



VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ENALAPRIL MALEATE AND DILTIAZEM HYDROCHLORIDE

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Abstract

A basic, specific, fast, exact and prudent converse stage HPLC technique has been produced for the concurrent assessment of Enalapril Maleate and Diltiazem Hydrochloride from mass. The created strategy was approved as far as exactness, accuracy, linearity, breaking point of recognition, cut off of quantitation. The proposed strategy can be utilized for the assessment of these medications in joined measurement structures in future. The present work emphasis on the novel techniques used till date and also guides the path for the further studies in which the work is undone. The proposed method can be used for the estimation of these drugs in biological fluids. The design, development, standardizing, and quality control of pharmaceutical drugs all depend rather precise and sensitive analytical procedures. Since the medication product involves human life, quality is crucial. Strong primary healthcare initiatives around the world must focus on the proper production and quality control of drugs. Quality is the complete sum of all elements which contribute directly or indirectly to the safety, efficacy and acceptability of the product.

Key-words: Enalapril Maleate, Diltiazem Hydrochloride, Chromatography, HPLC, Validation.

Introduction

Pharmaceutical analysis is a part of practical chemistry that entails a number of procedures for identifying, determining, quantifying, and purifying a substance, as well as the separation the components of a solution or mixture and the determination of chemical compound structure. It might be a single ingredient or a combination of compounds, and it could come in any dosage form. Animals, plants, microorganisms, minerals, and different synthetic materials are utilized as medications. Analytical techniques plays an important role in assuring and maintaining the quality of substance and are critical components of Q.A./Q.C. The sample to be analyzed is known as an analyte, and it can be classed as macro, semi micro, micro, sub micro, ultra-micro, trace analysis based on its

size. Among all, the semi micro analysis is widely used. The importance of quality assurance in determining the safety and efficacy of medications cannot be overstated. For the design, development, standardization, and quality control of pharmaceutical products, it has very specific and sensitive analytical methods. They're equally vital in pharmacokinetics and drug metabolism investigations, which are both crucial for determining bioavailability and clinical response.

Enalapril Maleate

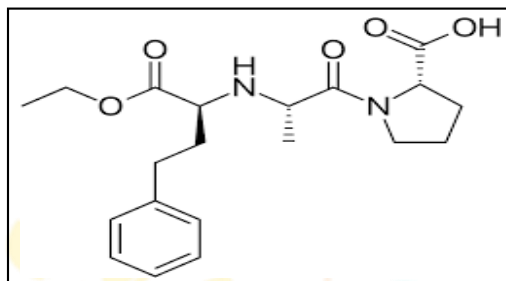


Fig. 1. Structure of Enalapril Maleate

The active pharmaceutical ingredient enalapril is applied in pharmaceutical oral formulations as a salt (1:1) with maleate to ensure its physicochemical stability. Angiotensin-converting enzyme normally converts angiotensin I to angiotensin II (ACE). Angiotensin II causes blood arteries to contract, which raises blood pressure. The active metabolite of enalapril, enalaprilat, inhibits ACE. Angiotensin II levels are reduced when ACE is inhibited, resulting in less vasoconstriction and lower blood pressure.

Diltiazem HCL

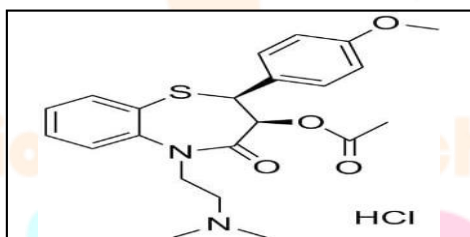


Figure 2. Structure of Diltiazem HCl

Diltiazem hydrochloride (DTZ) is a medication used not only in the treatment of hypertension but also to cure angina pectoris. Diltiazem HCl is a calcium channel blocker which is not dihydropyridine. Therapeutic effects are mediated by a variety of methods. Diltiazem works by preventing calcium ions from entering the cardiac smooth muscle during depolarization. Reduced intracellular calcium concentrations cause smooth muscle relaxation, which leads to arterial vasodilation and, as a result, lower blood pressure.

Material and Methods

The different materials and chemicals employed are listed. All were of pharmaceutical grade. Enalapril Maleate and Diltiazem HCl, were obtained as gift samples. All the HPLC grade solvents such as Acetonitrile (ACN), formic acid & water and the chemicals like Orthophosphoric acid (OPA) of Merck Life Sciences were from local vendor. High performance liquid chromatographic system (Waters e 2695 Gradient System) equipped with UV-visible

detector was used for the analysis. The data were recorded using Empower software 2.0 versions.

Preparation of Buffer solution: Mix 1ml of OPA and dissolved in 1litre of HPLC grade water. Filter through 0.45 μ nylon filter.

Preparation of Mobile Phase: Mobile phase was prepared by mixing 0.1% OPA and ACN taken in the ratio 50:50. It was filtered through 0.45 μ membrane filter to remove the impurities which may interfere in the final chromatogram.

Preparation of Diluent: Mobile phase was used as a diluent throughout the research work.

Preparation of standard stock solution: 180 mg of Diltiazem HCl and 5 mg of Enalapril Maleate accurately weighed and transferred into a working standard of a 100 ml clean dry volumetric flask, the Diluent was added and sonicate to dissolved it completely and make volume up to the mark with the same solvent (Stock solution) Further pipette 5 ml of the above stock solutions into a 50 ml volumetric flask and diluted up to the mark with diluent. (180ppm of Diltiazem HCl, 5ppm of Enalapril Maleate)

Preparation of Sample Solution and assay of Marketed formulation: 248 mg of sample accurately weighed and transferred into a 100mL clean dry volumetric flask and diluent added. The sample was dissolved by sonication for 30 min and centrifuge for 30min. Make volume up to the mark with the same solvent. Then it is filtered through 0.45 micron Injection filter (Stock solution). Further pipette 5 ml of the above stock solutions into a 50ml volumetric flask and diluted up to the mark with diluent. (180ppm of Diltiazem HCl, 5ppm of Enalapril Maleate). Inject 10 μ L of the standard, sample into the chromatographic system and measure the areas for Diltiazem HCl and Enalapril Maleate peaks and calculate the % Assay by using the formulae.

Method Development:

In order to optimize chromatographic conditions for the Diltiazem HCl and Enalapril Maleate several trials were carried out The wavelength of maximum absorption of the solution of the drugs in mixture of Acetonitrile and 1ml OPA in 1lt Water (50:50) were scanned using PDA Detector within the wavelength region of 200–400 nm against Acetonitrile and 1ml OPA in 1lt Water (50:50) as blank. The absorption curve shows isopiestic point at 236nm. Thus 236 nm was selected as detector wavelength for the HPLC chromatographic method.

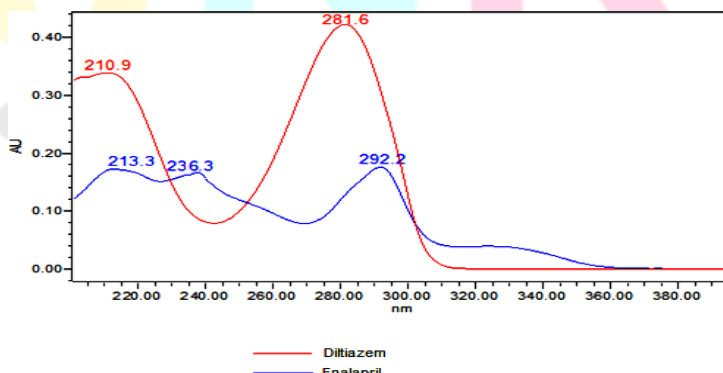


Fig. 3: Overlaid Spectrum of Diltiazem HCl & Enalapril Maleate

Assay of the Marketed Formulation:

The developed method was used to analyse the market formulation Teczem. The sample solution was

prepared as mentioned in above and injected into six replicates and the peak ratio was measured. Percentage assay was found using the formula described below. The percentage assay of Diltiazem HCl and Enalapril Maleate was found to be 99.7 percent w/w and 99.4 percent w/w respectively.

Formula for Assay:

$$\% \text{ Assay} = AT/AS * WS/DS * DT/WT * \text{Average weight/Label Claim} * P /100 * 100$$

Table No. 1: Assay of Diltiazem HCl & Enalapril Maleate

Brand	Drug	Avg. sample area (n=5)	Std. wt (µg/ml)	Sample wt. (µg/ml)	Label amount (mg)	Amount found (µg/ml)	% Assay
Teczem	Diltiazem HCl	3631547	180	248	180	179.51	99.7
	Enalapril Maleate	230648	5	248	5	4.97	99.4

Analytical Method validation

System suitability: System suitability testing is essential for the assurance of the quality performance of chromatographic system. Earlier prepared solutions for chromatographic conditions were tested for system suitability testing.



Specificity: Specificity of an analytical method is ability to measure specifically the analyte of interest without interference from blank and known impurities. For this purpose blank chromatogram, standard chromatogram and sample chromatogram were recorded. The chromatogram of blank shows no response at the retention times of drugs which confirms the response of drug was specific.

Precision: Precision is performed at three different level i.e. system precision, method precision, intermediate precision (intraday & interday precision).

Accuracy: In this study, successive analysis ($n = 3$) for three different concentrations of standard mixtures (50, 100 and 150 %) was carried out to determine the accuracy of proposed analytical method.

Linearity: Linearity has been performed on different concentrations within 2.5-15% of nominal standard concentration. The linearity of this proposed method was evaluated by using calibration curve to calculate the coefficient of correlation, slope and intercept values.

Robustness: Robustness is a capacity of the method to remain unaffected by small deliberate variations in method parameters. The effect of the following deliberate changes in chromatographic conditions was monitored, e.g. mobile phase concentration: ± 2 , flow rate: ± 0.2 ml.

Results and Discussion:

Development of analytical method is continuous process and very essential to confirm and maintain the quality of pharmaceutical finished product. Finalized chromatographic conditions were applied and performed analytical method validation.

1. Specificity

For specificity study blank, standard and sample solutions were injected into HPLC system and chromatogram were recorded. The chromatogram of blank, standard and sample are shown in Fig 4, 5,6 respectively. From the chromatogram it is clear that there was no interference from the blank at the retention time of Diltiazem HCl and Enalapril Maleate peak. From the result we conclude that this method is specific for Diltiazem HCl and Enalapril Maleate.

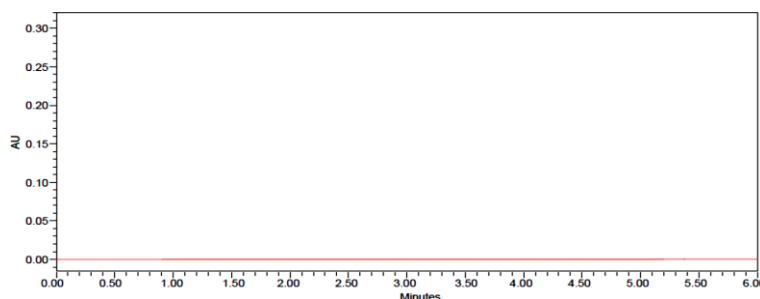
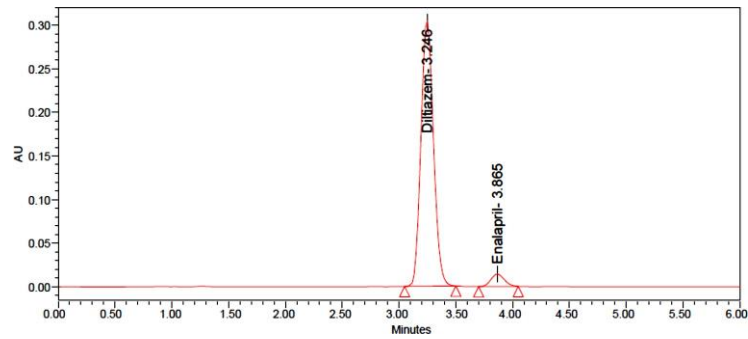
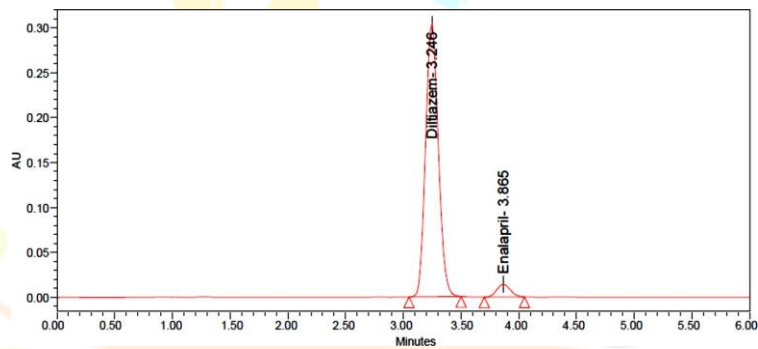


Figure 4: Chromatogram of Blank Solution**Figure 5: Chromatogram of Standard Solution****Figure 6. Chromatogram of Sample Solution**

2. System Suitability

The % RSD for the peak area, USP Tailing, plate count, and retention time for DiltiazemHCl was found to be 0.91, 0.43, 0.89 and for Enalapril Maleate was found to be 1.14, 0.16, 0.93 respectively. The system suitability parameters are tabulated in table no.6 and were found to be within the acceptable limit. So, it is concluded that the system suitability parameters are within limit.

Table No. 2: System suitability parameters for Diltiazem & Enalapril

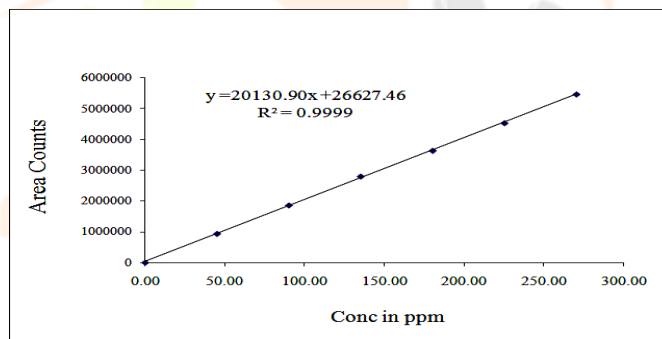
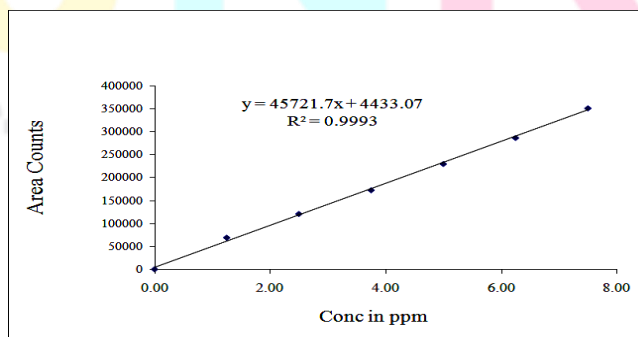
Sr. No.	Diltiazem HCl				Enalapril Maleate			
Parameter	Retention Time	Plate Count	Tailing Factor	Resolution	Retention Time	Plate Count	Tailing Factor	Resolution
	3.246	4152	1.11	-	3.865	5058	1.09	2.90
	3.198	4132	1.13	-	3.778	5073	1.07	2.86
	3.229	4162	1.12	-	3.821	5069	1.08	2.81
Mean	3.221	4150	1.12	-	3.821	5075	1.08	2.856
SD	0.292	18.03	0.01	-	0.043	8.326	0.01	0.0450
%RSD	0.91	0.43	0.89	-	1.14	0.16	0.93	1.58

Linearity and Range

A graph was plotted with concentration in $\mu\text{g/ml}$ on x axis and peak area on y axis which is shown in fig.5 and 19. Slopes, y intercept, correlation coefficient (R^2 value), were calculated which given in table 3. The R^2 was found to be 0.9999 and 0.9993 for Diltiazem HCl and Enalapril Maleate respectively, which is in the limit. From result it is concluded that this method is linear.

Table No. 3: The Linearity of Diltiazem HCl & Enalapril Maleate

Sr .No.	Diltiazem HCl		Enalapril Maleate	
	Conc.(µg/ml)	Peak area	Conc.(µg/ml)	Peak area
1	45.00	929639	1.25	69046
2	90.00	1859735	2.50	120773
3	135.00	2794721	3.75	172648
4	180.00	3632381	5.00	230091
5	225.00	4529283	6.25	286974
6	270.00	5464333	7.50	351696
Regression equation	y =20130.90x+26627.46		y =45721.77x+4433.07	
Slope	20130.90		45721.77	
Intercept	26627.46		4433.07	
R²	0.9999		0.9993	

**Figure 5: calibration curve for Diltiazem HCl at 236 nm****Figure 6: Calibration curve for Enalapril Maleate at 236nm****Chromatogram for Linearity 1:**

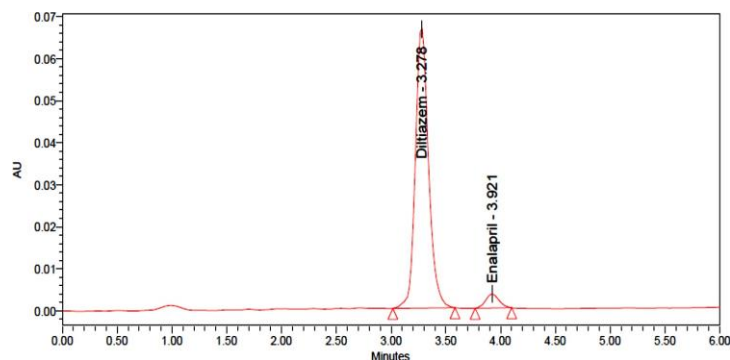


Figure7: Chromatogram for Linearity 1

Chromatogram for Linearity 2:

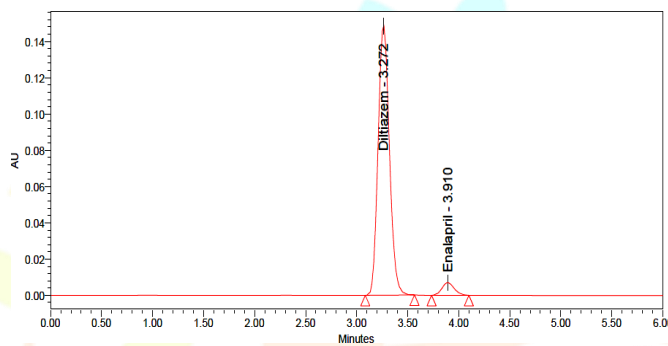


Figure 8: Chromatogram for Linearity 2

Chromatogram for Linearity 3:

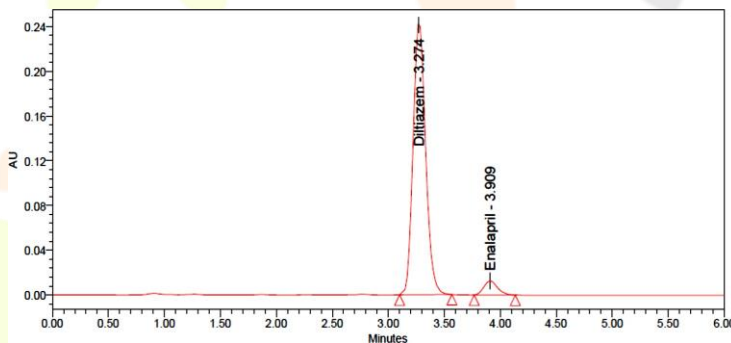


Figure 9: Chromatogram for Linearity 3

Chromatogram for Linearity 4:

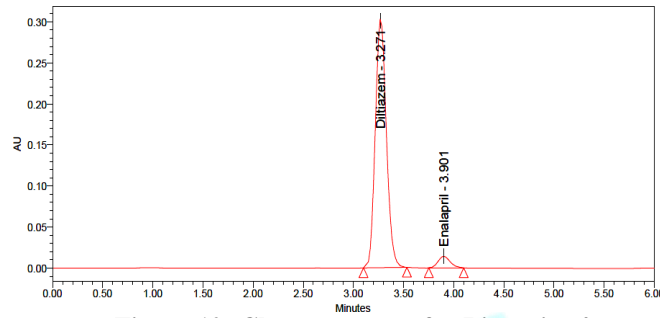


Figure 10: Chromatogram for Linearity 4

Chromatogram for Linearity 5:

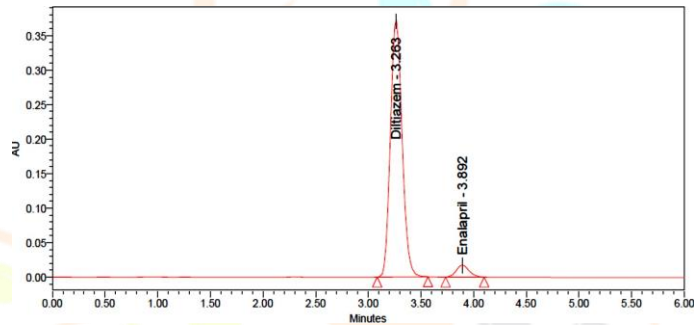


Figure 11: Chromatogram for Linearity 5

Chromatogram for Linearity 6:

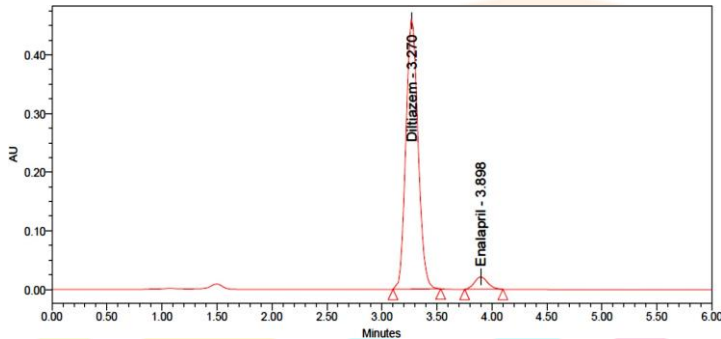


Figure 12: Chromatogram for Linearity 6

Precision:

System Precision:

The % RSD was found to be 0.318 and 0.531 for Diltiazem HCl and Enalapril Maleate respectively, which is in the limits. As the limit of Precision was less than '2' the System Precision was passed in this limit.

Table No. 4: System Precision

Sr. No.	Concentration Diltiazem HCl (µg/ml)	Area of Diltiazem HCl	Concentration Enalapril Maleate (µg/ml)	Area of Enalapril Maleate
1	180	3648402	5	231043
2	180	3629073	5	233617
3	180	3635956	5	230667
4	180	3659647	5	231479
5	180	3631617	5	233308
6	180	3644456	5	232627
Mean		3641525		232124
S.D.		11563.09		1232.18
% RSD		0.318		0.531

n=6

Method Precision: The % RSD was found to be 1.10 and 0.62 for Diltiazem HCl and Enalapril Maleate respectively, which is in the limits. As the limit of Precision was less than '2' the Method Precision was passed in this limit.

Table No. 5: Method Precision

S.NO.	Area of Diltiazem HCl	Area of EnalaprilMaleate
1	3702261	234747
2	3654589	231866
3	3625473	230541
4	3593675	232729
5	3670969	231376
6	3685239	232263
Average	3655367	232253
Standard Deviation	40087.32	1435.03
%RSD	1.10	0.62

Intermediate Precision:

The % RSD of concentration 135 µg/ml, 180 µg/ml, 225 µg/ml for intraday precision was found to be 0.09, 0.52, 0.67 respectively for Diltiazem HCl and 3.75 µg/ml, 5 µg/ml, 6.25 µg/ml was found to be 0.29, 0.41, 0.23 respectively for Enalapril Maleate.

The %RSD for intraday precision was found to be within the limits.

Table No. 6 : Intra-Day Precision

Sr NO	Diltiazem HCl					Enalapril Maleate				
	Con (µg/ml)	Peak Area	Mean	±SD	%RSD	Con (µg/ml)	PeakArea	Me an	±SD	%RSD
1.	135 .00	2794721	27928 23	2602.7 1	0.09 %	3.75	172648	173 022	506. 55	0.29 %
		2789856					173599			
		2793892					172821			
2.	180 .00	3632381	36202 270	18744. 82	0.52 %	5	230091	230 529	951. 846	0.41 %
		3598679					231621			
		3629751					229875			
3.	225 .00	4529283	44984 66	30259. 808	0.67 %	6.25	286974	286 665	668. 74	0.23 %
		4468796					285898			
		4497319					287124			

Inter Day Precision:

The % RSD of concentration 135 µg/ml, 180 µg/ml, 225 µg/ml for interday precision was found to be 0.56, 0.68, 0.45 respectively for Diltiazem HCl and 3.75 µg/ml, 5 µg/ml, 6.25 µg/ml was found to be 0.80, 0.92, 0.68 respectively for Enalapril Maleate. The % RSD for interday precision was found to be within the limits.

Table No. 7 : Inter-Day Precision

Sr.no	Day	Diltiazem HCl					Enalapril Maleate				
		Con (µg/ ml)	Peak Area	Mean	±SD	% RSD	Con (µg/ ml)	Peak Area	Mean	±SD	% RSD
1.	Day 1	135.00	2789763	2791395	1601.4	0.56%	3.75	181542	181254	1454	0.80%
	Day 2		2791458					179678			
	Day 3		2792964					182543			
2.	Day 1	180.00	3724712	3724482	25497.77	0.68%	5	235562	233910	2152	0.92%
	Day 2		3749864					234693			
	Day3		3698870					231476			

Accuracy (Recovery studies):

The % Recovery for Diltiazem HCl & Enalapril Maleate was within the range of 98.00 –100.8 % at all rates, which was found to be well within acceptance criteria. Recovery percentage was calculated and shown in table 7. Recovery % confirms that the method is accurate for determining Amitriptyline HCl and Enalapril Maleate.

Table No. 8 Accuracy Results of Diltiazem HCl

Level	Amount Present (mg)	Amount of Std Added (mg)	Amount Recovered (mg)	% Recovery	Mean	SD	% RSD
Level-1 (50%)	45	44.46	89.46	99.4	100.3	0.77	0.770
	45	45.42	90.40	100.5			
	45	45.81	90.81	100.9			
Level-2 (100%)	45	136.48	181.48	100.8	100.5	0.84	0.840
	45	137.18	182.18	101.2			
	45	134.28	179.28	99.6			
Level-3 (150%)	45	222.45	267.45	99.1	99.8	0.75	0.750
	45	226.47	271.47	100.5			
	45	224.62	269.62	99.9			

Table No. 9 : Accuracy Result of Enalapril Maleate

Level	Amount Present (mg)	Amount of Std Added (mg)	Amount Recovered (mg)	% Recovery	Mean	SD	%RSD
Level-1 (50%)	1.25	1.27	2.52	100.8	100.8	0.40	0.400
	1.25	1.26	2.51	100.4			
	1.25	1.28	2.53	101.2			
Level-2 (100%)	1.25	3.84	5.09	101.8	100.4	1.25	1.240
	1.25	3.72	4.97	99.4			

	1.25	3.75	5	100.0			
Level-3 (150%)	1.25	6.29	7.54	100.5	99.7	0.80	0.800
	1.25	6.17	7.42	98.9			
	1.25	6.23	7.48	99.7			

Robustness:

As a part of the robustness, deliberate change in the flow rate and organic phase ratio. The % RSD for retention time, USP Tailing, Peak area & plate count for Diltiazem HCl and for Enalapril Maleate was within the limit respectively. From this it is concluded that the developed method was robust.

Table no. 10 Robustness

Robustness Parameter		Diltiazem HCl				Enalapril Maleate			
		Retention Time (Rt)	Peak Area	Tailing	Plate Count	Retention Time (Rt)	Peak Area	Tailing	Plate Count
Flow (ml/min)	0.8 ml	3.98	3042222	1.17	4332	3.36	274157	1.05	4887
	1 ml	3.84	3035723	1.15	4232	3.45	276323	1.04	4865
	1.2 ml	3.91	3020159	1.13	4341	3.33	274978	1.06	4779
	Average	3.91	3032701	1.15	4301	3.38	275153	1.05	4843
	SD	0.07	11337.62	0.023	60.50	0.6244	1093.51	0.01	57.07
	% RSD	1.79	0.374	1.74	1.41	1.85	0.397	0.95	1.18

Table No. 11: Robustness

Parameter	Diltiazem HCl				Enalapril Maleate			
	Retention Time	Peak Area	Tailing	Plate Count	Retention Time	Peak Area	Tailing	Plate Count
Less Organic	4.62	4009598	1.17	4417	4.99	251565	1.05	5372

(45:50)								
Actual(50:50)	4.58	4019271	1.17	4392	4.82	252191	1.04	5412
More (55:50)	4.69	4008721	1.15	4427	4.91	249867	1.06	5496
Mean	4.63	4012530	1.063	4412	4.90	251207	1.05	5393
SD	0.0556	5854.32	0.011	18.02	0.850	12025	0.01	20.13
%RSD	1.20	0.15	0.99	0.41	1.73	0.48	0.95	0.37

Limit of Detection (LOD) and Limit of quantification (LOQ):

The LOD and LOQ were found to be 0.15 µg/ml & 5.4 µg/ml for Diltiazem HCl and 0.5 µg/ml & 1.5 µg/ml for Enalapril Maleate respectively. These values show the method is suitable for determining the lower concentration and confirmed the sensitivity of the proposed method for determined.

Table No. 12: LOD & LOQ

Name of drug	LOD(µg/ml)	LOQ(µg/ml)
Diltiazem HCl	0.15	5.4
Enalapril Maleate	0.5	1.5

Conclusion

The present work is to develop and validate the RP-HPLC method for estimating Diltiazem HCl & Enalapril Maleate in tablet dosage form with optimized chromatographic conditions. Agilent Eclipse XDB (250×4.6mm, 5µ) was used for separation and PDA detector used for detection. The various tests were performed as column by using ACN and 0.1% OPA in the ratio 50:50 as mobile phase. Filter through 0.45µ nylon filter. The flow rate was kept 1ml/min and injection volume was 10 µl detection wavelength was made at 236nm using PDA detector. The run time was 6min and the correlation coefficient of Diltiazem HCl was 0.9999 and for Enalapril Maleate was 0.9993. The percentage of recovery for Diltiazem HCl & Enalapril Maleate was found to be 99.77% w/w and 101.3% w/w respectively. The LOD & LOQ for Diltiazem HCl were found to be 0.15 µg/ml & 5.5 µg/ml respectively and for Enalapril Maleate were found to be 0.5 µg/ml & 1.5µg/ml. For validation parameters includes linearity, range, precision, accuracy, specificity, robustness, LOD, and LOQ, the approach has been assessed. The chromatographic method proposed for Diltiazem HCl & Enalapril Maleate is subsequently said to be quick, fast, sensitive, and reliable, and it may be utilized in routine analytical quality control.

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