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RP-HPLC Method Development and Validation for Determination of Lisinopril and Amlodipine in Tablet Dosage form

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ABSTRACT: Analytical method development involves screening various column and eluent conditions, method optimization includes iterative testing of various separation conditions of the HPLC method and is performed to achieve the best possible resolution, speed, and reproducibility, robustness testing and method validation.
Objective: The present study was aimed to develop a rapid, accurate, linear, and sensitive and validate high performance liquid chromatographic [RP-HPLC] method for determination of lisinopril and amlodipine in pharmaceutical dosage form. Methods: The chromatographic separation was performed on kromasil-C18 column [4.5 x 250 mm; 5 µm] using a mobile phase consisting of Methanol: 0.1% OPA in water (70:30 v/v). The flow rate is 1.0 ml/min and the detection was carried out at 210nm.
Results: The chromatographic condition, the peak retention time of lisinopril and amlodipine were found to be 1.82 min and 2.68 min respectively. The method was validated as per ICH Q2 R1 guidelines. The calibration curve was found to be linear in the concentration range of 2-30 µg/ml for lisinopril and amlodipin. The limit of detection and quantification was found to be 0.219µg/ml and 0.665µg/ml for lisinopril and 0.228µg/ml and 0.691µg/ml for amlodipine respectively.
Conclusion: A new sensitive, simple reverse-phase high-performance liquid chromatography [RP-HPLC] method has been developed and validated for the determination of amlodipine and lisinopril. The proposed method can be used for routine determination of amlodipine and lisinopril.

Keywords: Lisinopril, Amlodipine, Method validation, RP-HPLC.

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 (Budawari, 1996; Sweetman, 1999). Several spectroscopic (Nejres *et al.*, 2023; Gidwani and Patel 2017) RP-HPLC (Bistra & Peikova1 2013; More & Pawar 2021; Andhale & Kharat 2019; Gowri Sankar & Manju Latha 2014; Bhaskara & Rao 2011). HPTLC (Ramyasree *et al.*, 2018), 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 angiotens in converting enzyme inhibitor that is used in the treatment of hypertension and heart failure. It is used alone or in combination with other medications to treat high blood pressure in adults and children 6 years of age and older. It is used in combination with other medications to treat heart failure. Lisinopril is also used to improve survival after a heart attack. 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.

MATERIALS AND METHODS

The liquid chromatographic system consisted of the following components: Chromatographic analysis was performed using Spinchrom software on a Kromasil C18 with dimension (250 mm × 4.6 mm i.d.) 5µm. The Shimadzu electronic balance (AX 200) was used for weighing purpose. Analytically pure Lisinopril and Amlodipine were obtained as gift samples from Trumac Healthcare Ltd., (Mumbai, India) Panchkula Hariyana. Acetonitrile, methanol, water (E. Merck, Mumbai, India) were of HPLC grade, while ortho-phosphoric acid (S.D. Fine Chemicals, Mumbai, India) was of Analytical grade used for the preparation of mobile phase. Tablet formulation *Amlocure* L containing labeled amount 5 mg Lisinopril and 5 mg Amlodipine were procured from local market.

Preparation of stock solutions: Weigh accurately 13.87mg Amlodipine besylate (Equivalent to 10 mg of Amlodipine) and 10.90mg Lisinopril dihydrate (Equivalent to 10mg of Lisinopril) transferred into 20 ml volumetric flask, added15 ml of methanol and sonicated to dissolve the standard completely and

diluted up to the mark with methanol (500 PPM). Further diluted 0.8 mL to 20 mL with methanol. (20 PPM)

Selection of analytical wavelength for HPLC method development: Analytical wavelength for the examination was selected from the Q-point from the spectrophotometric analysis and it was 210 nm**.**

Determination of Lisinopril and amlodipine bisylate in their combined dosage forms. Weighed 20 tablets transferred in mortar pestle and crushed to fine powder. Mixed the contents with butter paper uniformly. Weighed the powder material equivalent to 10 mg of Lisinopril and 10 mg of Amlodipine (440.8 mg of powder material). Transfer it in a clean and dried 100 mL of volumetric flask, added 70 ml of methanol sonicated it for 15 minutes with intermittent shaking. Made the volume up to the mark with methanol. Filter the solution through suitable 0.45 µ syring filter discarding 3-5 mL of filtrate. Further diluted 2 ml of filtrate to 10 ml with diluent (20 PPM of Lisinopril and 20 PPM of Amlodipine).

Accuracy. Take clean and dried 9 volumetric flask of 100 mL. Weighed aprrox 416.04 mg of placebo and transferred in each 100 mL volumetric flask. Weighed Lisinopril Dihydrate and Amlodipine besylate API as per accuracy level and transferred in same 100 ml volumetric flask. Added 70 mL of methanol and sonicated it for 15 minutes with intermittent shaking. Made the volume up to the mark with methanol. Filter the solution through 0.45 µ PVDF syring filter discarding 3-5 mL of filtrate. Further dilute 2 ml of filtrate to 10 ml with diluent.

Acceptance criteria. 1. % Recovery for each sample and Mean recovery and overall recovery should be in the range of 98-102%.

2. The Relative Standard Deviation should not be more than 2.0%.

Precision. Weighed 20 tablets transferred in mortar pestle and crushed to fine powder. Mixed the contents with butter paper uniformly. Weighed the powder material equivalent to 10 mg of Lisinopril and 10 mg of Amlodipine (440.8 mg of powder material). Transfer it in a clean and dried 100 mL of volumetric flask; added 70 ml of methanol sonicated it for 15 minutes with intermittent shaking. Made the volume up to the mark with methanol. Filter the solution through suitable 0.45 µ syring filter discarding 3-5 mL of filtrate. Further diluted 2 ml of filtrate to 10 ml with diluent (20 PPM of Lisinopril and 20 PPM of Amlodipine). Six samples prepared.

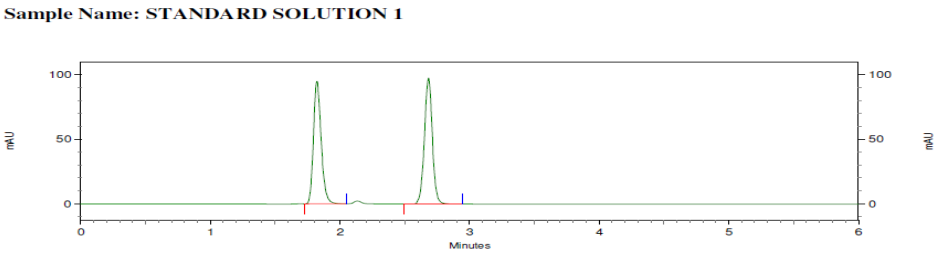
Specificity. Commonly used excipients (starch, microcrystalline cellulose and magnesium stearate) were spiked into a pre weighed quantity of drugs. The chromatogram was taken by appropriate dilutions and the quantities of drugs were determined.

Detection limit and quantitation limit. A calibration curve was prepared using concentrations in the range of 2-30 µg/ml for Lisinopril and 1-15 µg/ml for amlodipine (expected detection limit range). The standard deviation of y-intercepts of regression lines were determined and kept in following equation for the determination of detection limit and quantitation limit. Detection limit= 3.3σ /s; quantitation limit= 10σ/s; where σ is the standard deviation of y intercepts of regression lines and s is the slope of the calibration curve.

Robustness. Robustness of the method was studied by changing the composition of organic phase by ± % and the pH by ±0.2, and also by observing the stability of the drugs for 24 h at 35° temperature in the mobile phase.

RESULTS AND DISCUSSION

Optimization of mobile phase was performed based on resolution, asymmetric factor and peak area obtained for both Lisinopril and amlodipine. The mobile phase Methanol: 0.1% OPA in water (70:30 v/v) adjusted to pH 4 was found to be satisfactory and gave two symmetric and well-resolved peaks for both drugs. The retention time for Lisinopril and amlodipine were 1.82 min and 2.68 min, respectively (Fig. 1). The asymmetric factors were 1.30 and 1.48, respectively. Overlain UV spectra showed that both the drugs absorbs appreciably at 210 nm so, 210 nm was selected as the detection wavelength in liquid chromatography (Fig. 2).



VWD: Signal A, 210 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)	Resolution (USP)
Lisinopril	1.83	6064620	1.26	6431	0.00
Amlodipine	2.67	6572853	1.05	8363	7.83
Totals		12637473			

Fig. 1. Typical chromatogram of Lisinopril and amlodipine.

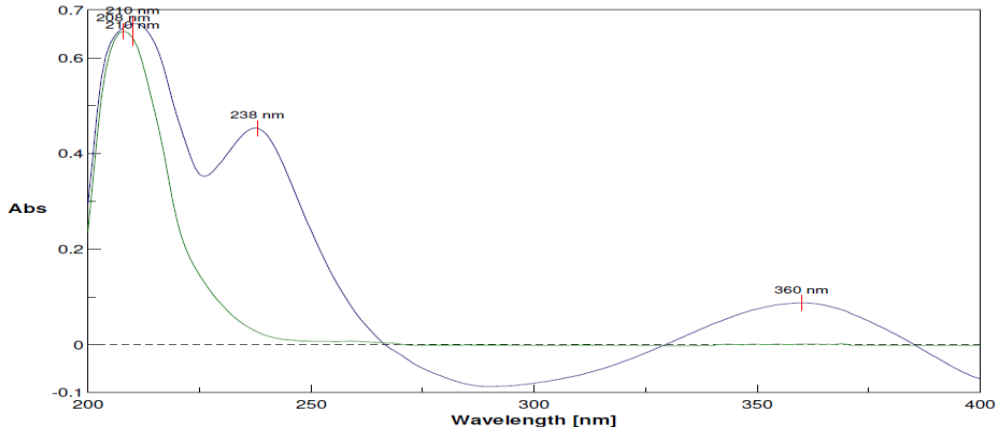


Fig. 2. Overlay UV spectrum of Lisinopril & Amlodipine

The calibration curve for Lisinopril was obtained by plotting the peak area versus the concentration over the range of 2-30 µg/ml, and it was found to be linear with r= 0.9982. Similarly, the calibration curve for amlodipine was obtained over the range of 2-30µg/ml and was found to be linear with r= 0.9993. The detection limit for Lisinopril and amlodipuine were 0.219 µg/ml and 0.228 µg/ ml, respectively. The quantitation limit were 0.665 µg/ml and 0.691 µg/ml, respectively, which suggest that a nanogram quantity of both the compounds can be estimated accurately. The validation parameters are summarized in Table 1.

Table 1: Summary of validation parameters.

Parameters	LSN	AMD
Detection limit (µg/ ml)	0.219	0.228
Quantitation limit (µg/ ml)	0.665	0.691
Accuracy (%)	98.52-100.08	98.28-101.42
Precision (RSDa, %)		
Intraday (n=3)	0.97-1.4	0.81-0.65
Interday (n=3)	1.01-1.42	0.71-1.19
Repeatability (RSDa,n=3)	0.56-0.91	0.23-0.31

The recoveries of Lisinopril and amlodipine were found to be in the range of 98.2–100.68% and 98.68–101.82%, respectively.

The system suitability test parameters are shown in Table 2. The liquid chromatographic method was applied to the determination of both the drugs in their combined dosage forms. The results for Lisinopril and amlodipine were comparable with the corresponding labeled amounts (Table 3).

Table 2: System suitability test parameters for LSN and AMD by the proposed method.

System suitability Parameters	LSN	AMD
Retention time (min)	1.82	2.68
Resolution	7.83	-
Theoretical plates	6431	8363
Tailing factor (asymmetric factor)	1.26	1.05

Table 3: Assay results of combined dosage form using proposed method.

Trial No.	Labelled amount (mg)		Amount obtained (mg)b		% Recovery b	
	LSN	AMD	LSN	AMD	LSN	AMD
1	10.47	10.24	10.42	10.23	99.52	99.9
2	10.47	10.24	10.32	10.06	98.56	98.24
3	10.47	10.24	10.17	10.02	97.13	97.85

DISCUSSION

In most of analytical method development for Lisinopril and amlodipine were carried out by using acetonitrile: phosphate buffer as a mobile phase. Acetonitrile is expensive solvent which will cost a method development, therefore method was developed using mobile phase Methanol: 0.1% OPA in water (70:30 v/v), wavelength 210 n, flow rate 1.0 ml/min. The column used was a Kromasil C18 column (250 mm × 4.6 mm, 5 mm). The method is simple, powerful and accurate, well insulated and usable for commercial analysis

CONCLUSIONS

Proposed study describes a new RP-HPLC method for the estimation of Lisinopril and amlodipine combination in mixture using simple mobile phase with low buffer concentration compared to the reported method. The method gives good resolution between both the compounds with a short analysis time (<10

min). The method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipient used in the formulation. Therefore, the proposed method can be used for routine analysis of Lisinopril and amlodipine in their combined dosage form.

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Conflict of Interest. None.

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