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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF LISINOPRIL AND AMLODIPINE BESYLATE IN TABLET DOSAGE FORM

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ABSTRACT

A simple, specific, precise, and accurate reversed-phase HPLC method was developed and validated for simultaneous estimation of lisinopril and amlodipine besylate in tablet dosage forms. The separation was achieved by Hypersil ODS-BP C_{18} column (250 mm × 4.6 mm; 5.0 µm) using methanol: phosphate buffer at pH 6 adjusted with orthophosphoric acid (30: 70, v/v) as eluent, at a flow rate of 1 mL/min. Detection was carried out at wavelength 212 nm. The retention times of lisinopril and amlodipine besylate were 3.88 min and 2.716 min, respectively. The linearity was established over the concentration ranges of 20–80 µg/mL and 20–80 µg/mL with correlation coefficients (r^2) 0.9999 and 0.9993 for lisinopril and amlodipine besylate respectively. The mean recoveries were found to be in the ranges of 98.33–101.37% and 98.90–100.70% for lisinopril and amlodipine besylate respectively. The proposed method has been validated as per ICH guidelines and successfully applied to the estimation of lisinopril and amlodipine besylate in their combined tablet dosage form.

Key words: Lisinopril, Amlodipine Besylate, ICH Validation, RP-HPLC

INTRODUCTION

Lisinopril (LSNP) 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 myocardialinfarction 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] (Figure 1).
Amlodipine (AMD) is a longacting 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] (Figure 2). The review of literature revealed that a few spectroscopic [3-5], HPLC [6-9], HPTLC[10], LC-MS[11,12] and CE[13]

methods have been 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 [14].

EXPERIMENTAL

Materials and Reagents: The reference sample of lisinopril and amlodipine besylate was supplied by Cipla Pharmaceutical Ltd., Mumbai, India. HPLC grade water and methanol were purchased from E. Merck (India) Ltd., Mumbai, India. Potassium dihydrogen phosphate and orthophosphoric acid of AR Grade were obtained from S.D. Fine Chemicals Ltd.,Mumbai, India. Tablet of LSNP and AMD in combined dosage form, AMLOPRESS-L, with 5mg LSNP and 5mg AMD lable claim manufactured by Cipla Pharmaceutical Ltd.

Instrumentation: An isocratic HPLC system (Analytical Technologies Limited) consisted of P2230 plus HPLC pump, variable wavelength programmable UV 2230 plus detector system, Rhenodyne valve with 20µL fixed loop and Analchrom 2006 as operating software. The chromatographic column used was Hypersil ODS-BP C_{18} column (250 mm × 4.6 mm; 5.0 µm). Analytical balance (wensar) was used for weighing purpose.

Chromatographic Condition: A mixture of Methanol: Phosphate Buffer at pH 6 adjusted with orthophosphoric acid (30:70v/v) was used as mobile phase and was filtered through 0.45µ membrane filter prior to use. The flow rate of mobile phase was maintained at 1mL/min. Detection was carried out at 212nm at the ambient temperature. Total run time 7min was used with injection volume of 20 µL.

Preparation of mobile phase and Standard Stock Solution: Accurately weighed potassium dihydrogen phosphate (13.6gm) into 1000mL beaker, dissolved and diluted to 1000mL with HPLC water and pH adjusted to 6.0 with orthophosporic acid. 700mL of this solution mixed with 300mL of methanol. The solution was sonicated for 10minand filtered through 0.45µ membrane filter. 100mg of standard LSNP and AMD were accurately weighed and transferred separately to a 50 mL volumetric flask and dissolved with mobile phase. The flask was sonicated for 10min. The flask was shaken and volume was made up to the mark with mobile phase to give a solution containing 1000µg/mL LSNP and AMD respectively. Appropriate volume of aliquot from LSNP and AMD standard stock solution was further diluted with mobile phase to obtain final concentration of 100µg/mL respectively.

Determination of LSNP and AMD from Combined Dosage Form: A powder quantity equivalent to 100 mg LSNP and AMD was accurately weighed and transferred to volumetric flask of 100 mL capacity. 50mL of solvent (methanol: phosphate buffer (30:70)) was transferred to this volumetric flask and sonicated for 10min. The flask was shaken and volume was made up to the mark with mobile phase. The above solution was filtered through membrane filter (0.45µ). From this solution 10mL was transferred to volumetric flask of 100mL capacity. Volume was made up to the mark to give a solution containing 100µg/mL. From this solution 4mL was transferred to volumetric flask of 100mL capacity. Volume was made up to the mark to give a solution containing 40µg/mL. The resulting solution was analyzed by proposed method. The prepared sample solution was chromatographed for 7 minutes using mobile phase at a flow rate of 1.0mL/min. From the peak area obtained in the chromatogram the amounts of both drugs were calculated.

Method Validation [15]

The proposed method has been extensively validated in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and system suitability. The accuracy was expressed in terms of percent recovery of the known amount of the standard drugs added to the known amount of the pharmaceutical dosage forms. The precision (% RSD) was expressed with respect to the repeatability, intraday, and interday variation in the expected drug concentrations. After validation, the developed methods have been applied to pharmaceutical dosage form.

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

Linearity: Appropriate volume of aliquot form LSNP and AMD standard stock solution was transferred to same volumetric flask of 10mL capacity. The volume was adjusted to the mark with mobile phase to give a solution containing LSNP (20, 30, 40, 50, 60, 70 and 80µg/mL) and AMD (20, 30,

40, 50, 60, 70 and 80µg/mL). The mixed standard solution was chromatographed using above chromatographic condition (n=6). All solutions were filtered through 0.45µm filter prior to use. Calibration curve were constructed by plotting average peak area versus concentrations for both drugs. Straight line equations were obtained from these calibration curves.

Accuracy: Accuracy was assessed by determination of the recovery of the method by addition of standard drug to pre-analyzed test sample preparation at three different levels 50, 100 and 150% taking into consideration percentage purity of added bulk drug samples. Each concentration was chromatographed 3 times and average recoveries were measured.

Precision: The repeatability was evaluated by assaying 6 times of test samples prepared for assay determination. The intraday and interday precision study of LSNP and AMD was carried out by estimating different concentrations of LSNP and AMD 3 times on the same days and on 3 different days and the results are reported in terms of % RSD.

Detection Limit and Quantitation Limit: ICH guideline describes several approaches to determine the detection and quantitation limits. These include visual evaluation signal-to-noise ratio and use of standard deviation of the response and the slope of the calibration curve. In the present study the LOD and LOQ were based on the third approach and were calculated according to the $3.3\sigma/s$ and $10\sigma/s$ criterions respectively, where σ is the standard deviation of y-intercepts of regression lines and s is the slope of the calibration curve.

Robustness: The robustness of the method was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions. For the proposed method it was done by changing the column temperature and variation of flow rate. There was no significant impact on the retention time and tailing factor.

System Suitability: The suitability of the chromatographic system was tested before each stage of validation. Five replicate injections of standard preparation were injected and tailing factor, USP plate count and %RSD (Relative Standard Deviation) of peak area were determined.

Determination of LSNP and AMD from combined Dosage Form: Sample solution was injected six times at above chromatographic conditions. An average peak area was measured from chromatograms. The quantitation was carried out by keeping these values to the straight line equation of calibration curve.

RESULT AND DISCUSSION

Optimizations of chromatographic conditions were performed to obtain the best peak and parameter (asymmetry, theoretical plates). For the selection of mobile phase initially methanol-water, acetonitrile – water and acetonitrile – phosphate buffer have been tried in different ratio which gave poor peak shape. Then methanol: phosphate buffer adjusted to pH 6.0 in different ratio have been tried. Finally methanol: phosphate buffer at pH 6 adjusted with orthophosphoric acid (30:70v/v) was found to be satisfactory and gave two symmetrical peaks for LSNP and AMD at flow rate of 1mL/min. The average retention times for LSNP and AMD were 3.8833 and 2.716 minutes respectively. The tailing factors for LSNP and AMD 1.06 and 1.17 respectively. For the selection of detection wavelength overlain UV spectrum of LSNP and AMD was taken which revealed that at 212nm both the drugs possess significant absorbance (Figure 3 and 4; Table 6). Summary of validation parameters for proposed method was given in Table 1.

The developed HPLC method was validated. The linear range, correlation coefficient, detection limit and standard deviation for LSNP and AMD by HPLC method are shown in Table 2 and Figure 5 and 6). Accuracy was determined by calculating the recovery. The method was found to be accurate with % recovery 98.33 – 101.37% for LSNP and 98.90 – 100.70 for AMD respectively in Table 4).

Precision was calculated as repeatability and intra and interday variation for both durgs. The method was precise with %RSD 0.12 – 1.17 for intraday (n=6) and %RSD 0.26 – 0.58 for interday (n=6) for LSNP and 0.06 – 0.18 for intraday (n=6) and %RSD 0.34 – 1.36 for interday (n=6) for AMD respectively. The method was specific as no interference observed when the drugs were estimated in presence of excipients. The method was also robust as there was no change in area up to 24 hours preparation of solution in methanol: phosphate buffer at pH 6 adjusted with orthophosporic acid (30:70v/v) in Table 3. The LOD for LSNP and AMD was found to be 0.0012 and 0.027 respectively. The LOQ for LSNP and AMD was found to be 0.037 and 0.083 respectively. Summary of validation parameters is tabulated in Table 1. Marketed formulation was analyzed by the proposed method and assay result of marketed formulation was shown in Table 5.

CONCLUSION

The proposed HPLC method provide simple, specific, precise, accurate and reproducible quantitative analysis for simultaneous analysis of LSNP and AMD in combined dosage form. The method was validated as per ICH guidelines in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and limits of quantification (LOQ), robustness and reproducibility. The proposed method can be used for routine analysis and quality control assay of LSNP and AMD in combined dosage form.

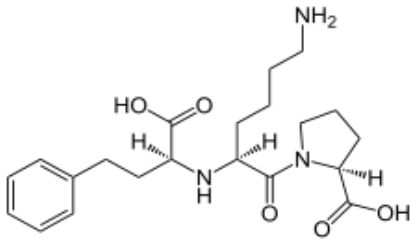

Figure 1: Structure of Lisinopril

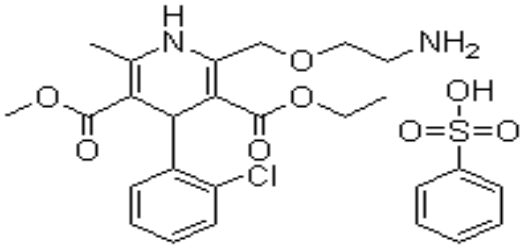

Figure 2: Structure of Amlodipine besylate

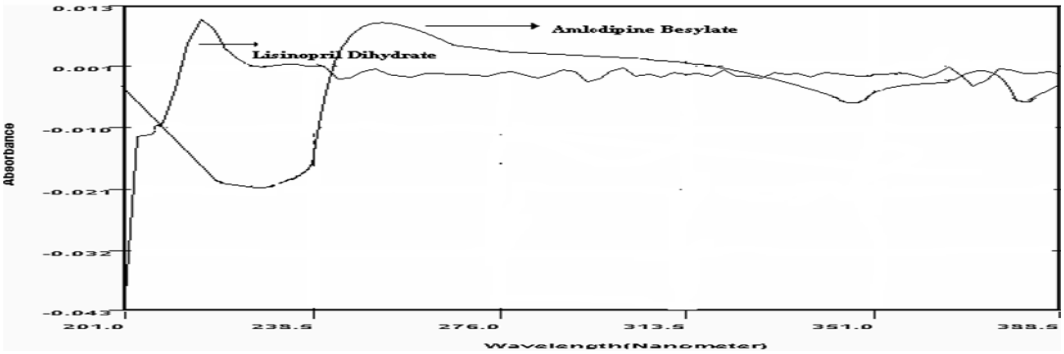

Figure 3: UV spectrum of lisinopril and amlodipine besylate

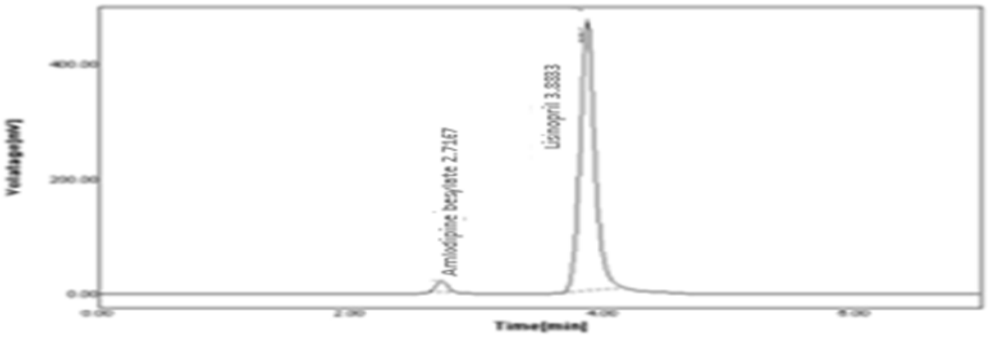

Figure 4: Chromatogram of Sample formulation

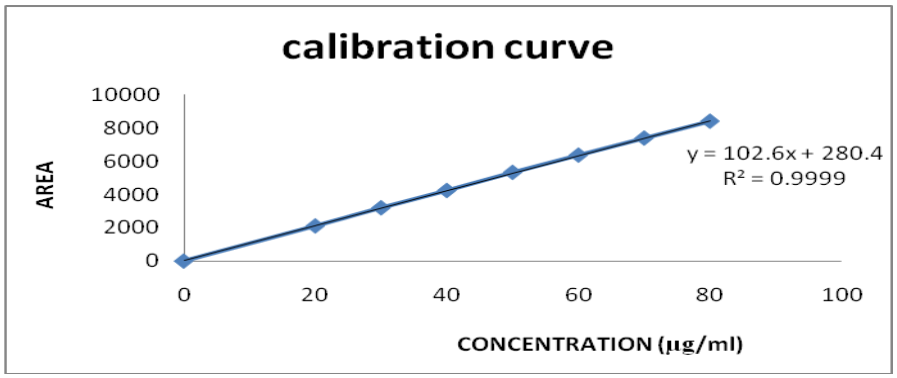

Figure 5: Linearity plot of lisinopril

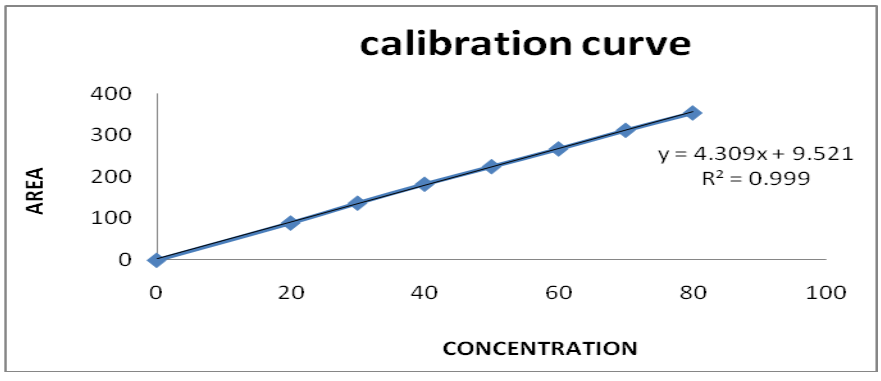

Figure 6: Linearity of Amlodipine besylate

Table 1: Summary of Validation parameters

Parameters	Lisinopril	Amlodipine Besylate
Recovery %	98.33 – 101.37	98.90 – 100.70
Precision- Intraday (%RSD)	0.12 – 1.17	0.06 – 0.18
Precision- Interday (%RSD)	0.26 – 0.58	0.34 – 1.36
Limit of detection (µg/ml)	0.012	0.027
Limit of quantification (µg/ml)	0.037	0.083
Specificity	Specific	Specific
Robustness	Robust	Robust
Solvent stability	Solvent stable for 24 hrs	Solvent stable for 24 hrs

Table 2: Calibration data for LSNP and AMD

Parameter	LSNP	AMD
Detection Wavelength (nm)	212	212
Linear range (µg/ml)	20 – 80	20 – 80
Intercept	280.4	9.521
Slop	102.6X	4.309X
Correlation co-efficient (r^2)	0.9999	0.999
Linearity Regression equation (y=mx+c)	Y=102.6x+280.4	Y=4.309x+9.521

Table 3: Robustness study for LSNP and AMD

Condition varied	Changed condition	Area (n=6)		% Assay	
		LSNP±S.D	AMD±S.D	LSNP	AMD
Flow rate (ml/min)	0.8	4393.0335±0.007	184.79±0.035	100.19	101.68
	1.0	4393.1357±0.013	184.60±0.041	100.21	101.57
	1.2	4392.9927±0.018	184.18±0.037	100.20	101.38
Temperature (^oC)	20	4393.3025±0.004	184.25±0.167	100.21	101.37
	25	4393.1032±0.008	184.39±0.105	100.21	101.37
	30	4393.3396±0.005	184.19±0.096	100.21	101.33

Table 4: Recovery studies of LSNP and AMD

Parameter	LSNP			AMD		
Level of Recovery(%)	50%	100%	150%	50%	100%	150%
Peak Area*	2307.5974	4366.9026	6483.2063	94.2615	184.2429	264.7747
%RSD	0.29	1.17	0.12	0.186	0.06	0.07
Mean % Recovery	98.33	101.37	98.72	98.9	99.5	100.7

* Mean of three trials

Table 5: Assay results of marketed formulation

Formulation	Actual amount (mg/tablet)		Amount obtained (mg/tablet)		% Recovery	
	LSNP	AMD	LSNP	AMD	% LSNP	%AMD
Tablet	5	5	4.97	5.04	99.4	100.8

Table 6: System suitability parameters

Parameters	LSNP	AMD
Retention time (Rt) (min)	3.88	2.71
Tailing factor	1.06	1.1
Plate count	6800	7458
%RSD	1.18	0.56

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