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A VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF AMLODIPINE AND LISINOPRIL IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A reverse phase high performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of amlodipine and lisinopril in marketed formulation is developed. The determination was carried out on a Phenomenex C18 (250 x 4.6 mm, 5 µm) column using a mobile phase of 0.02 M phosphate buffer solution: methanol (75:25v/v, pH 7.0). The flow rate was 1.0ml/min with detection at 212 nm. The retention time for amlodipine was 4.11 min and for lisiopril 7.29 min. Amlodipine and Lisinopril showed a linear response in the concentration range of 10-110 µg/ml. The correlation co-efficient (' *r* ' value) for amlodipine and lisinopril was 0.9991 and 0.9992, respectively. The results of analysis have been validated statistically and by recovery studies. The percentage recoveries obtained for amlodipine and lisinopril ranges from 100.04 to 100.57%.

INTRODUCTION: Amlodipine (AMD) is chemically a 2-[(2-Aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridine dicarboxylic acid- 3-ethyl 5-methyl ester and it belongs to the class of calcium channel blocker [1, 2]. Several spectroscopic [3, 4], RP-HPLC [5,6], HPTLC [7], LC-MS/MS [8] and LC-MS [9] have been reported for the estimation of amlodipine individually and in combination with other drugs. Lisinopril(LSNP), (S)-1-[*N*2-(1-Carboxy-3-phenylpropyl) -L-lysyl]-L-proline dihydrate is an angiotensin converting enzyme inhibitor that is used in the treatment of hypertension and heart failure [10]. Various spectrophotometric methods have been reported for the determination of lisinopril in pharmaceutical tablets using different reagents [11]. First and second derivative spectrophotometric [11, 12] and spectrofluorometric methods [11] were developed. The chromatographic techniques of analyses, HPLC [12], micellar electro kinetic chromatography [13] and gas liquid chromatography [14] have been reported for the estimation of lisinopril individually and in combination with other drugs. But no method is developed so far for the combination of AMD and LSN. 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 AMD and LSN from tablet formulations.

Experimental:

Instrumentation: A Gradient HPLC (Shimadzu) with LC-2010CHT double reciprocating pump, SPD-M20A detector , and RP-C18 column (5 µm particle size) was used. The RP-HPLC system was equipped with LC solution software for data processing.

Chemicals and Reagents: Lisinopril (LSN) reference standard and amlodipine (AMD) reference standard

were received as gift sample from Cipla Pharmaceutica Ltd, India. The pharmaceutical preparations of combination of lisinopril and amlodipine that is AMLOPRESS-L (Cipla) contains 5 mg of Lisinopril and 5 mg of amlodipine equivalent to amlodipine besylate was purchased from local market. Methanol of analytical reagent grade was purchased by Finar Chemical Ltd (India).

Chromatographic condition: Method was developed using a Phenomenex C18 (250 x 4.6 mm, 5 µm) column. Mobile phase used was 0.02 M phosphate buffer solution : methanol (75:25 v/v, pH 7.0). Flow rate employed was 1.0 ml/min. Detection was carried out at 212 nm.

Standard stock solution: About 100 mg of each of reference standard of AMD and LSN was weighed accurately and transferred to two separate 100 ml volumetric flask. Both drugs were dissolved in 50 ml of mobile phase with shaking and volume was made upto the mark with mobile phase to get 1000 µg/ml of standard stock solution of each drug. These stock solutions were filtered through vacuum filter.

Calibration curves: For each drug, appropriate aliquots were pipetted out from each standard stock solution into a series of 10 ml volumetric flasks. The volume was made upto mark with mobile phase to get set of solutions having concentration range 10, 20, 30, 40, 50, 60, 70, 80, 90,100 and 110 µg/ml for each drug. Triplicate dilutions of each concentration of each drug were prepared separately. From these triplicate solutions, 20 µl injections of each concentration of each drug were injected into the RP-HPLC system separately and chromatographed under the conditions as described above. Evaluation of both drugs was performed with UV detector at 212nm. Peak areas were recorded for all the peaks and peak areas were plotted against the concentrations to obtain the standard calibration curves.

Analysis of the marketed formulation: Twenty tablets were weighed and crushed to fine powder. The tablet powder equivalent to 5 mg of amlodipine and 5 mg of lisinopril was transferred to a 100 ml volumetric flask and dissolved in mobile phase and the content was kept in ultrasonicator for 30 min. Finally, the volume was made up to the mark with mobile phase. The solution was filtered through 0.2 µm Nylon 6, 6(N_{66}) membrane filter paper. This solution was further diluted with mobile phase and standard stock solution of AMD was added to obtain mixed sample solution containing 5 mg amlodipine and 5 mg lisinopril.

A 20 µl of sample solution was injected into sample injector for six times under chromatographic condition as described above. Area of each peak was measured at 212 nm. The amount of each drug present in the sample (n = 6) was determined from peak area of AMD and LSN present in the pure mixture and percent label claim and standard deviation (SD) was calculated. The results are given in **Table 1**. Typical chromatogram of AMD and LSN present in tablet formulation is given in **Figure 1**.

TABLE 1: RESULT OF MARKETED FORMULATION ANALYSIS

Marketed Formulation	Drug	Label claim (mg/tablet)	Estimated % of labeled claim ± SD*	%Recovery ± SD*		
				80	100	120
Amlopress-L (Cipla Pvt. Ltd)	**AMD**	5	100.51±0.342	100.13±0.853	100.57±0.514	100.51±0.405
	LSN	5	100.91±0.689	100.54±0.536	100.06±0.626	100.04±0.127

*Average of six determination

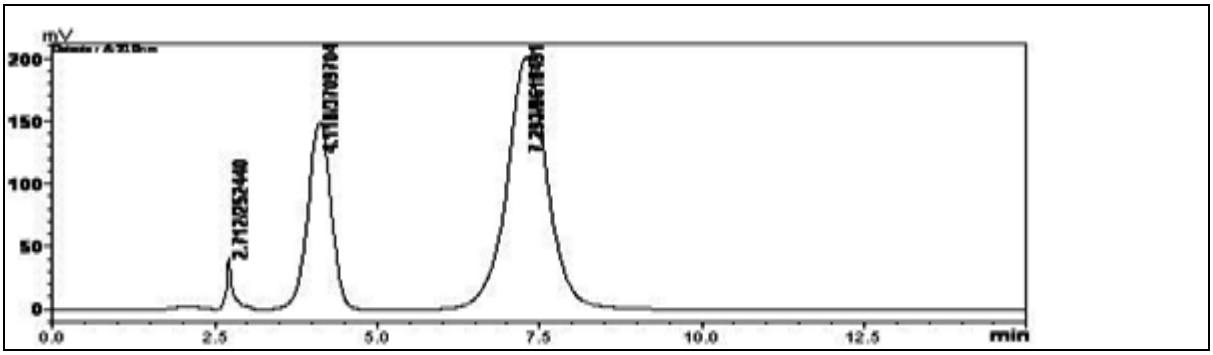

FIG. 1: TYPICAL CHROMATOGRAM OF AMLODIPINE (AMD) RT (4.118) AND LISINOPRIL (LSN) RT (7.293)

Validation of HPLC method: The proposed RP-HPLC method was validated as per ICH guidelines.

Specificity: The specificity of the RP-HPLC method was determined by comparison of the chromatogram of mixed standards and sample solutions. The parameters like retention time (*t* R), resolution (*R* S) and tailing factor (*T* f) were calculated. Good correlation was found between the results of mixed standards and sample solutions.

Precision: Precision study was performed to find out intra-day and inter-day variations. The %relative standard deviation (RSD) for intra-day precision was 0.138% for AMD and 0.129% for LSN and for inter-day precision was 0.590% for AMD and 0.414% for LSN, respectively which is less than 2% indicating high degree of precision.

Accuracy: Recovery studies were performed by standard addition method at three levels i.e., 80%, 100% and 120%. Known amounts of standard AMD and MET were added to pre-analyzed samples and they were subjected to proposed HPLC method. Results of recovery studies are shown in Table 1.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): The LOD and LOQ were separately determined based on the calibration curves. The standard deviation of the y-intercepts and slope of the regression lines were used. Results of LOD and LOQ are given in **Table 2**.

TABLE 2: SYSTEM SUITABILITY PARAMETERS

PARAMETERS	AMD	LSN
Tailing factor	1.066	1.171
Resolution (Rs)	3.682	3.814
Separation factor	1.56	
Capacity Factor	2.424	5.061
Limit of detection (µg/ml)	0.029	0.025
Limit of quantitation (µg/ml)	0.090	0.075

Robustness: The robustness study was done by making small changes in the optimized method parameters like ±0.1 change in pH, ±1% change in mobile phase ratio and column temperature. There was no significant impact on the retention time and tailing factor.

Ruggedness: The ruggedness study was done by the two analysts. The %RSD for analyst-I was 0.1088% for AMD and 0.1078% for LSN and for analyst-II was 0.3208% for AMD and 0.7329% for LSN, respectively.

RESULT AND DISCUSSION: The present work describes RP-HPLC method for estimation of AMD and LSN in tablets. Both the drugs were resolved on Phenomenex C18 (250 x 4.6 mm, 5 µm) column using 0.02 M phosphate buffer solution: methanol (75:25v/v, pH 7.0) as mobile phase with a flow rate of 1.0 ml/min, UV detection was performed at 212 nm. Linearity response was found in the concentration range of 10-110 µg/ml for both the drugs. The correlation co-efficient (' *r* ' value) for AMD and LSN was 0.9991 and 0.9992, respectively.

The %RSD for the tablet analysis and recovery studies was less than 2% indicating high degree of accuracy. The %RSD of AMD and LSN for intra-day precision and inter-day precision was less than 2% indicating high degree of precision. The results of the robustness study also indicated that the method is robust and is unaffected by small variations in the chromatographic conditions. The results of ruggedness study was found to be satisfactory. Hence, it can be concluded that the developed RP-HPLC method is accurate, precise, and selective and can be employed successfully for the estimation of AMD and LSN in both bulk and multicomponent formulation.

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