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Research Article

DEVELOPMENT OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF CANDESARTAN CILEXETIL AND HYDROCHLOROTHIAZIDE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, sensitive, and inexpensive high performance liquid chromatographic method has been developed for simultaneous estimation of Hydrochlorothiazide and Candesartan cilexetil in pharmaceutical formulations. Chromatographic separation was achieved on a Inertsil CN, 250 x 4.6 mm, 5µm column with a mixture of 55:45 potassium dihydrogen phosphate buffer solution pH 3.0 and Acetonitrile as mobile phase. Detection was at 258 nm. Response was a linear function between concentration and area of peak over the range from 50% to 150% of assay concentration for Hydrochlorothiazide and Candesartan cilexetil; the correlation coefficients were 0.9997 and 0.9992, respectively. Hydrochlorothiazide elutes as first major peak followed by Candesartan Cilexetil as major peak the retention time for Hydrochlorothiazide is 4.3 min and Candesartan Cilexetil is 16 min. Total elution time for the two components was less than 25 min.

Keywords: Hydrochlorothiazide, Candesartan cilexetil, High performance liquid chromatography.

INTRODUCTION

"Pharmaceutical analytical chemistry may be defined as the branch of practical chemistry which deals with resolution, separation, identification, determination and purification of a given sample of a medicine or a pharmaceutical; the detection and estimation of impurities that may be present therein is also included." The sample may be a single compound or a mixture of compounds, and may be in the form of a vegetable drug, tablet, pill, capsule, ampoule, liquid, mixture or an ointment.[1]

Analytical chemistry deals with methods for determining the chemical composition of samples of matter. Analytical Chemistry plays an important role in the resolution of a chemical compound into its proximate or ultimate parts, determination of its elements or of the foreign substances it may contain. Its application extends to all parts of an industrial society[2].

Qualitative inorganic analysis yields information about the identity of atomic or molecular species in a sample. Qualitative organic analysis yields information about the identity of functional group in a sample. Quantitative analysis provides numerical information as to the relative amount of one or more of components in the sample. Chromatography is probably the most powerful and versatile analytical technique available to the modern chemist. In broader sense chromatography is a technique for separating a sample into various fractions and then measuring or identifying the fractions in some manner. Its power arises from its capacity to determine quantitatively many individual components present in a mixture in one single analytical procedure. Its versatility comes from its capacity to handle a very wide variety of sample; they may be gaseous, liquid or solid in nature. In addition the sample

can range in complexity from single substance to a multi component mixture containing widely different chemical species. Another aspect of versatility of the technique is that the analysis can be carried out at one extreme, on a very costly and complex instrument and at the other on a simple, inexpensive thin layer plate.

Analysis is important in every product but it is vital in medicines as it involves life. The assurance of quality is achieved through analysis of the drug product. Now days, the pharmaceutical dosage form of combinational drugs are very much useful in multiple therapies, rather than the use of single drug formulation due to multiple action, fewer side effects and quicker relief. Thus, manufacturers market multiple formulations containing several drugs with similar chemical behavior. Review of literature revealed that although there are few methods reported for estimation of CDC and HCT singly and combined. [1-13]

Table 1: Classification of column chromatographic methods

General classification	Specific method	Stationary phase	Type of equilibrium
Liquid chromatography (Mobile phase: liquid)	Liquid-liquid, or partition	Liquid adsorbed on a solid	Partition between immiscible liquids
	Liquid-bonded phase	Organic species bonded to a solid surface	Partition between liquid and bonded surface
	Liquid-solid or adsorption	Solid	Adsorption
	Ion exchange	Ion-exchange resin	Ion exchange
	Size exclusion	Liquid in interstices of a polymeric solid	Partition/sieving
Gas chromatography (Mobile phase: gas)	Gas-liquid	Liquid adsorbed on a solid	Partition between gas and liquid
	Gas-bonded phase	Organic species bonded to a solid surface	Partition between liquid and bonded surface
	Gas-solid	Solid	Adsorption
Supercritical fluid chromatography	-	Organic species bonded to a solid surface	Partition between super critical fluid and bonded surface

Table 2: Plate number for well-packed HPLC columns under optimized test conditions

Particle Diameter (um)	Column Length (cm)	Plate Number N
10	15	6,000-7,000
10	25	8,000-10,000
5	10	7,000-9,000
5	15	10,000-12,000
5	25	17,000-20,000
3	5	6,000-7,000
3	7.5	9,000-11,000
3	10	l2,000- 14,000
3	15	7,000-20,000

MATERIALS AND METHODS

MATERIALS

Candesartan Cilexetil, Hydrochlorothiazidel were obtained from Zydus Research centre, Ahmedabad, India as gift samples. All the other solvents, reagents and chemicals used were of either Pharamcopoeial or analytical grade. Different instruments, HPLC with UV Detector- Agilent Technologies/Waters, HPLC with PDA Detector -Agilent Technologies, Weighing Balance-Mettler Toledo, pH Meter-Eutech Instruments.

Table 3: Instruments used

Instrument used	Make
HPLC with UV Detector	Agilent Technologies/Waters
HPLC with PDA Detector	Agilent Technologies
Weighing Balance	Mettler Toledo
pH Meter	Eutech Instruments

Determination of λ_{max}
Weigh accurately and transfer about 25mg of Hydrochlorothiazide WS and 32 mg of Candesartan Cilexetil WS in 100 mL volumetric flask, add dissolved in and dilute with methanol to volume and Mix.

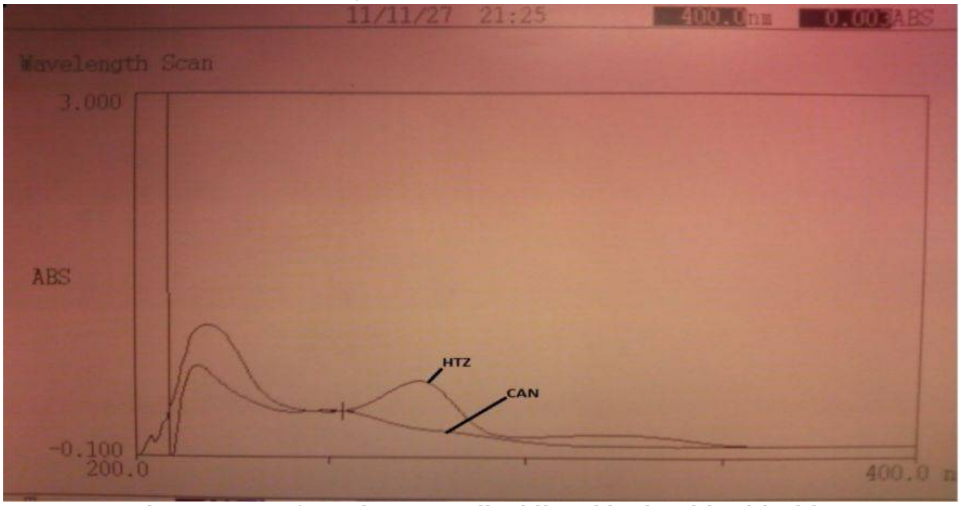

Fig. 1: Scan of candesartan cilexitil and hydrochlorthiazide

Selection of diluent
Main criteria for diluent selection are solubility and stability, i.e. drug should be soluble as well as stable for sufficient time in selected diluent. For present work methanol has been selected as diluent.

Preparation of Buffer solution
Dissolve about 7.8 g of sodium dihydrogen phosphate in to 1000ml water, stir to dissolve adjust the pH 3.0 with trifluroacetic acid, filter through membrane filter.

Preparation of Mobile phase
Prepare a mixture of 550 ml buffer solution and 450ml Acetonitrile mix well and degas. Make adjustment if necessary.

METHOD
Specificity
Selectivity
Procedure
Selectivity study was performed for Candesar – H Tablets as per the validation protocol.

Acceptance Criteria
The peak due to Hydrochlorothiazide and Candesartan Cilexetil should be well resolved from any other peak. The chromatogram obtain from the diluent blank solution, Excipient blend solution should not show any peak at the retention time of the Hydrochlorothiazide and Candesartan Cilexetil.

Preparation of Blank solution
Use diluent as blank solution.

Preparation of placebo solution
Weigh and transfer placebo sample equivalent to 25 mg of Hydrochlorothiazide of test sample into a 100mL volumetric flask. Add about 25 mL of diluent, sonicate for 15 minutes, with stirring at 5 minute interval and make up to the volume with diluent and mix well. Pass a portion of this solution through a filter having a 0.45µm or finer porosity.

Preparation of standard solution for Candesar – H Tablets
Weigh accurately and transfer about 25mg of Hydrochlorothiazide WS and 32 mg of Candesartan Cilexetil WS in 100 mL volumetric flask, add dissolved in and dilute with diluent to volume and Mix.

Preparation of Test solution for Candesar – H Tablets
Weigh and powder 20 tablet transfer test sample equivalent to 25 mg of Hydrochlorothiazide of test sample into a 100mL volumetric flask. Add about 25 mL of diluent, sonicate for 15 minutes, with stirring at 5 minute interval and make up to the volume with diluent and mix well. Pass a portion of this solution through a filter having a 0.45µm or finer porosity.

Procedure
Inject diluent solution, standard solution and test solution, record the chromatogram. Disregard any peak due to diluent in the test solution. % Relative standard deviation for five replicate injections of standard solution should be not more than 2.0. Resolution between hydrochlorothiazide and Candesartan Cilexetil is not less than 2.0.Calculate the % Assay for Candesartan Cilexetil and Hydrochlorothiazide for Candesar – H. Hydrochlorothiazide elutes as first major peak followed by Candesartan Cilexetil as major peak.

Precision
System precision
This experiment was performed as an analytical part of system precision.

Procedure
The system precision was performed by injecting diluent blank solution, and five replicate injections of standard solution and the chromatograms were reviewed for the system suitability criteria.

Method precision
Procedure
Six dfferent test solutions of Candesar – H Tablets were prepared. The samples were prepared and analyzed as per as method of analysis.
The % RSD of assay results of each six test solutions was calculated.

Intermediate precision
Procedure
Six different test solutions of Candesar – H Tablets were prepared. The samples were prepared and analyzed by a different analyst on a different day using different HPLC column of the same make but having different serial number and different HPLC system. (Other than used for method precision).
The % RSD of assay results of twelve test solutions (six test solutions from method precision and six test solutions from intermediate precision) were calculated.

Accuracy (% Recovery)
Procedure
Add to each of the flasks containing the excipients blend and a quantity of Hydrochlorothiazide and Candesratan Cilexetil standard to produce solutions having concentrations equivalent to 50%, 100% and 150% of assay concentration of Hydrochlorothiazide and Candesartan Cilexetil The % recovery was evaluated at each concentration level.

Filter validation
Procedure
The filter validation was performed by preparing unfiltered and filtered test solutions of Candesar – H Tablets as per the validation protocol.

Robustness
Procedure
The samples were analyzed with the following changes:
1. Change in column lot (Same make, different serial number)
2. Change in flow rate (±10 % mL/min)
3. Change in wavelength (± 2 nm)

The results obtained with every changed parameter were evaluated against the method precision results.

Change in wavelength to 256 nm
(Normal Experimental Condition: 258 nm)
The system suitability was found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table 19 for system suitability results).

Stability of analytical solution
Procedure
The test solutions of Candesar – HTablets and standard preparation were prepared at the beginning of this exercise i.e. on 1^{s} (72 hrs), 2nd (48 hrs), 3rd(24 hrs), day of experiment. These were then analyzed on 4th day with freshly prepared standard solution and freshly prepared Candesar – HTablets Test solution. The % assay was calculated & results of stored samples were compared with freshly prepared sample.

Linearity and Range
Procedure
For the linearity study five standard solutions of Hydrochlorothiaze and Candesartan Cilexetil standard were prepared from the range starting from 50% to 150% of the specified assay concentration. The diluent blank solution, standard solution and the linearity standard solutions were injected as per the protocol. The linearity graph of concentration (ppm) against peak response was plotted.

RESULT AND DISCUSSION
Observation
The system suitability was found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table 2 for system suitability results).

Table 4: System suitability for Selectivity

Sr. No.	Hydrochlorothiazide	Candesaratan Cilexetil
1	4591972	5184487
2	4570927	5095727
3	4555058	5071424
4	4552162	5088423
5	4524198	5118547
Mean	**4558863**	**5111722**
Standard Deviation	**25013.94**	**44056.61**
Relative Standard Deviation (%)	**0.55**	**0.86**

Resolution between Hydrochlorothiazide and Candesartan Cilexetil is 25.68
All the selective chromatograms are presented in Figure 2 to 7.

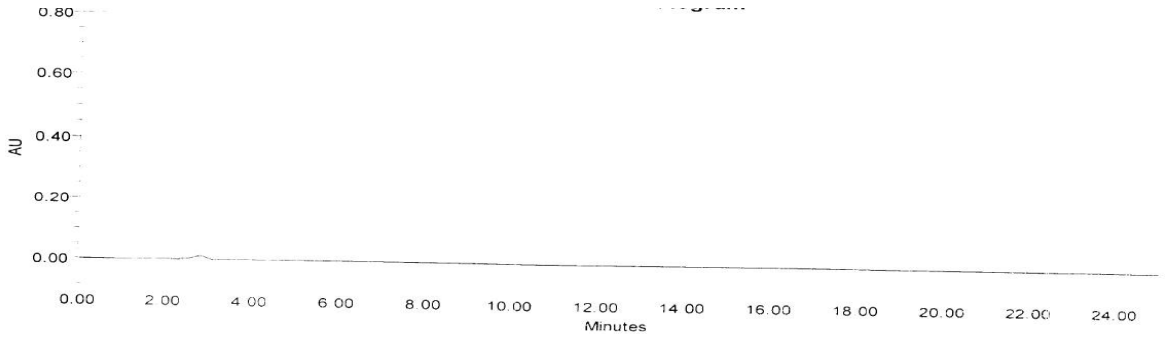

Fig. 2: Selectivity – Diluent blank Solution

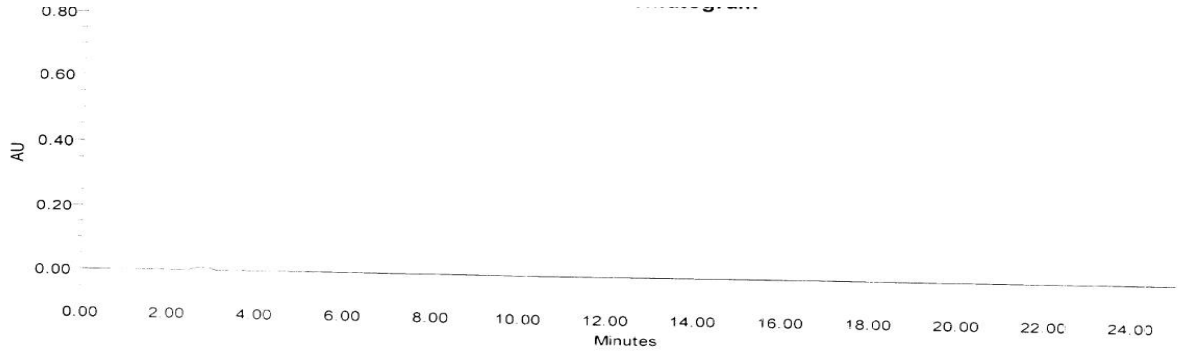

Fig. 3: Selectivity – Excipient Blend

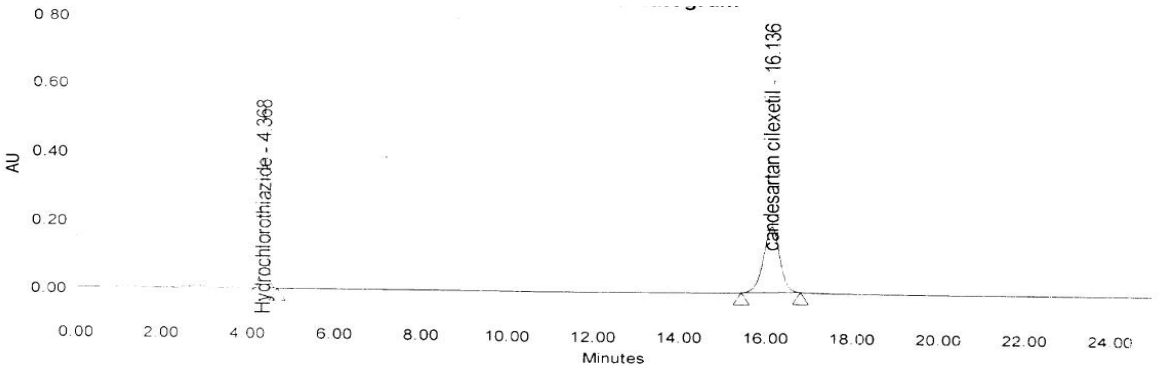

Fig. 4: Selectivity – Standard Solution

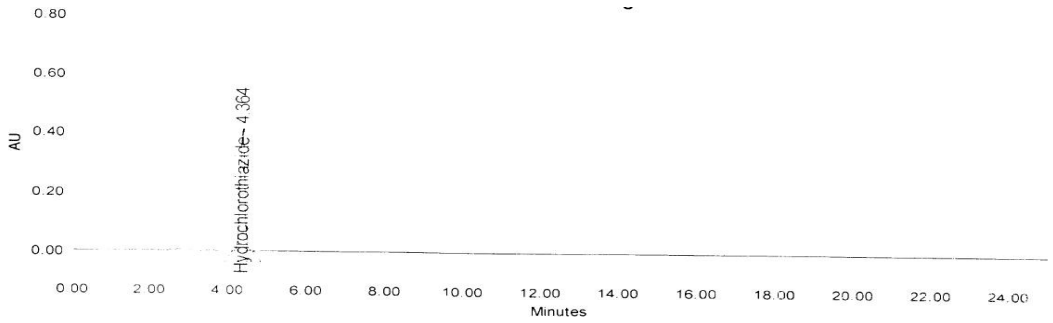

Fig. 5: Selectivity – Hydrochlorothiazide Standard

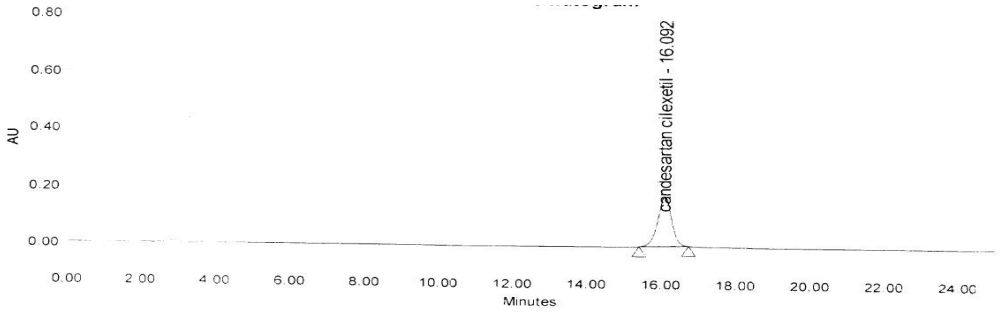

Fig. 6: Selectivity – Candesartan Cilexetil Standard

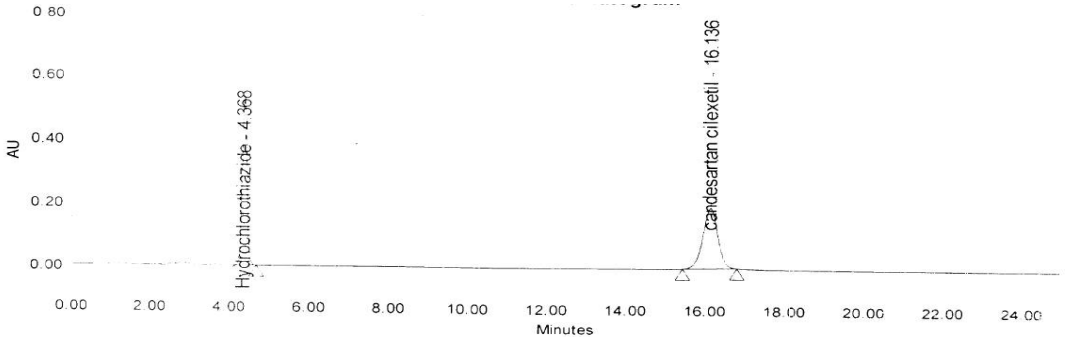

Fig. 7: Selectivity – Test solution

Precision Results

The system suitability was found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table 5 for system suitability results).

Table 5: System suitability for System precision

Sr. No.	Hydrochlorothiazide	Candesartan Cilexetil
1	4490973	5094479
2	4470995	5025773
3	4452063	4978419
4	4452063	4978419
5	4424185	5016546
Mean	**4458056**	**5018727**
Standard Deviation	**24849.66**	**47545.34**
Relative Standard Deviation (%)	**0.56**	**0.95**

Resolution between Hydrochlorothiazide and Candesartan cilexetil peak is 25.66

Method precision Results

The system suitability was found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table 6, 7 for system suitability results).

Table 6: System suitability for method precision Candesar – H Tablets

Sr. No.	Hydrochlorothiazide	Candesaratan Cilexetil
1	4490973	5094479
2	4470995	5025773
3	4452063	4978419
4	4452063	4978419
5	4424185	5016546
Mean	**4458056**	**5018727**
Standard Deviation	**24849.66**	**47545.34**
Relative Standard Deviation (%)	**0.56**	**0.95**

Resolution between Hydrochlorothiazide and Candesartan cilexetil peak is 25.66.

The results of assays obtained from six test solutions of Candesar – H Tablets are recorded in Table 7.

Table 7: Assay results of Method Precision for Candesar – H Tablets

Sample Preparation	% H	% C
Test solution -1	98.07	99.68
Test solution -2	98.09	99.45
Test solution -3	98.29	99.53
Test solution -4	98.57	98.24
Test solution -5	98.25	98.51
Test solution -6	98.90	100.81
Mean	**98.36**	**99.37**
Standard Deviation	**0.29**	**0.84**
Relative Standard Deviation (%)	**0.29**	**0.85**

% H : % Assay of Hydrochlorothiazide
% C: % Assay of Candesartan Cilexetil

Intermediate precision

Table 8 : System suitability for Intermediate precision Candesar – H Tablets

Sr. No.	Hydrochlorothiazide	Candesaratan Cilexetil
1	4390911	5094479
2	4470995	5025773
3	4452063	4978242
4	4452063	5078425
5	4424185	5018514
Mean	**4438043**	**5039087**
Standard Deviation	**31195**	**47219**
Relative Standard Deviation (%)	**0.70**	**0.94**

Resolution between Hydrochlorothiazide and Candesratan Cilexetil is 24.98

The results obtained from six test solutions of Candesar – H Tablets mg are recorded in Table 9.

Table 9: Intermediate Precision – Assay Results Candesar – H Tablets

Sample Preparation	% H	% C
Test solution -1	98.63	99.67
Test solution -2	100.90	99.64
Test solution -3	101.10	99.73
Test solution -4	99.50	99.43
Test solution -5	98.66	98.69
Test solution -6	99.45	100.79
Mean	**99.71**	**99.66**
Standard Deviation	**0.98**	**0.62**
Relative Standard Deviation (%)	**0.98**	**0.62**

% H: % Assay of Hydrochlorothiazide
% C: % Assay of Candisetran Cilexetil

% RSD of assay results of twelve test solutions (six of method precision and six of intermediate precision) of Candesar – H Tablets is as given in Table 10.

Table 10: Comparison of results of twelve samples of (Six of method precision & six of intermediate precision)

Analysis performed during method precision study		
Sr. No.	**%H**	**%C**
Test solution-1	98.07	99.68
Test solution-2	98.09	99.45
Test solution-3	98.29	99.53
Test solution-4	98.57	98.24
Test solution-5	98.25	98.51
Test solution-6	98.90	100.81
Analysis performed during intermediate precision study		
Test solution-1	98.63	99.67
Test solution-2	100.90	99.64
Test solution-3	101.10	99.73
Test solution-4	99.50	99.43
Test solution-5	98.66	98.69
Test solution-6	99.45	100.79
Mean of twelve samples	**99.03**	**99.51**
Standard Deviation	**1.03**	**0.78**
Relative Standard Deviation (%)	**1.04**	**0.78**

% H: % Assay of Hydrochlorothiazide
% C: % Assay of Candisetran Cilexetil

Accuracy (% Recovery) Results

The system suitability was found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table 11 for system suitability results).

Table 11: System suitability for Accuracy (% Recovery)

Sr. No.	Hydrochlorothiazide	Candesaratan Cilexetil
1	4490973	5094479
2	4470995	5025773
3	4452063	4978419
4	4452063	4978419
5	4424185	5016546
Mean	**4458056**	**5018727**
Standard Deviation	**24849.66**	**47545.34**
Relative Standard Deviation (%)	**0.56**	**0.95**

Resolution between Hydrochlorothiazide and Candesratan Cilexetil is 25.66

The results of accuracy recorded in Table 12 for Hydrochlorothiazide and Table 13 for Candesartan Cilexetil.

Table 12: Accuracy (%Recovery) For Hydrochlorothiazide

Level	Preparation	Amount added (mg)	Amount found (mg)	% Recovery	Mean % Recovery	% RSD
50 % Level	Preparation-1	12.60	12.58	99.8	100.4	0.51
	Preparation-2	12.88	12.98	100.8		
	Preparation-3	12.85	12.92	100.5		
100 % Level	Preparation-1	25.24	25.01	99.1	99.5	0.47
	Preparation-2	25.56	25.38	99.3		
	Preparation-3	25.41	25.41	100.0		
150 % Level	Preparation-1	37.52	37.80	100.7	100.2	0.44
	Preparation-2	37.29	37.24	99.9		
	Preparation-3	37.24	37.24	100.0		

Table 13: Accuracy (%Recovery) For Candesartan Cilexetil

Level	Preparation	Amount added (mg)	Amount found (mg)	% Recovery	Mean % Recovery	% RSD
50 % Level	Preparation-1	16.25	16.11	99.1	99.8	0.76
	Preparation-2	16.52	16.62	100.6		
	Preparation-3	16.09	16.03	99.6		
100 % Level	Preparation-1	32.25	32.19	99.8	100.0	0.53
	Preparation-2	32.28	32.16	99.6		
	Preparation-3	32.56	32.76	100.6		
150 % Level	Preparation-1	48.42	48.73	100.6	99.7	0.90
	Preparation-2	48.15	48.02	99.7		
	Preparation-3	48.05	47.49	98.8		

Filter validation

Procedure

The filter validation was performed by preparing unfiltered and filtered test solutions of Candesar – H Tablets as per the validation protocol.

Results

The system suitability result meets the pre-established acceptance criteria as per the analytical method. **(Refer to Table 14)**

Table 14: System suitability for (Filter validation)

Sr. No.	Hydrochlorothiazide	Candesaratan Cilexetil
1	4490973	5094479
2	4470995	5025773
3	4452063	4978419
4	4452063	4978419
5	4424185	5016546
Mean	**4458056**	**5018727**
Standard Deviation	**24849.66**	**47545.34**
Relative Standard Deviation (%)	**0.56**	**0.95**

Resolution between Hydrochlorothiazide and Candesartan Cilexetil: 25.66

Robustness Results

Change in Column lot

The experiment was performed as the part of intermediate precision using different serial number of column. Refer to intermediate precision results data.

Change inflow rate: 0.80 mL/min

(Normal Experimental Condition: 1.00 mL/min)

The system suitability was found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table 15 for system suitability results).

Table 15: System suitability for Change in flow rate 0.80 mL/min

Sr. No.	Hydrochlorothiazide	Candesaratan Cilexetil
1	4891972	5384234
2	4871287	5426928
3	4892215	5428218
4	4852215	5518525
5	4825298	5514528
Mean	**4866597**	**5454487**
Standard Deviation	**28436.85**	**59352.4**
Relative Standard Deviation (%)	**0.58**	**1.09**

Resolution between Hydrochlorothiazide and Candesartan Cilexetil: 26.88

The results for change in flow rate (0.80 mL/min) are as given in Table 16.

Table 16: Results for change in flow rate: 0.80 mL/min

Parameter	Test solution	% H	%C
Method precision	1	98.07	99.68
	2	98.09	99.45
	3	98.29	99.53
	4	98.57	98.24
	5	98.25	98.51
	6	98.90	100.81
Change in flow rate 0.80 mL/ min.	1	99.11	98.83
	2	98.35	98.47
Mean		**98.45**	**99.19**
Standard deviation		**0.38**	**0.85**
Relative standard deviation (%)		**0.39**	**0.86**

% H: % Assay of Hydrochlorothiazide
%C: % Assay of Candesartan Cilexetile

Change inflow rate: 1.20 mL/min
(Normal Experimental Condition: 1.00 mL/min)
The system suitability was found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table 19 for system suitability results).

Table 17: System suitability for Change in flow rate 1.20mL/min

Sr. No.	Hydrochlorothiazide	Candesaratan Cilexetil
1	4291574	4985254
2	4291258	4926825
3	4292257	4928419
4	4251118	4918515
5	4225198	4914729
Mean	**4270281**	**4934748**
Standard Deviation	**30724.86**	**28800.85**
Relative Standard Deviation (%)	**0.72**	**0.58**

Resolution between Hydrochlorothiazide and Candesartan Cilexetil: 23.54
The results for change in flow rate (1.20 mL/min) are as given in Table 18.

Table 18: Results for change in flow rate : 1.20 mL/min

Parameter	Test solution	% H	% C
Method precision	1	98.07	99.68
	2	98.09	99.45
	3	98.29	99.53
	4	98.57	98.24
	5	98.25	98.51
	6	98.90	100.81
Change in flow rate 1.20 mL/ min.	1	100.42	101.08
	2	100.22	100.64
Mean		**98.85**	**99.74**
Standard deviation		**0.95**	**1.05**
Relative standard deviation (%)		**0.96**	**1.05**

% H: % Assay of Hydrochlorothiazide
%C: % Assay of Candesartan Cilexetile

Change in wavelength to 256 nm
The system suitability was found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table 19 for system suitability results).

Table 19: System suitability for Change in wavelength to 256 nm

Sr. No.	Hydrochlorothiazide	Candesaratan Cilexetil
1	4299971	4783215
2	4271985	4725972
3	4192254	4778215
4	4252265	4878515
5	4225195	4712528
Mean	**4248334**	**4775689**
Standard Deviation	**41598.02**	**65378.32**
Relative Standard Deviation (%)	**0.98**	**1.37**

Resolution between Hydrochlorothiazide and Candesartan Cilexetil: 25.45
The results for change in wavelength to 256 nm are as given in Table 20.

Table 20: Results for change in wavelength to 256 nm

Parameter	Test solution	%H	%C
Method precision	1	98.07	99.68
	2	98.09	99.45
	3	98.29	99.53
	4	98.57	98.24
	5	98.25	98.51
	6	98.90	100.81
Change in wavelength to 256 nm	1	99.18	99.93
	2	99.30	99.77
Mean		**98.58**	**99.49**
Standard deviation		**0.49**	**0.81**
Relative standard deviation (%)		**0.50**	**0.81**

% H: % Assay of Hydrochlorothiazide
%C: % Assay of Candesartan Cilexetile

Change in wavelength to 260 nm
(Normal Experimental Condition: 258 nm)
The system suitability was found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table 21 for system suitability results).

Table 21: System suitability for Change in wavelength to 260 nm

Sr. No.	Hydrochlorothiazide	Candesaratan Cilexetil
1	4699882	5284356
2	4671715	5225917
3	4691189	5279259
4	4652217	5378445
5	4627145	5314524
Mean	**4668430**	**5296500**
Standard Deviation	**29518.23**	**55826.82**
Relative Standard Deviation (%)	**0.63**	**1.05**

Resolution between Hydrochlorothiazide and Candesartan Cilexetil: 25.45
The results for change in wavelength to 260 nm are as given in Table 22.

Table 22: Results for change in wavelength to 260 nm

Parameter	Test solution	%H	%C
Method precision	1	98.07	99.68
	2	98.09	99.45
	3	98.29	99.53
	4	98.57	98.24
	5	98.25	98.51
	6	98.90	100.81
Change in wavelength to 260 nm	1	98.97	99.48
	2	99.05	98.35
Mean		**98.52**	**99.26**
Standard deviation		**0.40**	**0.86**
Relative standard deviation (%)		**0.41**	**0.87**

% H: % Assay of Hydrochlorothiazide
%C: % Assay of Candesartan Cilexetile

Stability of analytical solution Results

The system suitability was found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table 23 for system suitability results).

Table 23: System suitability for solution stability

Sr. No.	Hydrochlorothiazide	Candesaratan Cilexetil
1	4590912	5194570
2	4570910	5125712
3	4552162	5098428
4	4552165	5158425
5	4494184	5119545
Mean	**4552067**	**5139336**
Standard Deviation	**36105.58**	**37636.24**
Relative Standard Deviation (%)	**0.79**	**0.73**

Resolution between Hydrochlorothiazide and Candesartan Cilexetil: 25.87

The cumulative % RSD results for standard solution obtained with solution stability study are as given in Table 24 and Table 25.

Table 24: Cumulative % RSD results of standard preparation stability study up to three days (72 hrs) for Hydrochlorothiazide

Standard preparation	Corrected Area of Hydrochlorothiazide	Cumulative %RSD
Freshly	4519526	NA
3rd Day prepared (24 hrs)	4476037	0.68
2nd Day prepared (48 hrs)	4450397	0.78
1st Day prepared (72 hrs)	4475780	0.64

Table 25: Cumulative % RSD results of standard preparation stability study up to three days (72 hrs) for Candesartan Cilexetil

Standard preparation	Corrected Area of Candesartan Cilexetil	Cumulative %RSD
Freshly	5105829	NA
3rd Day prepared (24 hrs)	5112568	0.09
2nd Day prepared (48 hrs)	5089997	0.23
1st Day prepared (72 hrs)	5079193	0.30

The assay results of test solution for solution stability study are as given in Table 26 and Table 27

Table 26: Assay results of test solutions for Hydrochlorothiazide

Test solution	%Assay for Hydrochlorothiazide	Cumulative % RSD
Freshly	99.56	NA
3rd Day prepared (24 hrs)	98.82	0.52
2nd Day prepared (48 hrs)	98.47	0.57
1st Day prepared (72 hrs)	98.52	0.51

Table 27: Assay results of test solutions for Candesartan Cilexetil

Test solution	%Assay for Candesartan Cilexetil	Cumulative % RSD
Freshly	100.07	**NA**
3rd Day prepared (24 hrs)	99.44	**0.45**
2nd Day prepared (48 hrs)	98.94	**0.57**
1st Day prepared (72 hrs)	98.65	**0.62**

Linearity and Range Results

The system suitability was found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table 28 for system suitability results).

Table 28: System suitability for Linearity

Sr. No.	Hydrochlorothiazide	Candesaratan Cilexetil
1	4490973	5094479
2	4470995	5025773
3	4452063	4978419
4	4452063	4978419
5	4424185	5016546
Mean	**4458056**	**5018727**
Standard Deviation	**24849.66**	**47545.34**
Relative Standard Deviation (%)	**0.56**	**0.95**

Resolution between Hydrochlorothiazide and Candesartan Cilexetil peak is 25.66

The average area of Hydrochlorothiazide and Candesartan Cilexetil at each concentration level was determined and the linearity graph was plotted against the concentration (ppm). The results of linearity study are as given in Table 29 for Hydrochlorothiazide and Table 30 for Candesartan Cilexetil.

Table 29: Linearity of Hydrochlorothiazide

Linearity Level	Standard concentration	Concentration of H (ppm)	Mean area (n = 3)	Regression coefficient (R^2)
Level – 1	50%	125.07	2214895	0.9997
Level – 2	80%	200.11	3555165	
Level – 3	100%	250.14	4455385	
Level – 4	120%	300.17	5286019	
Level – 5	150%	375.21	6703359	

H: Hydrochlorothiazide

The linearity plot of average peak area of Hydrochlorothiazide versus concentration (ppm) is as given in Figure 8.

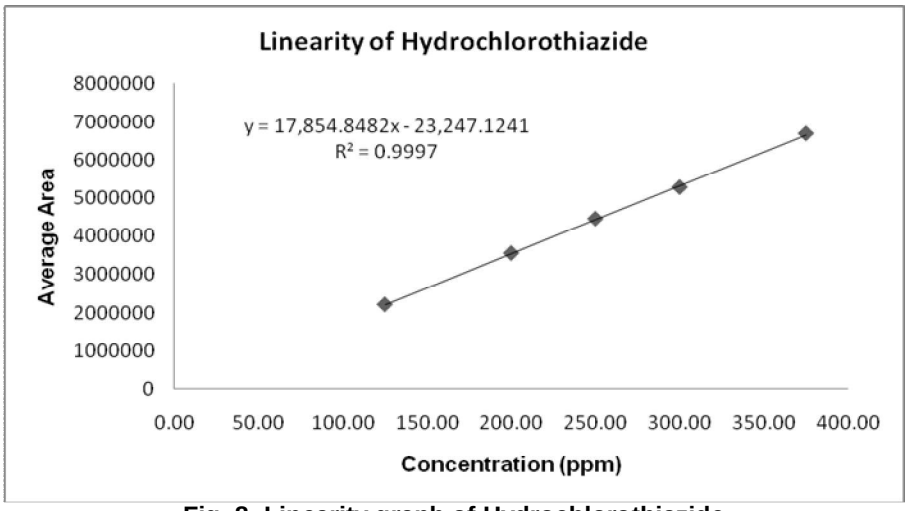

Fig. 8: Linearity graph of Hydrochlorothiazide

Table 30: Linearity of Candesartan Cilexetil

Linearity Level	Standard concentration	Concentration of C (ppm)	Mean area (n = 3)	Regression coefficient (R^2)
Level – 1	50%	159.48	2346815	0.9992
Level – 2	80%	255.17	3959112	
Level – 3	100%	318.96	4932290	
Level – 4	120%	382.75	5887215	
Level – 5	150%	478.44	7330756	

C: Candesartan Cilexetil

The linearity plot of average peak area of Candesartan Cilexetil versus concentration (ppm) is as given in Figure 9.

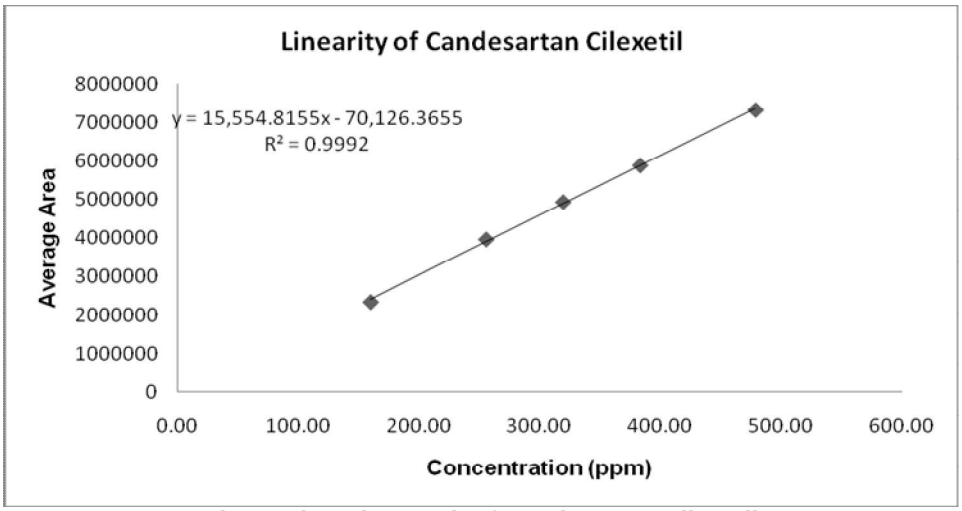

Fig. 9: Linearity graph of Candesartan Cilexetil

Validated the method as per ICH and FDA [14-18] guidelines with parameters like specificity, precision, accuracy, robustness, linearity and range.

System suitability for selectivity
The results are summarized in the following table for system suitability for selectivity

Table 31: System suitability for Selectivity

Sr. No.	Hydrochlorothiazide	Candesaratan Cilexetil
1	4591972	5184487
2	4570927	5095727
3	4555058	5071424
4	4552162	5088423
5	4524198	5118547
Mean	**4558863**	**5111722**
Standard Deviation	**25013.94**	**44056.61**
Relative Standard Deviation (%)	**0.55**	**0.86**

The results of comparative precision study
The results of system precision are summarized in the following table

Table 32: Six of method precision & six of intermediate precision

Analysis performed during method precision study		
Sr. No.	**%H**	**%C**
Test solution-1	98.07	99.68
Test solution-2	98.09	99.45
Test solution-3	98.29	99.53
Test solution-4	98.57	98.24
Test solution-5	98.25	98.51
Test solution-6	98.90	100.81
Analysis performed during intermediate precision study		
Test solution-1	98.63	99.67
Test solution-2	100.90	99.64
Test solution-3	101.10	99.73
Test solution-4	99.50	99.43
Test solution-5	98.66	98.69
Test solution-6	99.45	100.79
Mean of twelve samples	**99.03**	**99.51**
Standard Deviation	**1.03**	**0.78**
Relative Standard Deviation (%)	**1.04**	**0.78**

% H: % Assay of Hydrochlorothiazide
% C: % Assay of Candisetran Cilexetil

Accuracy (% Recovery)
Results of the accuracy study of Hydrochlorothiazide and Candesartan cilexetil are summarized in the following tables.

Table 33: Accuracy (%Recovery) For Hydrochlorothiazide

Level	Preparation	Amount added (mg)	Amount found (mg)	% Recovery	Mean % Recovery	% RSD
50 % Level	Preparation-1	12.60	12.58	99.8	100.4	0.51
	Preparation-2	12.88	12.98	100.8		
	Preparation-3	12.85	12.92	100.5		
100 % Level	Preparation-1	25.24	25.01	99.1	99.5	0.47
	Preparation-2	25.56	25.38	99.3		
	Preparation-3	25.41	25.41	100.0		
150 % Level	Preparation-1	37.52	37.80	100.7	100.2	0.44
	Preparation-2	37.29	37.24	99.9		
	Preparation-3	37.24	37.24	100.0		

Table 34: Accuracy (%Recovery) For Candesartan Cilexetil

Level	Preparation	Amount added (mg)	Amount found (mg)	% Recovery	Mean % Recovery	% RSD
50 % Level	Preparation-1	16.25	16.11	99.1	99.8	0.76
	Preparation-2	16.52	16.62	100.6		
	Preparation-3	16.09	16.03	99.6		
100 % Level	Preparation-1	32.25	32.19	99.8	100.0	0.53
	Preparation-2	32.28	32.16	99.6		
	Preparation-3	32.56	32.76	100.6		
150 % Level	Preparation-1	48.42	48.73	100.6	99.7	0.90
	Preparation-2	48.15	48.02	99.7		
	Preparation-3	48.05	47.49	98.8		

Results of Calibration Curve

Results of the Calibration Curve obtained by RP-HPLC are summarized in Table 35

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Table 35: Results of the Calibration Curve obtained by first order derivative method

Parameter	Candesartan cilexetil	Hydrochlorothiazide
Linearity range (ppm)	159.48-478.44	125.07-375.21
Slope	15554.8155x	17,854.8482x
Intercept	70,126.3655	23,247.1241
Regression coefficient (r2)	0.9992	0.9997

Results of Validation Parameters

Accuracy

Accuracy of method was ascertained by recovery studies performed at different levels of concentrations i.e. 80%, 100% and 120%. The Percentage of recovery in the range of 97-100% and 99-100%, SD at different levels of concentration is 0.4, 0.1356, 0.06519 and 0.11023, 0.1529, 0.009 for RAM & AMB respectively. The CV at different levels of concentration is 0.4106, 0.1356, 0.06530 and 0.11060, 0.1532, 0.09017 for RAM & AMB respectively.

The results of Intra-day precision by first derivative method shows mean 99.29%, SD values 0.52725 and CV value 0.5310 for RAM and for AMB mean 98.56%, SD values 0.5069 and CV value 0.5143.

The results of inter-day precision by first derivative method shows mean 99.17% & 99.102%, SD 0.38987 & 0.3024, CV 0.3931 & 0.3054 for RAM and AMB respectively.

CONCLUSION

The described methods give accurate and precise results for determination of Candesartan cilexetil and Hydrochlorothiazide mixtures in tablets without prior separation and are easily applied for routine analysis. The most striking features of the RP-HPLC method is its simplicity and rapidity. This method also provides simple and reproducible quantitative analysis without any interference from the excipients.

The % RSD values in precision shows that proposed methods provide acceptable variation of Candesartan cilexetil and Hydrochlorothiazide. The % RSD of proposed method was found to be less than 2% shows its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The low values of % RSD indicate the method is precise and accurate.

From the experimental studies it can be conclude that colorimetric method developed for the estimation of Ramipril in its dosage form. The Proposed method for the selected drugs was found to be accurate and precise. This method is economical, easy and can be applied to estimate Ramipril in the dosage form. It is also more sensitive and specific method. Result of validation parameter demonstrates that the analytical procedure is suitable for its intended purpose and meets the criteria defined in ICH.

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