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METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF AMLODIPINE AND ENALAPRIL MALEATE IN ACTIVE PHARMACEUTICAL INGREDIENT AND COMBINED TABLET DOSAGE FORM BY RP-HPLC

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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 Altima C_{18}. Mobile phase containing Methanol: TEA Buffer pH 4.5: Acetonitrile (50:25:25) 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 350 nm. Run time was selected to be 7 min because analyze gave peak around 2.102, 3.537 ±0.02min respectively and also to reduce the total run time. **Results**: Retention time of Amlodipine besylate and Enalapril maleate were found to be 2.120 min and 3.536 min. The % purity of Amlodipine besylate and Enalapril maleate was found to be 99.6 % and 99.6 % respectively. The system suitability parameters for Amlodipine besylate and Enalapril maleate such as theoretical plates were found to be 5432 and 5987. The linearity study for Amlodipine besylate and Enalapril maleate was found in concentration range of 15-25 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 99.6 % and 100 %, %RSD for repeatability was 0.42 and 0.18 %. The precision tudy was precise, robust and repeatable. LOD value was 0.2, 2.3 and LOQ value was 0.8 and 7.04 respectively. **Conclusion**: The results of study showed that the proposed HPLC 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, HPLC, 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 C26H31ClN2O8S. 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 nonsulphydryl antihypertensive agent that suppresses the renin-angiotensin-aldosterone system to lower blood pressure. It was developed from targeted research programmed using molecular modeling.[3] Being a prodrug, enalapril is rapidly biotransformed 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.

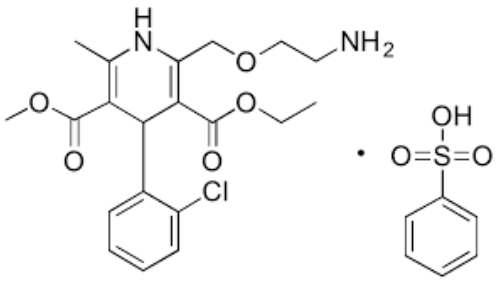

Figure 1: Structure of Amlodipine besylate.

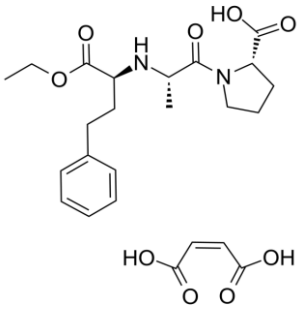

Figure 2: Structure of Enalapril maleate.

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 HPLC 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 HPLC. 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 from Sura labs, India. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: WATERS Alliance 2695 separation module, software: Empower 2, 996 PDA detector**.** Analysis was carried out at 350 nm with column Altima C18 (4.6×150mm, 5µ), dimensions at 40^0C temperature. The optimized mobile phase consists of Methanol: TEA buffer: ACN (50:25:25 v/v). Flow rate was maintained at 1 ml/min.

Preparation of solutions

Preparation of Triethylamine (TEA) buffer (pH-4.5)

Dissolve 1.5ml of Ttiethyl amine in 250 ml HPLC water and adjust the pH 4.5. Fliter and sonicate the solution by vaccum filtration and ultrasonication.

Preparation of mobile phase

Accurately measured 400 ml (40%) of Methanol, 200 ml of Triethylamine buffer (20%) and 400 ml of Acetonitrile (40%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

Preparation of standard solution

Accurately weigh and transfer 10 mg of Amlodipine and Enalapril maleate working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.1ml of the above Amlodipine and 0.375ml of the Enalapril maleate stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol: TEA Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Methanol: TEA Buffer: ACN in proportion 50:25:25 v/v respectively

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 10 µL of standard into Altima C18 (4.6×150mm, 5µ), the mobile phase of composition Methanol: TEA buffer: ACN (50:25:25 v/v) 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 1,2.

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-3.

Validation of Analytical method

Linearity: The linearity study was performed for the concentration of 5-25µg/ml of Amlodipine besylate and 12.5-50µg/ml of Enalapril maleate. 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 4 and figure 6,7.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150% and 50%, 100%, 150% 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 5 & 6.

Precision Studies: precision was calculated from Coefficient of variance for five replicate injections of the standard. The standard solution was injected for five times and measured the area for all five Injections in HPLC. The %RSD for the area of five replicate injections was found. The results are shown in table 7 & 8.

Intermediate precision: Intermediate 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 HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 9 & 10.

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 results are shown in table 11 to 12.

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 13.

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

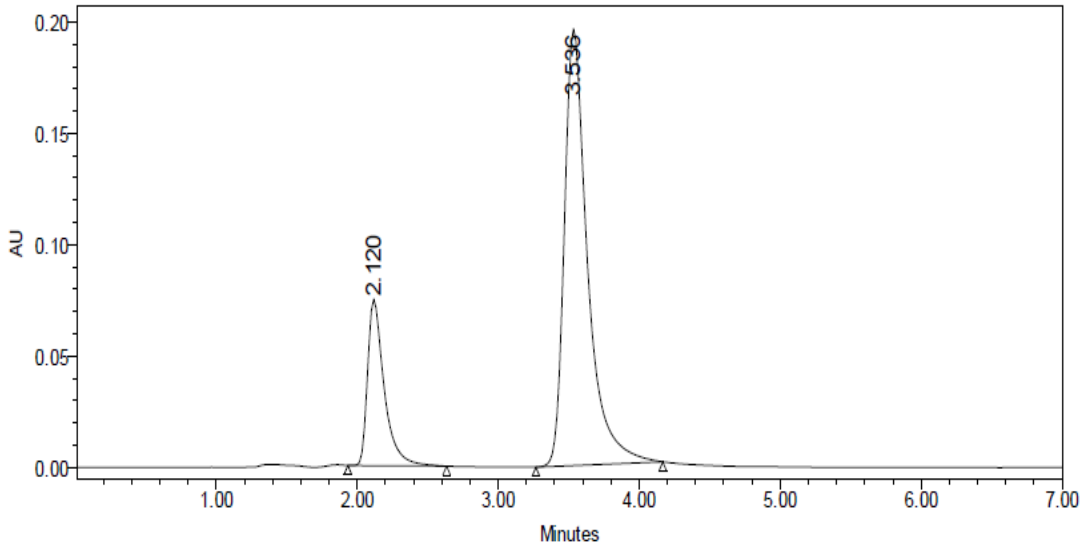

Figure 3: Standard chromatogram.

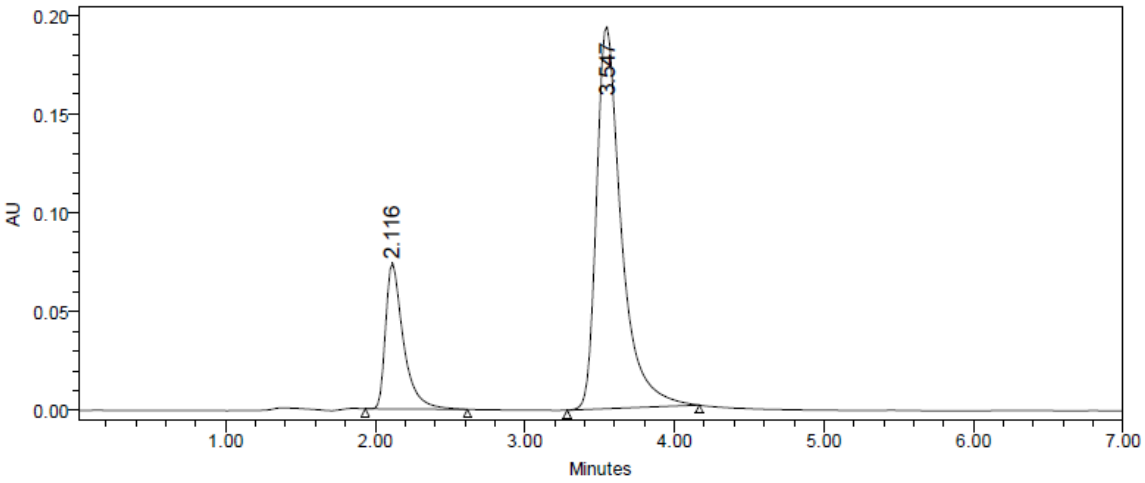

Figure 4: Sample chromatogram.

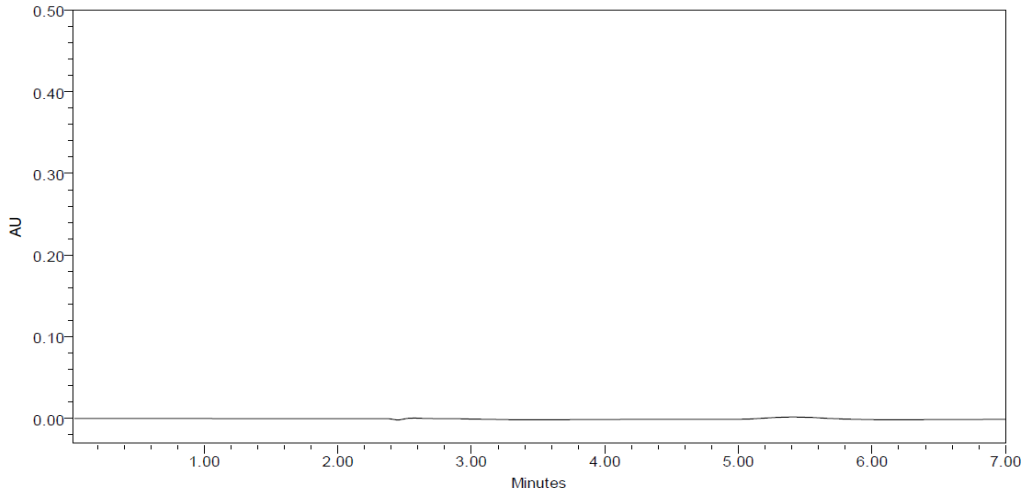

Figure 5: Blank chromatogram.

Table 1: Results of system suitability for Amlodipine.

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Amlodipine	2.117	608452	71498	5643	1.9
2	Amlodipine	2.118	606820	126412	5432	1.6
3	Amlodipine	2.116	608452	126471	5123	1.6
4	Amlodipine	2.109	595267	129859	5207	1.7
5	Amlodipine	2.102	596608	124691	5481	1.6
Mean			603119.8			
Std. Dev			6607.31			
% RSD			1.09			

Table 2: Results of system suitability for Enalapril maleate.

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Enalapril maleate	3.547	2234724	188631	5043	1.2	2.07
2	Enalapril maleate	3.539	2240080	2614821	5432	1.4	2.05
3	Enalapril maleate	3.547	2234724	2321451	5987	1.5	2.0
4	Enalapril maleate	3.565	2204466	2324710	5845	1.6	2.01
5	Enalapril maleate	3.537	2209574	2531247	5371	1.6	2.01
Mean			2224714				
Std. Dev			16399.05				
% RSD			0.73				

Table 3: Assay results for Amlodipine besylate and Enalapril maleate

	Label Claim (mg)	% Assay
Amlodipine besylate	10	99.6
Enalapril maleate	10	99.6

Table 4: Linearity results of Amlodipine besylate and Enalapril maleate.

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	5	205035
66.6	10	381239
100	15	561128
133.3	20	740162
166.6	25	909922
Concentration Level (%)	**Concentration µg/ml**	**Average Peak Area**
33	12.5	757881
66	12.5	757881
100	25	1458941
133	37.5	2132457
166	50	2901811

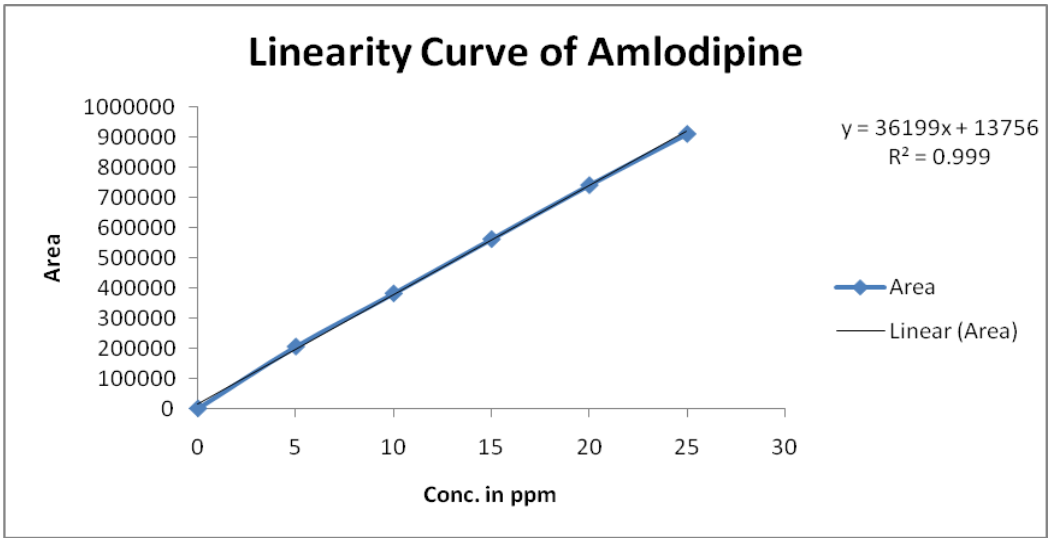

Figure 6: Linearity graph for Amlodipine besylate.

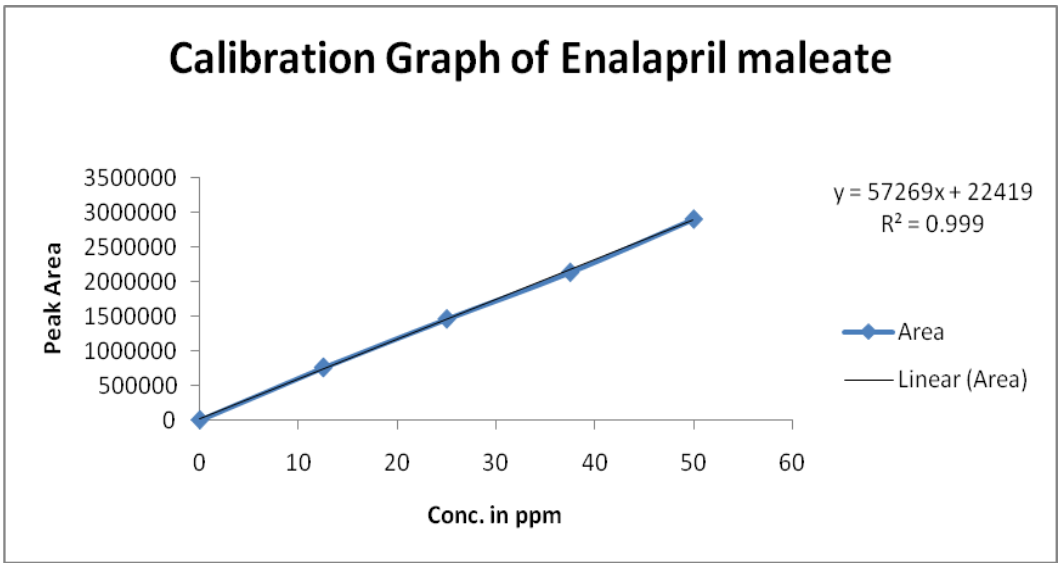

Figure 7: Linearity graph for Enalapril maleate.

Table 5: The accuracy results for Amlodipine.

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	287774	7.5	7.56	100.8	99.6%
100%	551495	15	14.8	98.6	
150%	825175	22.5	22.4	99.5	

Table 6: The accuracy results for Enalapril maleate

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1104782	18.75	18.73	100%	100%
100%	2105321	37.5	37.4	99.9%	
150%	3211306	56.25	56.21	100%	

Precision results for Amlodipine besylate and Enalapril maleate

Table 7: Results of repeatability for Amlodipine.

S. No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Amlodipine	2.108	602223	128898	2586	1.6
2	Amlodipine	2.105	607748	129233	2947	1.4
3	Amlodipine	2.113	607302	127409	2468	1.6
4	Amlodipine	2.109	608674	127047	2146	1.9
5	Amlodipine	2.109	607376	129859	2307	1.7
Mean			606665			
Std. Dev			2542.3			
% RSD			0.42			
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 8: Results of method precession for Enalapril maleate:

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Enalapril maleate	3.552	2220333	2231111	1.6	2371
2	Enalapril maleate	3.550	2221573	2674210	1.6	2841
3	Enalapril maleate	3.564	2215483	2231261	1.5	2816
4	Enalapril maleate	3.564	2217379	2421301	1.5	2872
5	Enalapril maleate	3.565	2211255	2324710	1.6	2845
Mean			2217205		1.6	2841
Std. Dev			4100.8			
% RSD			0.18			

Intermediate precision

Table 9: Results of Intermediate precision for Amlodipine.

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Amlodipine	2.108	596608	128898	2547	1.6
2	Amlodipine	2.105	598959	129233	2944	1.4
3	Amlodipine	2.113	595728	127409	2361	1.6
4	Amlodipine	2.109	594485	127047	2546	1.9
5	Amlodipine	2.109	595267	129859	2207	1.7
6	Amlodipine	2.102	596608	124691	2481	1.6
Mean			596209			
Std. Dev			1718.7			
% RSD			0.29			

Table 10: Results of Intermediate precision for Enalapril maleate.

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Enalapril maleate	3.552	2207732	2231134	8371	1.5	2.04
2	Enalapril maleate	3.550	2202266	2674210	6841	1.6	2.03
3	Enalapril maleate	3.564	2209375	2247461	7816	1.6	2.01
4	Enalapril maleate	3.564	2204037	2454301	8872	1.6	2.05
5	Enalapril maleate	3.565	2204466	2324710	4845	1.6	2.02
6	Enalapril maleate	3.537	2209574	2531247	8371	1.6	2.03
Mean			2205575				
Std. Dev			2899.8				
% RSD			0.13				

Robustness results Amlodipine besylate and Enalapril maleate

Table 11: Robustness results of Amlodipine besylate.

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	607323	2.102	5586	1.7
Less Flow rate of 0.9 mL/min	674735	2.330	5231	1.7
More Flow rate of 1.1 mL/min	1408920	1.950	5234	1.7
Less organic phase	606093	2.290	5643	1.4
More organic phase	603559	1.998	5298	1.5

Table 12: Robustness results of Enalapril maleate.

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	558777	3.537	5371	1.6
Less Flow rate of 0.9 mL/min	2505636	3.885	5324	1.7
More Flow rate of 1.1 mL/min	1408920	3.263	5098	1.7
Less organic phase	2239255	4.435	5239	1.2
More organic phase	2300346	3.009	5647	1.0

Table 13: LOD, LOQ of Amlodipine besylate and Enalapril maleate.

S.NO	Drug	LOD (µg/ml)	LOQ (µg/ml)
1	Amlodipine besylate	0.2	0.8
2	Enalapril maleate	2.3	7.04

CONCLUSION

The Developed HPLC 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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