of the method was determined by calculating the LOD and LOQ for atenolol and amlodipine. The LOD values were established as 0.2 µg/mL for atenolol and 0.1 µg/mL for amlodipine, while the LOQ values were 0.6 µg/mL and 0.3 µg/mL, respectively. These low values demonstrate that the method is sensitive enough to detect and quantify trace amounts of both drugs, making it suitable for applications requiring high sensitivity.

Results shows that the calculated LOD for atenolol was 0.2 µg/mL, and the LOQ was 0.6 µg/mL. For amlodipine, the LOD was 0.1 µg/mL, and the LOQ was 0.3 µg/mL. These results indicate that the method is highly sensitive, capable of detecting and quantifying very low concentrations of both drugs in pharmaceutical formulations, ensuring its applicability for trace analysis.

EXAMPLE 6; Specificity

The specificity of the method was demonstrated through forced degradation studies, where samples of atenolol and amlodipine were subjected to stress conditions such as acid, base, oxidative, and thermal degradation. The chromatograms showed that the degradation products were well-resolved from the peaks of the main analytes, with no interference at the retention times of atenolol and amlodipine. This confirms that the method is specific and can accurately quantify these drugs even in the presence of potential impurities or degradation products.

Results shows that the forced degradation studies showed that atenolol and amlodipine were stable under various stress conditions, including acid, base, oxidative, and thermal degradation. The degradation products formed during these conditions were well-separated from the analyte peaks, with no interference observed at the retention times of atenolol (2.8 minutes) and amlodipine (4.6 minutes). These results confirm the method's specificity and its ability to accurately analyze the drugs in the presence of degradation products.

EXAMPLE 7:Robustness

The robustness of the method was assessed by introducing deliberate small changes to critical chromatographic parameters, such as the composition of the mobile phase (±2%), flow rate (±0.1 mL/min), and column temperature (±2°C). These variations did not significantly affect the retention times, peak areas, or resolution of atenolol and amlodipine. The %RSD values remained below 2%, confirming that the method is robust and capable of delivering consistent results under slightly altered experimental conditions.

Results shows that the robustness study demonstrated that small deliberate changes in the mobile phase composition, flow rate, and column temperature did not significantly affect the retention times, peak areas, or resolution of atenolol and amlodipine. For instance, changes in the flow rate from 1.0 mL/min to 0.9 mL/min and 1.1 mL/min resulted in a %RSD of 1.4% for atenolol and 1.3% for amlodipine.

EXAMPLE 8: System Suitability

System suitability tests were conducted prior to analysis to ensure the optimal performance of the chromatographic system. Parameters such as theoretical plates (≥2000 for both atenolol and amlodipine), tailing factor (≤1.5), resolution (≥2.0), and retention time reproducibility were evaluated. The results met the acceptance criteria, demonstrating that the HPLC system was well-suited for the analysis of atenolol and amlodipine.

Result shows that the System suitability tests showed theoretical plate numbers of 3100 for atenolol and 2900 for amlodipine, tailing factors of 1.3 for both drugs, and a resolution of 5.2 between the peaks.

EXAMPLE 9: Ruggedness

The ruggedness of the method was tested by analyzing the same samples under different conditions, including variations in analysts, instruments, and laboratories. The results were consistent, with no significant differences in peak areas, retention times, or resolution. The %RSD values remained below 2%, indicating that the method is rugged and reliable when applied across different operational settings.

The ruggedness study showed consistent results across different analysts, instruments, and laboratories. The %RSD for atenolol was 1.6%, and for amlodipine, it was 1.5%. The retention times and peak areas were comparable, confirming that the method is rugged and produces reliable results under varying operational conditions.

EXAMPLE 10: Stability of Analytical Solutions

The stability of atenolol and amlodipine in the prepared analytical solutions was evaluated over 24 hours under room temperature and refrigerated conditions. The solutions were re-analyzed at regular intervals, and no significant changes were observed in peak areas or retention times. The results confirmed that the prepared solutions are stable for at least 24 hours, ensuring the reliability of the method for routine analysis.

The stability study revealed that the analytical solutions of atenolol and amlodipine were stable for up to 24 hours at both room temperature and refrigerated conditions. The %RSD for peak areas during the stability period was 1.2% for atenolol and 1.1% for amlodipine.

EXAMPLE 11: Peak Purity

The peak purity of atenolol and amlodipine was assessed using a photodiode array (PDA) detector. The purity indices for both analytes were greater than 0.999, indicating that the peaks were free from co-eluting impurities or degradation products. This ensures that the method is capable of accurate quantification, even in the presence of excipients or minor impurities.

Peak purity analysis using a photodiode array detector confirmed purity indices of 0.9998 for atenolol and 0.9999 for amlodipine. These high purity values indicate that the chromatographic peaks were free from co-eluting impurities or degradation products, ensuring accurate and specific quantification of the analytes.

While considerable emphasis has been placed herein on the specific features of the preferred embodiment, it will be appreciated that many additional features can be added and that many changes can be made in the preferred embodiment without departing from the principles of the disclosure. These and other changes in the preferred embodiment of the disclosure will be apparent to those skilled in the art from the disclosure herein, whereby it is to be distinctly understood that the foregoing descriptive matter is to be interpreted merely as illustrative of the disclosure and not as a limitation.

We Claim,

1. A method for the simultaneous analysis of atenolol and amlodipine in pharmaceutical formulations using reverse-phase high-performance liquid chromatography (rp-hplc), comprising:
    a) using a c18 reverse-phase column with dimensions of 250 mm × 4.6 mm and a particle size of 5 µm;
    b) preparing a mobile phase consisting of methanol and phosphate buffer (50 mm, ph adjusted to 3.0 with orthophosphoric acid) in a 60:40 v/v ratio;
    c) maintaining a flow rate of 1.0 ml/min and a detection wavelength of 235 nm;
    d) injecting a 20 µl volume of sample solution into the hplc system;
    e) separating atenolol and amlodipine with retention times of approximately 2.8 minutes and 4.6 minutes, respectively; and
    f) quantifying atenolol and amlodipine by integrating peak areas using calibration curves.
2. The method as claimed in claim 1, wherein the column temperature is maintained at 30°C to ensure optimal separation and reproducibility of results.
3. The method as claimed in claim 1, wherein the sample preparation involves dissolving a powdered tablet formulation containing atenolol and amlodipine in methanol, followed by sonication for 15 minutes and filtration through a 0.45 µm membrane filter.
4. The method as claimed in claim 1, wherein the method is validated for linearity, accuracy, precision, robustness, specificity, and sensitivity according to ICH guidelines.
5. The method as claimed in claim 1, wherein the linearity is demonstrated over a concentration range of 5–50 µg/mL for both atenolol and amlodipine, with correlation coefficients (R^2) greater than 0.999.
6. The method as claimed in claim 1, wherein the limits of detection (LOD)

and quantification (LOQ) are 0.2 µg/mL and 0.6 µg/mL for atenolol, and 0.1 µg/mL and 0.3 µg/mL for amlodipine, respectively.

7. The method as claimed in claim 1, wherein the robustness is demonstrated by maintaining consistent peak areas and resolution under small deliberate changes to chromatographic parameters, including flow rate, mobile phase composition, and column temperature.
8. The method as claimed in claim 1, wherein the method is capable of detecting atenolol and amlodipine in the presence of degradation products, ensuring specificity for stability and forced degradation studies.

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TITLE: METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ANALYSIS OF ATENOLOL-AMLODIPINE BY RP-HPLC

ABSTRACT

The present invention relates to a robust reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous analysis of atenolol and amlodipine in pharmaceutical formulations. The method employs a C18 column (250 mm × 4.6 mm, 5 µm), a mobile phase of methanol and phosphate buffer (60:40 v/v, pH 3.0), a flow rate of 1.0 mL/min, and UV detection at 235 nm. Atenolol and amlodipine are eluted at retention times of 2.8 and 4.6 minutes, respectively. The method is validated according to ICH guidelines for linearity, accuracy, precision, sensitivity, robustness, and specificity. It demonstrates a linear range of 5–50 µg/mL, LOD of 0.2 µg/mL (atenolol) and 0.1 µg/mL (amlodipine), and high recovery rates (98–102%). The method is suitable for routine quality control and stability studies, offering rapid, accurate, and cost-effective analysis of atenolol and amlodipine in combination drug products.