Research Article

Novel Validated RP-HPLC Method for Simultaneous Estimation of Lisinopril and Amlodipine in Bulk and Tablet Dosage Form

[1]Manju Latha Y B*, [2]Gowri Sankar D.

[1]University college of Pharmaceutical Sciences, Andhra university, Visakhapatnam,(A.P), India
[2]Sri Vasavi Institute of Pharmaceutical Sciences, Tadepalligudem-534 101.(A.P), India

ABSTRACT

A simple, reproducible and efficient reverse phase high performance liquid chromatographic method was developed for simultaneous determination of lisinopril(LSN) and amlodipine (AMD) in tablets. A column having 150×4.6 mm in isocratic mode with mobile phase containing acetonitrile: phosphate buffer (55:45; adjusted to pH 3.0) was used. The flow rate was 0.6 ml/min and effluent was monitored at 216 nm. The retention time 8 (min) and linearity range (μg/ml) for LSN and AMD were and (20-60,10-30), respectively. The developed method was found to be accurate, precise and selective for simultaneous determination of LSNand AMD in tablets.

Key words: Lisinopril(LSN), Amlodipine, (AMD) , RP-HPLC, Validation

INTRODUCTION

Lisinopril is a potent, competitive inhibitor of angiotensin-converting enzyme (ACE), the enzyme responsible for the conversion ofangiotensin I (ATI) to angiotensin II (ATII). ATII regulates blood pressure and is a key component of the renin-angiotensin aldosteron system (RAAS). Lisinopril may be used to treat hypertension and symptomatic congestive heart failure, to improve survivalin certain individuals following myocardial infarction and to prevent progression of rena ldisease in hypertensive patients with diabetes mellitus and micro albuminuria orovert nephropathy. Lisinopril is chemically(2S)-1-[(2S)-6-amino-2-{[(1S)-1-carboxy-3-phenylpropyl]amino}hexanoyl] pyrrolidine 2-carboxylicacid[1,2]. Amlodipine is a longacting 1,4-dihydropyridine calcium channelblocker. It acts primarily on vascular smooth muscle cells by stabilizing voltage-gated Ltypecalcium channels in their inactive conformation. By inhibiting the influx ofcalcium in smooth muscle cells, amlodipine prevents calcium-dependent myocytecontraction and vasoconstriction. A second proposed mechanism for the drug'svasodilatory effects involves pH-dependent inhibition of calcium influx via inhibition ofsmooth muscle carbonic anhydrase. Some studies have shown that amlodipine also exerts inhibitory effects on voltage-gated N type calcium channels. N-type calcium channels located in the central nervous system may be involved in nociceptivesignaling and pain sensation. Amlodipine is used to treat hypertension and chronic stableangina. Amlodipine is chemically 3-ethyl-5-methyl-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4dihydropyridin3,5dicarboxylate1,2.

A few spectroscopic[3-5,] HPLC[6-9], HPTLC[10,] LC-MS[11,12] and CE[13] methods were reported earlier for the individual determination of amlodipine and lisinopril in pharmaceutical dosage forms. But no method is developed so far for the combination of amlodipine and lisinopril. A successful attempt is made to estimate the two drugs simultaneously. Therefore it was thought worth while to develop an accurate and rapid RP-HPLC method for simultaneous estimation of amlodipine and lisinopril from tablet formulations

EXPERIMENTAL

Chemicals and reagents: The reference sample of lisinopril and amlodipine was supplied by Torrent Pharmaceutical Industries Ltd., Ahmedabad. HPLC grade water and acetonitrile were purchased from
E. Merck(India) Ltd., Mumbai. Potassium dihydrogen phosphate and orthophosphoric acid of AR Grade were obtained from S.D. Fine Chemicals Ltd., Mumbai.

Chromatographic conditions: The analysis of the drug was carried out on a Waters HPLC system equipped with a reverse phase Xterra C18 column (150mmx4.6mm; 5μm), a 2695 binary pump, a 20 μl injection loop and a 2487 dual absorbance detector and running on Waters Empower software.

Preparation of phosphate buffer (pH 3.0): Seven grams of KH_2PO_4 was weighed into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC water and pH adjusted to 3.0 with orthophosporic acid.

Preparation of mobile phase and diluents: 450 ml of the phosphate buffer was mixed with 650 ml of acetonitrile.

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*Author for Correspondence

The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µ filter under vacuum.

Table 1: Calibration data of Lisinopril

Linearity Level	Concentration	Area
I	20ppm	1251642
II	30ppm	1877463
III	40ppm	2503284
IV	50ppm	3129105
V	60ppm	3754926
Correlation Coefficient		0.999

Table 2: Calibration data of Amlodipin

Concentration	Area
10ppm	583214
15ppm	874821
20ppm	1166428
25ppm	1458035
30ppm	1749642

Table 3: Precision studies for Lisinopril

Injection	Area
Injection-1	4124947
Injection-2	4072032
Injection-3	4025835
Injection-4	4006997
Injection-5	4115028
Average	4068968
Standard Deviation	52357.9
%RSD	1.29

Table 4: Precision studies for Amlodipine

Injection	Area
Injection-1	1880375
Injection-2	1845837
Injection-3	1834928
Injection-4	1827094
Injection-5	1879444
Average	1853536
Standard Deviation	24981.4
%RSD	1.35

Procedure: A mixture of buffer and acetonitrile in the ratio of 45:55 v/v was found to be the most suitable mobile phase for ideal separation of lisinopril and amlodipine. The solvent mixture was filtered through a 0.45 µ membrane filter and sonicated before use. It was pumped through the column at a flow rate of 0.5 ml/min. The column was maintained at ambient temperature. The pump pressure was set at 800 psi. The column was equilibrated by pumping the mobile phase through the column for at least

30 min prior to the injection of the drug solution. The detection of the drug was monitored at 216 nm. The run time was set at 8 min. Under these optimized chromatographic conditions the retention time obtained for the drugs lisinopril and amlodipine was 4.120 min and 3.086 min. A typical chromatogram showing separation of the drug is given in Fig. 1.

Calibration plot: About 10 mg of lisinopril and 5 mg of amlodipine was weighed accurately, transferred into a 10 ml volumetric flask and dissolved in 7 ml of a 45:55 v/v mixture of phosphate buffer and acetonitrile. The solution was sonicated for 15 min and the volume made up to the mark. From this, a working standard solution of the drugs (20 ppm of lisinopril and 10 ppm of amlodipine) was prepared by diluting 0.2 ml of the above solution to 10 ml in a volumetric flask. Further dilutions ranging from 20-60 ppm for lisinopril and 10-30 ppm for amlodipine were prepared from the solution in 10 ml volumetric flasks using the above diluent. 20 µl of each dilution was injected six times into the column at a flow rate of 0.5 ml/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed. The calibration graph constructed by plotting concentration of the drug against peak area was found to be linear in the concentration range of 20-60ppm for lisinopril and 10-30ppm for amlodipine. The relevant data are furnished in Table 1&2. The regression equations of this curves was computed. This regression equation was later used to estimate the amount of lisinopril and amlodipine in tablets dosage forms.

Validation of the proposed method: The specificity, linearity, precision, accuracy, limit of detection, limit of quantification, robustness and system suitability parameters were studied systematically to validate the proposed HPLC method for the determination of lisinopril and amlodipine. Solution containing 20 ppm of lisinopril and 20 ppm of amlodipine was subjected to the proposed HPLC analysis to check intra-day and inter-day variation of the method and the results are furnished in Table 3&4. The accuracy of the HPLC method was assessed by analyzing solutions of lisinopril and amlodipine at 50%, 100% and 150% concentrated levels by the proposed method. The results are furnished in Table 5&6. The system suitability parameters are given in Table 7.

Estimation of lisinopril and amlodipine in tablet dosage forms: Two commercial brands of tablets were chosen for testing the suitability of the proposed method to estimate lisinopril and amlodipine in tablet formulations. Twenty tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 192 mg of lisinopril and amlodipine was transferred into a 100 ml

Table 5: Accuracy studies for Lisinopril

%Concentration (at specification Level)	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	4.8	4.73	98.6%	
100%	9.8	9.63	98.3%	98.6%
150%	15.0	14.8	98.9%	

Table 6: Accuracy studies for Amlodipine

%Concentration (at specification Level)	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	2.46	2.42	98.6%	
100%	5.0	4.96	98.0%	98.3%
150%	7.3	7.17	98.2%	

volumetric flask and dissolved in 25 ml of a 45:55 v/v mixture of phosphate buffer and acetonitrile. The contents

Table 7: System suitability parameters

parameter	Result (Lisinopril)	Result (Amlodipine)
Linearityppm)	20-60	10-30
Correlation coefficient	0.9990	0.9991
Theoretical plates (N)	2160.1	2147.7
Tailing factor	1.4	1.6
LOD (ppm)	0.032	0.034
LOQ (ppm)	0.1	0.1

Table 8: Assay and recovery studies for Lisinopril

Formulation	Label claim (mg)	Amount found (mg)	% Amount found
Brand 1	5.0	4.99	99.8
Brand 2	5.0	4.98	99.6

Table 9: Assay and recovery studies for Amlodipine

Formulation	Label claim (mg)	Amount found (mg)	% Amount found
Brand 1	2.5	2.49	99.6
Brand 2	2.5	2.48	99.2

of the flask were sonicated for 15 min and a further 25 ml of the diluent was added, the flask was shaken continuously for 15 min to ensure complete solubility of the drug. The volume was made up with the diluent and the solution was filtered through a 0.45 µ membrane filter. This solution was further diluted to get the required concentrations. The solution containing 40 µg/ml of lisinopril and 20 µg/ml of amlodipine was injected into the column six times. The average peak area of the drugs was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug. The relevant results are furnished in Table 8&9

RESULTS AND DISCUSSION
In the proposed method, the retention time of lisinopril and amlodipine was found to be 4.120min and 3.086 min. Quantification was linear in the concentration range of 20-60µg/ml for lisinopril and 10-30µg/ml for amlodipine. The regression equation of the linearity plot of concentration of lisinopril and amlodipine over its peak area was found to be Y =0+62582.1x (r2=0.9990) for lisinopril and Y=0+58327.4X (r2=0.999) for amlodipine, where X is the concentration of lisinopril and amlodipine (ppm) was

2025.9 for lisinopril and 2037.2 for amlodipine, which indicates efficient performance of the column. The limit of detection and limit of quantification for lisinopril were found to be 0.032 ppm and 0.1 ppm and for amlodipine were found to be 0.034ppm and 0.1 ppm respectively, which indicate the sensitivity of the method. The use of phosphate buffer and acetonitrile in the ratio of 45:55 v/v resulted in peak with good shape and resolution. The high percentage of recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drug by the proposed HPLC method.
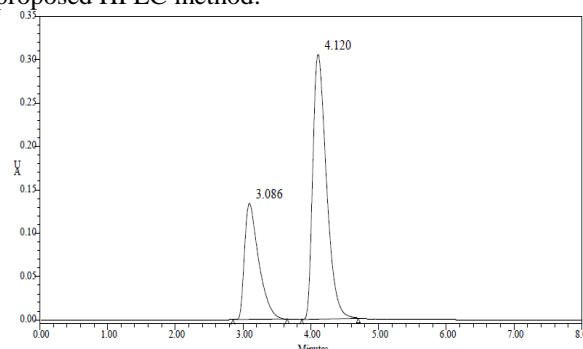

Fig. 1: Typical chromatogram of lisinopril and amlodipine

CONCLUSION
The proposed HPLC method is rapid, sensitive, precise and accurate for the determination of lisinopril and amlodipine and can be reliably adopted for routine quality control analysis of lisinopril and amlodipine in its tablet dosage forms.

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