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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF DILTIAZEM HCL AND ENALAPRIL MALEATE

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Abstract

A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Diltiazem HCl and Enalapril Maleate in pharmaceutical dosage form. Chromatographic separation of Diltiazem HCl and Enalapril Maleate was achieved on Waters Alliance-e2695 by using Inertsil ODS (250x 4.6mm, 5µ) column and the mobile phase containing Acetonitrile: 0.1% Formic acid in the ratio of 40:60% v/v. The flow rate was 1.0 ml/min; detection was carried out by absorption at 272nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Diltiazem HCl and Enalapril Maleate were NLT 2000 and should not more than 2 respectively. % Relative standard deviation of peak areas of all measurements always less than 2.0. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate & robust method for quantitative analysis of Diltiazem HCl and Enalapril Maleate study of its stability.

Keywords: RP-HPLC, Diltiazem HCl, Enalapril Maleate, simultaneous estimation.

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Introduction

Diltiazem, with the chemical name [(2*S*,3*S*)-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3-dihydro-1,5-benzothiazepin-3-yl] acetate hydrochloride, is used to treat hypertension and angina. Diltiazem belongs to the family of drugs known as calcium-channel blockers. It reduces cardiac output by easing tension in the blood vessels. It improves the heart's oxygen and blood flow as well [1]. (*Z*)-but-2-enedioic acid;(2*S*)-1-[(2*S*)-2-[[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino] propanoyl]pyrrolidine-2-carboxylic acid is Enalapril. Treating hypertension is the function of enalapril. Reduced risk of cardiovascular disease, renal disease, and stroke is associated with lowering blood pressure. Also, it helps avoid heart failure in patients with a specific cardiac condition (left ventricular dysfunction) and is used to treat heart failure in such patients.

An RP-HPLC approach for the measurement of Diltiazem HCl and Enalapril Maleate has been attempted, with the goal of developing a validated stability indicator. A review of the relevant literature uncovered a dearth of published analytical techniques, either alone or in conjunction with other medications. The objective is to create an RP-HPLC technique that is easy to utilize, quick and specific enough to measure Diltiazem HCl and Enalapril Maleate in bulk and prescription dose forms. To ensure that the suggested approaches are legitimate, they must meet the analytical criteria outlined in the ICH recommendations. These criteria include system appropriateness, accuracy, precision, specificity, linearity, robustness, limit of detection and limit of quantification.

Materials and Methods

Diltiazem HCl and Enalapril Maleate active pharmaceutical ingredients (API) were obtained from Spectrum Pharma Research Solutions, Hyderabad as gift samples along with their analytical reports. Acetonitrile, Methanol, Heptane Sulfonic acid and Ortho Phosphoric acid was obtained from Rankem and Commercial tablets of Diltiazem HCl and Enalapril Maleate were procured from the local drug market.

Chromatographic condition

The mobile phase consisted of Acetonitrile: 0.1% Formic acid (40:60) at a flow rate of 1.0 ml/min. Inertsil C-18 column (4.6 x250mm, 5µ particle size) was used as the stationary phase. Although the Diltiazem HCl and Enalapril Maleate have different λ max, but considering the chromatographic parameter, sensitivity and selectivity of method for both drugs, 272 nm was selected as the detection wavelength for PDA detector [2].

Preparation of standard stock solution

30 milligrams of diltiazem HCl and 10 milligrams of enalapril maleate (the working standard) was taken into a 10-milliliter clean, dry volumetric flask. Add the diluent and sonicate until fully dissolved. Fill the flask to the mark using the same solvent (Stock solution). The additional pipette Put 1 milliliter of each stock solution into a 10-milliliter volumetric flask and fill it up with diluent until it reaches the mark. (300ppm of Diltiazem HCl, 100ppm of Enalapril Maleate)

Sample preparation

198 milligrams of diltiazem HCl and 112 milligrams of enalapril maleate were accurately weighed and taken into a 10 milliliter clean and dry volumetric flask. Add the diluent and sonicate for 30 minutes to dissolve. Centrifuge for 30 minutes to thoroughly dissolve it. Finally, fill up the flask to the mark using the same solvent. The next step is an injection filter with a pore size of 0.45 microns. (Stock solution) The additional pipette transfer 1 milliliter of each stock solution to a 10-milliliter volumetric flask and fill the rest of the way with diluents. (300ppm of Diltiazem HCl, 100ppm of Enalapril Maleate)

Method validation

System suitability tests

The maximum allowable tailing factor for peaks in standard solution caused by diltiazem HCl and enalapril maleate is 2.0. There should be no fewer than 2000 theoretical plates for the peaks of Diltiazem HCl and Enalapril Maleate in the standard solution. The resolution of the peaks in the standard solution corresponding to Diltiazem HCl and Enalapril Maleate must not be lower than [2].

Linearity

By appropriate aliquots of the standard Diltiazem HCl and Enalapril Maleate solutions with the mobile phase, six working solutions ranging between 75-375 µg/mL and 25-125 µg/mL respectively were prepared. Each experiment was performed in triplicate according to optimized chromatographic conditions[3]. The peak areas of the chromatograms were plotted against the concentration of Diltiazem HCl and Enalapril Maleate to obtain the calibration curve.

Accuracy

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method [4]. Previously analyzed samples of Diltiazem HCl and Enalapril Maleate to which known amounts of standard Diltiazem HCl and Enalapril Maleate corresponding to 50%, 100% and 150% of label claim were added. The accuracy expressed as the percentage of analyte recovered by the proposed method.

Precision

Precision was determined as repeatability and intermediate precision, in accordance with ICH guidelines [5]. The repeatability and intermediate precision were determined by analyzing the samples of Diltiazem HCl and Enalapril Maleate. Determinations were performed on the same day as well as well as on consequent days.

Limit of detection and the limit of quantification

Limit of detection (LOD) and limit of quantification (LOD) of Diltiazem HCl and Enalapril Maleate were determined by calibration curve method[6]. Solutions of both Diltiazem HCl and Enalapril Maleate were prepared in linearity range and injected in triplicate. Average peak area of three analyses was plotted against concentration. LOD and LOQ were calculated by using following equations. LOD = (3.3 ×Syx)/b, LOQ= (10.0×Syx)/b.

Where Syx is residual variance due to regression; b is slope.

Robustness

The robustness of the method was performed by deliberately changing the chromatographic conditions[7]. The organic strength was varied by ±5%, column temperature was varied by ±50 c and the flow rate ±0.1mL.

Degradation Studies

Preparation of stock

After carefully weighing 198 milligrammes of Diltiazem HCl and 112 milligrammes of Enalapril Maleate, put the mixture to a 10 millilitre clean, dry volumetric flask. Add the diluent and sonicate for 30 minutes to dissolve. Centrifuge for 30 minutes to thoroughly dissolve the sample. Finally, add enough of the same solvent to fill the flask to the mark. A 0.45 micron injection filter is then used to further filter it (Stock solution).

Acid degradation, Alkali degradation, Thermal degradation, Peroxide degradation, Reduction degradation, Photolytic degradation and Hydrolysis degradation were carried out [8,9] (Table 9).

Result and Discussion

Method development: Initially reverse phase liquid chromatography separation was tried to develop using various ratios of Acetonitrile: 0.1% Formic acid as mobile phases, in which both the drugs did not responded properly, and the resolution was also poor. With 40:60 Acetonitrile: 0.1% Formic acid both drugs eluted with better separation at a flow rate of 1.0 ml/min. Inertsil C-18 column (4.6 x150mm, 5µ particle size) was used as the stationary phase was selected to improve resolution and the tailing of both peaks were reduced considerably and brought close to 1. To analyze both drugs detection were tried at various wavelengths from 210nm to 280nm. The

wavelength at which both Diltiazem HCl and Enalapril Maleate showed maximum absorption at 272nm was selected as the detection wavelength for PDA detector. The retention times were found to about 2.4 min and 3.8 min for Diltiazem HCl and Enalapril Maleate, respectively. The obtained chromatogram was shown in the figure 1.

Method Validation:

System suitability: System suitability parameters such as number of theoretical plates, retention time and peak tailing were determined. The results obtained were shown in table 1.

Linearity: Diltiazem HCl and Enalapril Maleate were showed a linearity of response between 75-450 µg/mL and 25-150 µg/mL (Figure 2 & Figure 3) and the linearity were represented by a linear regression equation.

Accuracy: The percentage recoveries of Diltiazem HCl and Enalapril Maleate were 99.7% and 100.0%, respectively. These results were summarized in table 2 & 3.

Repeatability: Six replicates of standard concentrations were analyzed in same day for repeatability and results were found within acceptable limits. These results were summarized in table 4.

Intermediate Precision: Six replicates of standard concentrations were analyzed on two different days and by two analysts for day to day and analyst to analyst variation and results were found within acceptable limits. These results were summarized in table 5.

Robustness: As per ICH norms, small, but deliberate variations, by altering the Flow rate, column temperature and concentration of the mobile phase were made to check the method's capacity to remain unaffected. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature (Table 6 & 7).

LOD and LOQ: LOD and LOQ for Diltiazem HCl were 0.54 µg/mL and 1.8 µg/mL and for Enalapril Maleate were 0.1 µg/mL and 0.6 µg/mL respectively.

Tablet Analysis: Content of Diltiazem HCl and Enalapril Maleate found in the tablets by the proposed method are shown in Table 8. The low values of RSD indicate that the method is precise and accurate.

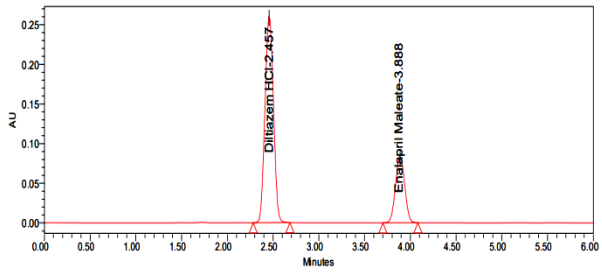

Figure 1. Optimized chromatogram

Table 1: System suitability of Diltiazem HCl and Enalapril Maleate

S.no	Parameter	Diltiazem HCl	Enalapril Maleate
1	Retention time	2.457	3.888
2	Theoretical plates	16610	8935
3	Tailing factor	1.02	1.15
4	Resolution	----	7.75
5	%RSD	0.28	0.14

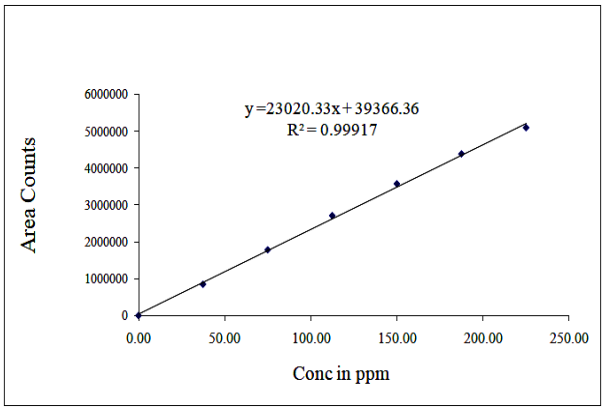

Figure 2. Calibration curve of Diltiazem

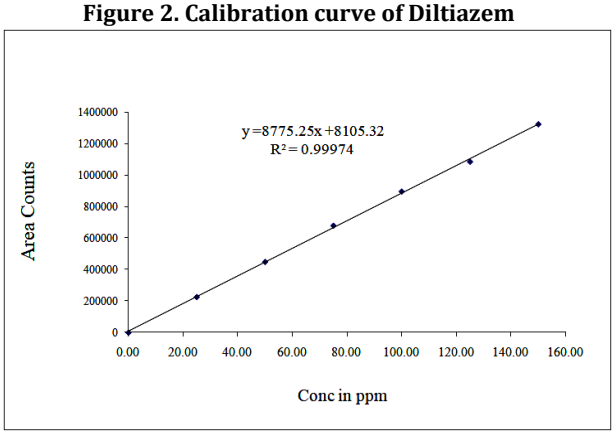

Figure 3. Calibration curve of Enalapril

Table 2: Results of Recovery Experiments of Diltiazem HCl

%Concentration	Response	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1322884	15	14.906	99.4	99.5
	1314564	15	14.812	98.7	
	1335638	15	15.049	100.3	
100%	2666958	30	30.050	100.2	99.9
	2635412	30	29.695	99.0	
	2674582	30	30.136	100.5	
150%	3986574	45	44.919	99.8	99.7
	3995287	45	45.017	100.0	

	3958691	45	44.605	99.1	

Table 3: Results of Recovery Experiments of Enalapril

% Concentration	Response	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	449152	5	5.015	100.3	99.9
	447633	5	4.998	100.0	
	445548	5	4.974	99.5	
100%	896541	10	10.009	100.1	100.0
	891022	10	9.948	99.5	
	898794	10	10.035	100.4	
150%	1348769	15	15.058	100.4	100.0
	1352078	15	15.095	100.6	
	1331612	15	14.867	99.1	

Table 4: Repeatability data of Diltiazem HCl and Enalapril Maleate

S. No.	Diltiazem HCl Response	Enalapril Maleate Response
1	2668943	896421
2	2625241	895705
3	2669583	896978
4	2652417	890042
5	2678594	893130
6	2655396	898915
Average	2658362	895199
Standard Deviation	18912.129	3149.547
%RSD	0.71	0.35

Table 5: Intermediate Precision for Diltiazem HCl and Enalapril Maleate

S. No.	Diltiazem HCl Response		Enalapril Maleate Response	
	Day-1	Day-2	Day-1	Day-2
1	2665894	2675414	896582	894025
2	2653256	2663491	892541	893369
3	2645879	2645787	892257	897241
4	2635209	2656562	897589	895823
5	2685481	2643050	899263	891277
6	2674583	2641279	894526	894095
Average	2660050	2654264	895460	894305
Standard Deviation	18741.116	13445.924	2825.118	2053.865
%RSD	0.70	0.51	0.32	0.23

Table 6: Robustness results of Diltiazem

Parameter	Diltiazem HCl					
	Condition	Retention time(min)	Response	Tailing	Theoretical plates	%RSD
Flow rate Change (mL/min)	Less flow (0.9ml)	2.629	2510623	0.98	16582	0.31
	Actual (1.0ml)	2.457	2655471	1.02	16610	0.28
	More flow (1.1ml)	2.312	2740107	1.07	16743	0.50
Organic Phase change	Less Org (36:64)	2.807	2467819	1.01	16524	0.46
	Actual (40:60)	2.456	2671348	1.05	16625	0.28
	More Org (44:56)	2.185	2955346	1.10	16790	0.20

Table 7: Robustness results of Enalapril

Parameter	Enalapril Maleate						
	Condition	Retention time (min)	Response	Resolution	Tailing	Theoretical plates	%RSD
Flow rate Change (mL/min)	Less flow (0.9 ml)	4.001	874623	7.42	1.10	8876	0.10
	Actual (1.0 ml)	3.888	895634	7.75	1.15	8935	0.14
	More flow (1.1	3.676	906243	7.35	1.19	9012	0.25

	ml)						
Organic Phase change	Less Org (36:64)	4.169	865241	7.31	1.13	8839	0.26
	Actual (40:60)	3.884	895201	7.71	1.18	8921	0.14
	More Org (44:56)	3.542	915487	7.24	1.23	9061	0.15

Table 8: Assay of Diltiazem HCl and Enalapril Maleate

Medication	Response	Avg sample area (n=2)	Std. Conc. (µg/ml)	Sample Conc. (µg/ml)	Label amount (mg)	Std purity	Amount found (µg/ml)	% assay
Diltiazem HCl	3521649	3528264	150	150	150	99.9	147.75	98.5
	3534878							
Enalapril Maleate	2458476	2464957	100	100	100	99.8	100.28	100.3
	2471438							

Table 9: Forced Degradation results for Diltiazem HCl and Enalapril Maleate

% Degradation	Diltiazem HCl					Enalapril Maleate				
	Response	% Assay	% Deg	Purity Angle	Purity Threshold	Area	% Assay	% Deg	Purity Angle	Purity Threshold
Control	2653487	100	0	1.058	7.345	895746	100	0	0.676	3.954
Acid	25930008	97.4	2.6	1.062	7.362	886478	98.9	1.1	0.654	3.936
Alkali	1147 9	6.9	3.1	054	27	8514	8.0	2.0	617	51
Peroxide	2254103	84.7	15.3	1.004	7.352	7775 96	86.8	13.2	0.637	3.974
Reduction	2604722	97.9	2.1	1.026	7.339	871325	97.2	2.8	0.661	3.928
Thermal	2384704	89.6	10.4	1.078	7.321	865402	96.6	3.4	0.641	3.935
Photolytic	2581987	97.0	3.0	1.083	7.368	882461	98.5	1.5	0.674	3.949
Hydrolysis	2619521	98.4	1.6	1.055	7.343	888238	99.1	0.9	0.682	3.905

Conclusion

The HPLC approach that has been devised for the measurement of certain medications is quick, easy, precise, accurate, reliable, and cost-effective. The solvents and mobile phase are cheap, easy to make, dependable, sensitive, and quick to prepare. The sample recoveries were consistent with the promises made on the labels, which means that the formulation receivers did not interfere with the estimate. This means that the medications may be routinely tested in labs. It has been concluded that the suggested methods, which are both simple and brief, would be the most useful for analysis because the system validation parameters of the HPLC method have demonstrated satisfactory, accurate, and repeatable results (without recipient interference, of course). The present study found that the RP-HPLC stability indicating test technique was easy to use, yielded correct results, was highly specific, and did not interact with either the placebo or degradation products. Therefore, they may be used for the normal evaluation of Diltiazem HCl and Enalapril Maleate.

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Not Declared.

Conflict of Interest

No Conflict of interest

Informed Consent and Ethical Statement

Not Applicable.

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