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Optimized RP-HPLC Method for the Quantification and Validation of Amlodipine and Irbesartan

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KEYWORDS
High-Performance Liquid Chromatography (HPLC), Amlodipine, Irbesartan, Method Validation, Pharmaceutical Analysis

ABSTRACT:
This study details the optimization and validation of an HPLC method for the simultaneous quantification of Amlodipine and Irbesartan in tablet formulations. Using a Methanol: Water (0.1% Orthophosphoric Acid) mobile phase in a 25:75 ratio and an Agilent C18 column, the method was optimized for effective separation. Validation was conducted according to ICH guidelines, with the method exhibiting excellent linearity for both drugs ($R^2 > 0.997$), precise results with low %RSD, and high accuracy as evidenced by recovery studies. The method proved robust under various tested conditions, confirming its suitability for routine quality control of Amlodipine and Irbesartan in pharmaceutical products.

Introduction

Analytical chemistry is a dynamic field focused on understanding the composition and properties of matter. Unlike other branches of chemistry, it's not limited to specific compounds or reactions. Analytical methods, which are specific applications of techniques to solve problems, often involve sophisticated instrumentation. [1] These instruments are vital for various scientific and industrial applications, from developing new products to ensuring consumer safety and environmental protection.

In pharmaceutical analysis, both qualitative and quantitative methods are essential. Qualitative analysis identifies the components present in a sample, while quantitative analysis measures the relative amounts of those components.[2,3] Analytical method development is crucial for both new and existing products, involving optimization, validation, and sometimes proposing alternate methods for better efficiency.

Analytical methods can be classified into instrumental and non-instrumental categories. Instrumental methods rely on measuring physical properties, like current or optical density, while non-instrumental methods use conventional physicochemical properties.[4,5]

Various analytical techniques are employed, including spectroscopy, electrochemistry, chromatography, and more. Chromatography, in particular, is a powerful method for separating components in a sample based on their interaction with a stationary phase and a mobile phase. [6,7]

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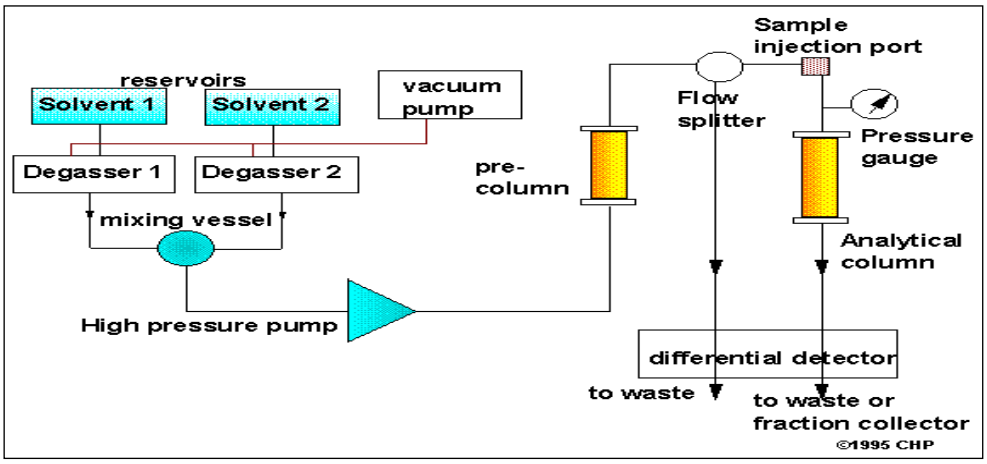

Figure 1. Schematic presentation of HPLC

High-performance liquid chromatography (HPLC) is widely used in pharmaceutical analysis due to its speed, resolution, and accuracy. It relies on maintaining a constant mobile phase composition (isocratic elution) or changing it continuously (gradient elution) during separation. [8,9] · HPLC instrumentation consists of components like pumps, injection systems, columns, and detectors. These components work together to achieve efficient separation and detection of analytes. Method development in HPLC involves careful selection of columns, mobile phases, and detection wavelengths to optimize analysis time, resolution, selectivity, and sensitivity. Sample preparation is crucial and should not damage the column or affect the analysis. [10,11]

The steps involved in HPLC method development include defining validation protocols, performance parameters, and acceptance criteria, as well as conducting validation experiments and documenting results.

Overall, HPLC offers high resolving power, speed, accuracy, and automation, making it a preferred method for various analytical applications, especially in pharmaceutical analysis.

Table 1. Steps involved and separation goals in HPLC methods**.** [12,13]

Goal	Comment
Resolution	Precise and rugged quantitative analysis requires that Rs be greater than 1.5
Separation time	< 5-10 min is desirable for routine procedures
Quantitation	<2% (1 SD) for assay < 5% for less demanding analyses < 15 % for trance analyses
Pressure	<150 bar is desirable <200 bar is usually essential
Peak height	Narrow peaks are desirable for large signal/noise ratios
Solvent consumption	Minimum mobile phase use per run is desirable

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Drug Profile [14,15,16]

Irbesartan is an angiotensin II receptor blocker (ARB) primarily used to treat hypertension and diabetic nephropathy. It functions by blocking the binding of angiotensin II to the AT1 receptor, which inhibits vasoconstriction and aldosterone effects, promoting vasodilation and reducing blood pressure. Irbesartan is well-absorbed orally with a bioavailability of 60-80% and is metabolized in the liver, with a biological half-life of 11-15 hours. It is excreted via both biliary and renal routes.

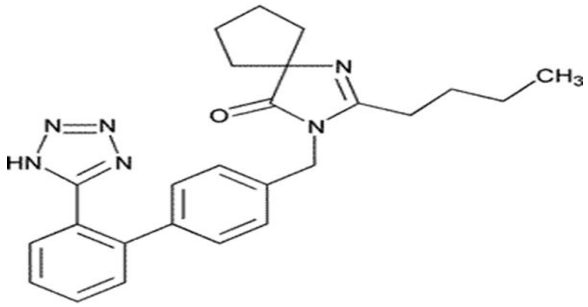

Figure 2 Structure of Irbesartan

Amlodipine is a calcium channel blocker used to manage hypertension and coronary artery disease, including conditions like stable and vasospastic angina. It works by inhibiting the influx of calcium ions into vascular smooth muscle and cardiac cells, reducing arterial resistance and blood pressure. Amlodipine has a bioavailability of 64-90%, undergoes hepatic metabolism, and has an elimination half-life of 30-50 hours, contributing to its ability to maintain effect over 24 hours. Common side effects include peripheral edema, dizziness, and palpitations, particularly at higher doses.

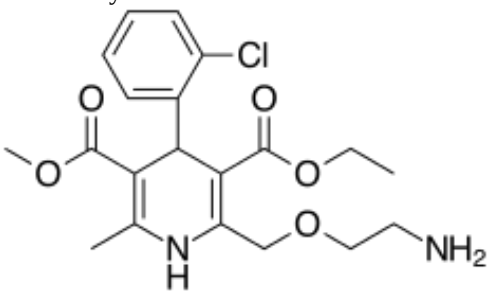

Figure 3 Structure of Amlodepine

Materials and Methods

Chemicals and Reagents

In the method development and validation of preservatives, the following chemicals and reagents were employed:

Ingredients:

Irbesartan, API grade, supplied by Sanofi.

Amlodipine, API grade, obtained from Hanmi Pharmaceuticals.

Orthophosphoric acid (OPA), HPLC grade, provided by Avantor Performance Materials India Ltd., Thane, Maharashtra.

Methanol, HPLC grade, sourced from Merck Specialties Pvt. Ltd., Shiv Sager Estate ‘A’, Worli, Mumbai.

Water, HPLC grade, also supplied by Merck Specialties Pvt. Ltd., Shiv Sager Estate ‘A’, Worli, Mumbai.

List of Marketed Formulations:

The study includes marketed formulations of the combination of Irbesartan and Amlodipine, specifically

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the AMIX R TABLET (100 mg Irbesartan and 10 mg Amlodipine) manufactured by Sanofi.

Instrumentation: [17,18,19]

The analysis was conducted using an Agilent (S.K.) Gradient System with a UV Detector.

The system was equipped with a Reverse Phase (Agilent) C18 column (4.6mm x 250mm; 5µm), an SP940D pump, a 20µl injection loop, a UV740D Absorbance detector, and utilized Chem Station software.

Chromatographic Conditions:

HPLC System: Agilent Gradient System with UV Detector.

Column: Agilent C18 (4.6mm x 250mm; 5µm).

Mobile Phase: Methanol: Water (0.1% with Orthophosphoric Acid) at a ratio of 25:75.

Detection Wavelength: 240 nm.

Flow Rate: 1 ml/min.

Temperature: Ambient.

Injection Volume: 20 µl.

Run Time: 15 min.

Filter Paper: 0.45 µm.

Method Development of HPLC:

Various mobile phase compositions were tested to optimize separation and peak symmetry:

60% 0.1% Orthophosphoric Acid + 40% Methanol.

70% Orthophosphoric Acid Water + 30% Methanol.

80% Orthophosphoric Acid Water + 20% Methanol.

75% Orthophosphoric Acid Water + 25% Methanol.

The selected mobile phase for routine analyses consisted of 75% water and 25% methanol, both adjusted with 0.1% Orthophosphoric Acid to enhance peak resolution and reproducibility.

Preparation of Standard Solutions:[20,21]

Irbesartan Standard Stock Solution (Stock I): 5 mg of Irbesartan was accurately weighed and dissolved in methanol to a final volume of 10 ml in a volumetric flask, resulting in a solution of 500 µg/ml.

Amlodipine Standard Stock Solution (Stock II): 75 mg of Amlodipine was accurately weighed and dissolved in methanol to a final volume of 10 ml in a volumetric flask, creating a solution of 7500 µg/ml.

Combination Stock Standard Solution (Stock III): 5 mg of Irbesartan and 75 mg of Amlodipine were accurately weighed, transferred to a 10 ml volumetric flask, dissolved in methanol, and volume adjusted to the mark with the same solvent, producing standard solutions of 500 µg/ml and 7500 µg/ml, respectively. The mixture was sonicated for 15 minutes to ensure complete dissolution and degassing.

Calibration and Validation: [22,23]

The method was calibrated and validated according to ICH guidelines for linearity, accuracy, precision, and robustness. Calibration curves were constructed for concentrations ranging from 5 to 25 µg/ml for Irbesartan and 75 to 375 µg/ml for Amlodipine, based on peak areas recorded at a detection wavelength of 240 nm.

The developed method was rigorously validated to confirm its suitability for the quantitative determination of Irbesartan and Amlodipine in combined dosage forms, ensuring reliability and reproducibility of the results.

Optimization of HPLC Method [24]

The optimization of the HPLC method for the simultaneous estimation of Irbesartan and Amlodipine involved a systematic evaluation of various chromatographic conditions to achieve optimal separation, resolution, and reproducibility of the analyte peaks. The optimization process included adjustments to the mobile phase composition, flow rate, column type, and detection wavelength. Here are the details of the steps involved in the method optimization:

Selection of Column:[25]

The choice of column is critical in HPLC method development. A Reverse Phase C18 column (Agilent,

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4.6mm x 250mm, 5µm particle size) was selected due to its robustness and effectiveness in separating a wide range of compounds, including drugs like Irbesartan and Amlodipine.

Mobile Phase Composition: [26]

The mobile phase plays a crucial role in controlling the separation and elution of compounds. Several compositions were tried, and the following were evaluated for best results:

60% 0.1% Orthophosphoric Acid + 40% Methanol

70% Orthophosphoric Acid Water + 30% Methanol

80% Orthophosphoric Acid Water + 20% Methanol

75% Orthophosphoric Acid Water + 25% Methanol

The optimal mobile phase composition selected was Methanol: Water (0.1% Orthophosphoric Acid) at a ratio of 25:75. This composition provided the best balance between peak resolution and analysis time, with sharp and well-resolved peaks.

Optimization of Flow Rate:

The flow rate affects both the analysis time and the resolution of peaks. A flow rate of 1 ml/min was found to be optimal for providing adequate contact time between the analytes and the stationary phase, resulting in better separation.

Detection Wavelength:

The detection wavelength was set at 240 nm based on the maximum absorbance (λmax) of both Irbesartan and Amlodipine. This wavelength provided the best sensitivity for detecting both compounds.

pH of the Mobile Phase:

The pH was adjusted to 3.0 using Orthophosphoric Acid. This acidic environment ensured better peak shapes and improved the resolution of the analytes by suppressing ionization, which is particularly important for drugs like Amlodipine.

Column Temperature:

The column temperature was maintained at ambient conditions. Stability tests indicated that temperature control did not significantly impact the separation, thus simplifying the method and reducing the need for specialized equipment.

Injection Volume:

An injection volume of 20 µl was chosen to balance between sensitivity and peak size. This volume was sufficient to achieve detectable and quantifiable peaks without causing column overload.

Run Time:

A total run time of 15 minutes was established to ensure complete elution of both analytes, as well as any impurities, without unnecessarily prolonging the analysis time.

Table 2 Instrumentation of HPLC

Item Number	Name of Instrument	Description/Specifications
1	Software	Ezchrom Elite, used for controlling the HPLC system and processing data
2	Pump	Agilent LC20AT, provides consistent and precise flow of mobile phase
3	Column	Inertsil CN-3.5µm (4.6 x 250 mm), used for effective separation with superior peak symmetry
4	Detector	UV/VIS (UV-2489), detects and analyzes compounds at specific wavelengths

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5	Injector	Rheodyne, used for introducing the sample into the flow stream of the HPLC system
6	Temperature Control	Ambient, the system operates under ambient temperature conditions

Method Validation as per ICH Guidelines [27,28,29]

Method validation is a critical requirement for ensuring the reliability and robustness of an analytical technique. According to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), method validation involves a series of experiments to assess the analytical method's performance characteristics and to ensure the quality, reliability, and consistency of analytical results. Below are the key elements required for method validation according to the ICH guidelines:

1. Specificity

The ability of the method to measure the analyte unequivocally in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Specificity is generally confirmed by demonstrating that the analyte and interferences do not co-elute.

2. Linearity

The method must demonstrate that it can obtain test results proportional to the concentration of analyte within a given range (i.e., the method's dynamic range). This is usually established by the calibration curve, which should exhibit a linear relationship between concentration and signal response. Linearity is typically assessed by a linear regression analysis, which should provide an acceptable correlation coefficient (R^2 value), slope, and intercept.

3. Accuracy

The accuracy of an analytical method is the closeness of the test results obtained by the method to the true value. Accuracy is usually expressed as the percentage of recovery by the assay of known, added amounts of analyte. This parameter is often validated by spiking known concentrations of analyte into the matrix and then measuring the recovery rates.

4. Precision

Precision reflects the reproducibility of the method under the same operating conditions over a short interval of time. Precision is divided into:

Repeatability (intra-day): Variation observed when the same operator, using the same equipment, and over a short interval of time, repeats measurements.

Intermediate Precision (inter-day): Variability observed when different operators, instruments, and days are involved in the analysis.

Reproducibility: Precision under changed conditions that covers different laboratories and different equipment, etc.

5. Detection Limit and Quantitation Limit

Detection Limit (DL): The lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value.

Quantitation Limit (QL): The lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. The QL is used for determining the minimum concentration at which the analyte can not only be reliably detected but also quantitatively measured.

6. Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small, but deliberate, variations in method parameters and provides an indication of its reliability during normal usage. Changes in conditions include variations in pH, temperature, flow rates, and sample injection volume.

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7. Range

The range of an analytical method is the interval between the upper and lower levels (including these levels) of analyte that have been demonstrated to be determined with precision, accuracy, and linearity using the method as intended.

Table 3 Results of Linearity of Detector Response

Concentration (µg/ml)	Drug	Mean Peak Area (µV.sec)	Standard Deviation of Peak Area	% RSD of Peak Area
75	Amlodipine	494.21	0.40	0.08
150	Amlodipine	917.285	0.12	0.01
225	Amlodipine	1358.58	0.34	0.02
300	Amlodipine	1842.235	3.10	0.17
375	Amlodipine	2270.88	1.26	0.06
5	Irbesartan	107.56	-	-
10	Irbesartan	198.46	-	-
15	Irbesartan	294.98	-	-
20	Irbesartan	402.16	-	-
25	Irbesartan	496.00	-	-

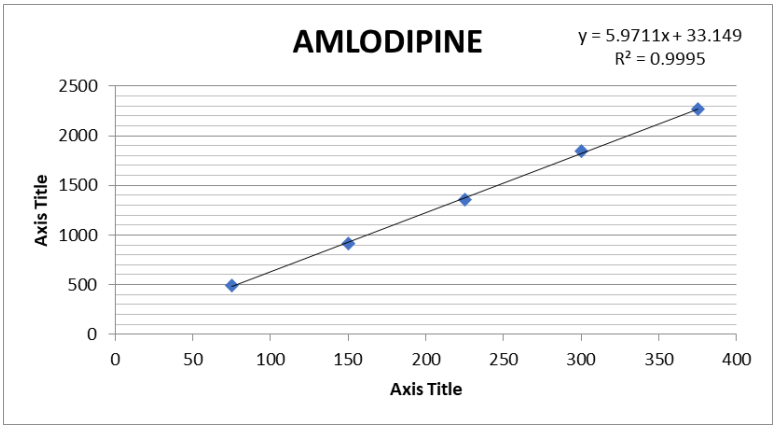

Figure 4 Calibration curve of Amlodepine

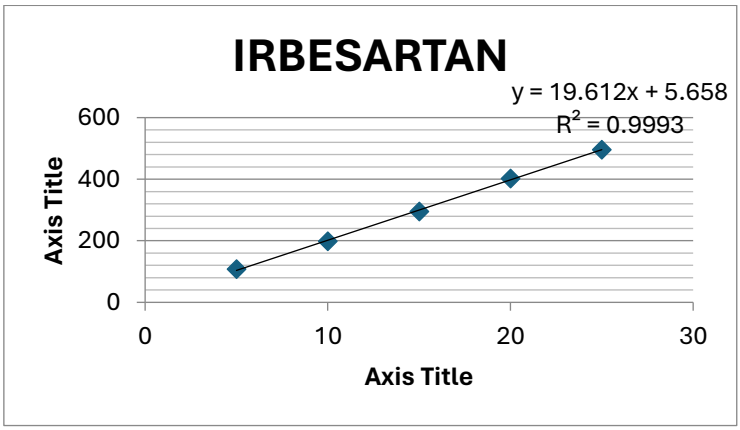

Figure 5 Calibration curve of Irbesartan

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Table 4 Accuracy for Amlodipine and Irbesartan

Drug	Level (% of nominal)	Amount Added (µg/ml)	Amount Recovered (µg/ml)	% Recovery	Mean % Recovery
Amlodipine	80%	60	59.8	99.67	
Amlodipine	100%	75	74.6	99.47	99.53
Amlodipine	120%	90	89.5	99.44	
Irbesartan	80%	4	3.98	99.50	
Irbesartan	100%	5	4.97	99.40	99.47
Irbesartan	120%	6	5.96	99.33	

Assay of Amlodipine and Irbesartan in Tablet Formulation

The assay of Amlodipine and Irbesartan in their tablet formulations involves quantifying the amounts of each drug per tablet. This is done by extrapolating the area values obtained from the HPLC analysis to the established calibration curve for each drug. The procedure was repeated six times to ensure accuracy and reproducibility of the results. The calculated amounts of Amlodipine and Irbesartan per tablet are reported in the following table:

Table 5 Result**s** of % Assay of Amlodipine and Irbesartan

Drug	Label Claimed (mg)	Amount Found (mg)	% Label Claim	Standard Deviation (SD)	% RSD
Amlodipine	10	9.94	99.40	0.31	0.02
Irbesartan	10	10.25	102.50	1.10	0.12
Amlodipine	10	9.95	99.50	0.01	0.07
Irbesartan	10	10.18	101.80	0.05	0.01

Table 6 Summary of Validation Parameters by HPLC Method for Amlodipine and Irbesartan

Validation Parameters	Amlodipine	Irbesartan
System Suitability		
Tailing Factor	1.16	1.08
% RSD	0.59	0.29
Theoretical Plates	7954	9610
Resolution	N.A	5.44
Linearity		
Correlation Coefficient	0.997	0.999

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Slope	14107	77067
Precision		
% RSD	0.18	0.28
Accuracy		
Mean % Recovery (50%, 100%, 150%)	98.80, 96.80, 98.50	96.65, 96.73, 97.65
Specificity	No interference	No interference
Robustness		
Flow rate by ± 10%	All parameters within limit	All parameters within limit
Column Oven Temperature by ± 5°C	All parameters within limit	All parameters within limit
pH of Buffer Solution by ± 0.2 units	All parameters within limit	All parameters within limit
Wavelength of Analysis ± 5 nm	All parameters within limit	All parameters within limit
Organic Composition of Mobile Phase by ± 5%	All parameters within limit	All parameters within limit
LOD (µg/ml)	0.85	1.37

Conclusion

The study successfully developed and validated an optimized HPLC method for the simultaneous quantification of Amlodipine and Irbesartan in pharmaceutical formulations. The method employed a mobile phase consisting of Methanol: Water (0.1% Orthophosphoric Acid) at a 25:75 ratio, with an Agilent C18 column facilitating effective separation. The method demonstrated robustness, precision, and accuracy, adhering to ICH guidelines for method validation.

The chromatographic conditions optimized for this method resulted in satisfactory resolution and reproducibility of the analyte peaks, as evidenced by the system suitability tests, which showed theoretical plates of 7954 for Amlodipine and 9610 for Irbesartan, and tailing factors within the acceptable limits. The method exhibited excellent linearity with correlation coefficients (R^2) of 0.997 for Amlodipine and 0.999 for Irbesartan, validating the method's capability to achieve consistent and reliable results across a specified range.

The accuracy studies revealed mean percent recoveries well within the acceptable limits for pharmaceutical analysis, confirming the method's ability to perform under varied conditions without significant deviation. Precision, as reflected by the %RSD values, was found to be less than 0.3% for both drugs, underscoring the method's reproducibility.

Furthermore, robustness testing affirmed that the HPLC method remained unaffected by small deliberate variations in method parameters such as pH, flow rate, and column temperature, thereby illustrating its reliability for routine quality control analysis of Amlodipine and Irbesartan in combined dosage forms.

In conclusion, this validated HPLC method is efficient, reliable, and reproducible for the routine analysis of Amlodipine and Irbesartan in pharmaceutical industries, ensuring quality control and compliance with pharmacopeial standards. The method's high sensitivity, accuracy, and short run time also enhance its applicability in high-throughput settings, making it a valuable tool for the pharmaceutical analysis of these widely used cardiovascular drugs.

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