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Research Article

Method development and validation for assay of candesartancilexetil and hydrochlorothiazide in tablet dosage form by RP-HPLC

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ABSTRACT

A new analytical RP-HPLC chromatographic method was developed and validated for simultaneous estimation of Candesartan cilexetil (CANC) and Hydrochlorothiazide (HCT) in bulk and pharmaceutical dosage form. The method was validated as per ICH guidelines. The separation was carried out by using a mobile phase consisting of methanol:acetonitrile:phosphate buffer pH 3.5 in the ratio of 50:20:30. The column used was X-Terra C8 column with (150mm length and 4.6mm internal diameter with 3.5µm particle size) with flow rate of 1.0ml /min using PDA detector at wavelength of 225 nm and temperature was maintained at 25 °C. The retention times of Candesartan cilexetil and hydrochlorothiazide were 6 min and 2 min respectively. The linearity for Candesartan cilexetil and Hydrochlorothiazide were in the range of 12.8 to 64 mcg/ml and 10 -50 mcg/ml respectively with value of LOD found to be 0.03µg/ml and 0.06µg/ml and LOQ were found to be 0.1µg/ml and 0.02µg/ml respectively, which were linear enough showing correlation coefficient of 0.999 in both cases. The present method was specific, sensitive, reproducible, precise, rapid and simple.

Keywords: Candesartan cilexetil; Hydrochlorothiazide; RP-HPLC; LOD & LOQ.

INTRODUCTION

Candesartan cilexetil, a prodrug, is hydrolyzed to candesartan during absorption from the gastrointestinal tract. Candesartan is a selective AT_1 subtype angiotensin II receptor antagonist. Candesartan cilexetil, a non-peptide, is chemically described as (±)-1-Hydroxyethyl 2-ethoxy-1-[p-(o-1Htetrazol-5-ylphenyl) benzyl]-7-benzimidazolecarboxylate, cyclohexyl carbonate (ester). The chemical structure shown in figure 1. (United States Pharmacopeia, Sweetmam sc Martindale, 2009 the Merck Index, British Pharmacopoeia, 2014). It is used as a angiotensin II receptor antagonist.

Hydrochlorothiazide diuretic often considered the prototypical member of this class and chemically 6-chloro-1,1-dioxo-3,4-dihydro-2H-1S{6},2,4-benzothiadiazine-7-sulfonamide. The HCT chemical structure shown in figure 2 (Unites States Pharmacopoeia, Sweetmam sc Martindale, 2009, Merck Index, Indian Pharmacopoeia, 2007). It reduces the reabsorption of electrolytes from the renal tubules. This results in increased excretion of water and electrolytes, including sodium, potassium, chloride, and magnesium. It has been used in the treatment of several disorders including edema, hypertension, diabetes insipidus, and hypoparathyroidism. USFDA approved two drugs combination therapy (candesartan cilexetil 16 mg and hydrochlorothiazide 12.5mg) as a single pill regimen. Although many HPLC methods were available for the determination of CANC alone (Revathi R. et al; 2011; Vairappan K. et al; 2011) and in combination with Atorvastatin Calcium, Olmesartan Medoxomil, Candesartan, and Chlorthalidone (Mhaske R.A. et al; 2012; Candesartan cilexetil and Hydrochlorothiazide (Mathrusri Annapurna et al; 2012), they were all carried out with combination of two mobile phases. Hence sincere attempt was made to develop a isocratic mode by using a combination of three mobile phases for estimation of CANC and HCT in combined dosage forms.

Instrumentation

Method development and validation was performed on High performance liquid chromatography equipped with Waters 2695 HPLC system with PDA detector and Waters column X-Terra C8 column 4.6 x 150mm, 3.5µm column was used. A Rheodyne injector with a 20 µl loop was used for the injection of sample. The data processing was used Empower software.

Chromatographic Conditions

The separation was carried out by using a mobile phase consisting of methanol:acetonitrile:phosphate buffer pH 3.5 in the ratio of 50:20:30. The column used was waters X Terra C8 column with (150mm length and

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4.6mm internal diameter with 3.5µm particle size) with flow rate of 1.0ml /min using PDA detector with the detection wavelength at 225 nm and temperature was maintained at 25°C. The retention times of CANC and HCT were 6 min and 2 min, respectively. The chromatographic conditions were given Table 1**.**

Selection of Wavelength

The known concentration of CANC and HCT were weighed in different volumetric flasks and dissolved in methanol. The resulting solutions were scanned in the range of 190nm to 400nm. The obtained two spectra were overlaid to get an isobestic point of two drugs. The maximum absorbance was found at 225nm with characteristic peak as shown in the figure 3.

Preparation of Mobile Phase

Mix a mixture of buffer 300ml (30%), 500ml of Methanol (50%) and 200ml of ACN (20%) and degas in ultrasonic water bath for 5minutes. Filter through 0.45µ filter under vacuum filtration.

Preparation of Standard

Accurately weigh and transfer 12.8mg and 10mg of CANC and HCT working standard in two different 10ml clean dry volumetric flasks add about 7ml of methanol and sonicate to dissolve it completely and make up the volume to the mark with the same solvent.

Preparation of Standard Solution

Pipette out 0.3ml of CANC and HCT from standard stock solution into two separate 10ml volumetric flasks and make up the volume to the mark with diluents.

Mix Standard Solution

Pipette out 0.3ml of CANC stock solution (30µg/ml) and 0.3ml HCT stock solution (37.5µg/ml) into 10ml volumetric flasks, add 7ml of diluents and mix. Finally made it up to the mark with diluents.

Preparation of Sample Stock Solution

Weigh accurately and transfer 59.8 mg of CANC and HCT Tablet powder into a 10ml clean dry volumetric flask and add about 7ml of diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further dilute 3ml of the supernatant solution to 100ml with diluents.

Method Validation

Linearity and Range

The linearity of a method is its ability to obtain test results that are directly proportional to the sample concentration over a given range. The peak area and concentration were plotted to get a standard calibration curve. The correlation co-efficient and regression co-efficient were calculated. The linearity for CANC and HCT were in the range of 12.8 to 64 mcg/ml and 10 -50 mg/ml. From the linearity studies calibration curve was plotted and concentrations were subjected to least square regression analysis to calculate regression equation. The Correlation coefficient value for calibration plot of CANC and HCT was 0.999 it shows good linearity for both the drugs. The calibration curves were given in figures 6 (a and b)

Accuracy

The accuracy of a method is the closeness of the measured value to the true value for the sample. Accuracy is usually determined by recovery studies. The percentage recovery of CANC was found to be 101.53%, 100.10% and 99.10% for accuracy 50%, 100% and 150% samples respectively. The %RSD of the samples was found to be less than 2 and HCT was found to be 99.95%, 100.10% and 99.07% for accuracy 50%, 100% and 150% samples respectively. The %RSD of the samples was found to be less than 2. The results of accuracy were given in Table 4.

Precision

The precision studies were studied by a series of measurements are done with CANC and HCT. Six replicate injections of the specific standard at various time intervals on the same day and on different days were done The % RSD value indicates a good degree of precision within the specified range. The results for precision were given in Table 5.

Limit of Detection and Limit of Quantification

LOD and LOQ were calculated by using standard deviation and slope values obtained from calibration curve. The LOD value for CANC and HCT was found to be 0.03µg/ml and 0.06µg/ml and The LOQ value for CANC and HCT was found to be 0.1µg/ml and 0.02µg/ml respectively. The LOQ values were determined by the formulae LOD=10σ/s. The results obtained were satisfactory and good agreement as per the ICH guidelines.

Robustness and Ruggedness

All the system suitability parameters are within limits for variation in flow rate Effect of change in mobile phase flow (±10% of actual flow) chromatogram CANC and HCT for robustness (less flow -10% change in actual flow rate). Chromatogram of CANC and HCT for robustness (more flow +10% change in actual flow rate). Effect of change in mobile phase composition (±10% of change in organic phase). Hence there is no significant effect on the result by doing small deliberate changes in the system as well as in method parameters. Ruggedness is the degree of reproducibility of results obtained by the analysis of the same sample under a variety of normal test conditions i.e. different analysts, laboratories, instruments, reagents, assay temperatures, small variations in mobile phase, different days etc. The interday precision studies were studied by five replicate injections of CANC and HCT on different days. The intra-day and inter-day variation was calculated in terms of percentage relative standard deviation and were within the acceptance criteria of not more than

Table 1: Parameters optimized conditions

Parameters	Optimized Conditions
Chromatograph	HPLC Waters
Column	Waters X Terra C8, column with 150 × 4.6mm , 3.5µm
Mobile Phase	Methanol: Acetonitrile: Phosphate buffer pH 3.5 (50:20:30)
Flow Rate	1 ml/min
Detection wavelength	225
Injection Volume	20 µl
Column Temperature	25°C
Detector Type	PDA

Table 2: Results and statistical data for estimation of Candesartan cilexetil and Hydrochlorothiazide in marketed formulation

S. No.	Weight of STD (mg)		Wt. of the sample (mg)	Peak area of STD		Peak area of the sample		% Label claim	
	CAN	HCZ		CAN	HCZ	CAN	HCZ	CAN	HCZ
1	12.80	10.00	59.80	2552370	4663840	2533197	4608443	99.05	98.72
2			60.00			2536893	4612490	99.20	98.80
3			59.89			2529694	4635426	98.92	99.30
								99.057	98.94
							SD	0.14	0.315
							% RSD	0.14	0.317

Table 3: System suitability parameters

Parameters	Candesartan cilexetil	Hydrochlorothiazide
Linearity range	12.8 - 64.0 µg/ml	10 - 50 µg/ml
Correlation coefficient	0.9999	0.9999
Slope	10392	36532
Retention time	6 min	2 min
Resolution factor	12.2	--
Plate count (USP)	2348	3589
Tailing (USP)	1.1	1.3
Limit of Detection (LOD)	0.03 µg/ml	0.6µg/ml
Limit of Quantitation (LOQ)	0.1 µg/ml	0.02µg/ml

Table 4: Recovery data

Parameters	Candesartan cilexetil		Hydrochlorothiazide	
	% Estimated	% RSD	% Estimated	% RSD
50 %	101.53	0.31	99.95	0.63
100 %	100.10	0.29	100.10	0.31
150 %	99.10	0.70	99.10	0.39

*Mean six determination (n = 6)

Table 5: Precision study data

Drug	Intraday		Inter day	
	% Obtained	% RSD	% Obtained	% RSD
CAN	100.4%	0.12	99.6 %	0. 34
HCZ	99.6%	0.09	100.2%	0.69

*Mean of six determinations (n=6)

2%. The results for robustness and ruggedness were given in Tables 6 (a and b).

RESULTS AND DISCUSSION

Several trials were conducted to achieve optimized chromatographic conditions. The isobestic point for two drugs was determined by spectrophometric method. The initial attempt was to employ as much low proportion of organic solvents for elution of the compounds. More part of aqueous solvents in mobile phase resulted in prolonging of retention time of both the compounds, especially for HCT. The most accepta

Table 6a: Effect of change in mobile phase flow rate

S. No.	System Suitability parameter		Observations			Limits
			As Such	Less flow	More flow	
1	Theoretical plates	HCT	2348	2386	2149	NLT 2000
		CANC	3589	4020	3584	
2	Tailing factor	HCT	1.3	1.1	1.1	NMT 2.0
		CANC	1.1	1.1	1.1	
3	Resolution	HCT	NA	NA	NA	NLT 2.0
		CANC	12.7	13.1	12.2	

Table 6b: Effect of change in mobile phase composition

S. No.	System Suitability parameter		Observations			Limits
			As Such	Less Organic Phase	More Organic Phase	
1	Theoretical plates	HCT	2348	2913	2561	NLT 2000
		CANC	3589	4236	3895	
2	Tailing factor	HCT	1.3	1.1	1.1	NMT 2.0
		CANC	1.1	1.1	1.1	
3.	Resolution	HCT	NA	NA	NA	NLT 2.0
		CANC	12.7	13.1	12.2	

Table 7: Linearity of Hydrochlorothiazide and Candesartan cilexetil

S. No.	Linearity Level	Hydrochlorothiazide		Candesartan cilexetil	
		Conc. (µg/mL)	Average Area	Conc. (µg/mL)	Average Area
1	25	10	1850783	12.8	992568
2	50	20	3355816	25.6	2055471
3	75	30	4856490	38.4	3123297
4	100	40	6367158	51.2	4000104
5	125	50	7843757	64.0	5130663
	Correlation Coefficient	0.99999		0.99941	
	Slope	36536.2690		103929.080	
	Intercept	-0.2228		0.154	

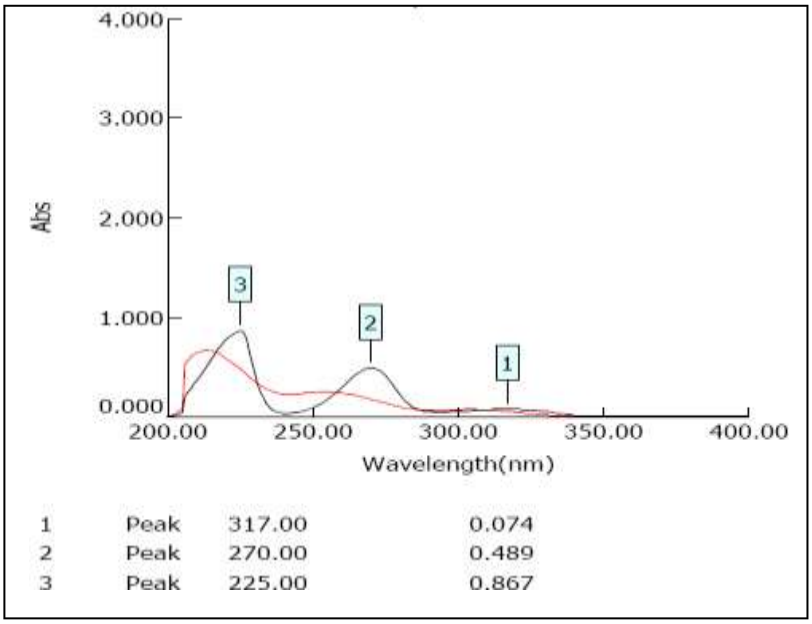

Figure 1: Isobestic point of Candesartan cilexetil and Hydrochlorothiazide

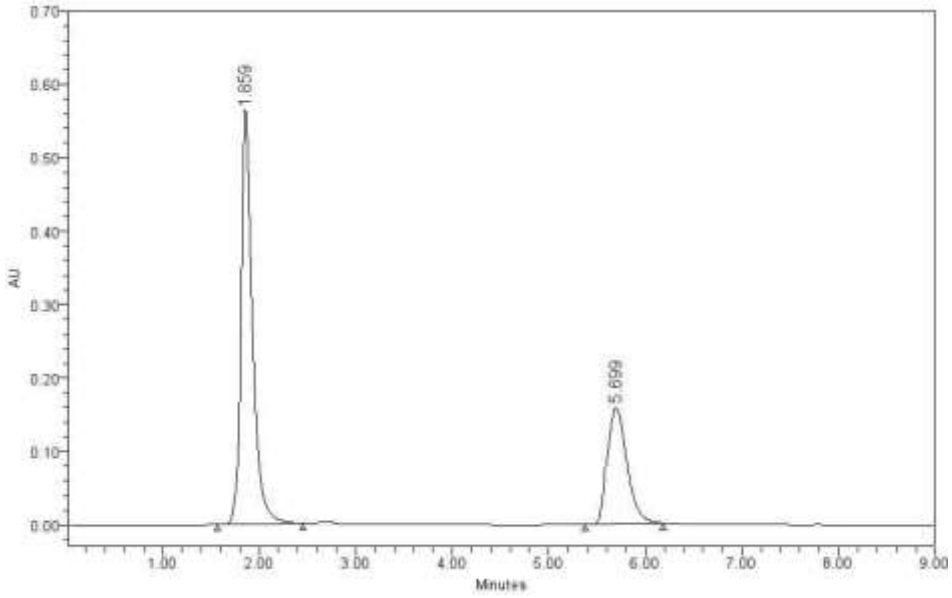

Figure 2: Standard chromatogram of Candesartan cilexetil and Hydrochlorothiazide

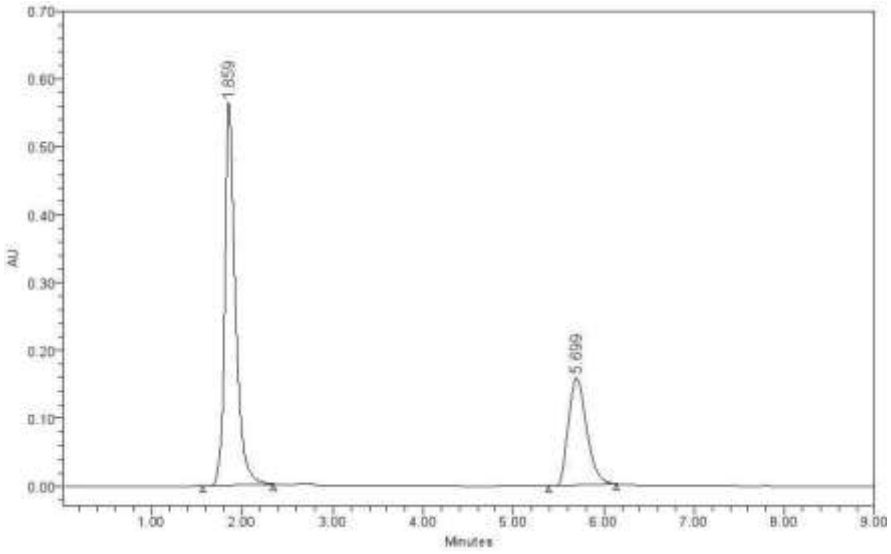

Figure 3: Chromatogram of Marketed Preparation of Candesartan cilexetil and Hydrochlorothiazide

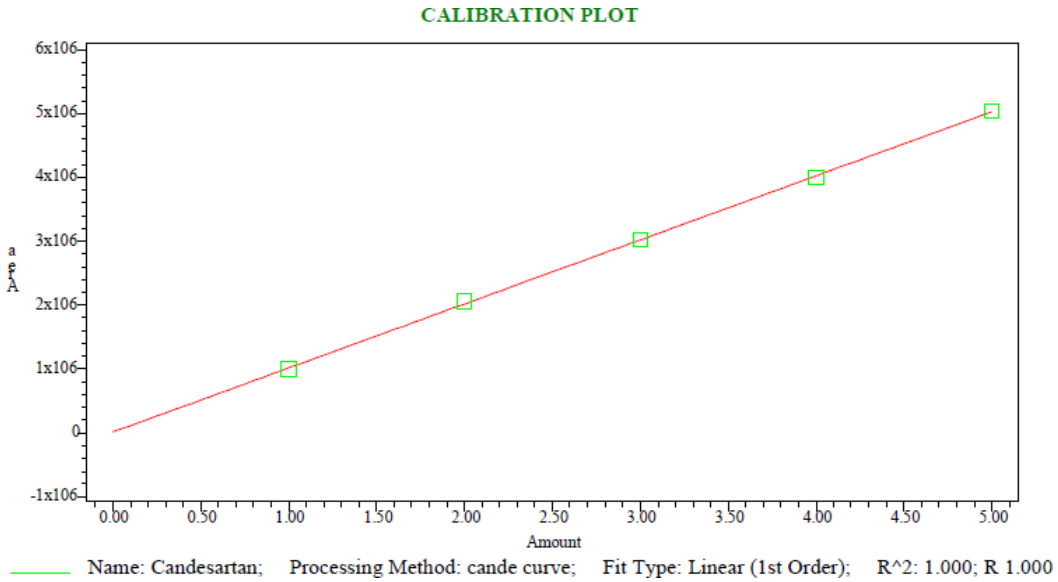

Figure 4a: Calibration plot of Candesartan cilexetil

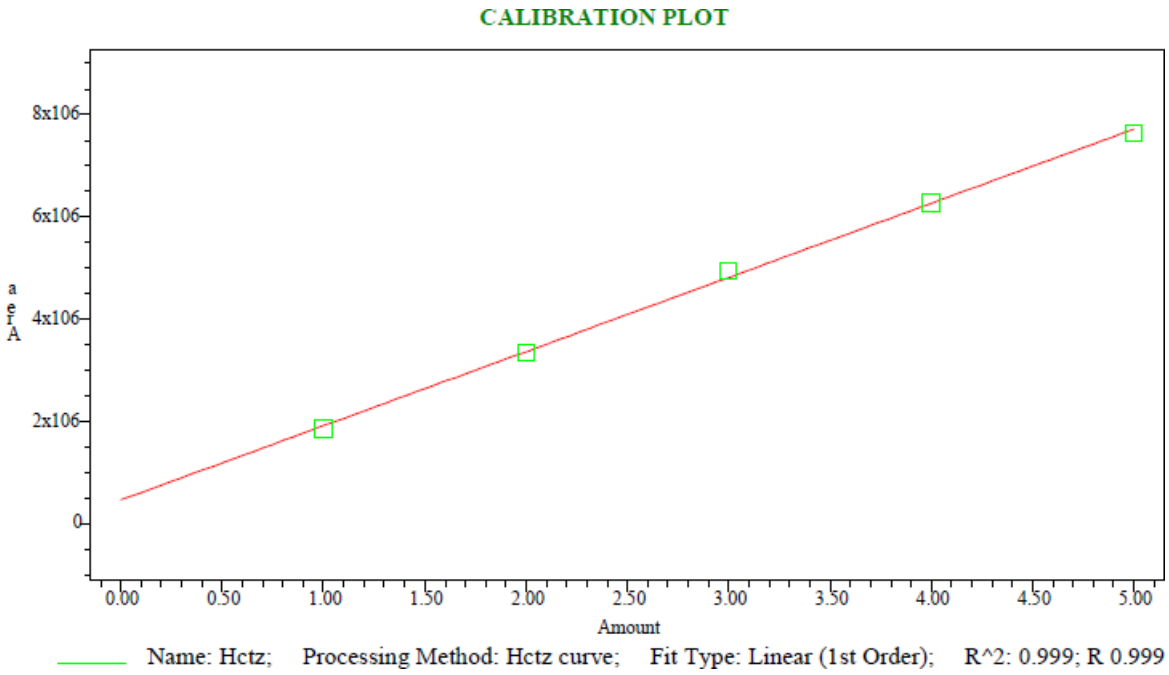

Figure 4b: Calibration plot of Hydrochlorothiazide

ble results were achieved by the use of proposed chromatographic conditions. The proposed method is simple, rapid and statistically validated for its accuracy. Hence no interfering peaks were found in the chromatograms indicating that anyone excipient is not interfered during analysis of these drugs.

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