RESEARCH ARTICLE

LIQUID CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS QUANTITATIVE DETERMINATION OF CANDESARTAN CILEXETIL AND HYDROCHLORTHIAZIDE IN PHARMACEUTICAL DOSAGE FORMS

M. Mathrusri Annapurna[a*], A. Narendra[b], K. Ravi Kumar[b]

[a]* Dept Pharmaceutical Analysis & Quality Assurance, GITAM Institute of Pharmacy, GITAM University, Visakhapatnam-530045, India

[b]Dept Pharmaceutical Analysis & Quality Assurance, Roland Institute of Pharm Sciences, Berhampur, Orissa-760010, India

[a] Corresponding author's E mail: mathrusri2000@yahoo.com Tel: 91-9010476308*

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ABSTRACT

A simple and sensitive RP-HPLC method was developed and validated for the determination of Candesartan cilexetil and Hydrochlorthiazide in pharmaceutical dosage forms. The separation of components was achieved on a SHIMADZU Hypersil ODS-C_{18} column (250 × 4.6 mm, 5 µm) with UV detection at 270 nm. Isocratic elution with a mobile phase consisting of 10 mM (pH 3.37) Tetra butyl ammonium hydrogen sulphate: methanol (15:85, V/V), at a flow rate 1.0 mL min^{-1} was employed. Linearity was observed in the concentration range 0.625-62.5 µg/mL for Hydrochlorthiazide and 0.8-80 µg/mL for Candesartan cilexetil respectively. The linear regression equation was found to be Y=64002X-1412.6 for Hydrochlorthiazide and Y=24649X-6701.8 for Candesartan cilexetil respectively with correlation coefficients greater than 0.999. The LOD was found to be 0.1385 and 0.1892 µg/mL for Hydrochlorthiazide and Candesartan cilexetil respectively where as the LOQ was found to be 0.4394 and 0.6187 µg/mL for Hydrochlorthiazide and Candesartan cilexetil respectively. The mean analytical recovery in determination of Candesartan cilexetil and Hydrochlorthiazide tablets was 99.31-100.08% Hydrochlorthiazide and 99.58-100.39% for Candesartan cilexetil respectively. Thus, the proposed method is applicable for routine determination of Candesartan cilexetil and Hydrochlorthiazide in pharmaceutical formulations.

Keywords: Candesartan cilexetil, Hydrochlorthiazide, Liquid Chromatography, LOD, LOQ, Tablets

INTRODUCTION

Candesartan cilexetil [1] (CST), 1-[[(cyclohexyloxy) carbonyl]oxy]ethyl 2-ethoxy-1-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1H-benzimidazole-7-carboxylate is an angiotensin II receptor antagonist, selective for AT1 receptors, with tight binding to and slow dissociation from the receptor (Figure 1). It has no agonist activity. It is rapidly converted to the active substance, candesartan, by ester hydrolysis during absorption from the gastrointestinal tract.

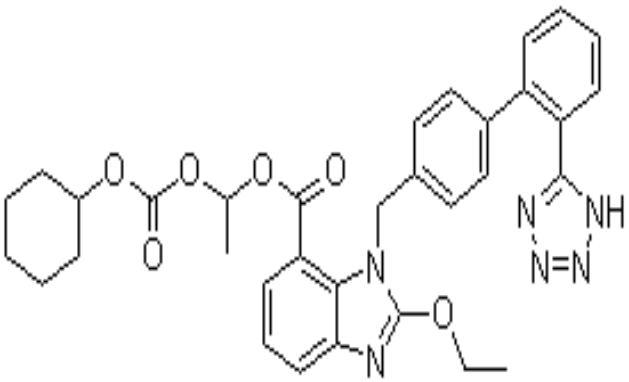

Figure 1: Structure of Candesartan cilexetil (CST)

Chemically, Hydrochlorothiazide [2] (HCT) is 6-chloro-1, 1-dioxo-3, 4-dihydro-2*H*-1, 2, 4-benzothiadiazine-7 sulfonamide which is a first line diuretic drug of the thiazide class (Figure 2). It acts by lowering blood pressure initially by increasing sodium and water excretion. This causes a decrease in extracellular volume, resulting in a decrease in cardiac output and renal blood flow. With long-term treatment, plasma volume approaches a normal value, but peripheral resistance decreases.

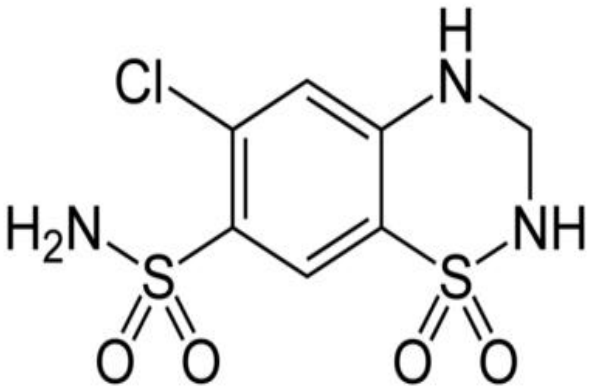

Figure 2: Structure of Hydrochlorothiazide (HCT)

Literature survey reveals that spectrophotometric [3], LC [4] and LC-MS [5] methods were developed for the analysis of Candesartan cilexetil alone and three HPLC methods were proposed for the estimation of Hydrochlorothiazide [6-8] alone in biological fluids.

Very few analytical methods such as spectrophotometric [9], HPLC [10-12] and HPTLC [13] method have been developed till

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now for the simultaneous determination of Candesartan cilexetil and Hydrochlorothiazide in pharmaceutical formulations and biological fluids. The objective of this investigation is to develop an efficient, simple, rapid, validated and reliable method for the routine quality control analysis for the simultaneous determination of Candesartan cilexetil and Hydrochlorthiazide in pharmaceutical preparations.

MATERIALS AND METHODS

Instrumentation

A Shimadzu HPLC instrument (LC-10AT Vp) equipped with UV-Visible detector, manual injector with 20 µl sample loop and A Hibar 250 x 4.6 mm LiChrospher 100 C_{18} column (250 mm x 4.6 mm i.d., 5 µm particle size) was used. The output signal was monitored and integrated using Shimadzu Class-Vp version 6.12 SP1 software was used for separation.

Reagents and Materials

Ranbaxy Laboratories Limited, India, kindly gifted Candesartan cilexetil and Hydrochlorthiazide pure powder with 99.98% and 99.98% purity respectively. HPLC grade methanol was purchased from Merck, India. The water for HPLC was obtained from TKA Gen Pure (Pacific) system (Germany). Tetra butyl ammonium hydrogen sulphate was purchased from S.D. Fine Chemicals, Ahmedabad, India and was of analytical grade. CANDELONG-H® (Candesartan cilexetil 8 mg and Hydrochlorthiazide 12.5 mg) and CANDESAR-H® (Candesartan cilexetil 16 mg and Hydrochlorthiazide 12.5 mg) brands are available as tablet.

Chromatographic Conditions

A Shimadzu C_{18} column (250 mm x 4.6 mm i.d., 5 µm) was used at ambient temperature. The mobile phase comprised of 0.01 M Tetra butyl ammonium hydrogen sulphate (pH 3.37) and methanol (15:85 v/v) was prepared, filtered through nylon 0.45 mm membrane filter and degassed before use. The mobile phase was pumped at a flow rate of 1 mL/min and the elution was monitored at 270 nm. The injection volume was 20 µL.

Preparation of Hydrochlorthiazide and Candesartan cilexetil Standard Solutions

Accurately weighed Hydrochlorthiazide (6.25 mg) and Candesartan cilexetil (8.0 mg) were transferred to a 100 mL volumetric flask, dissolved in and diluted to the mark with mobile phase to obtain a standard solution having a concentration of Hydrochlorthiazide (62.5 µg/mL) and Candesartan cilexetil (80 µg/mL). This solution was further diluted to obtain working standard solutions with Hydrochlorthiazide (0.625 -62.5 µg/mL) and Candesartan cilexetil (0.8-80 µg/mL) for the HPLC method.

Preparation of Sample Solutions

Powder of each of 20 tablets (2 brands, CANDESAR-H® and CANDELONG-H®) were weighed and analyzed as follows. A mass of tablet powder equivalent to the powder of one tablet was weighed from each brand and transferred in to a 100 mL volumetric flask separately and methanol (80 mL) was added. It was sonicated for 15 minutes and final volume was made up to the mark with methanol to get a solution with Hydrochlorthiazide (62.5 µg/mL) and Candesartan cilexetil (80 µg/mL) for brand I (CANDESAR-H®) and Hydrochlorthiazide (62.5 µg/mL) and Candesartan cilexetil (40 µg/mL) for brand II (CANDELONG-H®). Each mixture was then filtered separately through a nylon membrane filter.

Method Validation

Specificity (Selectivity)

The selectivity of the RP HPLC method was checked by comparison of chromatograms obtained from samples and the corresponding placebo. Additives in tablets are sparingly soluble in methanol or the mobile phase, whereas the active constituents are freely soluble.

Linearity

Calibration curves were constructed by plotting peak areas versus concentrations of Hydrochlorthiazide and Candesartan cilexetil and the regression equations were calculated. A calibration curve was plotted over a concentration range 0.625-625 µg/mL for Hydrochlorthiazide and 0.8-80 µg/mL for Candesartan cilexetil respectively. Accurately measured standard working solutions of Hydrochlorthiazide (0.625-62.5 µg/mL) and Candesartan cilexetil (0.8-80 µg/mL) were prepared in a series of 10 mL volumetric flasks and diluted to the mark with mobile phase. 20 µL of these solutions were injected under operating chromatographic conditions at UV detection 270 nm and the peak area was recorded.

Precision

The precision was checked by repeatedly (n = 3) injecting standard solutions of three different concentrations of Hydrochlorthiazide (12.5, 25 and 50 µg/mL) and Candesartan cilexetil (16, 32 and 64 µg/mL) respectively.

Intermediate Precision (Reproducibility)

The intra-day and inter-day precisions of the proposed methods were determined by estimating the corresponding responses 3 times on the same day and on 3 different days, over a period of 1 week, for 3 different concentrations of 12.5, 25 and 50 µg/mL Hydrochlorthiazide and 16, 32 and 64 µg/mL for Candesartan cilexetil. The results are reported in terms of relative standard deviation.

The stability of standard solutions can also affect the robustness of analytical methods. The stability of the standard solutions of the drug substances used in these methods was tested over a long period of time. One portion of a standard solution was kept at room temperature and another portion was stored under refrigeration at approximately 48ºC, and the content of these solutions was regularly compared with that of a freshly prepared solution.

Accuracy (% Recovery)

The accuracy of the methods was determined by calculating recoveries of Hydrochlorthiazide and Candesartan cilexetil by the standard additions method. Known amounts of standard solution of Hydrochlorthiazide (0.020, 0.025, 0.030 mg mL^{-1}) and Candesartan cilexetil (0.0256, 0.032, 0.0384 mg/mL) for the HPLC method were added to a pre-quantified sample solution of tablet dosage forms (HCT = 0.025 mg/mL and CST = 0.032 mg/mL). The amounts of Hydrochlorthiazide

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and Candesartan cilexetil were estimated by applying these values to the regression equation of the calibration curve.

Limit of Detection and Limit of Quantification

Experimentally the detection limit is defined as the concentration of the analyte producing a signal which is at least three times the base line noise measured from peak to peak and the quantitation limit is defined as the concentration of the analyte producing the signal which is at least ten times the base line noise. The limit of detection and the limit of quantification of the drug were calculated using the following equations as per ICH [14] guidelines.

$$LOD = 3.3 \times \sigma / S$$

$$LOQ = 10 \times \sigma / S$$

Where σ = the standard deviation of the response, S = the standard deviation of y-intercept of regression lines.

Analysis of CST and HCT in Combined Tablet Dosage Form

Tablets containing Candesartan cilexetil and Hydrochlorthiazide of the two brands CANDESAR-H® and CANDELONG-H®) were purchased from the local market and the drugs were extracted using the mobile phase. The response of tablet dosage forms was measured at 270 nm for quantification of Candesartan cilexetil and Hydrochlorthiazide respectively as described above. The amount of Candesartan cilexetil and Hydrochlorthiazide present in sample solutions were determined by fitting the responses into the regression equation for Candesartan cilexetil and Hydrochlorthiazide.

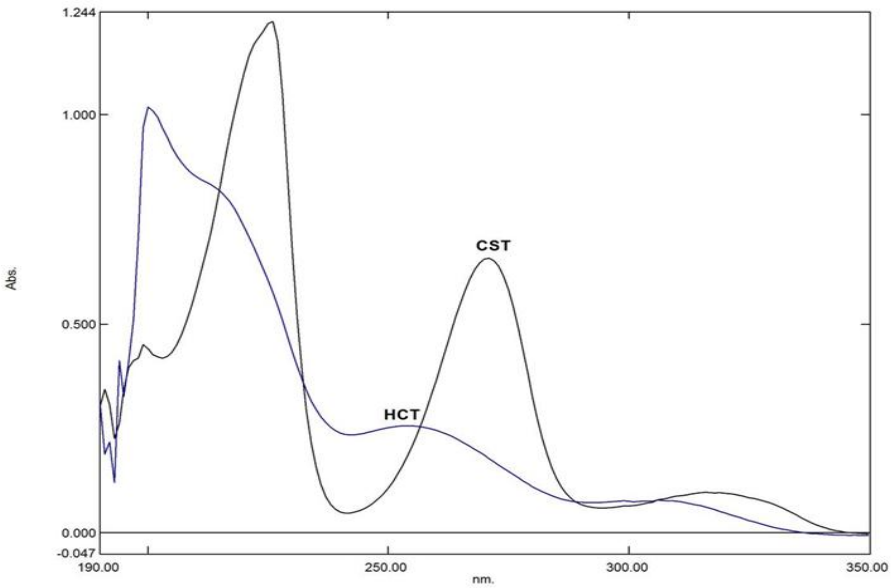

Figure 3: Overlay UV absorption spectrum of Candesartan cilexetil (10 µg/ml) and Hydrochlorthiazide (10 µg/ml)

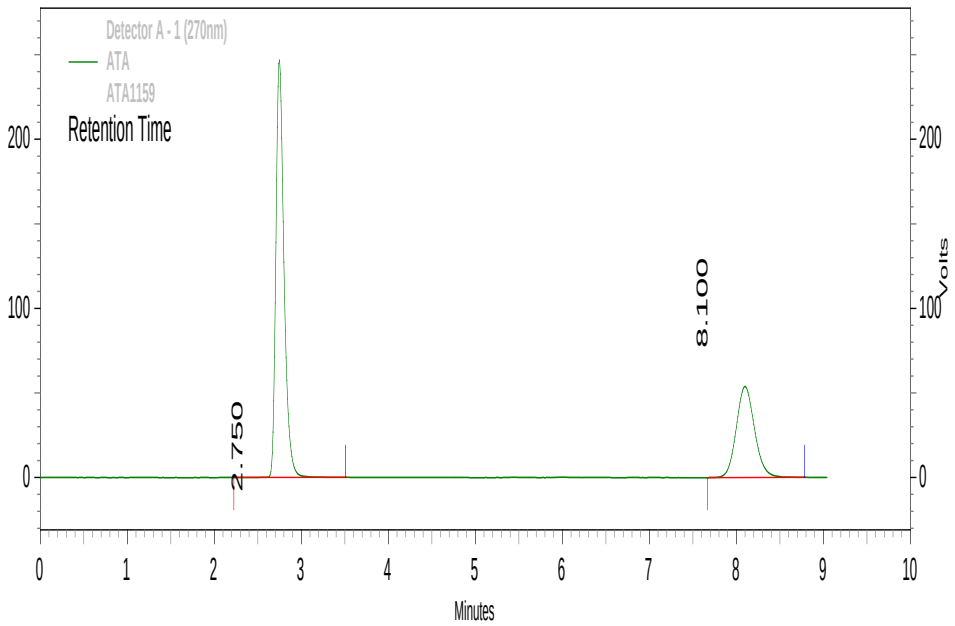

Figure 4: Representative chromatogram of Candesartan cilexetil (32 µg/mL) and Hydrochlorthiazide (25 µg/mL)

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RESULTS AND DISCUSSION

To optimize the HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and peak symmetry for Candesartan cilexetil and Hydrochlorthiazide were obtained with a mobile phase mixture consisting of 0.01 M (pH 3.37) Tetra butyl ammonium hydrogen Sulphate and methanol (15:85 v/v) to get better reproducibility and repeatability. Quantification was achieved with UV detection at 270 nm (Figure 3) based on peak area. The retention times were 8.100 and 2.750 mins for Candesartan cilexetil and Hydrochlorthiazide respectively. A good resolution of the peaks with clear base line separation was found (Figure 4). The present proposed method has been compared with the previously published liquid chromatographic methods in Table 1

Table 1: Comparison of the performance characteristics of the present liquid chromatographic method with the previous published methods

S. No.	Mobile phase / Mode	λ (nm)	Linearity (µg/mL)	Remarks	Ref.
1.	(0.02 M) potassium dihydrogen phosphate: methanol: triethylamine (25:75:0.2) (pH 6.0 ± 0.1) (Isocratic mode)	271	5-45 (HCT) 12-56 (CST)	Narrow range and more care for pH adjustment & Less sensitive	10
2	10 mM potassium dihydrogen phosphate : methanol : acetonitrile (2:80:18, v/v/v) (pH 2.5) ((Isocratic mode)	260	0.02-1.0 (HCT) 0.03-2.5 (CST)	Very narrow range (Biological method)	11
3	Acetonitrile : 0.02M sodium acetate (Gradient mode)	265	0.0125-1.25 (HCT) 0.016-0.2 (CST)	Very narrow range	12
4	Methanol: (10 mM) TBA HS (85:15 v/v) (Isocratic mode)	270	0.625-62.5 (HCT) 0.8-80 (CST)	Wide linearity range and no extra reagents for pH adjustment	Present work

Validation of the Proposed Method

Specificity (Selectivity)

The selectivity of the RP-HPLC method was checked by comparison of chromatograms obtained from samples and the corresponding placebo. No interference from additives was obtained.

Linearity

Linear correlation was obtained in the concentration range of 0.625-62.5 µg/mL for Hydrochlorthiazide and 0.8-80 µg/mL for Candesartan cilexetil (Table 2) respectively. The linear regression equation was found to be Y=64002X-1412.6 for Hydrochlorthiazide and Y=24649X-6701.8 for Candesartan cilexetil respectively with correlation coefficients greater than 0.999 (Figure 5 & 6).

Table 2: Linearity

CST		% RSD	HCT		% RSD
Conc. (µg/ml)	Mean peak area		Conc. (µg/ml)	Mean peak area	
0.8	19180 ± 44.114	0.23	0.625	44664 ± 169.72	0.38
1.6	32214 ±164.29	0.51	1.25	71311± 335.16	0.47
3.2	73947 ± 236.63	0.32	2.5	154098±1232.78	0.80
4	91489 ± 795.94	0.87	3.125	189825±1366.74	0.72
8	186392 ± 1211.55	0.65	6.25	389612±1402.60	0.36
16	396720 ± 2935.73	0.74	12.5	813233± 4228.81	0.52
32	759988 ± 6763.89	0.89	25	1608031± 11417.02	0.71
64	1551197 ± 10237.90	0.66	50	3214327± 22178.86	0.69
80	1988198 ± 15110.31	0.76	62.5	3981171± 25877.61	0.65

RSD = Relative standard deviation

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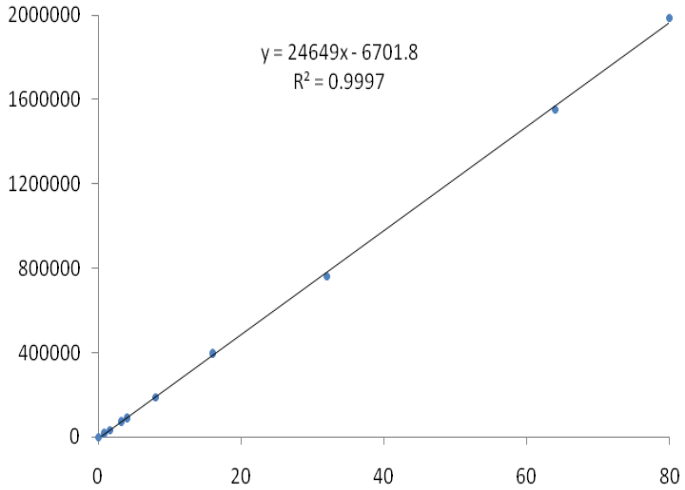

Figure 5: Calibration curve of Candesartan cilexetil

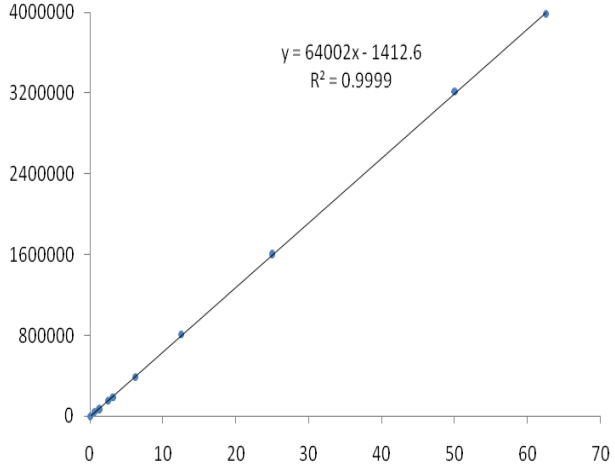

Figure 6: Calibration curve of Hydrochlorthiazide

Precision

The low % RSD values of inter-day was found to be 0.73-1.43 and 0.58-1.39 for Candesartan cilexetil and Hydrochlorthiazide respectively, while % RSD values of intra-day was found to be 0.194-0.564and 0.194–1.003 for Candesartan cilexetil and Hydrochlorthiazide respectively. The low % RSD values of intra-day and inter-day variations reveal that the proposed methods precise (Table 3 and 4).

Table 3: Intra-day and inter-day precision for Candesartan cilexetil (*n* = 3)

Conc. (µg/mL)	Intra-day precision		Inter-day precision	
	Mean area ± SD (n = 3)	RSD (%)	Mean area ± SD (n = 3)	RSD (%)
16	397898 ± 771.92	0.194	397729 ± 5687.52	1.43
32	760893 ± 4291.43	0.564	770981 ± 6322.04	0.82
64	1562437 ± 4718.56	0.302	1576879 ± 11511.22	0.73

SD = Standard deviation. RSD = Relative standard deviation

Table 4: Intra-day and inter-day precision for Hydrochlorthiazide (*n* = 3)

Conc. (µg/mL)	Intra-day precision		Inter-day precision	
	Mean area ± SD (n = 3)	RSD (%)	Mean area ± SD (n = 3)	RSD (%)
12.5	814563 ± 1580.25	0.194	820987 ± 11411.72	1.39
25	1611251 ± 16160.84	1.003	1723454 ± 10030.50	0.58
50	3197687 ± 9657.02	0.302	3238768 ± 28177.28	0.87

SD = Standard deviation. RSD = Relative standard deviation

Because the stability of standard solutions can also affect the robustness of analytical methods, the stability of the standard solutions of the drug substances used in these methods were tested over a long period of time. One portion of a standard solution was kept at room temperature and the other portion was stored under refrigeration at approximately 48C, and the content of these solutions was regularly compared with that of a freshly prepared solution. No changes in drug concentrations were observed for solutions stored under refrigeration. But, it is recommended that the standard and sample solutions must, therefore, be freshly prepared in amber colored flasks to protect from light for both of the methods.

Accuracy

The recovery experiments were carried out by a standard addition method. The percent recoveries obtained were 99.31-100.08 and 99.58-100.39 for Candesartan cilexetil and Hydrochlorthiazide respectively (Table 5). The low value of % RSD indicates that the method is accurate.

Table 5: Accuracy - Recovery data (n = 3)

Conc. (µg/mL)				Total Conc. (µg/mL)		Mean Peak Area ± SD		% RSD		Amount recovered (% Recovery)	
Pure drug		Formulation									
HCT	CST	HCT	CST	HCT	CST	HCT	CST	HCT	CST	HCT	CST
20	25.6	25	32	45	57.6	2869525.67 ± 16295.38	1403240.67 ± 4287.59	0.57	0.31	44.86 (99.68)	57.20 (99.31)
25	32	25	32	50	64	3211066 ± 34452.23	1572033 ± 3821.73	1.07	0.24	50.19 (100.39)	64.05 (100.08)
30	38.4	25	32	55	70.4	3503930.33 ± 34441.49	1727601.84 ± 19283.27	0.98	1.12	54.77 (99.58)	70.36 (99.94)

SD = Standard deviation. RSD = Relative standard deviation

Limit of Detection and Limit of Quantification

The LOD was found to be 0.1385 and 0.1892 µg/mL for Hydrochlorthiazide and Candesartan cilexetil respectively where as the LOQ was found to be 0.4394 and 0.6187 µg/mL for Hydrochlorthiazide and Candesartan cilexetil respectively.

System suitability

As system suitability test is an integral part of chromatographic methods development and it is used to verify that the system is adequate for the analysis to be performed, the parameter for Candesartan cilexetil and Hydrochlorthiazide was evaluated. The theoretical plates were found to be 4723 and 5435 (N >2000) for Candesartan cilexetil and Hydrochlorthiazide and the resolution was >1.5. The tailing factor was found to be 1.04 and 1.12) for Candesartan cilexetil and Hydrochlorthiazide respectively and the capacity factor (k') was found to be >2.0 for both.

Assay of the Tablet Dosage Forms

The proposed validated methods were successfully applied to determine Candesartan cilexetil and Hydrochlorthiazide in their combined tablet dosage form and a model chromatogram obtained from the marketed formulation was shown in Figure 5. The results obtained for Candesartan cilexetil and Hydrochlorthiazide was comparable with the corresponding labeled amounts (Table 6).

Table 6: Analysis of commercial formulation (Tablets)

Commercial Formulation	Labeled amount (mg)		Amount found (mg)		% Recovery	
	CST	HCT	CST	HCT	CST	HCT
CANDESAR-H®	16	12.5	15.93	12.41	99.56	99.28
CANDELONG-H®	8	12.5	7.96	12.52	99.50	100.16

CONCLUSION

The results of the analysis of pharmaceutical dosage forms by the proposed methods are highly reproducible, reliable, and are in good agreement with the label claims of the drug. The additives usually present in the pharmaceutical formulations of the assayed samples did not interfere with Candesartan cilexetil and Hydrochlorthiazide. It may be said that the proposed methods are precise, sensitive, and accurate, so that these can be used as standard pharmacopoeial methods for the simultaneous determination of Candesartan cilexetil and Hydrochlorthiazide in tablets using the HPLC systems. The advantages of the proposed method involve a simple procedure for sample preparation and relatively short time of analysis. Apart from this, it can be used for assays of Candesartan cilexetil and Hydrochlorthiazide in biological fluids or in pharmacokinetic investigations.

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