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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS DETERMINATION OF RAMIPRIL AND CILNIDIPINE BY USING RP-HPLC

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Abstract

A new reversed-phase HPLC method was developed and subsequently validated for simultaneous estimation of antihypertensive drugs Ramipril and cilnidipine in pharmaceutical dosage forms. Chromatography was carried out on Inertsil C-18 column (4.6 x 150mm, 5µ particle size) with a mobile phase composed of Acetonitrile: K2HPO4 pH-2.5/OPA (30:70) and the mobile phase was pumped at a flow rate of 1.0 mL/min. Detection was carried out using a PDA detector at 225nm. Parameters such as linearity, precision, accuracy, recovery, specificity and ruggedness were studied as reported in the International Conference on Harmonization guidelines. The retention times for Ramipril and cilnidipine were 2.3 min and 3.7 min respectively. The linearity range for Ramipril and cilnidipine was 25-150 µg/mL. The percentage recoveries of Ramipril and cilnidipine were 100.2% and 99.9%, respectively. This method can be employed for routine quality control of Ramipril and cilnidipine tablets in quality control laboratories and pharmaceutical industries.

Keywords: RP-HPLC, Ramipril, Cilnidipine, Simultaneous estimation.

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Introduction

Ramipril (RP), with the chemical name [(2*S*, 3a*S*, 6a*S*)-1-[(*S*)-2-[[(*S*)-1-(ethoxycarbonyl)-3-phenylpropyl] amino] propanoyl] octahydrocyclopenta [*b*] pyrrole-2-carboxylic acid, is an angiotensin-converting enzyme inhibitor (ACEI), which is widely used in the treatment of hypertension and congestive heart failure. RP plays an important role in inhibiting the conversion of the inactive angiotensin I to the active angiotensin II. However, it may cause hypotension, cough and other side effects [1,2,3]. Diesterified 1,4-dihydropyridine-3,5-dicarboxylic acid is Cilnidipine. As an antihypertensive, it is a calcium channel blocker. As a medication for cardiovascular health, it lowers blood pressure and acts as a calcium channel blocker. As a dihydropyridine, 2-methoxyethyl ester, and C-nitro molecule, it is really rather interesting.

An RP-HPLC approach for the measurement of Cilnidipine and Ramipril 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 Ramipril and Cilnidipine 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

Ramipril active pharmaceutical ingredient (API) and pharmaceutically pure sample of Cilnidipine were obtained from Spectrum Pharma Research Solutions, Hyderabad as gift samples along with their analytical reports. Acetonitrile, Potassium Phosphate (K_2HPO_4) and ortho phosphoric acid was obtained from Merck chemical division, Mumbai and Commercial tablets of Ramipril and Cilnidipine were procured from the local drug market.

Chromatographic condition

The mobile phase consisted of Acetonitrile: K_2HPO_4 pH-2.5/OPA (30:70) 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 Ramipril and Cilnidipine have different λ max, but considering the chromatographic parameter, sensitivity and selectivity of method for both drugs, 269 nm was selected as the detection wavelength for PDA detector[4].

Preparation of standard stock solution

10 mg of ramipril and ten milligrammes of cilnidipine, the working standard, should be transferred to a ten millilitre clean, dry volumetric flask by precise weighing. Fill the container to the specified level using the same solvent after adding the diluent and sonicating it to dissolve it entirely (Stock solution). Pipette Put 1 millilitre of each stock solution into a 10-milliliter volumetric flask and fill it up with diluent until it reaches the mark. (100ppm of Ramipril, 100ppm of Cilnidipine).

Sample Preparation

After carefully weighing 127 mg of Ramipril and 144 mg of Cilnidipine, 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). Pipette Put 1 millilitre of each stock solution into a 10-milliliter volumetric flask and fill it up with diluent until it reaches the mark. (100ppm of Ramipril, 100ppm of Cilnidipine).

Method validation

System suitability tests

The maximum allowable tailing factor for peaks in a standard solution caused by ramipril and cilnidipine is 2.0. No less than 2000 should be used as the theoretical plate value for the Ramipril and Cilnidipine peaks in the standard solution. In a typical solution, the resolution of the Ramipril and Cilnidipine peaks must be at least 2.

Linearity

By appropriate aliquots of the standard ramipril and cilnidipine solutions with the mobile phase, six working solutions ranging between 25-150 µg/mL were prepared. Each experiment was performed in triplicate according to optimized chromatographic conditions[5]. The peak areas of the chromatograms were plotted against the concentration of ramipril and cilnidipine 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[6]. Previously analyzed samples of ramipril and cilnidipine to which known amounts of standard ramipril and cilnidipine 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[7]. The repeatability and intermediate precision were determined by analyzing the samples of ramipril and cilnidipine. 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 ramipril and cilnidipine were determined by calibration curve method[8]. Solutions of both ramipril and cilnidipine 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[9]. 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 127 milligrammes of Ramipril and 144 milligrammes of Cilnidipine, 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[10,11] (Table 9).

Result and Discussion

Method Development: Initially reverse phase liquid chromatography separation was tried to develop using various ratios of Acetonitrile: K_2HPO_4 pH-2.5/OPA as mobile phases, in which both the drugs did not responded properly, and the resolution was also poor. With 30:70 Acetonitrile: K_2HPO_4 pH-2.5/OPA 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 Ramipril and cilnidipine showed maximum absorption at 269nm was selected as the detection wavelength for PDA detector. The retention times were found to about 2.3 min and 3.7 min for Ramipril and cilnidipine, 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: Ramipril and cilnidipine were showed a linearity of response between 25-150 µg/mL (Figure 2 & Figure 3) and the linearity were represented by a linear regression equation.

Accuracy: The percentage recoveries of Ramipril and Clinidipine were 100.2% and 99.9%, 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 Ramipril were 0.3 µg/mL and for cilnidipine were 0.1 µg/mL.

Tablet Analysis: Content of Ramipril and cilnidipine 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.

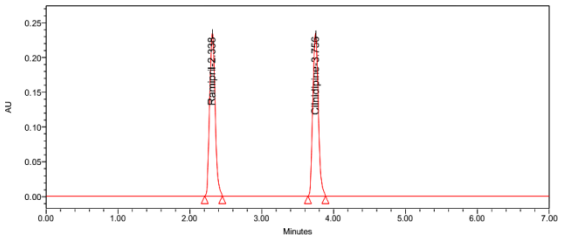

Figure 1. Optimized chromatogram

Table 1: System suitability of Ramipril & Cilnidipine

S.no	Parameter	Ramipril	Cilnidipine
1	Retention time	2.338	3.756
2	Theoretical plates	9865	5082
3	Tailing factor	1.08	0.96
4	Resolution	----	5.58
5	%RSD	0.42	0.44

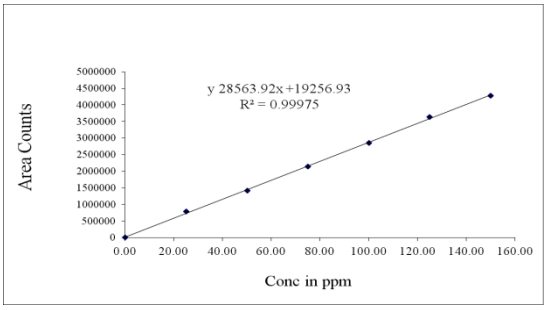

Figure 2. Calibration curve of Ramipril

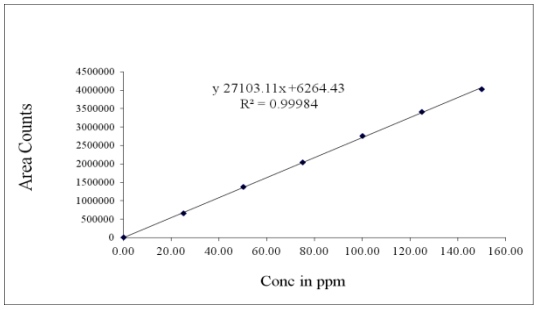

Figure 3. alibration curve of Clinidipine

Table 2: Results of Recovery Experiments of Ramipril

%Concentration	Response	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1422537	5.00	5.02	100.4	100.2
	1425489	5.00	5.03	100.6	
	1410374	5.00	4.98	99.6	
100%	2841487	10.00	10.03	100.3	100.3
	2856312	10.00	10.09	100.9	
	2822683	10.00	9.97	99.7	

150%	4230024	15.00	14.94	99.6	100.2
	4277287	15.00	15.10	100.7	
	4264526	15.00	15.06	100.4	

Table 3: Results of Recovery Experiments of Clinidipine

%Concentration	Response	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1378451	5.00	5.014	100.3	100.2
	1377624	5.00	5.011	100.2	
	1375147	5.00	5.002	100.0	
100%	2753562	10.00	10.016	100.2	99.4
	2733647	10.00	9.944	99.4	
	2713965	10.00	9.872	98.7	
150%	4132013	15.00	15.030	100.2	100.2
	4148631	15.00	15.091	100.6	
	4109896	15.00	14.950	99.7	

Table 4: Repeatability data of Ramipril & Cilnidipine

S. No.	Area for Ramipril	Area for Cilnidipine
1	2826357	2751454
2	2815434	2759745
3	2822235	2765963
4	2817459	2764415
5	2854147	2738971
6	2833542	2741523
Average	2828196	2753679
Standard Deviation	14276.564	11593.092

Table 5: Intermediate Precision for Ramipril and Cilnidipine

S. No.	Ramipril Response		Cilnidipine Response	
	Day-1	Day-2	Day-1	Day-2
1	2874593	2856321	2765302	2745869
2	2869127	2815047	2758571	2768365
3	2874851	2846303	2725823	2739365
4	2878873	2835986	2758965	2752168
5	2810240	2885614	2735471	2746318
6	2856317	2827589	2745632	2721243
Average	2860667	2844477	2748294	2745555
Standard Deviation	25922.786	24735.779	15396.236	15453.790
%RSD	0.91	0.87	0.56	0.56

Table 6: Robustness results of Ramipril

Parameter	Ramipril					
	Condition	**Retention time(min)**	**Response**	**Tailing**	**Theoretical plates**	**% RSD**
Flow rate Change (mL/min)	Less flow (0.9ml)	2.576	2723325	1.05	9779	0.30
	Actual (1.0ml)	2.338	2842994	1.08	9865	0.42
	More flow (1.1ml)	2.158	2965328	1.09	9919	0.66
Organic Phase change	Less Org (27:73)	2.630	2592514	1.00	9736	0.47
	Actual (30:70)	2.334	2836391	1.04	9877	0.42
	More Org (33:67)	2.052	3150235	1.07	9984	0.52

Table 7: Robustness results of Cilnidipine

Parameter	Cilnidipine						
	Condition	**Retention time(min)**	**Response**	**Resolution**	**Tailing**	**Theoretical plates**	**% RSD**
Flow rate Change (mL/min)	Less flow (0.9ml)	3.834	2645896	4.64	0.94	4996	0.45
	Actual (1.0ml)	3.756	2745898	5.58	0.96	5082	0.44
	More flow (1.1ml)	3.545	2925649	5.10	0.99	5135	0.40
Organic Phase change	Less Org (27:73)	4.087	2558455	5.97	0.92	4922	0.45
	Actual (30:70)	3.752	2754625	5.63	0.95	5068	0.44
	More Org (33:67)	3.447	3076524	5.21	0.97	5187	0.25

Table 8: Assay of Ramipril and Cilnidipine

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**
Ramipril	2841268	2846989	100	100	10	99.8	10.5	100.5
	2852710							
Cilnidipine	2774874	2779506	100	100	10	99.9	10.1	101.1
	2784137							

Table 9: Forced Degradation results for Ramipril and Cilnidipine

% Degradation	Ramipril					Cilnidipine				
	Response	% Assay	% Deg	Purity Angle	Purity Threshold	Response	% Assay	% Deg	Purity Angle	Purity Threshold
Control	2833164	100	0	2.142	10.341	2752262	100	0	1.822	12.568
Acid	2522469	89.0	11.0	2.175	10.352	2392469	86.9	13.1	1.824	12.547
Alkali	2485476	87.7	12.3	2.166	10.347	2435476	88.5	11.5	1.856	12.535
Peroxide	2415647	85.3	14.7	2.185	10.388	2355647	85.6	14.4	1.877	12.581
Reduction	2736752	96.6	3.4	2.177	10.369	2642236	96.0	4.0	1.893	12.574
Photolytic	2714758	95.8	4.2	2.138	10.333	2618459	95.2	4.8	1.829	12.597
Thermal	2575687	90.9	9.1	2.189	10.387	2422350	88.0	12.0	1.847	12.530
Hydrolysis	2747513	97.0	3.0	2.126	10.394	2708475	98.4	1.6	1.863	12.512

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 Cilnidipine and Ramipril.

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Not Declared.

Conflict of Interest

No Conflict of interest

Informed Consent and Ethical Statement

Not Applicable.

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