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Research Article

NOVEL VALIDATED RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF LISINOPRIL AND AMLODIPINE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A simple, reproducible and efficient reverse phase high performance liquid chromatographic method was developed for simultaneous determination of lisinopril and amlodipine in tablets. A column having 150 × 4.6 mm i.d. in isocratic mode with mobile phase containing acetonitrile: phosphate buffer (60:40; adjusted to pH 3.0) was used. The flow rate was 0.5 ml/min and effluent was monitored at 215 nm. The retention time (min) and linearity range (µg/ml) for lisinopril and amlodipine were (4.111, 3.097) and (20-60, 10-30), respectively. The developed method was found to be accurate, precise and selective for simultaneous determination of lisinopril and amlodipine in tablets.

Key words: Lisinopril, Amlodipine, RP-HPLC, Validation.

INTRODUCTION

Lisinopril is a potent, competitive inhibitor of angiotensin-converting enzyme (ACE), the enzyme responsible for the conversion of angiotensin I (ATI) to angiotensin II (ATII). ATII regulates blood pressure and is a key component of the renin-angiotensin-aldosterone system (RAAS). Lisinopril may be used to treat hypertension and symptomatic congestive heart failure, to improve survival in certain individuals following myocardial infarction and to prevent progression of renal disease in hypertensive patients with diabetes mellitus and microalbuminuria or overt nephropathy. Lisinopril is chemically (2S)-1-[(2S)-6-amino-2-{[(1S)-1-carboxy-3-phenylpropyl]amino}hexanoyl] pyrrolidine-2-carboxylic acid[1,2]. Amlodipine is a long-acting 1,4-dihydropyridine calcium channel blocker. It acts primarily on vascular smooth muscle cells by stabilizing voltage-gated L-type calcium channels in their inactive conformation. By inhibiting the influx of calcium in smooth muscle cells, amlodipine prevents calcium-dependent myocyte contraction and vasoconstriction. A second proposed mechanism for the drug's vasodilatory effects involves pH-dependent inhibition of calcium influx via inhibition of smooth 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 nociceptive signaling and pain sensation. Amlodipine is used to treat hypertension and chronic stable angina. Amlodipine is chemically 3-ethyl-5-methyl-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydro pyridine-3,5-dicarboxylate[1,2]. 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 worthwhile 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 C_{18} 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. The UV spectrum of the drugs was taken using an Elico SL-159 UV-Visible spectrophotometer.

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

400 ml of the phosphate buffer was mixed with 600 ml of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µ filter under vacuum.

Procedure

A mixture of buffer and acetonitrile in the ratio of 40:60 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 215 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 4.111 min and 3.097 min. A typical chromatogram showing the separation of the drug is given in Fig. 1.

Calibration plot

About 100 mg of lisinopril and 100 mg of amlodipine was weighed accurately, transferred into a 100 ml volumetric flask and dissolved in 25 ml of a 40:60 v/v mixture of phosphate buffer and acetonitrile. The solution was sonicated for 15 min and the volume made up to the mark with a further quantity of the diluent to get a 1000 µg/ml solution. From this, a working standard solution of the drugs (40 µg/ml for lisinopril and 20 µg/ml for amlodipine) was prepared by diluting the above solution to 10 ml in a volumetric flask. Further dilutions ranging from 20-60 µg/ml for lisinopril and 10-30 µg/ml 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 20-60 µg/ml for lisinopril and 10-30 µg/ml 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 40 µg/ml for lisinopril and 20 µg/ml for 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.

Table 1: Calibration data of lisinopril

Concentration (µg/ml)	Mean peak area (n=5)
20	1412395
30	2824780
40	4237186
50	5649581
60	7061977

Table 2: Calibration data of amlodipine

Concentration (µg/ml)	Mean peak area (n=5)
10	647464
15	1294928
20	1942392
25	2589856
30	3237320

Table 3: Precision studies for lisinopril

Concentration of lisinopril (40µg/ml)	Peak area	
	Intra-day	Inter-day
Injection-1	4097171	4075618
Injection-2	4048225	4063216
Injection-3	4002185	4054321
Injection-4	3983963	4025620
Injection-5	4095695	4078963
Average	4045448	4059548
Standard Deviation	52105.2	21372.13
% RSD	1.29	0.52

Table 4: Precision studies for amlodipine

Concentration of amlodipine (20µg/ml)	Peak area	
	Intra-day	Inter-day
Injection-1	1789162	1878830
Injection-2	1790181	1856558
Injection-3	1792231	1833398
Injection-4	1793146	1821962
Injection-5	1791853	1875957
Average	1791315	1853340
Standard Deviation	1612.6	25267.8
% RSD	0.09	1.36

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 100 mg of lisinopril and 100 mg of amlodipine was transferred into a 100 ml volumetric flask and dissolved in 25 ml of a 40:60 v/v mixture of phosphate buffer and acetonitrile. The contents 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 4.111 min and 3.097 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=-1412402.2+141239.65X (r^2=0.9990) for lisinopril and Y=-647464+129492.8X (r^2=1) for amlodipine, where X is the concentration of lisinopril and amlodipine (µg/ml) and Y is the corresponding peak area. The number of theoretical plates calculated 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 0.03 µg/ml and 0.1 µg/ml and for amlodipine were found to be 0.038 µg/ml and 0.12 µg/ml respectively, which indicate the sensitivity of the method. The use of phosphate buffer and acetonitrile in the ratio of 40:60 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.

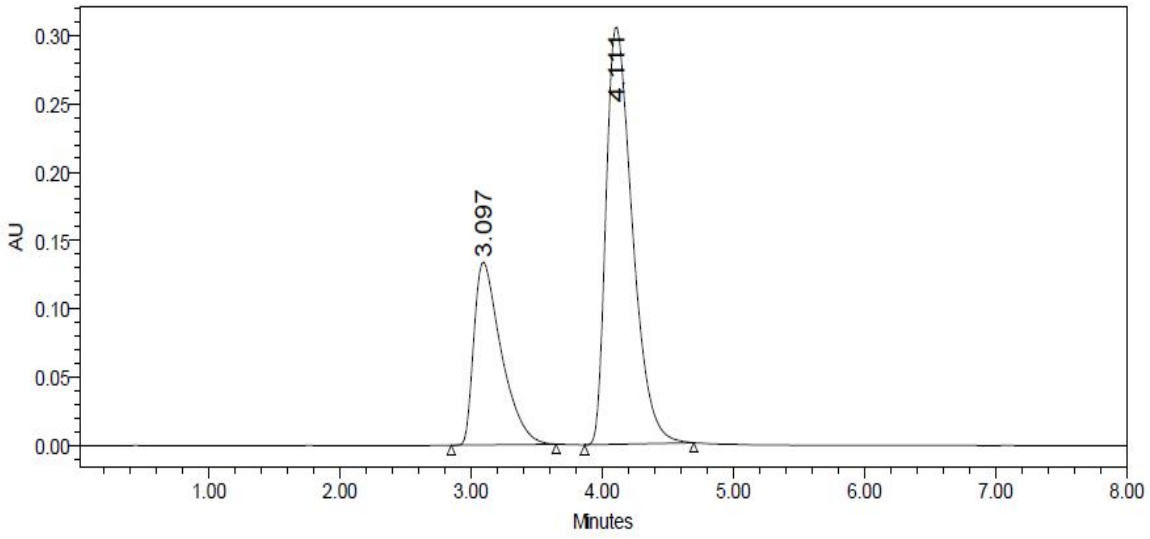

Fig. 1: Typical chromatogram of lisinopril and amlodipine

Table 5: Accuracy studies for lisinopril

%Concentration (at specification level)	Amount added (mg)	Amount found (mg)	% Recovery	% Mean recovery
50%	19.95	20.25	101.50	
100%	39.95	39.96	100.03	100.48
150%	60.01	59.95	99.91	

Table 6: Accuracy studies for amlodipine

%Concentration (at specification level)	Amount added (mg)	Amount found (mg)	% Recovery	% Mean recovery
50%	9.97	10.10	101.34	
100%	20.08	19.98	99.53	100.87
150%	29.96	30.42	101.562	

Table 7: System suitability parameters

Parameter	Result (Lisinopril)	Result (Amlodipine)
Linearity (μg/ml)	20-60	10-30
Correlation coefficient	0.9990	1
Theoretical plates (N)	2025.9	2037.2
Tailing factor	1.5	1.7
LOD (μg/ml)	0.03	0.038
LOQ (μg/ml)	0.1	0.12

Table 8: Assay and recovery studies for lisinopril

Formulation	Label claim (mg)	Amount found (mg)	% Amount found
Brand 1	40	40.48	101.20
Brand 2	40	39.45	98.62

Table 9: Assay and recovery studies for amlodipine

Formulation	Label claim (mg)	Amount found (mg)	% Amount found
Brand 1	20	20.45	101.05
Brand 2	20	19.34	98.35

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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