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A Stability Indicating RP-HPLC Method for the Determination of Lisinopril and Amlodipine Besylate in Bulk and Combined Pharmaceutical Dosage Form

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Abstract
The simple, selective, accurate, efficient, and reproducible Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for analysis of Lisinopril and Amlodipine besylate in bulk and tablet dosage form. The separation was carried out on Younglin (S.K) Gradient System UV Detector UV 730 D & SP930 D (150 mm x 4.6ID, Particle size: 5 micron) in isocratic mode column using the mobile phase composition was acetonitrile: phosphate buffer (25:75 % v/v) adjusted to pH 3.0 using triethylamine. The injection volume was 20µl at flow rate of 1.0 mL/min, effluent was detected at 211 nm with a sharp peak obtained for Lisinopril and Amlodipine besylate at a retention time of 5.8333 ± 0.01 min and 4.4167 ± 0.01 min resp. Linearity was observed in the concentration range from 05 to 30 µg/mL and 2.5 to 15 µg/mL for Lisinopril and Amlodipine besylate with a correlation coefficient of (r^2) 0.9993 and 0.9994, respectively. The recoveries of Lisinopril and Amlodipine besylate were found within limit 98.85-99.28% and 100.48-101.58 %. The percentage relative standard deviation for accuracy and precision was found to be less than 2%. The method was validated according to 'The International Council for Harmonisation' guidelines in terms of linearity, accuracy, precision, and specificity. Hence, the proposed method can be utilized for routine quality control of Lisinopril and Amlodipine besylate in bulk and tablet dosage form.

Keywords
The International Council for Harmonisation' (ICH) guidelines, Amlodipine besylate (AML), Lisinopril (LIS), ultraviolet detection, validation, reverse phase high performance liquid chromatography.

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INTRODUCTION
Amlodipine besylate chemically 3-Ethyl-5-methyl (4RS)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate benzene sulphonate. AML a long-acting calcium channel blocker used for hypertension and angina pectoris [2-4]. Amlodipine besylate block the inward movement of calcium by binding to L-

Type calcium channels in the heart and in smooth muscle of the coronary and peripheral vasculature relaxing the smooth muscle and dilating arterioles thereby decreasing peripheral resistance. Hence improving blood pressure; in angina it improves blood flow to the myocardium. Lisinopril dihydrate (LIS) chemically (2S)-1-[(2S)-6-amino-2-[{(1S)-1-carboxy-3-phenylpropyl} amino] hexanoyl] pyrrole-2-carboxylic acid is an ACE inhibitor which acts by directly blocking the formation of AT-II & at the same time by increasing bradykinin level.

In one of research studies reported the effects of Amlodipine besylate and Lisinopril on left ventricular mass and E/A ratio after 1 year of treatment in patients with previously untreated mild to moderate hypertension are similar.

A detailed literature survey suggests various methods available for estimation of AML and LIS in bulk, blood plasma and urine such as spectrophotometry, gas-liquid chromatography capillary electrophoresis and polarography. Several High-pressure liquid chromatography HPLC methods have been used for the analysis of lisinopril in human plasma. However, all these methods required laborious experimental work high consumption of organic solvents and these methods were developed on single column. The present research was undertaken to develop novel, accurate, precise, fast and chief liquid chromatographic method for the estimation of Lisinopril and Amlodipine besylate in bulk and tablet dosage form.

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To optimize the reverse phase HPLC (RP-HPLC) parameters, several mobile phases of different compositions were tried. A satisfactory separation and good peak symmetry for Lisinopril and Amlodipine besylate was obtained with a mobile phase consisting of acetonitrile: phosphate buffer (25:75 % v/v) adjusted to pH 3.0 using (TEA) triethylamine. Quantification was achieved with UV detection at 211 nm based on peak area and retention time was found 5.8333 ± 0.01 min and 4.4167 ± 0.01 min resp. Suitability of chromatographic system was monitored by calculating tailing/asymmetry factor and theoretical plates.[1,2,4,6]

MATERIALS AND METHODS

Equipment used:

HPLC Younglin (S.K) Gradient System UV Detector; Detector & pump No. was UV 730 D & SP930 D. The system was controlled through Autochro-3000 software using C18 - COSMOSIL (150 mm x 4.6ID, Particle size: 5 micron) column.

Reagents and chemicals:

Pharmaceutical grade pure Lisinopril and Amlodipine besylate were obtained as gift samples. Marketed formulation AMLOPRESS-L with dose of 05 mg each of LIS and AMLO were procured from local market. HPLC grade HPLC grade water and acetonitrile were purchased from E. Merck (India) Ltd., Mumbai.

Chromatographic conditions:

The system was controlled through Autochro -3000 software using C18 – COSMOSIL (150 mm x 4.6ID, Particle size: 5 micron) column maintained at ambient temperature and at flow rate 1.0 mL/min. The measurements were done with UV detection at 211 nm. The mobile phase was composed of acetonitrile: phosphate buffer (25:75 % v/v). The mobile phase was kept in ultrasonicator for 30 min and filtered through a 0.45 µm nylon membrane filter with 8 min run time.

Preparation of standard solutions:

05 mg & 05 mg each of LIS and AMLO were accurately weighed and transferred into two 25mL volumetric flasks, dissolved using mobile phase and the volume was made up with the same solvent to obtain primary stock solutions A (LIS) and B (AMLO) of concentration 500 µgm/mL and 1000µg/mL, respectively. From the primary stock solutions, 0.05 ml were pipetted out from A and B respectively, transferred to a 50ml volumetric flask and the volume was made up with the mobile phase to obtain final concentrations.

Preparation of sample solution

Twenty tablets of AMLOPRESS-L (Cipla Pvt. Ltd) (average weight 0.033 Grams/Tab) were weighed and crushed. Each tab content equivalent to 5mg LIS and 5mg AML was weighed accurately and transferred to a 50ml volumetric flask. The content was dissolved with 10mL of solvent and then sonicated for 15min. The volume was made up with the mobile phase and filtered with 0.45µ membrane filter.

Validation of the RP-HPLC method

Validation of the optimized method was performed according to the ICH Q2 (B) guidelines. The method was validated for linearity, precision (inter-day, intra-day and intermediate precision), accuracy and specificity. Standard plots were constructed for both Lisinopril and Amlodipine besylate in the range of 05-25µg/mL and 05-15 µg/mL, respectively. The

experiment was repeated thrice on the same day and additionally on two consecutive days to determine intra- and inter-day precision, respectively.

Experimental Work:

A simple reversed phase HPLC Method has been developed for simultaneous determination of Amlodipine besylate (AML) and Lisinopril (LIS). The method was based on reversed phase liquid chromatography using C18 - COSMOSIL (150 mm x 4.6ID, Particle size: 5 micron) column with UV detection 211 nm. The mobile phase consisting of acetonitrile: phosphate buffer (25:75 % v/v) at pH 3 adjusted using TEA and flow rate was 1.0 mL/min. The method was linear over the concentration range for 05-25 µg/mL Lisinopril and Amlodipine 2.5-15 µg/mL; the recoveries of Lisinopril and Amlodipine were found within limit 98.85-99.28% and 100.48-101.58 %. The method was validated.

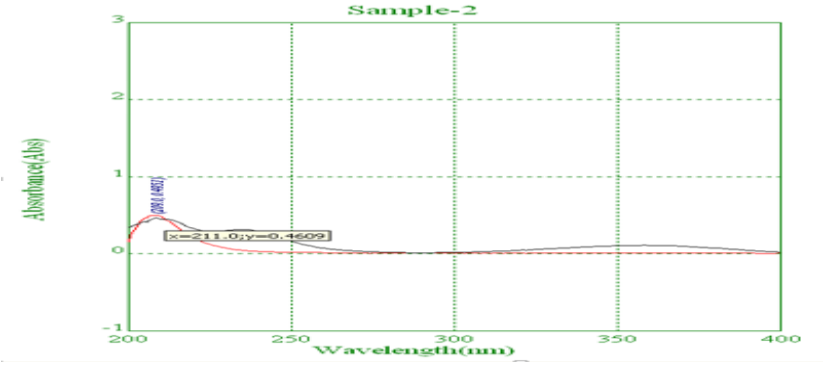

Figure 1: UV spectrum of Lisinopril and Amlodipine

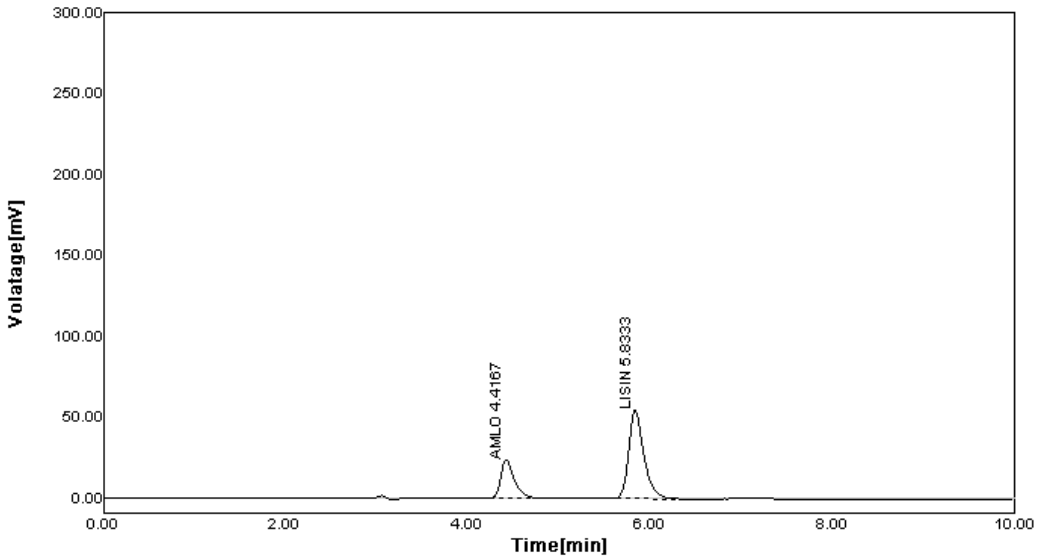

Figure 2: Typical chromatogram of Lisinopril and Amlodipine

Table 1: System Suitability

Parameter	Lisinopril	Amlodipine
Linearity Range (µg/mL)	05 to 30 µg/mL	2.5 to 15 µg/mL
Correlation of coefficient (r^2)	0.9993	0.9994
Limit of Detection (µg/ml)	0.4304	0.1401
Limit of Quantitation (µg/ml)	1.3044	0.4280
Retention Time (min)	5.8333	4.4167
Tailing Factor	1.3000	1.5000
Theoretical plates	5613.9	3894.1

Table 2: Linearity of Lisinopril at 211 nm

Sr. no	Concentration (µg /mL)	Peak area
1	5	639.9467
2	10	1133.07
3	15	1574.828
4	20	2096.975
5	25	2528.796

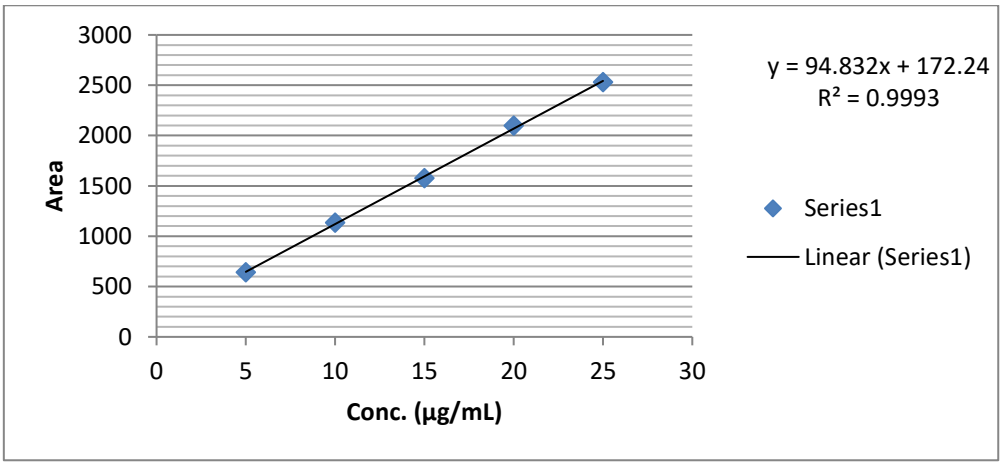

Figure 3: Linearity plot or calibration curve for Lisinopril at 211 nm

Table 3: Linearity Results of Amlodipine at 211 nm

Sr. no	Concentration (µg / mL)	Peak area
1	2.5	271.9494
2	5	456.3426
3	7.5	638.687
4	10	831.1917
5	12.5	993.5098
6	15	1200.6985

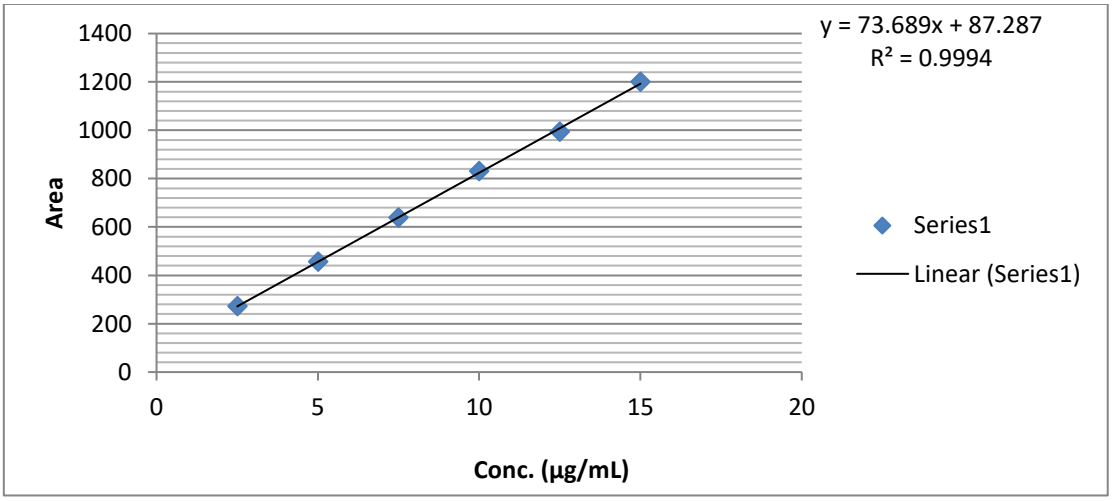

Figure 4: Linearity plot or calibration curve for Amlodipine at 211 nm

Accuracy

The accuracy of the method was determined by calculating recovery of Amlodipine besylate and Lisinopril by the standard addition method. The accuracy of the method was determined by preparing solutions of different concentrations 80, 100 and 120% level to prequantified sample solutions of Amlodipine and Lisinopril (2.5, 7.5, 15 µg/mL and 05, 15, 30 µg/mL respectively). The solutions were prepared in triplicates and the accuracy was indicated by percentage recovery.

Table 4: Accuracy studies

Sr. No.	Level of % Recovery	% Mean Recovery*		% R.S.D.*	
		Amlodipine	Lisinopril	Amlodipine	Lisinopril
1	50	100.48	99.77	1.24	0.02
2	100	100.49	99.06	1.49	0.64
3	150	101.58	101.58	0.21	0.41

***Avg. of six determinations for 50 & 150, three determinations for 100%, R.S.D. is relative standard deviation.**

Precision

The precision of the method was checked by inter day and intraday repeatability and reproducibility. The repeatability of method was analysed by repeatedly injecting (n=3) solutions of Amlodipine and Lisinopril (2.5, 7.5, 15 µg/mL and 05, 15, 30 µg/mL respectively) into the HPLC system. The results are shown in the table 6 and 7, which indicates that the proposed method is good with high precision. Moreover, the low RSD values indicate the high degree of correctness of method.

Table 5: Repeatability

Sr No.	Conc µg/mL	Area			Mean	Amount Found µg/mL	% Amount Found µg/mL	SD	%RSD
		I	II	III					
1	5	465.4239	452.8001	465.8327	461.35	5.07	101.34	7.41	1.61

Table 6: Interday and Intraday Precision Amlodipine

Intraday

Sr No.	Conc µg/mL	Morning	Evening	Mean	Amount Found µg/mL	% Amount Found µg/mL	SD	%RSD
1	2.5	270.6305	270.9279	270.78	2.49	99.60	0.21	0.08
2	7.5	640.3	652.82	646.56	7.50	100.00	8.85	1.37
3	15	1218.32	1208.83	1213.55	15.28	101.87	6.71	0.55

Interday

Sr No.	Conc µg/mL	Day 1	Day 2	Mean	Amount Found µg/mL	% Amount Found	SD	%RSD
1	2.5	271.235	270.23	270.73	2.48	99.20	0.71	0.26
2	7.5	645.25	640.39	642.82	7.53	100.40	3.44	0.53
3	15	1208.12	1215.69	1211.91	15.28	101.87	5.35	0.44

Table 7: Interday and Intraday Precision Lisinopril

Intraday

Sr No.	Conc µg/mL	Morning	Evening	Mean	Amount Found µg/mL	% Amount Found	SD	%RSD
1	5	634.2227	648.176	641.20	4.94	98.91	9.87	1.54
2	15	1570.9308	1582.5063	1576.72	14.81	98.73	8.19	0.52
3	30	3007.7366	3014.4646	3011.10	29.93	99.77	4.76	0.16

Interday

Sr No.	Conc µg/mL	Day 1	Day 2	Mean	Amount Found µg/mL	% Amount Found	SD	%RSD
1	5	634.2227	638.176	638.18	4.91	98.20	2.80	0.44
2	15	1550.9308	1572.5063	1561.72	14.65	97.67	15.26	0.98
3	30	3010.7366	3004.4646	3007.60	29.89	99.63	4.43	0.15

Robustness

Robustness is the ability of method to remain unaffected by small changes in parameters. Here in this study different flow rate (± 2 mL/min), mobile phase compositions and wavelength variations (± 1 nm) were investigated.

The results revealed that the method is robust enough.

Table 8: Robustness study for Amlodipine

Varied Conditions	Changed conditions	Concentration (µg/mL)	Area	Mean	SD	%RSD
Flow rate (mL/min)	0.9 mL/min	10	967.1725	955.84	16.03	1.68
		10	944.5093			
	1.1 mL/min	10	898.0843	893.84	6.00	0.67
		10	889.5958			
Mobile Phase Composition	ACN 24+76	10	813.6263	817.1	4.95	0.61
		10	820.6304			
	ACN 26+74	10	830.7922	833.035	0.79	0.12
		10	831.9136			
Wavelength (nm) change	212nm	10	765.4468	766.6	1.67	0.22
		10	767.81			
	210nm	10	794.215	800.28	8.58	1.07
		10	806.3531			

Table 9: Robustness study for Lisinopril

Varied Conditions	Changed conditions	Concentration (µg/mL)	Area	Mean	SD	%RSD
Flow rate (mL/min)	0.9 mL/min	20	2147.897	2150.47	3.63	0.17
		20	2153.0339			
	1.1 mL/min	20	2090.7631	2082.70	11.41	0.55
		20	2074.6272			
Mobile Phase Composition	ACN 24+76	20	2022.699	2017.7	7.04	0.35
		20	2012.7444			
	ACN 26+74	20	2102.3904	2110.12	0.79	0.52
		20	2117.8462			
Wavelength (nm) change	212nm	20	2072.73	2067.7	7.05	0.34
		20	2062.7532			
	210nm	20	2090.3318	2088.78	2.20	0.11
		20	2087.2197			

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentration of standard solution by using HPLC method. The LOD and LOQ were calculated using the following equation as per ICH guidelines. LOD=3.3×σ/S and LOQ=10×σ/S, where σ is slope and S is standard deviation.

Table No.10 Assay Results

Formulation	Label Claim(mg)	% Assay ± S.D. (n =5)
AMLOPRESS -L	5mg Amlodipine & 5mg Lisinopril	99.40 ± 0.689 Amlodipine & 99.91 ± 0.342 Lisinopril

Forced Degradation Studies

Drug product and placebo were subjected to forced degradation at various stressed conditions like acid, base, hydrolysis, peroxide, heat, photo light, U.V light and Humidity. All the samples were analysed for peak purity of Amlodipine besylate and Lisinopril peak. In all the samples, Peak purity meets the acceptance limits. Furthermore, studies on degradation products is under investigation for more specifications and justifications.

Table 11: Forced Degradation at various stressed conditions of Lisinopril.

Sr. No	Forced Degradation at various stressed conditions	Area	Amount Found MCG
1	1 N NaOH After 1 Hr	2013.59	**19.41**
2	1 N NaOH After 2 Hr	1922.84	**18.46**
3	1 N NaOH After 3 Hr	1943.95	**18.68**
4	10 % H_2O_2 After 1 Hr	2050.86	**19.81**
5	30 % H_2O_2 After 1 Hr	1882.098	**18.03**
6	0.1 N HCl After 1 Hr	2004.8	**19.23**
7	1 N HCl After 1 Hr	2046.78	**19.73**
8	1 N. HCl After 2 Hr	1941.21	**18.65**
9	1 N HCl After 3 Hr	1829.49	**17.47**
10	NEUTRAL After 2 Hr	2014.02	19.42

Table 12: Forced Degradation at various stressed conditions of Amlodipine.

S.No	Forced Degradation at various stressed conditions	Area	Amount Found MCG
1	1 N NaOH After 1 Hr	829.1	**10.06**
2	1 N NaOH After 2 Hr	751.0624	**9.00**
3	1 N NaOH After 3 Hr	649.9	**7.63**
4	10 % H_2O_2 After 1 Hr	786.2	**9.48**
5	30 % H_2O_2 After 1 Hr	736.42	**8.81**
6	0.1 N HCl After 1 Hr	723.87	**8.63**
7	1 N HCl After 1 Hr	784.91	**9.46**
8	1 N. HCl After 2 Hr	705.49	**8.39**
9	1 N HCL After 3 Hr	675	**7.97**
10	NEUTRAL After 2 Hr	781.41	**9.42**

CONCLUSION:

A simple, precise, selective, sensitive, and rapid RP-HPLC method with UV detection for Amlodipine besylate and Lisinopril in pharmaceutical dosage form has been developed and validated. The method has been found best than from few methods reported, because of use of a less economical and readily available mobile phase, lack of extraction procedures. The method would be extensively used for the estimation of Amlodipine besylate and Lisinopril in bulk and pharmaceutical formulation. Drug product and placebo were subjected to forced degradation at various stressed conditions like acid, base, hydrolysis, peroxide, heat, photo light, U.V light and Humidity. All the samples were analysed for peak purity of Amlodipine besylate and Lisinopril peak. In all the samples, Peak purity meets the acceptance limits.

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