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Development of Robust RP-HPLC Method for Concurrent Analysis of Aliskiren and Amlodipine in Combined Tablet Dosage Form

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Abstract

Present research focuses on development & validation of a robust RP-HPLC method for the concurrent estimation of Aliskiren & Amlodipine in collective tablet dosage forms. The mixture of Aliskiren, selective renin inhibitor, & Amlodipine, calcium channel blocker, is commonly prescribed for the management of hypertension. The primary purpose of research was to establish a fast, accurate, & stability-indicating RP-HPLC method that can reliably quantify both active pharmaceutical ingredients (APIs) in a single analytical run. The method was validated as per ICH guidelines, confirming its precision, linearity, accuracy, & sensitivity. Optimized chromatographic conditions included an acetonitrile and hexane sulfonic acid mixture (55:45 v/v) as mobile phase, which provided adequate resolution & quick retention times for both compounds. Method demonstrated excellent linearity with Aliskiren concentration ranging from 15 to 225 µg/mL & Amlodipine from 1 to 15 µg/mL. Retention times for Aliskiren & Amlodipine were 2.716 minutes & 7.351 minutes, respectively. Method proved suitable for routine analysis & stability testing, offering high sensitivity & reliable results for the quantification of Aliskiren & Amlodipine in tablet formulations under stressed conditions.

Keywords: Aliskiren, Amlodipine, Hypertension, RP-HPLC, Validation, Quality Control.

Introduction

Hypertension, a condition characterized by persistently high blood pressure. It is significant danger issue for cardiac conditions, stroke, and kidney failure, affecting millions globally. Among the many treatment options for managing hypertension, a combination of drugs targeting different pathways in the body is often prescribed for optimal blood pressure control. Aliskiren (ALN) (Figure 1), a direct renin inhibitor (1), and Amlodipine (AML) (Figure 2), a calcium channel blocker, are frequently used together to treat hypertension (2). Aliskiren works by inhibiting renin, an enzyme responsible for the production of angiotensin II, a potent vasoconstrictor. By lowering angiotensin II levels (3), Aliskiren helps in vasodilation, thus reducing blood pressure. Amlodipine, on the other hand, blocks calcium channels in the smooth muscle of blood vessels, leading to vasodilation and a subsequent reduction in peripheral resistance, which helps in lowering blood pressure (4-6).

Despite the effectiveness of Aliskiren and Amlodipine in managing hypertension, accurate and reliable methods for the simultaneous determination of both drugs in pharmaceutical formulations are essential for quality control and therapeutic monitoring. High-performance liquid chromatography (HPLC), particularly reverse-phase HPLC (RP-HPLC), is one of the most widely used analytical techniques for this purpose due to

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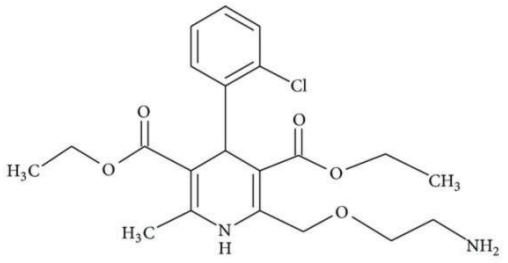

Figure 1: Structure of Aliskiren

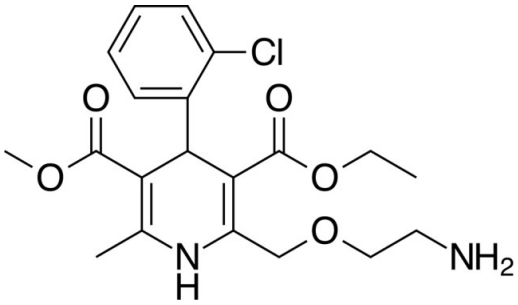

Figure 2: Structure of Amlodipine

its precision, sensitivity, and ability to separate and quantify multiple compounds in a single run (7-10).

In this study, the aim is to develop and validate a robust RP-HPLC method for the simultaneous quantification of Aliskiren and Amlodipine in combined tablet formulations. This method is designed to be simple, rapid, and precise while adhering to the stringent validation guidelines (11-20) set by the International Council for Harmonisation (ICH) (21,22). The method will also be stability-indicating, meaning it will be able to detect and quantify the drugs even under various stress conditions such as acid, base, oxidative, thermal, and photolytic degradation. This will ensure that the method can be used for routine quality control in the pharmaceutical industry.

Development of such a method is crucial for the assurance of the quality, safety, and efficacy of Aliskiren and Amlodipine-containing dosage forms, making it an essential tool for manufacturers and regulatory authorities alike. This research aims to bridge the gap in the current literature by providing a validated RP-HPLC method that is able to routinely useful for the examination of these drugs in combined formulations.

Materials and Methods

Materials

Pure ALN and AML were provided by Shree Icon Pharma Laboratories Ltd., Vijayawada, India. Tekamlo tablets containing both drugs were obtained from a local pharmacy. All reagents, including acetonitrile, hexane sulfonic acid, orthophosphoric acid, & high-purity water, were sourced from the Rankem Chemicals Ltd., Mumbai, India.

Instrumentation

An X-Bridge Phenyl C18 column was used on Waters HPLC e2695 system controlled with Empower software version 2.0. A setup including a 2695 pump with the degasser incorporated, a 2996 PDA detector, an auto injector with a 20 µL sample loop, and data acquisition were used. Sample injections were done by means of a 20 µL Hamilton syringe. It was done by Waters Empower 2 software. UV absorbance measurements were made using a double-beam UV-Visible spectrophotometer of Shimadzu UV-1700 model, using 10 mm quartz cuvettes at bandwidth of two nm. Ultrasonic bath sonicator (Unichrome UCA 701) was used to degas mobile phase.

Mobile phase and diluent

The acetonitrile and hexane sulfonic acid (55:45 v/v) was used, the same was used as diluent.

Stock Solution Preparation

Accurate amounts of ALN (150 mg) & AML (10 mg) were weighed & moved to volumetric flask of capacity 100 milli litre. To flask, 70 milli litre of diluent was added, and mixture was agitated using ultrasound for thirty minutes to ensure complete dissolution. Additional diluent was used to expand volume up to 100 mL. Resultant was then

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filtered by the aid of a 0.45-micron membrane filter. From this stock solution, 5 mL aliquot was diluted to 50 mL with diluent, yielding final concentration of 150 micro gram per milli litre for ALN and 10 micro gram per milli litre for AML.

Sample Solution Preparation

The each tablet weight was measured & exact transfer of required quantities of 150 milli gram of ALN & 10 milli gram of AML into 100 milli litre volumetric flask. However, sample solution was prepared in accordance with same procedure used to prepare the stock solution containing complete dissolution by sonication and dilution with the respective diluent. The solution was then filtered to remove any undissolved particles and the clear sample was then further analyzed.

Results and Discussion

Different trials were conducted using mobile phases composed of acetonitrile and water, acetonitrile and triethylamine, and acetonitrile and hexane sulfonic acid in various ratios for the separation of Aliskiren and Amlodipine. The optimized chromatographic conditions were determined to be a mobile phase consisting of acetonitrile and hexane sulfonic acid in a 55:45 v/v ratio, which provided the best separation and resolution of Aliskiren and Amlodipine and the results are furnished in Table 1. Figure 3 shows the corresponding chromatogram obtained under these conditions, where Aliskiren and Amlodipine are effectively separated from each other.

System Suitability Parameters

Standard solutions of ALN at 150 micro gram per milli litre & Amlodipine at 10 micro gram per milli litre were formulated & used to test system suitability. Six times prepared solutions were introduced into HPLC system for analysis & several important parameters, such as the tailing of the peaks, the resolution of the two peaks and the plate count, were evaluated. For the results, the tailing factor was showed to be

Table 1: Optimized chromatographic conditions

Mobile phase	Acetonitrile:Hexane sulphonic acid, 55:45v/v
Flow rate	1mL/min
Column	Waters X-Bridge Phenyl C18 (150 x 4.6mm, 3.5 µm)
Detector wave length	308nm
Column temperature	25°C
Injection volume	10 µL
Run time	10min
Diluent	Mobile phase
Retention time	Aliskiren: 2.716 min & Amlodipine: 7.351min
Theoretical plates	Aliskiren: 3069 & Amlodipine: 10987
Tailing factor	Aliskiren: 1.13 & Amlodipine: 1.06
Resolution	Aliskiren: -- & Amlodipine: 19.15

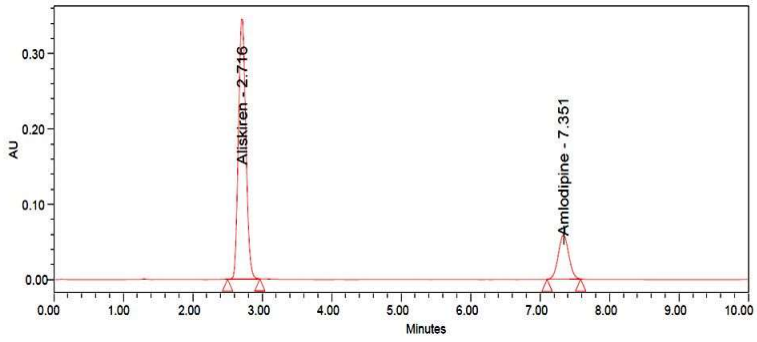

Figure 3: Optimized Chromatogram of Aliskiren & Amlodipine

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below 2, well resolved peak and the theoretical plate count satisfied the ICH guideline for system suitability.

Specificity

It was determined that the method was specific to avoid interference from diluent (blank injection), placebo, standard and sample solutions. The retention times of the target compounds were confirmed by no peaks at the retention times as no peaks appeared.

Linearity

ALN and AML standard solutions of various concentrations were formulated in order to determine their linearity. The calibration curves for both drugs are constructed (Figures 4 & 5), and outcomes are summarized in Table 2. Finally, linearity was tested, as a linear relationship to the concentration versus peak area was acquired for the ALN and AML solutions over tested range.

Precision

In three ways, precision for the same was assessed; system precision, repeatability and intermediate precision. Six consecutive injections of solution of standard were used to calculate %RSD which are given in Tables 3-5. The acceptable %RSD values, which resulted in high precision of the proposed method were also demonstrated by these results.

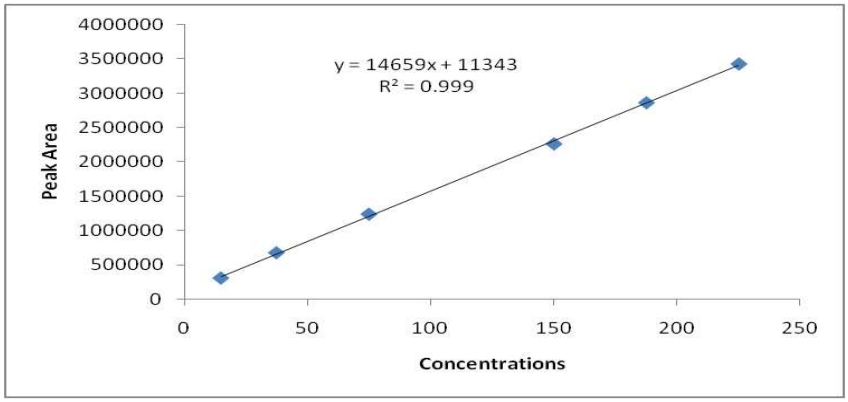

Figure 4: Calibration curve of Aliskiren

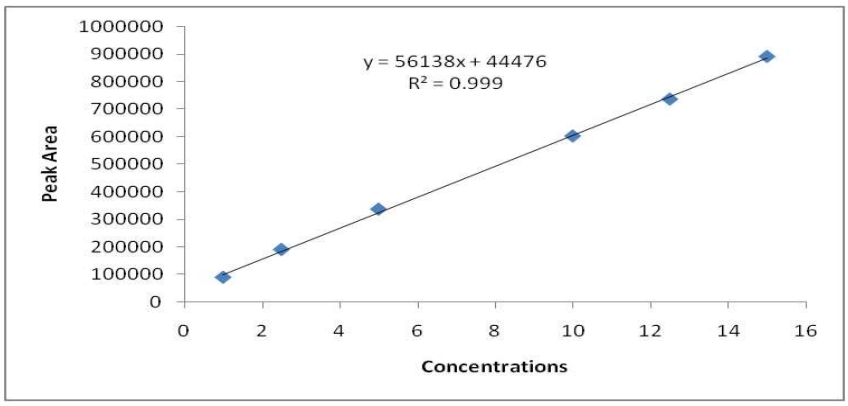

Figure 5: Calibration curve of Amlodipine

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Table 2: Linearity results for Aliskiren & Amlodipine				
S. No.	Concentration of Aliskiren (µg/ml))	Peak area	Concentration of Amlodipine (µg/ml)	Peak area
1	15	312533	1	68540
2	37.5	681295	2.5	300583
3	75	1242112	5	457194
4	150	2265157	10	603016
5	187.5	2864676	12.5	736954
6	225	3429409	15	924903
Slope		148994.11	Slope	60410.56
Intercept		74035.59	Intercept	1464.48
Correlation coefficient		0.9994	Correlation coefficient	0.9994

Table 3: System precision results for Aliskiren & Amlodipine		
S. No.	Aliskiren Peal area	Amlodipine Peal area
1	2264676	606954
2	2265147	606983
3	2268978	606899
4	2264461	606987
5	2265147	606978
6	2267974	606878
Mean	2266063	606946
SD	1914.02	46.8390
%RSD	0.08	0.01

Table 4: Repeatability results for Aliskiren & Amlodipine		
S. No.	Aliskiren Peal area	Amlodipine Peal area
1	2268676	606964
2	2265147	606791
3	2267978	606898
4	2268461	606979
5	2266147	606872
6	2268974	605790
Mean	2267563	606715
SD	1552.44	458.526
%RSD	0.07	0.08

Table 5: Intermediate precision results for Aliskiren & Amlodipine		
S. No.	Aliskiren Peal area	Amlodipine Peal area
1	2269083	606884
2	2266647	607794
3	2267478	607894
4	2268464	606973
5	2268147	606872
6	2268974	606773
Mean	2267802	607196
SD	653.34	645.26
%RSD	0.09	0.11

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Table 6: Accuracy results of Aliskiren

%Concentration (at specification level)	Peak area	Amount added (mg)	Amount found (mg)	%Recovery	%Mean Recovery
50%	1242136	75.0	75.0	100.00	100.12
100%	2265214	150	150.15	100.10	
150%	3429514	225.0	225.29	100.13	

Table 7: Accuracy results for Amlodipine

%Concentration (at specification level)	Peak area	Amount added (mg)	Amount found (mg)	%Recovery	%Mean Recovery
50%	457143	5.0	5.01	100.20	100.1
100%	603021	10.0	10.01	100.01	
150%	924912	15.0	15.01	100.01	

Accuracy

It was confirmed by the percentage recovery of ALN & AML when a pre-quantified sample solution was spiked with known amount of both drugs. It was found that mean recovery values from 99% to 101% demonstrates that the method is accurate and can produce useful results. The Tables 6 and 7 summarize these recovery data.

Sensitivity

To evaluate the method's sensitivity, LOD and LOQ for both ALN & AML were assessed. The LOD of ALN & AML was measured with the aid of standard deviation method & was obtained to be 0.15 micro gram per milli litre & 0.01 micro gram per milli litre respectively. In same manner, it was determined that LOQ numerical for ALN and AML were 0.45 micro gram per milli litre & 0.03 micro gram per milli litre, respectively, for high sensitivity of method in low concentration determination for both drugs.

Degradation studies

Acid Degradation Studies

A hydrochloric acid solution was prepared containing ALN and AML, which was subsequently treated at elevated temperatures to assess stability of the solution and of ALN and AML separately. In this degradation study, the aim here was to simulate the possible break down of the drugs under such acidic conditions, and therefore get some idea of the stability of the drugs exposed to such environments.

Alkali Degradation Studies

Similarly, the stock solutions were subjected to sodium hydroxide to study the stability of drugs in an alkaline medium. The success of this process was to help establish how drugs were susceptible to degradation when at basic conditions, since this is helpful to know about how they behave under different pH levels.

Reduction Degradation Studies

For the reduction degradation study, stock solutions of ALN and AML were also treated with sodium bisulfite. The purpose of this test was to assess the stability of drugs in reducing condition to determine if any changes in their structure or degradation occur under such reducing environments.

Oxidative Degradation Studies

Oxidative degradation studies were performed to determine effect of oxidative stress on ALN and AML using Hydrogen peroxide. The aim was to simulate the conditions in which the drugs may be exposed to oxidizing agents, in order to gain an insight into their stability and degradation under oxidative conditions.

Thermal Degradation Studies

The simultaneous thermal analysis (TGA) was performed to accesses thermal stability of test materail which were placed for

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Table 8: Degradation data of Aliskiren & Amlodipine

Condition	Aliskiren		Amlodipine	
	Peak area	%Degraded	Peak area	%Degraded
Control	2268781	-	606557	-
Acid	1915342	15.6	524214	13.5
Alkali	1906874	15.9	523795	13.6
Reduction	1984326	12.5	519687	14.3
Oxidative	1954522	13.8	526341	13.2
Thermal	1898532	16.3	515254	15
Photolytic	1859798	18	513420	15.3

an episode of 24 hours in hot air oven. In this study it was asked whether overexposure to high heat could cause some drug to degrade or lose potency.

Photolytic Degradation Studies

Photos stability of ALN and AML was observed by their exposure to ultraviolet (UV) light. In this degradation study, light exposure that a drug might see during storage or handling is simulated in order to determine (if) exposure to light might cause chemical breakdown or change in the composition of the drug.

The degradation studies have been summarized in Table 8 and the effects of acid, alkali, reduction, oxidation, heat and light have been considered.

Conclusion

The developed RP-HPLC process for concurrent quantification of ALN & AML in tablet formulations is innovative, straightforward, rapid, accurate, & precise. The reduced retention times lead to shorter analysis durations, enhancing efficiency & cost-effectiveness for routine quality control in the pharmaceutical industry.

Acknowledgement

Gratitude extends to V. V. Institute of Pharmaceutical Sciences, Gudlavalleru for providing all necessary facilities to perform the work as well as for their supportive atmosphere.

Conflicts of Interest

This research work has no conflicts of interest that need to be declared according to the authors.

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