Nat. Volatiles & Essent. Oils, 2021; 8(4): 9441-9451

Development And Validation Of RP-HPLC Method For The Determination Of Lisinopril And Amlodipine In Bulk And Multicomponent Pharmaceutical Cardiovascular Dosage Form.

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ABSTRACT

Objective: To develop a simple, accurate, linear, and precise RP-HPLC method for determination of Lisinopril and Amlodipine in bulk and multicomponent pharmaceutical cardiovascular tablet dosage form and validate as per the ICH guidelines.

Methods: In the methods used Phenomenex Luna C18(2) (250 x 4.6 mm, 5 µ) column, mobile phase Methanol: 0.1 % Perchloric Acid (52.5: 47.5%, v/v), the flow rate of 1 ml/min and the detection wavelength of 220 nm using PDA detector.

Results: The calibration curves were linear r^2 = 0.999 and 0.999 in the concentration range of 40 to 60 ug/ml for both Lisinopril and Amlodipine. The developed method resulted in elution of Lisinopril at 2.11 min and Amlodipine at 4.65 min. The % recovery was found to be 99.97% to 100.09 % and 99.42% to 100.06% for Lisinopril and Amlodipine. The limit of detection was found to be 1.44 ug/ml and 2.62 ug/ml for Lisinopril and Amlodipine. The limit of Quantification was found to be 4.35 ug/ml and 7.94 ug/ml for Lisinopril and Amlodipine sequentially.

Conclusion: The present method of RP-HPLC was found to be accurate, simple and easy, specific, precise, linear, quick, and inexpensive. Within the concise analysis time, this method gives a superior resolution between both the compounds. That's why the method to support good for the routine analysis of Lisinopril and Amlodipine in several pharmaceutical industries and also in academics.

Keywords: RP-HPLC, Lisinopril, Amlodipine, Validation, Method development

INTRODUCTION

Lisinopril is a class of angiotensin-converting enzymes i.e., ACE inhibitors. It is worked by reducing a certain chemical that secures the blood vessels that's why blood flow further smoothly. Its chemical name is N_2-[(1S)-1-Carboxy-3-phenylpropyl]-L-lysyl-L-proline. The angiotensin-converting enzyme is a peptidyl dipeptidase that works the conversion of angiotensin-I to vasoconstrictor material angiotensin-

II. This ACE inhibitor is used in the therapy of heart failure and hypertension. The angiotensin-II stimulates the cortex obstruction of ACE which outcome in reduced plasma angiotensin-I and consequently shows vasopressor activity and reduced aldosterone secretion, which finally reduce may result in portable enhancement of serum potassium [1,2].

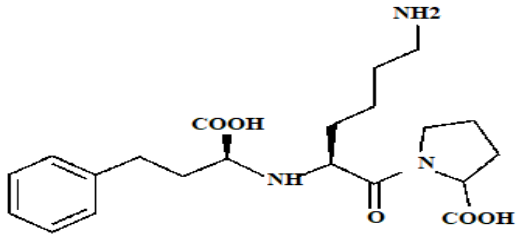


Fig. 1. Structure of Lisinopril

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[3,4] (dihydropyridine derivative) used as an anti-hypertensive and in the treatment of angina [5-6].

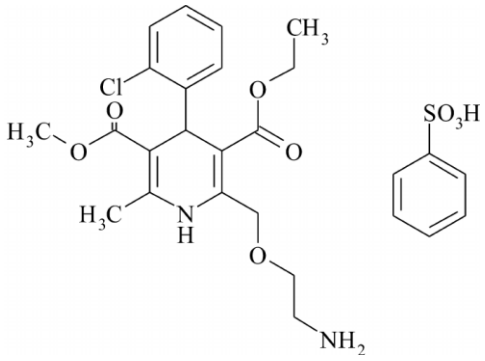


Fig.2: Structure of Amlodipine besylate

The detailed literature survey disclosed that there was a spectrophotometric method for concurrent estimation of Lisinopril with another combination. In bulk and pharmaceutical dosage forms some spectroscopic, Spectrofluorometric, LC methods have also been described earlier for the determination of lisinopril in bulk and pharmaceutical dosage form [7-13]. Literature review showed different analytical methods for the analysis of amlodipine in pharmaceutical preparations or biological fluids either as a single drug or in combination with other drugs. Those analytical methods include spectrophotometric methods with or without derivatization[14-20]. The derivative spectroscopy has also been applied for the determination of amlodipine and its photodegrade compound[21]. There were several RP-HPLC

techniques have been developed for the resolution of Lisinopril in combination with other drugs [22]. Although, there was no RP-HPLC technique that has been described for concurrent estimation of Lisinopril and Amlodipine in the bulk and pharmaceutical dosage form. In this study, we dispense easy, accurate, simple, rapid, and particular HPLC technique for simultaneous estimation of an RP-HPLC assay procedure for the analysis of Lisinopril and Amlodipine in the bulk and pharmaceutical dosage form. As per the ICH guidelines, the developed method was validated [23].

MATERIALS AND METHODS

Materials: The pharmaceutical gradeLisinopril was supplied by Micro Labs Limited, Mumbai-400072, and the Amlodipine drug was obtained from Cipla Pharmaceutical Ltd., (Goa), India. Commercial tablet AMLOPRESS-L (Cipla) of Lisinopril and Amlodipine (5mg & 5mg) was acquired from the local drug market. From Merck Methanol, Perchloric Acid, and HPLC grade water were obtained. All solvents used in this work are HPLC grade. RP-HPLC Shimadzu model with Spin chrome software was used in this method. Phenomenex Luna C18(2) (250 x 4.6 mm, 5 µ) analytical column used for separation of Analytes.

Methods:

Chromatographic conditions: The developed method used a Phenomenex Luna C18 (2) (250 ×4.6 mm, 5u), a mobile phase Methanol: 0.1 % Perchloric Acid (50: 50%, v/v), the flow rate of 1 ml/min, and a detection wavelength of 220 nm using a PDA detector.

Mobile phase preparation: The mixture of 52.5 volumes of Methanol and Perchloric Acid 47.5 volumes was prepared. To remove the all gasses mobile phase was sonicated for 10 min.

Diluent: The mobile phase was used as a diluent.

Standard solution preparation:

Lisinopril Standard Stock Solution-I (LSSS-I): Initially Prepare a Standard Stock Solution (LSSS-I) of Lisinopril by adding 5mg in 10 ml volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. (Conc. of Lisinopril = 500 µg/ml).

Amlodipine Standard Stock Solution-II (ASSS-II): Then prepare a Standard Stock Solution (SSS-II) of Amlodipine by adding 5 mg in 10 ml volumetric flask & adding 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. (Conc. of Amlodipine = 500 µg/ml).

Then add 1.0 ml of LSSS-I &1.0 ml ASSS-II in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluents.

Lisinopril Ion Pair Standard Stock Solution (LISSS-III):

Initially Prepare a Standard Stock Solution (LISSS-III) of Lisinopril- Ion pair by adding 5mg in 10 ml volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. (Conc. of Lisinopril = 500 µg/ml).

Then add 1.0 ml of LISSS-III & 1.0 ml ASSS-II in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluents.

Drug Product Sample Preparation for Assay:

Tablet Sample Solution (TSS): 10 Tablets were weighed and the average weight was calculated. And tablets were crushed & mixed in mortar and pestle. Powder weight equivalent to 5 mg Lisinopril and 5 mg Amlodipine was weighed into 10 ml volumetric flask & add 5 ml diluent, sonicate for 10 minutes and make the volume to 10 ml with diluent. (Conc. of Lisinopril = 500 µg/ml, Amlodipine = 500 µg/ml). Pipette out of 1 ml of above solution in 10 ml volumetric flask and add 5 ml diluents, Sonicate for 10 minutes and make the volume to 10 ml with diluent. (Conc. of Lisinopril = 50 µg/ml & Amlodipine =50 µg/ml).

RESULTS AND DISCUSSION

Method development: For better separation and resolution, the various chromatographic conditions were tried. Phenomenex Luna C18(2) (250 x 4.6 mm, 5 µ) column was found adequately. Peak purity of Lisinopril and Amlodipine was checked using a PDA detector at 220nm. It was examined satisfactorily for detecting both the drugs with sufficient sensitivity. In the several ratios, several solvents over an extensive range of pH were tried, yet likewise, peak shape was wide or resolution was not good. Frequent trials to get superior, sharp peaks with a systematic resolution between two peaks of Lisinopril and Amlodipine complete on a C18 column in isocratic HPLC gave acceptable results. The rum time was superior in an isocratic trial with mobile phase contain Methanol: 0.1 % Perchloric acid (52.5:47.5 %, v/v) and Phenomenex Luna C18(2) (250 x 4.6 mm, 5 µ) column, flow rate 1 ml/min, and detection

wavelength 220 nm gave the acceptable results in terms of retention time, resolution, sensitivity, and symmetry.

Method Validation: Afterwards method development the validation of the advanced method was accomplished in a period of the following variables like accuracy, precision, linearity and range, percentage recovery, robustness, the limit of detection (LOD), and limit of quantitation (LOQ).

System suitability: The standard solution was prepared by the test technique and injected into the chromatographic system. The system suitability variables such as resolution, theoretical plates, and asymmetric factors were evaluated. All variables were found to be within a limit. The parameters of system suitability were shown in table 1.

Table 1: System suitability parameters

Parameters	Acceptance limits	Lisinopril	Amlodipine
Retention time	-	2.11	4.65
Resolution	NLT 2	0.00	14.26
Theoretical plates	NLT 2000	5272	5987

Precision

Method precision: By precision method studies the precision of the technique was confirmed. At working concentration, the sample solution was prepared and analysis was accomplished at replicating. The sample solution of Lisinopril and Amlodipine was prepared as per the test method and injected 5 times into the column. The results of precision were shown in table 2. The average was taken and the percent RSD was calculated and described. The percent RSD values were within the limits and the technique was found to be precise.

Table 2: Precision data

n	Lisinopril	Hydrochlorothiazide

Rep 1	715783	1487770
Rep 2	715863	1487687
Rep 3	715724	1487274
Rep 4	715354	1487574
Rep 5	715322	1487547
Avg	**715609**	**1487570**
STDEV	**252.691**	**188.314**
RSD	**0.04**	**0.01**

Linearity

Linearity: The linearity of the test solution for the assay technique was prepared from Lisinopril and Amlodipine standard stock solution at five concentration levels 80% to 120 % of assay concentration. The peak area compared to concentration data was treated by least-squares linear regression analysis (fig.3 and 4). The results have manifested a magnificent correlation between peak areas and concentration within the concentration range 40-60 ug/ml for Lisinopril and 40-60 ug/ml for Amlodipine (table 3). For both drugs, the correlation coefficient was found to be 0.999 for Lisinopril and 0.999 for Amlodipine which meets the technique validation acquiescence criteria and that's why the method was said to be linear.

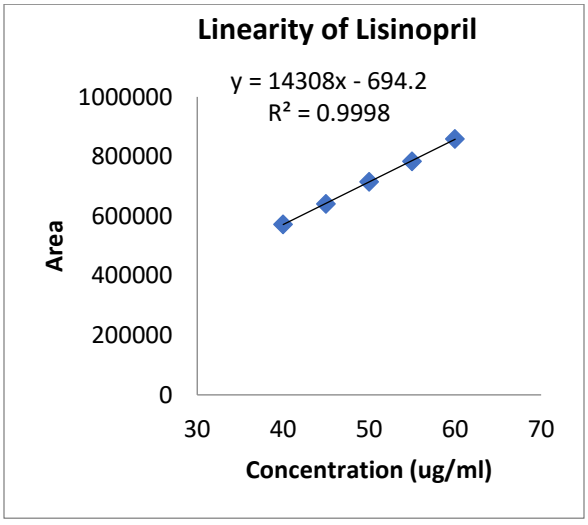


Fig.3: Linearity chart of Lisinopril

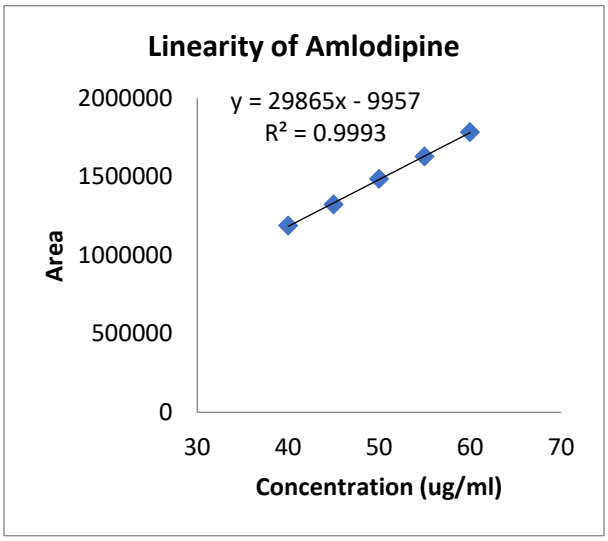
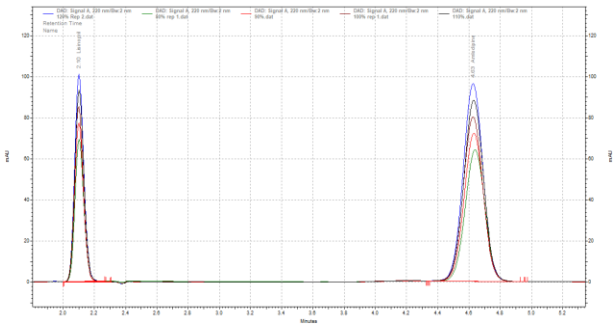


Fig.4: Linearity chart of Amlodipine

Table 3: Linearity data of both drugs

% Level	Lisinopril concentration (ug/ml)	Lisinopril peak area	Amlodipine concentration (ug/ml)	Hydrochlorothiazide peak area
80	40	572975	40	1190475
90	45	641176	45	1324419
100	50	715863	50	1487770
110	55	784558	55	1629127
120	60	858987	60	1784754



Accuracy

Fig.5: Linearity Overlay of Lisinopril and Amlodipine

Accuracy: The accuracy of the technique was resolute by recovery studies by the determination of percent mean recovery of Lisinopril and Hydrochlorothiazide at three dissimilar levels (80%, 100%, 120%). At the individual level, three determinations were performed. The drug percent recovery and mean percent recovery was shown in table 4. The perceived data were within the essential range, which shows superior recovery value and that's why the accuracy of the method developed.

Table 4: Accuracy (% recovery) results of both drugs

Level (%)	Lisinopril % recovery	% Mean	Amlodipine % recovery	% Mean
80	100.09	100.04	100.04	100.05
80	100.00		100.06	
100	100.04	100.03	100.01	100.01
100	100.02		100.01	
120	100.03	100.00	99.98	99.70
120	99.97		99.42	

Detection limit and Quantification limit: The Limit of detection (LOD) which constitutes a concentration of the analyte at an S/N ratio of 3.3 and Limit of Quantification (LOQ) at which S/N was 10 were decided analytically for the suggested technique. Therefore, the detection limit and quantification limit of both drugs were given an S/N ratio of 3.3 and 10 sequentially. The results of LOD and LOQ are shown in table 5

Table 5: Results of LOD and LOQ

Sample name	LOD	LOQ
Lisinopril	1.44 (ug/ml)	4.35 (ug/ml)
Hydrochlorothiazide	2.62 (ug/ml)	7.94 (ug/ml)

Assay of Lisinopril and Amlodipine in tablet formulation: Behind prosperous development and validation of all these methods, it was working for analysis of the Lisinopril and Amlodipine in composite tablet formulation. Between the two analytes, the method results in exemplary separation with superior resolution. Furthermore, the elevated percentage of recovery and non-interference of the formulation excipients in retention time of the drugs manifest the selectivity of the technique for assessment of both drugs in their combined dosage form. The mean percent approximate for Lisinopril **100.02 %** and Amlodipine **99.92 %** were in superior concurrence with the label claimed.

CONCLUSION

The present method of RP-HPLC was found to be accurate, simple and easy, specific, precise, linear, quick, and inexpensive. Within the concise analysis time, this method gives a superior resolution between both the compounds. That's why the method to support good for the routine analysis of Lisinopril/Lisinopril-Ion pair and Hydrochlorothiazide in several pharmaceutical industries and also in academics.

ACKNOWLEDGMENT

CONFLICT OF INTERESTS

The authors report no conflicts of interest

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