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RESEARCH ARTICLE

Development and Validation of RP-HPLC Method for Simultaneous Determination of Amlodipine and Lisinopril in Pharmaceutical Dosage Form

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ABSTRACT

A simple, accurate, sensitive and validated RP-HPLC method for simultaneous determination of Amlodipine Besylate and Lisinopril in combined tablet dosage form has been developed. Separation carried out on RP-HPLC system equipped with Zorbax SB-C8 Column (150 × 4.6 mm i.d., 3.5µm particle size) using mobile phase of Acetonitrile and phosphate buffer adjusted pH to 3.6 with orthophosphoric acid at a flow rate of 1 mL/min in the Gradient program with run time of 10 minutes and detection using UV/VIS detector was carried out at 210 nm. Results were linear in the range of 12 – 36 µg/mL for both Amlodipine besylate and lisinopril respectively. The method has been successfully applied for the analysis of drugs in pharmaceutical formulation. Results of analysis were validated statistically and by recovery studies.

KEYWORDS

RP-HPLC, Amlodipine Besylate, Lisinopril, Tablet Dosage Form

INTRODUCTION

Amlodipine besylate (AMB), 2-[(2- amino ethoxy)- methyl] - 4 - (2 – chloro phenyl) -1,4 -dihydro-6- methyl- 3,5- pyridine dicarboxylic acid 3 -ethyl- 5- methyl ester, benzene sulfonate, is a potent dihydro calcium channel blocker[1]. lisinopril dihydrate (LID), chemically (2S)-1- [(2S)-6-amino-2-[{(1S)-1-carboxy-3 pheny l propyl} amino] hexanoyl] pyrrole-2-carboxylic acid is an ACE inhibitor. Lisinopril is a potent, competitive inhibitor of angiotensin-converting enzyme (ACE)[2]. Literature survey reveals High Performance Liquid Chromatographic (HPLC)[3-5] for determination of Amlodipine besylate and Lisinopril combination are not official in Pharmacopeias of USP and BP.

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And their determination is official as single compound in Pharmacopeias. Various analytical methods have been reported for the assay of Amlodipine besylate and Lisinopril dihydrate alone or in combination with other antihypertensive agents in pharmaceutical formulations. They include UV spectroscopy[6-20], high performance liquid chromatography[19-34], high performance thin layer chromatography[35] and LC - MS/ MS.[36-38]

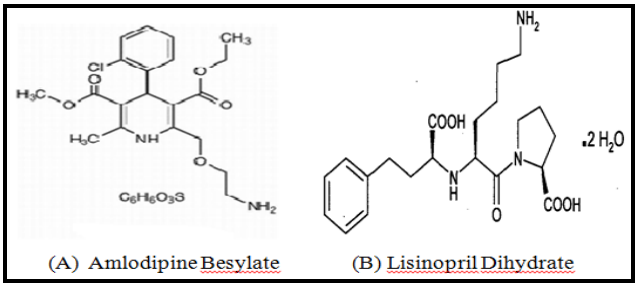

Figure 1: The Chemical Structures of API

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As on only few methods is available for their simultaneous determination, however, it is essential to develop a suitable analytical method for simultaneous estimation of Amlodipine besylate and Lisinopril in bulk and in pharmaceutical preparations, because HPLC methods have been widely used for routine quality control assessment of drugs, because of their accuracy, repeatability, selectivity, sensitivity and specificity. We have developed a simple, precise, Amlodipine besylate and Lisinopril in bulk and in pharmaceutical dosage forms. Because analytical methods must be validated before use by the pharmaceutical industry, the proposed HPLC- UV detection method was validated in accordance with International conference on Harmonization (ICH).

MATEIALS AND METHOD

Chemicals and Reagents

Pharmaceutically pure samples of Amlodipine besylate and Lisinopril were obtained as a gift samples from Dr. Reddy's, Hyderabad used as such without further purification. A combination of Amlodipine besylate (5 mg) and Lisinopril (5 mg) in tablet formulation (Amchek L) was procured from Indian market (Indoco Remedies Limited, Mumbai), HPLC grade methanol, Acetonitrile, water and sodium dihydrogen phosphate mono hydrate (AR grade) purchased from Merck Chemicals India Pvt. Limited, Mumbai, India.

Instrumentation and Chromatographic Conditions

Analysis was performed with a Waters 2695 separation module equipped with Empower-2 software and loop of injection capacity of 80µL, and waters-PDA detector set at 210 nm. Compounds were separated on a Zorbax SB-C8 Column (150 × 4.6 mm i.d., 3.5µm particle size) under reversed phase partition conditions. The mobile phase was a Acetonitrile and pH -3.6 phosphate buffer (50Mm, pH 3.60 ±0.05, adjusted with orthophosphoric acid). The flow rate was 1.0ml/min and the run time was 10 minutes with gradient elution. Samples were injected using Rheodyne injector with 20 µL loop and detection was carried out at 210 nm. Before analysis mobile phase were degassed by the use of a sonicator (Ultrasonic Cleaner, Power Sonic 420) and filtered through a 0.45µm Nylon membrane filter (Axiva). The identity of the compounds was established by comparing the retention times of compounds in the sample solution with those in standard solutions. Chromatography was performed in column temperature maintained at 50±5◦c. The UV spectrum of Amlodipine besylate and Lisinopril for selecting the working wavelength of detection was taken using a shimadzu UV-1800, With UV Probe software UV-Visible spectrophotometer (shimadzu, Kyoto, Japan). All Weighing were done on Shimadzu balance (Model AY-120).

Preparation of Standard Stock Solutions

(About 24 ppm for Amlodipine and about 24 ppm for Lisinopril)**:**

Weigh and quantitatively transfer about 28 mg of amlodipine besylate and 20 mg of Lisinopril dihydrate working standard or reference standard into a 50 mL volumetric flask. Add about 30mL of diluent(0.85 ml of conc. HCl (about 35% pure) in 1000mL of water), sonicate to dissolve the material for 5 min and dilute to volume with diluent and mix well and Transfer 3 mL of above solution into 50 ml volumetric flask and dilute to volume with diluent and mix well.

Procedure for Analysis of Tablet Formulation

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 50 mg of amlodipine and transferred in to a 250 ml volumetric flask and add about 150 ml of diluent. The contents were sonicated for 25 min, to ensure the complete solubility of drugs and volume was made up to the mark with the diluent. The solution was then centrifuged at 4000rpm for 10min and the clear supernatant was collected. From that, further dilutions were made by diluting 3 ml into 25ml with diluent; filtered through 0.45µm Nylon syringe filter.

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After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the tablet sample solutions were injected, chromatogram was obtained and the peak areas were recorded. The injections were repeated six times and the amount of each drug present per tablet was estimated from the respective calibration curves.

Method Validation

The method was validated for specificity, linearity, accuracy, intra-day and inter-day precision and robustness, in accordance with ICH guidelines.

RESULTS AND DISCUSSION

Method Development

Several tests were performed in order to get satisfactory separation-resolution amlodipine besylate and lisinopril in different mobile phases with various ratios of buffers and organic phases by using different columns. The ideal mobile phase was found to be an Acetonitrile and phosphate buffer (50Mm, pH 3.60±0.05, adjusted with orthophosphoric acid). This mobile phase used under gradient elution gave a very satisfactory and good resolution of amlodipine besylate and Lisinopril. Increasing or decreasing pH of mobile phase by ± 0.2 did not show significant change in retention time of each analyte. The retention time of amlodipine besylate and lisinopril on the analytical column was evaluated at a flow rate of 1.0 ml min. The injection volume was 20 µL. The retention time of standard and sample for amlodipine besylate and lisinopril were satisfactory with good resolution. This work was focused on optimization of the conditions for the simple and rapid as well as low cost effective analysis including a selection of the proper column or mobile phase to obtain satisfactory results. Solvent type, solvent strength (volume fraction of organic solvent(s) in the mobile phase and pH of the buffer solution), detection wavelength, and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimized so there was no interference from solvent and excipients. Finalized chromatographic conditions were mentioned on below Table-1.

To inject the standards on above finalized chromatographic conditions and their results was mentioned on below Table-2.

Table 1: Finalized chromatographic conditions

Flow rate:1.0 ml/min	**Wave length**:210 nm	**Injection Volume**:20µL
Column temperature: 50±5˚C	**Sample temperature**: Ambient	**Run time**:10 minutes
Gradient programme		
Time (in mins)	**Mobile phase-A (%v/v)** (pH 3.60 phosphate buffer)	**Mobile phase-B (%v/v)** (Acetonitrile)
0.0	93.0	7.0
5.0	40.0	60.0
7.0	40.0	60.0
7.1	93.0	7.0
10.0	93.0	7.0

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Table 2: Results from system suitability study of Amlodipine & Lisinopril

System Suitability Parameters	Results		Acceptance Criteria
	Amlodipine	Lisinopril	
Retention time	6.49	3.89	
%RSD for area of amlodipine & Lisinopril for five replicate injections of standard solution	0.2	0.1	NMT 2.0
Tailing factor for amlodipine & Lisinopril peak	1.1	1.2	NMT 2.0
Theoretical plates for amlodipine & Lisinopril peak	48020	11603	NLT 2000
Resolution Lisinopril	19.3		

Table 3: Precision studies

S.No	% Assay For (5 mg Label claim of amlodipine and 5 mg of Lisinopril)			
	Amlodipine		Lisinopril	
	Intraday precision (n=6)	Interday precision (n=6)	Intraday precision (n=6)	Interday precision (n=6)
1	98.2	100.2	99.7	101.8
2	99.5	99.5	100.1	101.0
3	98.8	100.3	99.4	101.2
4	99.1	99.9	100.8	101.1
5	98.4	99.8	100.4	100.9
6	99.5	99.7	99.4	101.4
Mean	98.9	99.9	100.0	101.4
%RSD	0.6	0.3	0.6	0.3
Over all % RSD(n=12)	0.7		0.8	

Table 4: Recovery studies of amlodipine and lisinopril

Level of % Recovery	% Mean Recovery*		% R.S.D.*	
	Amlodipine	Lisinopril	Amlodipine	Lisinopril
50	99.4	99.2	0.59	0.27
100	99.6	99.9	0.32	0.32
150	99.6	99.6	0.78	0.65

**Avg. of six determinations for 50 & 150, three determinations for 100%, R.S.D. is relative standard deviation*

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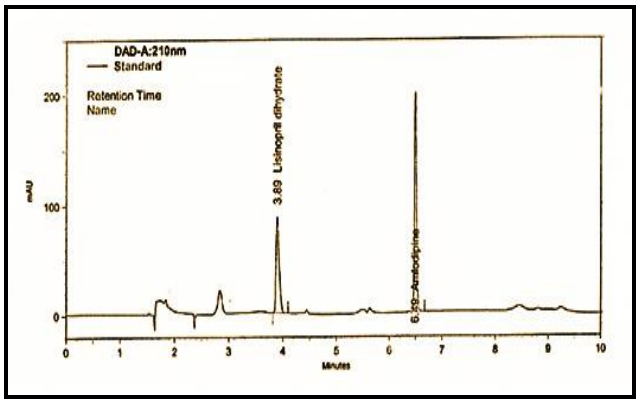

Figure 2: Optimized chromatogram for amlodipine (25 ppm) and Lisinopril (40ppm)

Linearity

Aliquots 1.5, 2.4, 3.0, 3.6 and 4.5 mL of stock solution of Working standard solution of amlodipine besylate and lisinopril were transferred in a series of 50 mL volumetric flasks and the volume was made up to the mark with the diluent. Two replicates per concentration were injected and chromatograms were recorded. The peak area ratios of amlodipine and lisinopril were calculated and respective calibration curves were plotted of response against concentration of each drug. Calibration curves for amlodipine and lisinopril were plotted separately of response against respective concentration of amlodipine besylate and Lisinopril. The slope and intercept value for calibration curve were y= 13,651.9517 x+ 6,620.6897 (R^2 = 0.9994) for amlodipine and y= 11,339.45012x - 1,062.06897 (R^2 = 0.99999) for lisinopril, where Y represents the peak area of analyte and X represents analyte concentration. The results are satisfactory, because there is a significant correlation between response factor and concentration of drugs within the concentration range. The calibration curves of amlodipine and lisinopril are given in Figures 3 and 4 respectively.

Precision

Precision of the method was confirmed by the repeated analysis of formulation for six times. The% RSD values were found to be satisfactory. The low % RSD values indicated that drugs showed good agreement with the label claim ensures the precision of the method.

Intraday and Interday precision was determined by preparing six (n=6) replicate samples and analyzed on same day for intraday and on different days for interday precision. (Table3). The peak areas were recorded and Relative standard deviation (RSD) was calculated for both series of analyses. The %RSD of intraday precision was 0.6, 0.6 for amlodipine and lisinopril, respectively. The %RSD of interday precision was 0.3, 0.3 for amlodipine and Lisinopril, respectively and overall %RSD for Amlodipine is 0.7 and Lisinopril is 0.8. (Table3)

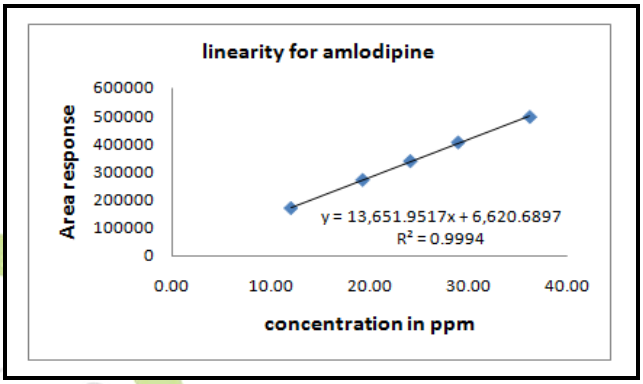

Figure 3: Linearity curve for Amlodipine

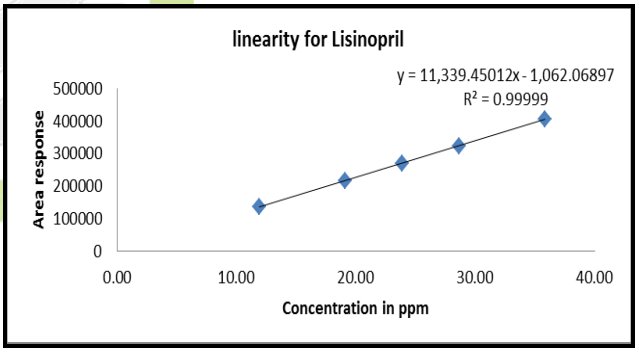

Figure 4: Linearity curve for Lisinopril

Accuracy

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100% and 150%. The percentages of recoveries were calculated, results of which are represented in Table 4.

LOD and LOQ

LOD and LOQ were calculated as 3.3 σ /S and 10 σ /S respectively; where σ is the standard deviation of the response (Y-Intercept) and S is the slope of the calibration plot.

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Robustness

As defined by ICH, The robustness of an analytical procedure describes to its capability to remain unaffected by small and deliberate variations in method parameters. Robustness was performed to injected the standard and samples by small variation in the chromatographic conditions and found to be unaffected by small variations like ± 2% variation in volume of mobile phase composition with respect to acetonitrile, ± 0.2 mL/min in flow rate of mobile phase ,± 0.2 variation in pH, different type of filters and ± 5 column temperature variation. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

Specificity

Specificity was tested against standard compounds and against potential interferences. Specificity was determined by comparing the responses of standard and sample solution. No interference was detected at the retention times of both amlodipine and lisinopril in sample solution.

Table 5: Summary of validation parameters of proposed RP-HPLC method

Parameters	Amlodipine	Lisinopril
Linearity range (µg/mL)	12 - 36	12 - 36
Correlation co-efficient	0.999	0.999
LOD [a] (µg/mL)	0.162	0.236
LOQ [b] (µg/mL)	0.493	0.717
Accuracy (% Recovery)	99.4 - 99.6	99.2 - 99.9
Precision (% RSD)[c]		
Intraday (n[d] = 6)	0.6	0.6
Interday (n[d] = 6)	0.3	0.3

[a] *LOD = Limit of detection.*
[b] *LOQ =Limit of quantitation.*
[c] *RSD = Relative standard deviation.*
[d] *n = Number of determination*

CONCLUSION

The validated RP-HPLC method employed here proved to be simple, fast, accurate, precise and robust, thus can be used for routine analysis of Amlodipine and Lisinopril in combined tablet dosage form.

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