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Original Article

Development and validation of HPLC method for simultaneous estimation of Amlodipine besylate and Enalapril maleate in solid dosage form

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ABSTRACT

A rapid, sensitive and specific HPLC method involving UV detection was developed and validated for determination and quantification of Amlodipine besylate and Enalapril maleate in tablet dosage form. The determination was carried out on a Phenomenex C18 (250 x 4.6 mm, 5 µm) column using filtered and degassed mixture of methanol: 1N HCl (1:1) as mobile phase at a flow rate of 1ml/min and effluent was monitored at 218nm. The retention time for Amlodipine besylate was 8.062 min and for Enalapril maleate 2.457 min. Amlodipine besylate and Enalapril maleate showed a linear response in the concentration range of 10-60µg/ml. The correlation co-efficient ('*r*' value) for Amlodipine besylate and Enalapril maleate was 0.9938 and 0.999, respectively. The method was validated in terms of linearity, precision, accuracy, specificity, robustness and solution stability. The proposed method can be useful in the quality control of bulk manufacturing and pharmaceutical dosage forms.

Key words: Amlodipine besylate, Enalapril maleate, Method development, Validation, HPLC.

INTRODUCTION

Amlodipine (AML) is chemically 3-ethyl 5-methyl (4*RS*)- 2- [(2-aminoethoxy) methyl]- 4- (2-chlorophenyl) -6-methyl- 1,4- dihydropyridine-3,5-dicarboxylate benzene sulphonate. It is used as Antihypertensive & antianginal agent[1]. Amlodipine act by blocking voltage-sensitive calcium channels (L-type). Amlodipine slow conduction in the SA and AV nodes where action potential propagation depends on slow inward Ca^{2+} current, slowing the heart and terminating SVT by causing partial AV block. It shortens the plateau of the action potential and reduces the force of contraction. Reduced Ca^{2+} entry reduces after depolarization and thus suppresses premature ectopic beats.[2-4] Enalapril is a prodrug which is hydrolysed in the body to Enalaprilate, which is an inhibitor of angiotensin-converting enzyme (ACE). It is indicated for treatment of hypertension, treatment of symptomatic heart failure and prevention of symptomatic heart failure in patients with asymptomatic left ventricular dysfunction (ejection fraction <35%). Chemically it is ((S)-1-{N-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl}-L-proline, (Z)-2-butenedioate (1:1), a derivative of two amino-acids, L-alanine and L-proline. It is a white to off-white crystalline, odourless powder which melts in the range of 143–144◦C. ACE is a peptidyl dipeptidase that catalyzes the conversion of angiotensin I to the vasoconstrictor substance, angiotensin II, which stimulates aldosterone secretion by the adrenal cortex. Blocking the conversion of the angiotensin I to the angiotensin II, leads to a reduction in vasopressin activity and a decrease in peripheral vascular resistance.[5-10]

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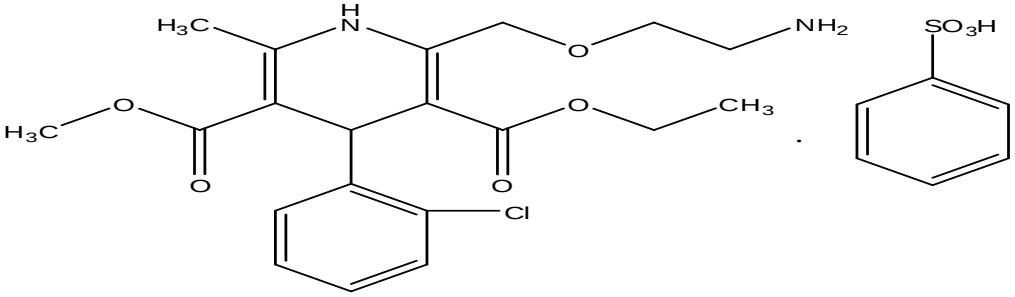

Figure: Structure of Amlodipine Besylate

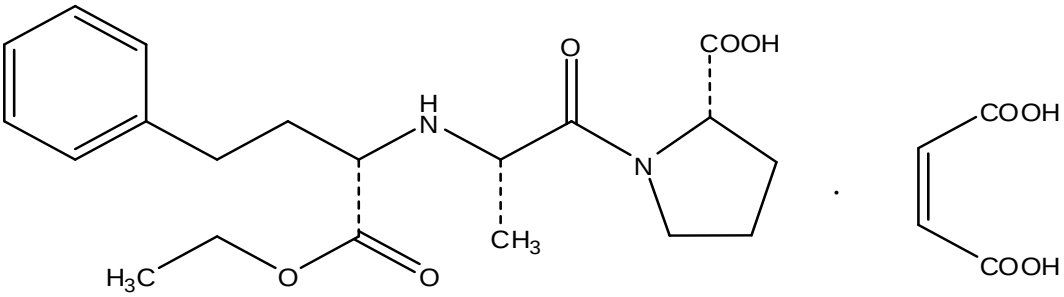

Figure: Structure of Enalapril Maleate

MATERIALS AND METHODS

Reagents and Chemicals: All solvents used were of HPLC grade. The reference standards of Amlodipine besylate and Enalapril maleate were obtained as gift samples from LUPIN Pharmaceutical Ltd. (Bhopal, India). The commercial fixed dose combination product Amtas E (Intas, Ahemdabad) containing Amlodipine 5 mg and Enalapril 5 mg was obtained from local pharmacy store. The solvents used were Methanol HPLC grade and Hydrochloric acid was procured from Cipla.

Preparation of Standard Stock solution: The standard stock solutions of AML (100µg/ml), ENA (100µg/ml) were prepared by transferring 10mg of Amlodipine besylate and 10mg of Enalapril maleate respectively in 100ml Volumetric flasks. The volume was made upto the mark using mobile phase (methanol : 1N HCl [1:1]). The solutions were sonicated for 15 min and filtered through Whatmann filter paper.

Preparation of Sample solution: Twenty tablets were weighed accurately, their average weight was determined and powdered. The powder of the tablets equivalent to 5 mg of AML and 5 mg of ENA was transferred into 50 ml volumetric flask. 25 ml of methanol : 1N HCl (1:1) was added into the volumetric flask and sonicated for 15 min to effect complete dissolution of the drugs. Then the volume was made upto the mark with mobile phase. The solution was filtered through the Whatmann filter paper and the aliquot portion of the filtrate was further diluted to get the final concentration of 100µg/ml. 10µl of the above solution was injected into the HPLC under the set chromatographic conditions.

Instrument and Chromatographic conditions: Chromatographic separation was carried out using Analytical Technologies Ltd HPLC system with UV-2230 UV-Vis detector and P-2230 HPLC pump. The elution was carried out isocratically.

Table 1: Optimized Chromatographic conditions

Parameter/Condition	Specification
Column	Phenomenex C18 (250 x 4.6 mm, 5 µm)
Mobile phase	Methanol:1N HCl (1:1)
Flow rate	1ml/min
Wavelength of detection	218nm
Sample load	10 µl
Column temperature	40ºC

RESULTS AND DISCUSSION

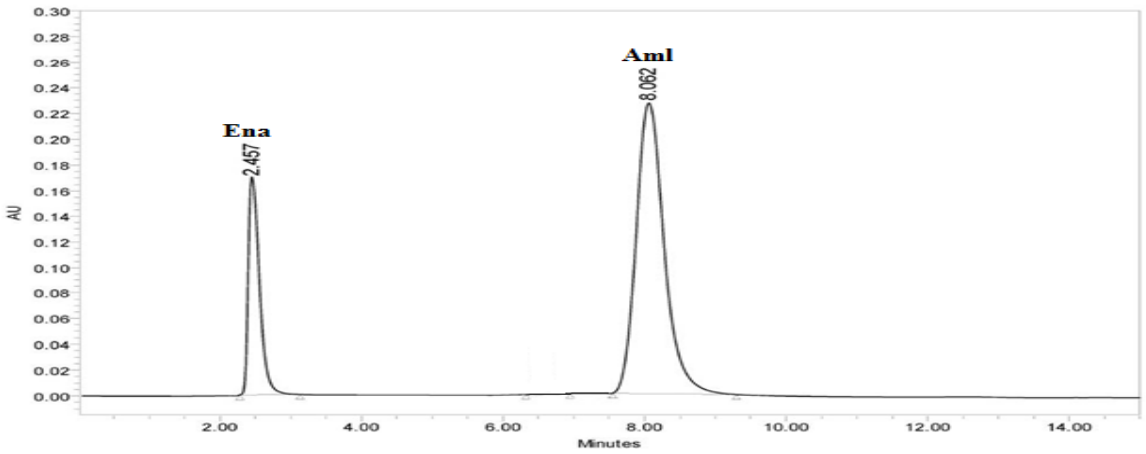

Fig : Chromatogram Report of Amlodipine besylate & Enalapril Maleate
For Amlodipine Besylate RT = 8.062 min; For Enalapril maleate RT = 2.457 min

Method Validation

Validation of any analytical method shall be done to establish by laboratory studies, that the performance of the method meet the requirement for the intended analytical application. The method was validated according to ICH guidelines to study linearity, accuracy and precision.[11]

Linearity: Several aliquots of standard solutions of AML and ENA were taken in different 10 ml volumetric flasks and the volume was made upto the mark with mobile phase such that final concentration of AML and ENA were 10-60 µg/ml, respectively. Evaluation was performed using the UV-Vis detector at 218 nm, peak area recorded for all the peaks, results are displayed in Table 2. Calibration curve was plotted as concentration against peak area as shown in Figure 3 & 4. The slope and intercept value for calibration curve were y = 3281.9x + 112554 (R^2 = 0.9938) for AML, y = 2934.4x + 129402 (R^2 = 0.99) for ENA.

Table 2: Linearity study

Concentration of AML (µg/ml)	Peak Area	Concentration of ENA (µg/ml)	Peak Area
10	141232	10	159228
20	176265	20	174269
30	219331	30	208384
40	244658	40	245685
50	278454	50	277459
60	304587	60	304510

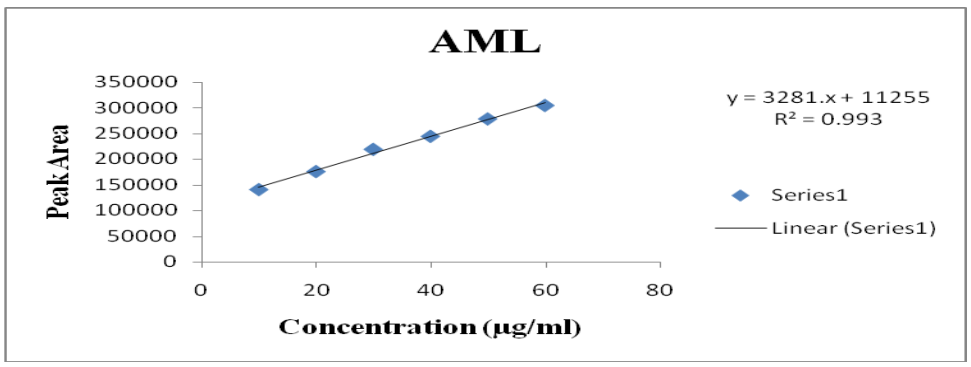

Fig 3 : Calibration curve of Amlodipine besylate (AML)

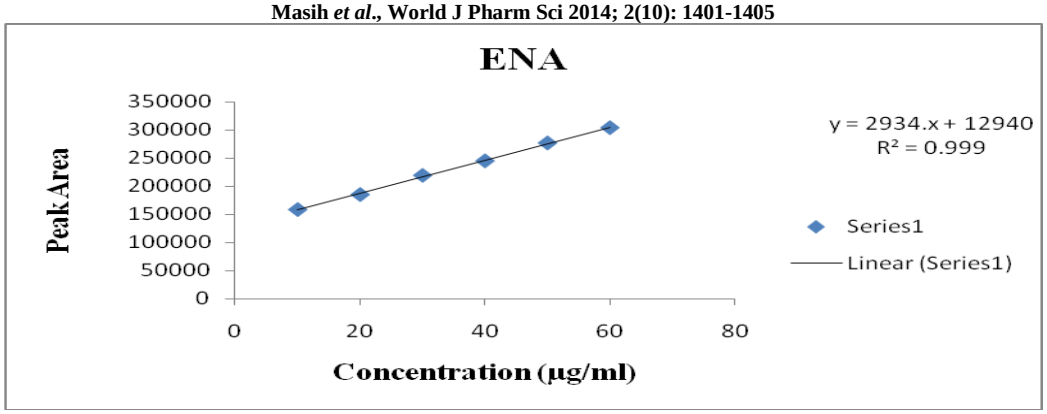

Fig 4 : Calibration curve of Enalapril maleate (ENA)

Recovery: Accuracy of the method was calculated by recovery studies at three levels (80%, 100% and 120%) by standard addition method. The accuracy was expressed as the percentage of the analyte recovered. Accuracy of proposed method was checked as per ICH guidelines. For AML, tablet powder equivalent to 5 mg AML was taken individually into three different 100 ml volumetric flasks and then 8 mg (80%), 10 mg (100%) and 12 mg (120%) of standard AML were added to each of the volumetric flasks. After that 25 ml of the mobile phase [methanol : 1N HCl (1:1)] was added to each of the volumetric flask and sonicated for 5 min. The solutions were then filtered and 1 ml of the filtrate from each was taken in 10 ml volumetric flasks individually and diluted upto the mark with mobile phase. The solutions were injected in triplicates into the chromatographic system and the peak area were evaluated to give Percent Recovery and Standard deviation. Similar procedure was repeated for other drug.

Table 3: Percent Recovery

Drug	Label Claim (mg)	Level (%)	Amount of std spiked (mg)	% Recovery ± SD	%RSD
Amlodipine Besylate	5	80%	4	97.08 ± 1.01	1.04
		100%	5	85.53 ± 0.61	0.71
		120%	6	97.82 ± 0.67	0.69
Enalapril maleate	5	80%	4	96.83 ± 1.12	1.16
		100%	5	94.33 ± 0.61	0.65
		120%	6	98.61 ± 0.53	0.54

Precision: To determine the precision of method, six replicates of the sample prepared from the commercial tablets were injected and assay was calculated to measure the repeatability of retention times and peak area of standard and sample. Precision of the method was verified by using tablet stock solution. Intraday and interday precision were determined by repeating assay six times in same day for intraday precision and on different days for interday precision studies. The results of these analyses are shown in Table 4.

Table 4: Precision

Drug	Intraday		Interday	
	% Obtained ± SD	%RSD	% Obtained ± SD	%RSD
Amlodipine Besylate	104.93 ± 1.48	1.41	105.82 ± 0.93	0.88
Enalapril Maleate	104.26 ± 1.34	1.29	105.71 ± 0.94	0.90

Robustness: The robustness of the proposed method was verified by varying the solvent ratio in the mobile phase, flow rate and wavelength range. Sample solutions were injected as 10 µl injection into the chromatographic system. The parameters studied were peak area and found their standard deviation & % RSD.

Limit of detection and Limit of quantification: The LOD and LOQ of the proposed method were determined by progressively injecting lower concentrations of the standard solutions under the set chromatographic conditions. The results obtained are displayed in Table 5.

L.O.D. = 3.3(SD/S)
L.O.Q. = 10(SD/S)
Where, SD = Standard deviation of the response,
S =Slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte.

Table 5: LOD and LOQ Results

Drug	LOD	LOQ
Amlodipine besylate	0.15	0.46
Enalapril Maleate	0.22	0.68

Table 6 : System suitability Parameters

Parameters	Observation	
	Amlodipine besylate	Enalapril Maleate
Linearity	10 – 60µg/ml	10 – 60µg/ml
Regression equation	y = 3281.9x + 112554	y = 2934.4x + 129402
Correlation coefficient	0.9938	0.99
Retention time	8.062 min	2.457 min
Resolution	13.03	16.54
Theoretical plates	54908.64	82173.57
Robustness	Robust	Robust
LOD	0.15	0.22
LOQ	0.46	0.68

CONCLUSION

The developed method gives good resolution between Amlodipine besylate and Enalapril maleate with short analysis time. The method is simple, accurate, rapid, precise and can be easily used for routine analysis of these drugs without involving any complicated sample preparation.

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