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UPLC method development and validation for simultaneous determination of amlodipine besylate and enalapril maleate in drug products

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***Abstract*---**Objective: A simple, Accurate, precise method was developed for the simultaneous estimation of the Amlodipine besylate and Enalapril maleate in pharmaceutical dosage form. Methods: Chromatogram was run through BEH C18 column (2.1 × 50mm i.d., 1.7 µm particle size). Mobile phase containing methanol: 1N HCl (1:1) was pumped through column at a flow rate of 1ml/min. Temperature was maintained at Ambient. Optimized wavelength for Amlodipine besylate and Enalapril maleate was 272 nm. Results: Retention time of Amlodipine besylate and Enalapril maleate were found to be 1.04 min and 0.59 min. The % purity of Amlodipine besylate and Enalapril maleate was found to be 100.03 % and 99.75 % respectively. The system suitability parameters for Amlodipine besylate and Enalapril maleate such as theoretical plates were found to be 5659.11 and 3214.07. the resolution was found to be 4.58,2.91. The linearity study for Amlodipine besylate and Enalapril maleate was found in concentration range of 10µg-50 µg and 10 µg-50 µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 97.41 % and 95.5 %, %RSD for repeatability was 0.73 and 0.66 %. The recision tudy was precise, robust and repeatable. LOD value was 10.06, 7.80 and LOQ value was 30.32 and 24.43 respectively. Conclusion: The results of study showed that the proposed UPLC method is a simple, accurate, precise, rugged, robust, fast and reproducible, which may be useful for the routine estimation of Amlodipine besylate and Enalapril maleate in pharmaceutical dosage form.

***Keywords*---**amlodipine besylate, enalapril maleate, UPLC, simultaneous estimation.

Introduction

Amlodipine is a popular antihypertensive drug belonging to the group of drugs called dihydropyridine calcium channel blockers. Due to their selectivity for the peripheral blood vessels, dihydropyridine calcium channel blockers are associated with a lower incidence of myocardial depression and cardiac conduction abnormalities than other calcium channel blockers [1]. Amlodipine is commonly used in the treatment of high blood pressure and angina. Amlodipine has antioxidant properties and an ability to enhance the production of nitric oxide (NO), an important vasodilator that decreases blood pressure [2]. The option for single daily dosing of amlodipine is an attractive feature of this drug. IUPAC Name is 3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5 dicarboxylate; benzenesulfonic acid. Molecular formula is $C_{26}H_{31}ClN_2O_8S$. Molecular weight is 567 g/mol. It is slightly soluble in water and sparingly soluble in ethanol.

Enalapril is a prodrug belonging to the angiotensin-converting enzyme (ACE) inhibitor drug class that works on the renin-angiotensin-aldosterone system, which is responsible for the regulation of blood pressure and fluid and electrolyte homeostasis. Enalapril is an orally-active and long-acting non sulfhydryl antihypertensive agent that suppresses the renin-angiotensin-aldosterone system to lower blood pressure. It was developed from targeted research programmed using molecular modelling [3]. Being a prodrug, enalapril is rapidly bio transformed into its active metabolite, enalaprilat, which is responsible for the pharmacological actions of enalapril. The active metabolite of enalapril competitively inhibits the ACE to hinder the production of angiotensin II, a key component of the renin-angiotensin-aldosterone system that promotes vasoconstriction and renal reabsorption of sodium ions in the kidneys. Ultimately, enalaprilat works to reduce blood pressure and blood fluid volume. IUPAC Name is (2S)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl] amino} propanoyl] pyrrolidine-2-carboxylic acid. Molecular formula is $C_{20}H_{28}N_2O_5$. Molecular weight is 376.44 g/mol. It is sparingly soluble in water, soluble in ethanol, and freely soluble in methanol.

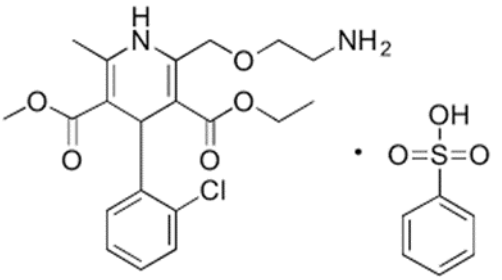

Figure 1: Structure of Amlodipine besylate

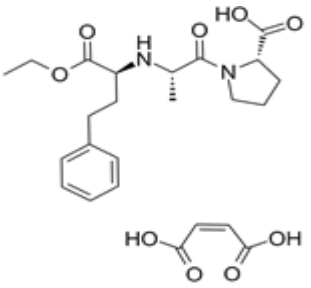

Figure 2: Structure of Enalapril maleate

The literature survey revealed that There are very few methods reported in the literature for analysis of Amlodipine besylate and Enalapril maleate alone or in combination with other drugs in the pure form and pharmaceuticals formulations [4-12]. In view of the need for a suitable, cost-effective UPLC method for routine analysis of Amlodipine besylate and Enalapril maleate Simultaneous estimation of in pharmaceutical dosage form. Attempts were made to develop simple, precise, accurate and cost-effective analytical method for the estimation of Amlodipine besylate and Enalapril maleate. The proposed method will be validated as per ICH guidelines. The objective of the proposed work is to develop a new, simple, sensitive, accurate and economical analytical method and validation for the Simultaneous estimation of Amlodipine besylate and Enalapril maleate in pharmaceutical dosage form by using UPLC. To validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form.

Materials and Methods

Chemicals and Reagents: Amlodipine besylate and Enalapril maleate were Purchased from Lupin Pharmaceuticals Ltd., Bhopal, M.P., India. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters Acquity UPLC system, equipped with an auto sampler, UV-visible PDA detector. Analysis was carried out at 272 nm with column BEH C18 column (2.1 × 50mm i.d., 1.7 µm particle size), dimensions at 25^0C temperature. The optimized mobile phase consists of methanol: 1N HCl (1:1). Flow rate was maintained at 1 ml/min.

Preparation of solutions
Preparation of Buffer

4.7 g of sodium dihydrogen orthophosphate and 1 ml of triethyl amine in 1000 ml of water and the pH of the solution was adjusted to 4.0± 0.05 with orthophosphoric acid.

Preparation of Mobile Phase (Methanol

The mobile phase consists of methanol: 1N HCl (1:1) and methanol: 0.1N HCl (1:1) as solvent. Mobile phase was filtered through a 0.22 µm membrane filter before use and degassed in an ultrasonic bath.

Preparation of Diluent: Mobile phase was used as diluent.

Preparation of Standard Stock Solution

Standard stock solution containing Amlodipine besylate (1000µg/ml) and Enalapril maleate (1000µg/ml) was prepared by transferring 100mg Amlodipine besylate 100mg Enalapril maleate working standard into a 100 ml volumetric flask. A 40 ml portion of diluent (methanol: 1N HCl, 1:1v/v) was added, sonicated and cooled to room temperature. The solution was diluted to the mark with diluent. Standard solution containing Amlodipine besylate (100µg/ml) and Enalapril maleate 0(01µg/ml) was prepared by pipetting 10 ml stock solution into a 100ml volumetric flask and diluted up to the mark with diluent.

Preparation of Test Stock Solution

Twenty tablets of marketed formulation Amtas E (Intas, Ahemdabad, Gujarat, India) containing Amlodipine besylate 5 mg and Enalapril maleate 5 mg were weighed and the average weight was calculated. The tablets were crushed with a mortar and pestle for 10 min. A portion of powder equivalent to the weight of 5 mg of Amlodipine besylate and 5 mg of Enalapril maleate was accurately weighed and transferred to a 100 ml volumetric flask. Approximately 50 ml diluent was added and the mixture was sonicated for 15 min with intermittent shaking. The contents were restored to room temperature and diluted to volume with diluent to furnish stock test solution. The stock solution was filtered through 0.45µm membrane filters and 10 ml of the filtered solution was transferred to a 100 ml volumetric flask and diluted to volume with diluents to give test solution containing 1000µg/ml Amlodipine besylate and 1000µg/ml Enalapril maleate.

Method

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters

To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 5 µL of standard into BEH C18 column (2.1 × 50mm i.d., 1.7 µm particle size), the mobile phase of composition methanol: 1N HCl (1:1) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation

The proposed validated method was successfully applied to determine Amlodipine besylate and Enalapril maleate in their pharmaceutical dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method

Linearity

The linearity study was performed for the concentration of 100ppm and 500ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 3.

Accuracy studies

The accuracy was determined by help of recovery study. The recovery method carried out at three level 80%, 100%, 120% and 80%, 100%, 120% Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Amlodipine besylate and Enalapril maleate and calculate the individual recovery and mean recovery values. The results are shown in table 4 & 5.

Precision Studies

Precision was calculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in UPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 6 & 7.

Robustness

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at +10% and -10% ml/min. The results are shown in table 8 to 10.

LOD and LOQ

The sensitivity of UPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 11.
LOD = 3.3 (SD/S) and
LOQ = 10 (SD/S), where
SD= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

Results and Discussion

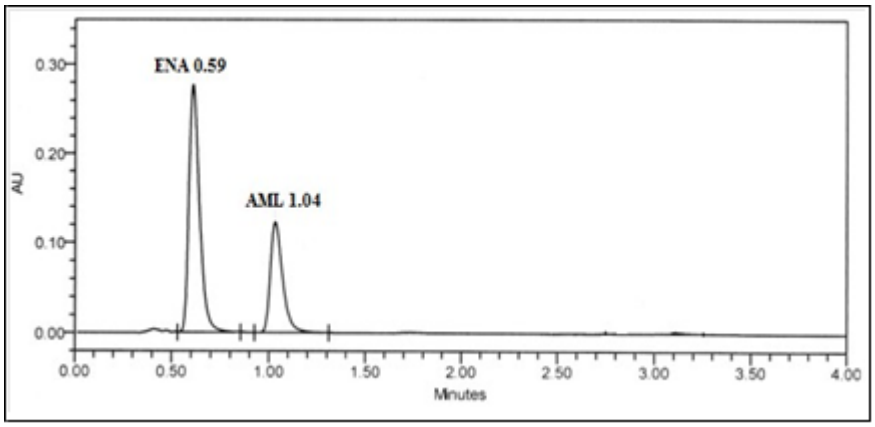

Figure 3: Standard chromatogram

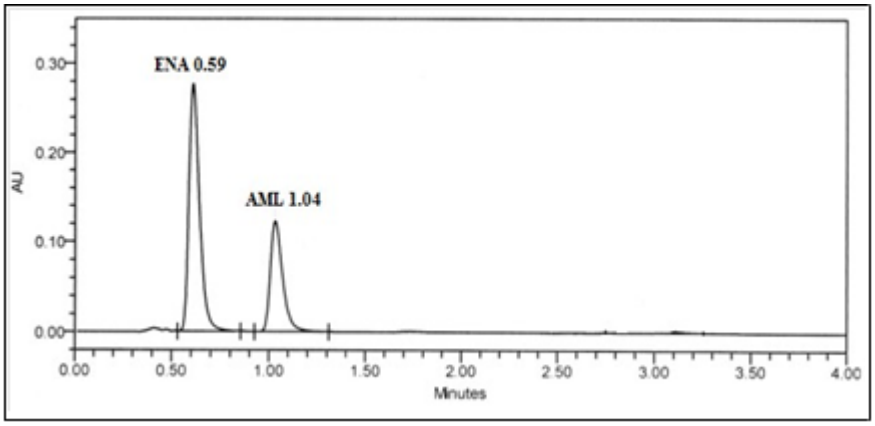

Figure 4: Sample chromatogram

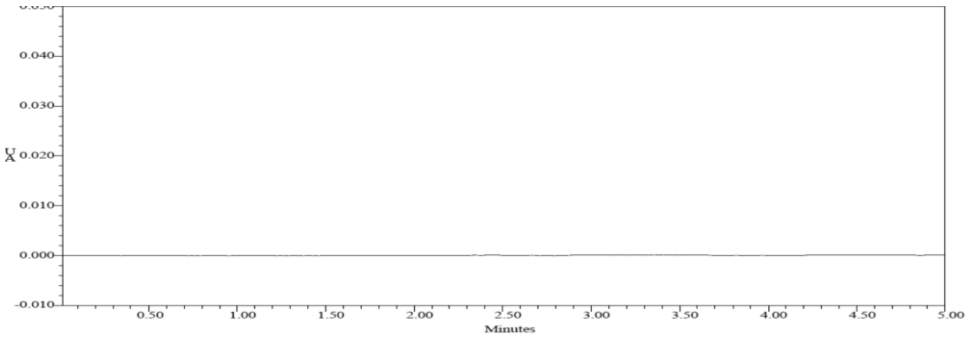

Figure 5: Blank chromatogram

Table 1: System suitability parameters

Parameter	Amlodipine besylate (n= 5)	Enalapril maleate (n= 5)
Retention time (Rt) (min)	1.04	0.59
Theoretical plates (N)	5679.11	3214.07

Table 2: Assay results for Amlodipine besylate and Enalapril maleate

	Label Claim (mg)	% Assay
Amlodipine besylate	100	100.03
Enalapril maleate	100	99.75

Table 3: Linearity results of Amlodipine besylate and Enalapril maleate

Conc. of mixed std		Area of Amlodipine besylate	Area of Enalapril maleate
Amlodipine besylate	Enalapril maleate		
10	10	537581	231916
20	20	537952	232459
30	30	538211	233124
40	40	538548	233781
50	50	538932	234467

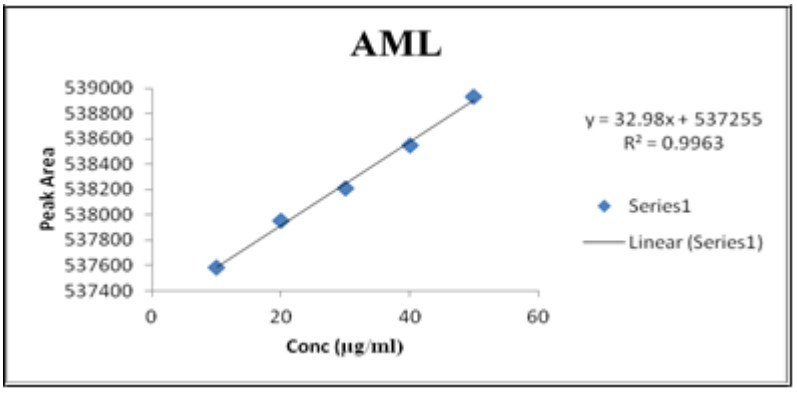

Figure 6: Linearity graph for Amlodipine besylate

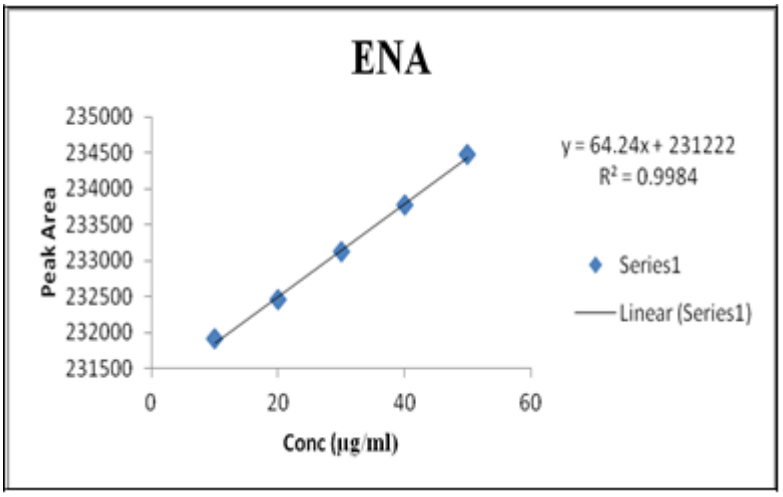

Figure 7: Linearity graph for Enalapril maleate

Table 4: Showing accuracy results for Amlodipine besylate

Level	Conc. of stock (Tab) (µg/ml)	Amount added (µg/ml)	Theoretical conc. (µg/ml)	Observed conc (µg/ml)	% Recovery	% Mean recovery	Std. Dev	% RSD
80%	5	4	9	8.92	98	97.41	0.52	0.53
	5	4	9	8.89	97.25			
	5	4	9	8.88	97			
100%	5	5	10	9.15	83	83.53	0.61	0.73
	5	5	10	9.21	84.2			
	5	5	10	9.17	83.4			
120%	5	6	11	10.81	96.86	97.82	0.60	0.62
	5	6	11	10.85	97.5			
	5	6	11	10.78	96.3			

Table 5: Showing accuracy results for Enalapril maleate

Level	Conc. of stock (Tab) (µg/ml)	Amount added (µg/ml)	Theoretical conc. (µg/ml)	Observed conc (µg/ml)	% Recovery	% Mean recovery	Std. Dev	% RSD
80%	5	4	9	8.77	94.25	93.33	0.80	0.86
	5	4	9	8.72	93			
	5	4	9	8.71	92.75			
100%	5	6	11	10.73	95.5	95.77	0.34	0.36
	5	6	11	10.77	96.16			
	5	6	11	10.74	95.66			
120%	5	5	10	9.85	97	97.46	0.64	0.66
	5	5	10	9.86	97.2			
	5	5	10	9.91	98.2			

Precision results for Amlodipine besylate and Enalapril maleate

Table 6: Precision Intraday

Set	Label claim (mg/tab)		Label claim estimated		% Label claim	
	Amlodipine besylate	Enalapril maleate	Amlodipine besylate	Enalapril maleate	Amlodipine besylate	Enalapril maleate
Initial	5	5	5.11	5.19	102.2	103.8
	5	5	5.19	5.23	103.8	104.6
	5	5	5.15	5.20	103	104
After 1 hr	5	5	5.21	5.17	104.2	103.4
	5	5	5.26	5.14	105.2	102.8
	5	5	5.28	5.23	105.6	104.6
After 2 hr	5	5	5.20	5.17	104	103.4
	5	5	5.21	5.18	104.2	103.6
	5	5	5.16	5.15	103.2	103
After 3 hr	5	5	5.18	5.22	103.6	104.4
	5	5	5.25	5.29	105	105.8
	5	5	5.26	5.25	105.2	105
After 4 hr	5	5	5.22	5.18	104.4	103.6
	5	5	5.13	5.28	102.6	105.6
	5	5	5.25	5.31	105	106.2
After	5	5	5.18	5.27	103.6	105.4
5 hr	5	5	5.17	5.24	103.4	104.8
	5	5	5.20	5.18	104	103.6
Mean					104.01	104.31
SD					0.94	1.001
%RSD					0.91	0.96

Table 7: Precision Interday

Set	Label claim (mg/tab)		Label claim estimated		% Label claim	
Day	Amlodipine besylate	Enalapril maleate	Amlodipine besylate	Enalapril maleate	Amlodipine besylate	Enalapril maleate
I	5	5	5.32	5.29	106.4	105.8
	5	5	5.25	5.26	105	105.2
	5	5	5.27	5.35	105.4	107
II	5	5	5.31	5.34	106.2	106.8
	5	5	5.28	5.25	105.6	105
	5	5	5.32	5.23	106.4	104.6
III	5	5	5.35	5.27	107	105.4
	5	5	5.29	5.22	105.8	104.4
	5	5	5.31	5.34	106.2	106.8
Mean					106	105.66
SD					0.60	0.98
%RSD					0.57	0.94

Robustness results Amlodipine besylate and Enalapril maleate

Table 8: Change of mobile phase ratio

No. of injection applied	Ratio of mobile phase has changed +1%		Ratio of mobile phase has changed -1%	
	Amlodipine besylate	Enalapril maleate	Amlodipine besylate	Enalapril maleate
1	538990	232618	537514	232527
2	538752	234207	538403	232381
3	538241	233581	538380	232521
Mean	538661	233468	538099	232476
SD	382.7	800.43	506.75	82.61
%RSD	0.07	0.34	0.09	0.04

Table 9: Change of flow rate

No. of injection applied	Flow rate has changed +10%		Flow rate has changed -10%	
	Amlodipine besylate	Enalapril maleate	Amlodipine besylate	Enalapril maleate
1	538929	234003	538430	233574
2	538081	233536	538412	233968
3	539001	233602	539629	233459
Mean	538670	233713	538823	233667
SD	511.64	252.73	697.49	266.94
%RSD	0.09	0.11	0.13	0.11

Table 10: Change of wavelength parameter

No. of injection applied	Wavelength has changed +2nm		Wavelength has changed -2nm	
	Amlodipine besylate	Enalapril maleate	Amlodipine besylate	Enalapril maleate
1	538432	234213	537518	233958
2	538245	232521	538384	232623
3	538083	233608	538419	233465
Mean	538253	233447	538107	233348
SD	174.64	857.36	510.38	675.06
%RSD	0.03	0.34	0.09	0.29

Table 11: LOD, LOQ of Amlodipine besylate and Enalapril maleate

S.NO	Drug	LOD	LOQ
1	Amlodipine besylate hydrochloride	10.006	30.32
2	Enalapril maleate	7.80	24.43

Conclusion

The Developed UPLC method was validated and it was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Amlodipine besylate and Enalapril maleate in its pure form and in its pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Amlodipine besylate and Enalapril maleate in pure and its pharmaceutical dosage forms.

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