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STANDADADIZATION AND STABILITY INDICATING STUDIES BY RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF CANDESARTAN CILEXETIL & HYDROCHLORTHIAZIDE IN TABLET DOSAGE FORM

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Keywords:

Candesartan Cilexetil & Hydrochlorthiazide , HPLC, Chromatography

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ABSTRACT: A rapid, selective, precise, accurate, rugged and robust high performance liquid chrmatgraphic (HPLC) method for the simultaneous determination of Candesartan Cilexetil & Hydrochlorthiazide in tablet dosage form .The method was validated according to ICH and FDA guidelines. The chromatography is performed on a Kromasil C8, 150 x 4.6 mm, 5µm, in a gradient mode with a mobile phase of Water, acetonitrile and Trifluracetic acid in different ratios. UV-Visible detector at 285 nm was found to be suitable for detection. Linearity was observed in the range of 70 -130µg/ml with correlation coefficient of 0.9999. Sensitivity, accuracy, range, precision, robustness, ruggedness, stability, specificity, limit of detection, limit of quantification and system suitability parameters were validated for the developed method. The developed method was successfully applied to estimate the amounts in pharmaceutical formulations and for the stability indicating studies.

INTRODUCTION: Candesar – H tablets (Ranbaxy Laboratories Ltd India) [1-3], which contain candesartan cilexetil [1-6] and hydrochlorothiazide [2-10], are one of the most commonly used formulations for treatment of high blood pressure when one medicine is not sufficiently effective. Hydrochlorothiazide: 6-chloro - 1, 1 - dioxo- 3, 4-dihydro-2H-1$l ^ {6}, 2, 4-benzothiadiazine-7-sulfonamide **(Fig.1)** with molecular Formula and Molecular weight of $C_7H_8ClN_3O_4S_2$ & 297.741; is a diuretic.

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Several analytical methods, including LC–MS [11] Voltametry [12], Spectrophotometry [13-16, 25] and HPLC [17-21, 24-26], have already been reported for its determination, either alone or in combination with other drugs. Candesartan cilexetil: 2-ethoxy-1-({4-[2-(2H-1, 2, 3, 4-tetrazol-5-yl) phenyl] phenyl} methyl)-1H-1, 3-benzodiazole-7-carboxylic acid **(Fig.2)**. With molecular weight formula and weight of $C_{33}H_{34}N_6O_6$ & 610.67 is a non-peptide angiotensin II receptor.

The literature contains very few methods for analysis of candesartan cilexetil; those reported include HPLC with fluorimetric detection [22] and spectrofluorimetry. HPLC [25] and ratio derivative Spectrophotometry methods have been used for simultaneous determination of the two compounds. The HPLC method used UV – VIS detection for simultaneous quantification of hydrochlorothiazide

and candesartan cilexetil. The retention time was 4.5 mins and 10.6 mins. And stability indicating studies proves here that this method is stabile. The main objective of this study is to produce a quick, reproducible and stable method for the routine, simultaneous analysis of candesartan cilexetil and hydrochlorothiazide.

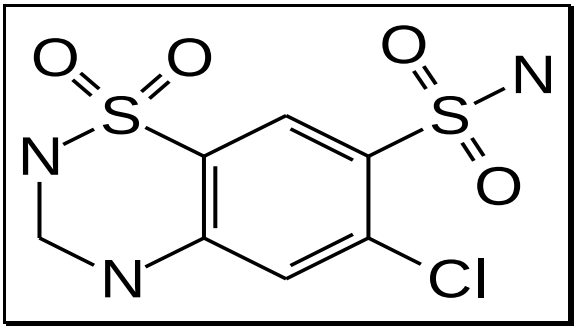


FIG 1. MOLECULAR STRUCTURE OF HYDROCHLOROTHIAZIDE

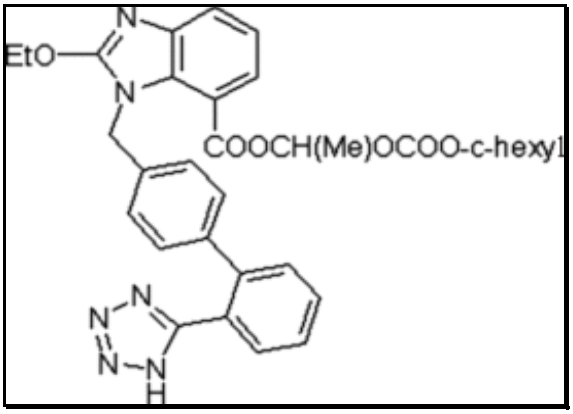


FIG 2. MOLECULAR STRUCTURE CANDESARTAN CILEXETIL

Mobile phase B: Prepare a mixture of Water, acetonitrile and Triflouroacetic acid in the ratio of 2**0:80:0.1%v/v/v** and sonicated to degas.

THE GRADIENT PROGRAMMING IS AS FOLLOWS:

Time (min.)	Flow (ml/min)	% Mobile phase A	% Mobile phase B
0.0	1.0	90	10
5.0	1.0	95	05
7.0	1.0	35	65
8.5	1.0	35	65
17.5	1.0	90	10
20.0	1.0	90	10

Injection volume: 10 µL

Column: Kromasil C8, 150 x 4.6 mm, 5µm

Column temperature: 30°C

Flow rate: 1.0 ml/min

Diluent: Methanol: water (80:20)

Detector wave length: 285nm for Hydrochlorothiazide and candesartan

Run time: 20mins

Observation:

Hydrochlorothiazide: 4.5 mins
Candesartan cilexetil: 10.6mins
Candesartan cilexetil and Hydrochlorothiazide peaks are found sharp and they are symmetrical.

Standard Preparation: Accurately weigh and transfer about 30mg of Candensartan Cilexetil working standard and 12.5mg of Hydrochlorothiazide workig standard in to a 50 ml volumetric flask. Add 30ml of diluent. Sonicate to dissolve and make up to the volume with diluent. Filter the solution through 0.45 µm nylon filter

Test Preparation: Transfer 10 tablets into a 500 ml volumetric flask. Add 150ml of diluent and sonicate for 25 minutes with intermediate shaking cool to room temperature and dilute to volume with Filter the solution through 0.45 µm nylon filter

System Suitability: From the Chromatogragram of standard solution USP Tailing for candensartan Cilexetil and Hydrochlorothiazide. Peak is not more than 2.0USP Tangent for candensartan Cilexetil peak is not less than 5000 and Hydrochlorothiazide. Peak is not less than 1500. The %RSD of the peak area of candensartan Cilexetil and Hydrochlorothiazide peak from six replicate injections should be not more then 2.0.

Procedure: Equilibrate the system with mobile phase A and mobile phase B at least 30 minutes. Inject Blank, Stadard solution six times and samples solution in duplicate.

Calculation: Calculate the amount of and candensartan Cilexetil Hydrochlorothiazide. present in % of tablet using the formula

% Assay of candensartan Cilexetil. =

$$\frac{AT1}{AS1} \times \frac{DS}{DT} \times \frac{P1}{100} \times \frac{100}{C}$$

Where,

AT1= Average area of candensartan Cilexetil peak in Sample Solution.

AS1 = Average area of candensartan Cilexetil peak in Standard Solution.

DS= Stanard Solution dilution,

DT=Test solution dilution

P = Potency of candensartan Cilexetil Working Standard on as is basis
C =Claim

Calculation: Calculate the amount of Hydrochlorothiazide present in % of tablet using the formula

$$\frac{AT2}{AS2}\times\frac{DS}{DT}\times\frac{P2}{100}\times\frac{100}{C}$$

Where,
AT2 = Average area of Hydrochlorothiazide peak in Sample Solution.
AS2 = Average area of Hydrochlorothiazide peak in Standard Solution.

DS= Stanard Solution dilution,

DT=Test solution dilution

P = Potency of Hydrochlorothiazide Working Standard on as is basis

C=Claim

METHOD VALIDATION SUMMARY [23]

System Suitability and System Precision:

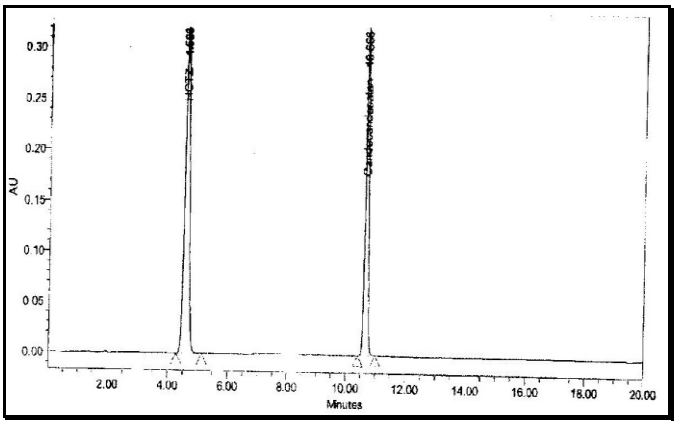
A Standard solution was prepared by using Candensartan Cilexetil and Hydrochlorothiazide working standard as per test method and was injected six times into the HPLC system.

The system suitability parameters were evaluated from standard chromatograms and found to be within the limits and results are shown in **Table 1** and **Table 2**. Calculated the % RSD from six replicate injections for Candensartan Cilexetil and Hydrochlorothiazide peak area.

SPECIFICITY

Placebo Interference:

A study to establish the interference of placebo was conducted. Samples were prepared in triplicate by taking the placebo equivalent to about the weight in portion of test preparation as per the test method. Chromatogram of placebo did not show any extra peaks. This indicates that the excipients used in the formulation do not interfere in estimation of Candensartan Cilexetil and Hydrochlorothiazide. No interference at the retention times of Candensartan Cilexetil and Hydrochlorothiazide and the results are shown in **Table 3**.



	Name of the Compound	Reten. Time [min]	Area [%]
1	Hydrochlorothiazide	4.56	99.94
2	Candesartan cilexetil	10.666	99.92

FIG 3: HPLC CHROMATOGRAM OF STANDARD CANDESARTAN CILEXETIL AND HYDROCHLOROTHIAZIDE

Precision of Test Method:

The precision of test method was evaluated by analysing six samples prepared as per test method. The assay and relative standard deviation of the individual Candensartan Cilexetil and Hydrochlorothiazide were calculated the results are shown in **Table 4, Table 5** and **Table 6** and they are within the limits.

TABLE 1 : RESULTS OF SYSTEM PRESION

Injection Number	Hydrochloro Thiazide Peak area	Candesartan Cilexetil Peak area
	16/12.5 mg Strength	16/12.5 mg Strength
01	3292304	2118089
02	3222673	2118014
03	3294617	2121766
04	3293364	2121529
05	3293156	2120580
06	3292391	2123691
Mean	3281417.5	2120612
% RSD	0.8	0.1

TABLE 2: RESULTS OF SYSTEM SUITABILITY

System Suitability Parameters	Observed value	
	16/12.5 mg Strength	Acceptance Criteria
% RSD for Hydrochlorothiazide peak areas from six replicate injections of standard solution	0.8	NMT 2.0
% RSD for Candesartan Cilexetil peak areas from six replicate injections of standard solution	0.1	NMT 2.0
% USP Tailing for Hydrochlorothiazide peak in the chromatogram of standard solution	1.0	NMT 2.0
% USP Tailing for Candesartan Cilexetil peak in the chromatogram of standard solution	1.0	NMT 2.0
USP Tangent for Hydrochlorothiazide peak in the chromatogram of standard solution	2500	NLT 1500

TABLE 3: RESULTS OF SYSTEM SUITABILITY

Sample No	Peak found at RT of Candesaratan Cilexetil and Hydrochlorothiazide (Yes/No)	
	16/12.5 mg Strength	Acceptance criteria
1.	No	Placebo should not show any peak at the retention time of analysis.

Accuracy:

A study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of Candensartan Cilexetil and Hydrochlorothiazide into each volumetric flask by spikeing API into the placebo each level to get the concentration of Candensartan Cilexetil and Hydrochlorothiazide equivalent to 75,100%,150% of the sample conc of Candensartan Cilexetil and Hydrochlorothiazide as per the test method. The average % recovery of Candensartan Cilexetil and Hydrochlorothiazide was found to be within the limits and the results are shown in **Table 7** and **Table 8**.

Linearity of Test Method:

A graph is plotted to "mg/ml of Candensartan Cilexetil and Hydrochlorothiazide added" versus mg/ml of Candensartan Cilexetil and hydrochlorothiazide found" in Accuracy section. The correlation coefficient was found to be 0.999. And the results are shown in **Table 4, Table 9** and **Figure 4**.

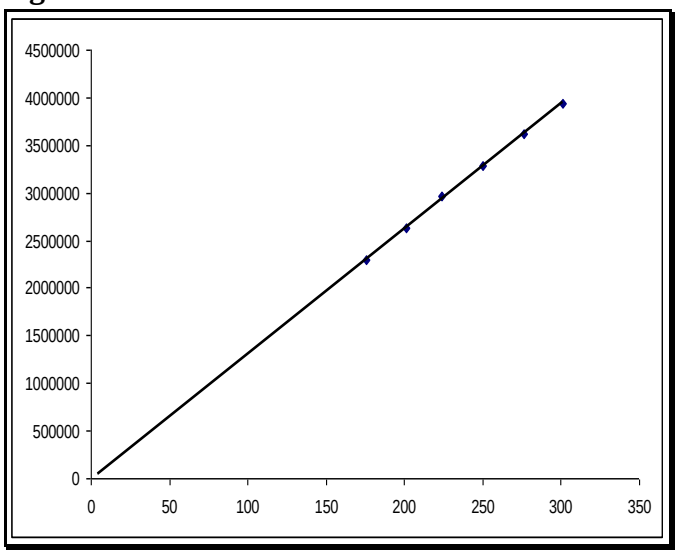


FIG 4. LINEARITY GRAPH OF HYDROCHLOROTHIAZIDE

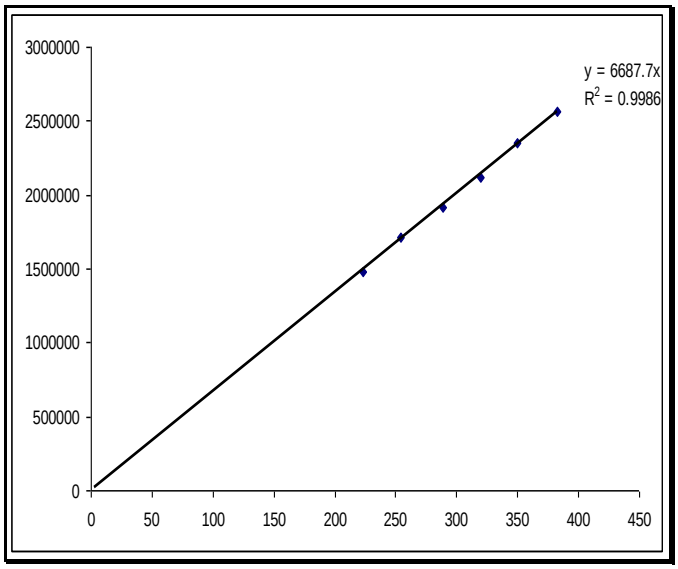


FIG 5. LINEARITY GRAPH OF CANDESARTAN CILEXETIL

From the above study it was established that the linearity of test method is from 70% to 130% of the labeled amount of Candensartan Cilexetil and Hydrochlorothiazide. The Correlation Coefficient shall be not less than 0.99. And the results are shown in **Table 4, Table 9** and **Figure 5**.

RUGGEDNESS OF TEST METHOD:

System to system /Analyst to Analyst/column to Column variability:

System to system /Analyst to Analyst/column to Column variability study was conducted on different HPLC system, different column and different analyst under similar conditions at

different times. Six samples were prepared and each were analysed as per test method.

The relative standard deviation for Candensartan Cilexetil and Hydrochlorothiazide were found to be below 2 % on the columns, systems and Analysts. Comparison of both the results obtained on two different HPLC systems, different column and different analysts shows that the related substances test method is rugged for System to system /Analyst to Analyst/column to Column variability.

TABLE 4: RESULTS OF PRECISION

Sl.no	% Assay of Hydrochlorothiazide	% Assay of Candesartan Cilexetil
1.	100.2	99.9
2.	101.0	100.8
3.	100.6	100.4
4.	98.2	98.3
5.	98.2	98.3
6.	98.7	98.9
Mean	99.5	99.4
% RSD	1.3	1.1

TABLE 5: RESULTS OF METHOD PRECISION

System Suitability Parameters	Observed Value 16/12.5 mg Strength Analyst-1	Analyst-2	Acceptance Criteria
% RSD for Hydrochlorothiazide peak areas from six replicate injections of standard solution	0.1	0.6	NMT 2.0
% RSD for Candesartan Cilexetil peak areas from six replicate injections of standard solution	0.1	0.6	NMT 2.0
% USP Tailing for Hydrochlorothiazide peak in the chromatogram of standard solution	1.0	1.0	NMT 2.0
% USP Tailing for Candesartan Cilexetil peak in the chromatogram of standard solution	1.0	1.0	NMT 2.0
USP Tangent for Hydrochlorothiazide peak in the chromatogram of standard solution	4101	4475	NLT 1500
USP Tangent for Candesartan Cilexetil peak in the chromatogram of standard solution	54969	50020	NLT 5000

Robustness:

Effect of variation in mobile phase composition:

A study was conducted to determine the effect of variation in composition of mobile phase. Standard solution prepared as per the test method was injected into the HPLC system using mobile phases. The system suitability parameters were evaluated and found to be within the limits

Effect of variation of flow rate:

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates 0.8ml/min and 1.2ml/min. The system suitability parameters were evaluated and found to be within the limits

Effect of variation of temperature:

A study was conducted to determine the effect of variation in temperature. Standard solution prepared as per the test method was injected into the HPLC system using temperatures 35ºC. The system suitability parameters were evaluated and found to be within the limits for temperatures 35ºC and the results are shown in **Table 10**.

Stability Indicating Studies

Forced Degradation

Degradation were carried out by attempting degradation of the sample with exposure to stressconditions like Acidic (1M HCL), Alkaline (1M NaOH), Peroxide, water and UV light.

With 0.1M NaOH

Crush the ten Tablets of both Candesartan Cilexetil and Hydrochlorothiazide, accurately weigh and transfer about 60mg of Candesartan Cilexetil, 25 mg of in to a 100 ml volumetric flask. Then add 10 ml of 0.1 NaOH and reflex for 30 min at 60^0C and cool to room temperature and add 10 ml of 0.1 N HCL to neutralize. Sonicate to dissolve and make up to the volume with diluent. Filter the solution through 0.45 µm nylon filter. Then it was injected once into the chromatographic system obtainthe chromatograms. Retention times were found.

With 0.1M HCL

Crush the ten Tablets of both Candensartan Cilexetil and Hydrochlorothiazide, accurately weigh and transfer about 60mg of Candensartan Cilexetil, 25 mg of in to a 100 ml volumetric flask. Then add 10 ml of 0.1 HCL and reflex for 30 min at 60^0C and cool to room temperature and add 10 ml of 0.1 N NaOH to neutralize. Sonicate to dissolve and make up to the volume with diluent.

Filter the solution through 0.45 µm nylon filter. Then it was injected once into the chromatographic system obtain the chromatograms. Retention times were found.

With 1 % M H_2O_2

Crush the ten Tablets of both Candensartan Cilexetil and Hydrochlorothiazide, accurately weigh and transfer about 60mg of Candensartan Cilexetil, 25 mg of in to a 100 ml volumetric flask. Then add 10 ml of 1 % M H_2O_2 and reflex for 30 min at 60^0C and cool to room temperature. Sonicate to dissolve and make up to the volume with diluent. Filter the solution through 0.45 µm nylon filter. Then it was injected once into the chromatographic system obtain the chromatograms. Retention times were found.

With M H_2O

Crush the ten Tablets of both Candensartan Cilexetil and Hydrochlorothiazide, accurately weigh and transfer about 60mg of Candensartan Cilexetil, 25 mg of in to a 100 ml volumetric flask. Then add 10 ml of H_2O and reflex for 30 min at 60^0C and cool to room temperature. Sonicate to dissolve and make up to the volume with diluent. Filter the solution through 0.45 µm nylon filter. Then it was injected once into the chromatographic system obtainthe chromatograms. Retention times were found.

With Heat

Take both Candensartan Cilexetil and Hydrochlorothiazide, accurately weigh and transfer about 60mg of Candensartan Cilexetil, 25 mg of in to a 100 ml volumetric flask and exposed to 105^0C for 6 hours. Add 20 ml of diluents and Sonicate to dissolve and make up to the volume with diluent. Filter the solution through 0.45 µm nylon filter. Then it was injected once into the chromatographic system obtainthe chromatograms. Retention times were found.

Bench top stability of standard and Test preparation:

A study to establish stability of Candesartan Cilexetil and Hydrochlorothiazide standard and test preparation on bench top was conducted over period of two days. Candesartan Cilexetil and Hydrochlorothiazide test preparation with target concentration spiking are injected initial, 1 day and 2 days. The difference in % of Candesartan Cilexetil and Hydrochlorothiazide from initial to 24 hours is within the limits. Candesartan Cilexetil and Hydrochlorothiazide standard was injected initial, 1 day and 2 days and the difference of the standard over period of one day was found stable.

From the above study, it was established that the Forced degradation studies with Acid, Base, Peroxide, Heat and Light The percentage assay is within the limits Were shown in the **Table 11**. And got the Retention time similar with standard sample.

TABLE 6: RESULTS OF PRECISION

Sample No	% Assay of Hydrochlorothiazide		% Assay of Candesartan Cilexetil	
	Analyst-1	Analyst-2	Analyst-1	Analyst-2
1	100.2	99.7	99.9	98.9
2.	101.0	99.9	100.8	99.0
3.	100.6	99.8	100.4	98.8
4.	98.2	98.3	98.3	97.9
5.	98.2	98.3	98.3	97.7
6.	98.7	99.0	98.9	98.5
Mean	99.5	99.2	99.4	98.5
% RSD	1.3	0.7	1.1	0.6
(Mean(n=12)	99.3		99.0	
%RSD(n=12)	1.0		1.0	

TABLE 7: RESULTS OF ACCURACY

S.No	% Spike level	"mg" added	"mg" found	% Recovery	Mean % Recovery	% RSD
1.	75%	120.00	119.18	99.3		
2.	75%	120.11	118.79	98.9	99.1	0.2
3.	75%	120.06	118.95	99.1		
1.	100%	159.88	157.42	98.5		
2.	100%	160.34	157.74	98.4	98.6	0.3
3.	100%	159.95	158.18	98.9		
1.	150%	239.77	235.35	98.2		
2.	150%	239.60	237.00	98.9	98.7	0.4
3.	150%	239.89	237.47	99.0		

TABLE 8: RESULTS OF ACCURACY

S.No	% Spike level	"mg" added	"mg" found	% Recovery	Mean % Recovery	% RSD
1.	75%	46.54	46.96	100.9		
2.	75%	46.55	46.83	100.6	100.7	0.2
3.	75%	46.60	46.91	100.7		
1.	100%	61.96	62.48	100.8		
2.	100%	62.29	62.61	100.5	100.7	0.2
3.	100%	62.29	62.78	100.8		
1.	150%	93.10	93.14	100.0		
2.	150%	92.92	93.79	100.9	100.6	0.5
3.	150%	93.14	94.04	101.0		

TABLE 9: RESULTS OF LINEARITY OF DETECTOR RESPONSE

Sl. No	Hydrochlorothiazide		Candesartan Cilexetil	
	Concentration (ug/ml)	Peak area	Concentration (ug/ml)	Peak area
1	176	2300471	223	1482306
2.	201	2629110	254	1715492
3.	224	2957748	289	1918679
4.	250	3286387	320	2121865
5.	276	3615026	350	2355052
6.	301	3943664	383	2568238
Slope		13126.37	Slope	6744.191
Intercept		-2007.43	Intercept	-17675.6
Co-efficient of Correlation		0.99	Co-efficient of Correlation	0.99

TABLE 10: RESULTS OF ROBUSTNESS SHOWING EFFECT OF VARIATION IN COMPOSITION OF ORGANIC PHASE IN MOBILE PHASE

S no.	Assay (mg/tablet)						
	Set- I	Set- II	Set- III	Set-IV	Set- V	Set-VI	Set-VII
1	12.4	12.3	12.2	12.4	12.5	12.4	12.3
2	12.3	12.4	12.5	12.4	12.3	12.6	12.4
3	12.5	12.6	12.7	12.5	12.4	12.5	12.5
4	12.4	-	-	-	-	-	-
5	12.3	-	-	-	-	-	-
6	12.5	-	-	-	-	-	-
Mean	12.40	12.43	12.47	12.43	12.40	12.50	12.40
SD	0.09	0.15	0.25	0.06	0.10	0.10	0.10
RSD (%)	0.721	1.229	2.019	0.464	0.806	0.800	0.806
Overall mean		12.41	12.42	12.41	12.40	12.43	12.40
Overall SD		0.105	0.148	0.078	0.087	0.100	0.087
Overall RSD (%)		0.849	1.193	0.630	0.698	0.804	0.698

Set I = Control (Proposed method)
Set II = Variation in flow rate (-10%)
Set III = Variation in flow rate (+10%)
Set IV = Column oven temperature (35°C)
Set V = Variation in wavelength (λ = 280m)
Set VI = Variation in wavelength (λ = 290m)
Set VII = Variation in organic content in mobile phase (-2%)
Set VIII = Variation in organic content in mobile phase (+2%)

TABLE 11: RESULTS OF FORCED DEGRADATION STUDIES

S.No	Name	Hydrochloro Thiazide RT	Hydrochloro Thiazide Peak area	Candesartan Cilexetil RT	Candesartan Cilexetil Peak area
		16/12.5 mg Strength	16/12.5 mg Strength		16/12.5 mg Strength
	ACID	4.60	3622304	10.70	2218089
	BASE	4.59	3622673	10.68	2218014
	PEROXIDE	4.62	3634617	10.67	2221766
	HEAT	4.60	3643364	10.72	2221529
	LIGHT	4.57	3633156	10.68	2220580
MEAN		4.596	3631223	10.69	2219996
ASSAY			98.07 %		

CONCLUSIONS: It is concluded from the RP-HPLC method development for the simultaneous qualitative determination of Candesartan Cilexetil and Hydrochlorothiazide is fast, reproducible, and simple. The proposed method is found to be specific, accurate, precise, linear, robust, and rugged. The developed method is stability indicating and can be used by a quality control department to determine assay of regular Candesartan Cilexetil and Hydrochlorothiazide commercial samples and also stability samples

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