Validation of Rp-HPLC Method for Simultaneous Estimation of Losartan and Amlodipine in Bulk and Tablet Dosage Form

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Abstract: A simple, precise, and validated Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) method was developed for the simultaneous estimation of Losartan and Amlodipine in bulk and tablet dosage forms. The cinematographic analysis was carried out using a Develosil C18 column (4.6 mm × 250 mm, 5 µm) maintained at ambient temperature. The mobile phase comprised Acetonitrile and Acetate buffer (pH 4.3) in the ratio of 35:65% v/v, delivered at a flow rate of 1 mL/min. Detection was performed at a wavelength of 238 nm, with an injection volume of 20 µL and a run time of 6 minutes. The method produced well-resolved peaks for both Losartan and Amlodipine with consistent retention times and no interference from excipients. It was validated in accordance with ICH Q2(R1) guidelines, demonstrating excellent linearity, precision, accuracy, specificity, and robustness. The results confirm that this RP-HPLC method is suitable for the routine quality control of Losartan and Amlodipine in combined pharmaceutical dosage forms.

Keywords: RP-HPLC, Losartan, Amlodipine, Develosil C18 column, linearity, accuracy.

I.INTRODUCTION

Hypertension, a chronic medical condition characterized by elevated blood pressure, is commonly managed through combination therapy involving drugs with complementary mechanism of action. Among these, Losartan potassium, an angiotensin II receptor antagonist, and Amlodipine besylate, a calcium channel blocker, are frequently co-administered due to their synergistic effects in lowering blood pressure and improving cardiovascular outcomes [1].

Analytical technique for estimating losartan and amlodipine in bulk and tablet dosage form has been extensively documented in the literature. High performance liquid chromatography (HPLC) reversed-phase HPLC have been estimated for Dosage form [2,3]. The challenge in developing a losartan and amlodipine in bulk and tablet dosage form was to develop an effective and stability indicating method. The method was developed by using column Develosil C18 (250mm X4.6 mm),5µm particle and HPLC-UV detector and to validate this method [4,5,6].

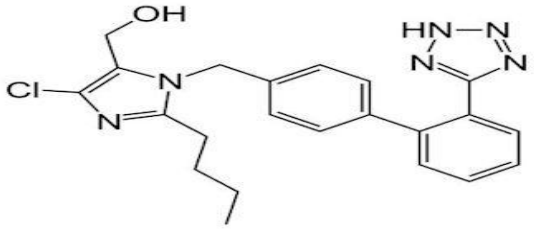

Fig 1: Structure of Losartan

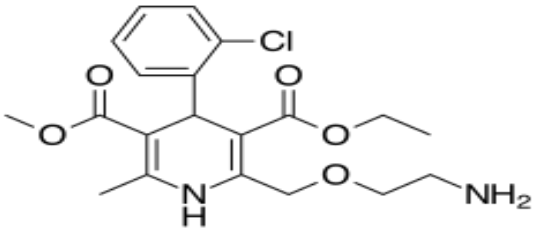

Fig 2: Structure of Amlodipine

High-Performance Liquid Chromatography also known as High Pressure Liquid Chromatography, is a type of column chromatography that is commonly used in biochemistry and analysis to separate, identify, and quantify active chemicals [7]. It is a popular analytical technique for separating, identifying, and quantifying each element of a

mixture. The distribution of the analyse (sample) between a mobile phase[8] and a stationary phase is the foundation of the HPLC separation principle (packing material of the column). The molecules travel through the stationary phase more slowly depending on the chemical makeup of the analytic. The duration of a sample's "on-column" time is determined by the specific intermolecular interactions between the sample's molecules and the packing material[9].

II.MATERIALS AND METHODS

Losartan and Amlodipine working standards were procured from Sun pharma, provided by sun pharma labs. Methanol and Water were obtained from LICHROSOLV. Acetonitrile from Merck.

HPLC WATERS (Alliance 2695 separation module, Software: Empower 2, 996 PDA detector.), pH meter (Lab India), Weighing machine (Sartorius), Volumetric flasks (Borosil), Pipettes and Burettes (Borosil), Beakers (Borosil), Digital ultra sonicator (Labman).

Optimized Chromatographic Conditions:

Column: Develosil C18 (250 mm × 4.6 mm, 5 µm)

Mobile Phase: Acetonitrile: Acetate buffer (pH 4.3) (35:65 v/v)

Flow rate: 1.0 ml/min

Wavelength: 238 nm

Injection volume: 20 µL

Run time: 6 min

Temperature: Ambient

Preparation of Mobile Phase: 350 ml of Acetonitrile and 650 ml of Acetate buffer (pH 4.3) were mixed, sonicated for 20 minutes, and filtered through a 0.45 µm membrane filter.

Preparation of Standard Solutions: 10 mg of each Losartan and Amlodipine working standard was accurately weighed and transferred into a 10 ml volumetric flask, dissolved in 7 ml of diluent, sonicated, and diluted to volume with the same solvent (Stock Solution). From the stock, 0.2 ml of Losartan and 0.6 ml of Amlodipine were further diluted to 10 ml with diluent.

Preparation of Sample Solutions: Tablets containing Losartan and Amlodipine were weighed and powdered. A quantity equivalent to 10 mg of each drug was transferred into a 10 ml volumetric flask, dissolved in 7 ml diluent, sonicated, and diluted to volume. The solution was filtered through a 0.45 µm syringe filter. From this, 0.2 ml and 0.6 ml of the respective sample stock solutions were diluted to 10 ml with diluent.

Validation Parameters:

System Suitability: The standard solution was injected five times. System suitability parameters such as %RSD of peak area, retention time, theoretical plates, and tailing factor were evaluated. The %RSD was found within acceptable limits.

Specificity: Specificity was evaluated by comparing chromatograms of blank, standard, and sample solutions to ensure no interference at the retention times of the analytes.

Linearity: Linearity was assessed at five concentration levels for each drug:

Losartan: 10 – 30 ppm

Amlodipine: 30 – 90 ppm

Each level was prepared by appropriate dilution of stock solution:

Level I: 0.1 ml Losartan & 0.3 ml Amlodipine

Level II: 0.15 ml Losartan & 0.45 ml Amlodipine

Level III: 0.2 ml Losartan & 0.6 ml Amlodipine

Level IV: 0.25 ml Losartan & 0.75 ml Amlodipine

Level V: 0.3 ml Losartan & 0.9 ml Amlodipine

Peak areas were recorded, and calibration curves were plotted. Correlation coefficients (R^2) exceeded 0.999 for both drugs.

Precision:

Repeatability: Five replicate injections of standard solutions were performed. The %RSD for peak area was within the limit of <2%.

Intermediate Precision (Ruggedness): Analysis was repeated on two different days under the same conditions. The %RSD for six replicate injections each day remained within limits.

Accuracy: Accuracy was determined by recovery studies at 50%, 100%, and 150% concentration levels. Known amounts of standards were added to per-analyzed samples, and recovery was calculated:

50%: 0.1 ml Losartan & 0.3 ml Amlodipine

100%: 0.2 ml Losartan & 0.6 ml Amlodipine

150%: 0.3 ml Losartan & 0.9 ml Amlodipine

Three replicate injections for each level were performed. Mean recovery values for both drugs were found within 98–102%.

Robustness: Robustness was evaluated by deliberately varying method parameters:

Flow Rate: ±0.1 ml/min (0.9 and 1.1 ml/min)

Mobile Phase Composition: Acetonitrile : Acetate buffer varied from 35:65 to 40:60 and 30:70

III.RESULTS AND DISCUSSION

The developed RP-HPLC method successfully separated Losartan and Amlodipine with good peak resolution and acceptable system suitability parameters. Validation results proved that the method is specific, linear, precise, accurate, and robust. Hence, it can be reliably used for the simultaneous estimation of these drugs in routine pharmaceutical analysis.

Table 1: System Suitability Parameters

Parameter	Losartan	Amlodipine	Acceptance Criteria	Results
Retention time	2.1 min	3.6 min	RSD < 2%	Within the limits
Tailing Factor	1.2	1.1	< 2.0	Within the limits
Theoretical plates (N)	4698	7985	> 2000	Within the limits
% RSD (area)	0.11	0.66	≤ 2%	pass

Table 2: Linearity Results

Concentration µg/mL	Losartan peak area	Concentration µg/mL	Amlodipine Peak area
10	245899	30	863094
15	365687	45	1249397
20	481526	60	1678592
25	589854	75	2050412
30	705882	90	2468444

Table 3: Accuracy Results for Losartan

Level (%)	Amount Added (ppm)	Amount found (ppm)	% Recovery	Acceptance Criteria
50 %	10	10.179	101.79 %	101.36 %
100 %	20	20.316	101.58 %	
150 %	30	30	100.72 %	

Table 4: Accuracy Results for Amlodipine

Level (%)	Amount Added (ppm)	Amount found (ppm)	% Recovery	Acceptance Criteria
50 %	30	30.114	100.38 %	100.26%
100 %	60	60.068	100.11 %	
150 %	90	90.268	100. 30 %	

Table 5: Precision Results

Parameter	Losartan (% RSD)	Amlodipine (%RSD)
Repeatability	0.03	0.53
Intermediate Precision (Day -1)	0.16	0.41
Intermediate Precision (Day -2)	0.04	0.11

Table 6: Robustness Study

Condition	Variation	Observation	Result
Flow rate	0.9 ml/min	No significant change	Pass
Flow rate	1.1 ml/min	No significant change	Pass
Mobile phase	40:60	Slight change in retention time	Acceptable
Mobile phase	30:70	Slight change in resolution	Acceptable

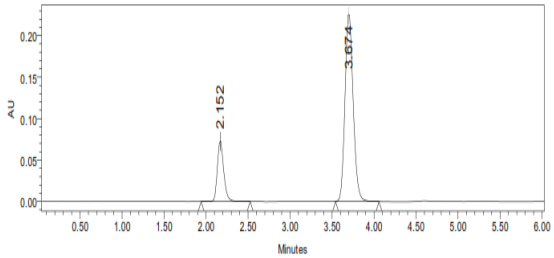

Fig. 3: Losartan and Amlodipine Standard Chromatogram

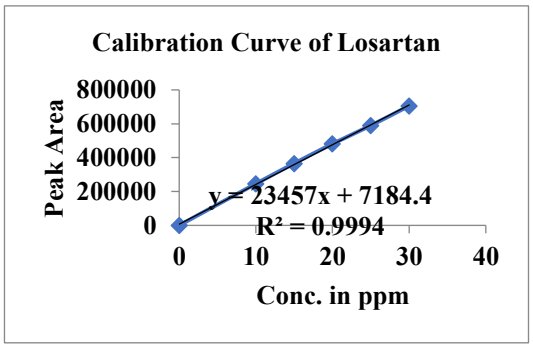

Fig. 4: Linearity Plot of Losartan

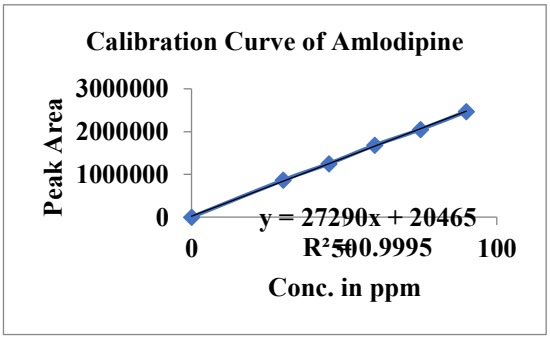

Fig. 5: Linearity Plot of Amlodipine

IV.CONCLUSION

A validated Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) method was developed for the simultaneous estimation of Losartan and Amlodipine in both bulk drugs and tablet dosage forms. The goal was to establish a simple, accurate, and reproducible analytical method that meets ICH validation guidelines and can be applied for routine quality control analysis.

Chromatographic separation was performed using a Develosil C18 column (4.6 mm × 250 mm, 5 µm particle size) under ambient temperature conditions. The optimized mobile phase consisted of Acetonitrile and Acetate buffer (pH 4.3) in a 35:65% v/v ratio, pumped at a flow rate of 1 mL/min. Detection was carried out at a wavelength of 238 nm, with an injection volume of 20 µL, and a total run time of 6 minutes. Under the optimized chromatographic conditions, both Losartan and Amlodipine were well separated with sharp, distinct peaks and consistent retention times. The method was validated according to ICH Q2(R1) guidelines, and it showed excellent results for linearity, precision, accuracy, specificity, and robustness, confirming the method's reliability and suitability for pharmaceutical analysis.

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