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"VALIDATED ANALYTICAL METHOD DEVELOPMENT AND STUDY OF DEGRADATION PROFILING OF CANDESARTAN AND HYDROCHLOROTHIAZIDE BY QBD APPROCH"

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Abstract:

RP-HPLC method was developed for the estimation of Candesartan cilexetil and Hydrochlorothiazide in tablet dosage form with the help of Quality by Design (QbD) approaches. In this method concentration of each drug was obtained by using the absorptivity values calculated for drug wavelength 262 nm and solving the equation. The RP-HPLC method was performed C18 (250mm x 4.6 mm,) 2.5 µm particle size in gradient mode, and the sample was analysed using Acetonitrile 25.0 ml and 75.0 ml (pH 2.7 0.1% OPA with water) as a mobile phase at a flow rate of 0.7 ml/min and detection at nm. By the retention time for Candesartan cilexetil and Hydrochlorothiazide found 3.046 and 6.161 min respectively. Validation related the method is specific, rapid, accurate, precise, reliable, and reproducible. Calibration plots by both HPLC were linear over the 16-80 and12.5-62.5 µg/ml for Candesartan and Hydrochlorothiazide respectively, and recoveries from tablet dosage form were between 99.02 and 100.00 %. The method can be used for routine of the quality control in pharmaceuticals. The degradation profiling of Candesartan and Hydrochlorothiazide were also carried out.

Keywords: Hydrochlorothiazide, Candesartan cilexetil, Reverse phase HPLC, Quality by Design, Validation, Degradation.

Introduction:

Analytical chemistry is the science and practise of determining the composition of materials in terms of the elements that make up that composition. Medical product design, development, standardisation, and quality control all depend on analytical techniques. They play an equal role in pharmacokinetics and drug metabolism research. Both of these factors are critical in determining bioavailability and the length of a therapeutic response. Analytical equipment is crucial in the development and testing of new goods, as well as in the production of consumers and the environment [1]. Pharmaceutical analysis is an essential component of pharmaceutical research. The research analyst in the pharmaceutical analysis department is in charge of three key tasks:

- ✓ Development of an investigational technique for the product's raw materials, active components, and chemical intermediates.
- ✓ Develop inspective techniques for selective analysis of drugs, excipients, degradation products, and contaminants, as well as identification of degradation product, degradation route, and amount of degradation while kept at ambient and accelerated temperatures.
- ✓ Development of a method to investigate micro and semi micro amounts of medicines and their metabolites in a biotic system [2].

Method development in HPLC:

The process of showing that a developed chromatographic method is suitable for use in the development and manufacture of pharmaceutical drug ingredients and drug products is known as analytical method development [3].

Steps involve in Method Development [4-6]:

1 Understand the physicochemical properties of drug molecule.

1. Set up HPLC conditions.
2. Preparation of sample solution for method development.
3. Method optimization.
4. Validation of method.

Validation of Methods:

An analytical technique's validation (assessment of appropriateness) is a method for acquiring experimentally justified proof of the technique's capacity to generate findings with the requisite precision and accuracy. All analytical procedures employed in the creation of medicines and the determination of their quality features must be validated. It is not required to examine the appropriateness of techniques defined and detailed in the State Pharmacopoeia if the analyses are carried out with full adherence to the text of each individual article. In most other situations, especially when the drug composition, synthesis scheme, or analysis process is changed, it is important to re-evaluate the analytical techniques' appropriateness [3].

Parameters for Method Validation [3]:

The various validation parameters are

1. Accuracy
2. Precision (repeatability and reproducibility)
3. Linearity
4. Range
5. Limit of Detection (LOD)
6. Limit of Quantitation (LOQ)
7. Selectivity/ specificity
8. Robustness
9. Ruggedness
10. System Suitability Studies

QbD Approach:

Definition [ICH Q 8(R1)] A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management [7].

Definition [FDA PAT Guidelines, Sept. 2004] A system for designing, analyzing and controlling manufacturing through timely measurements (i.e. during processing) of critical quality and performance attributes of new and in-process materials and processes, with the goal of ensuring final product safety[8].

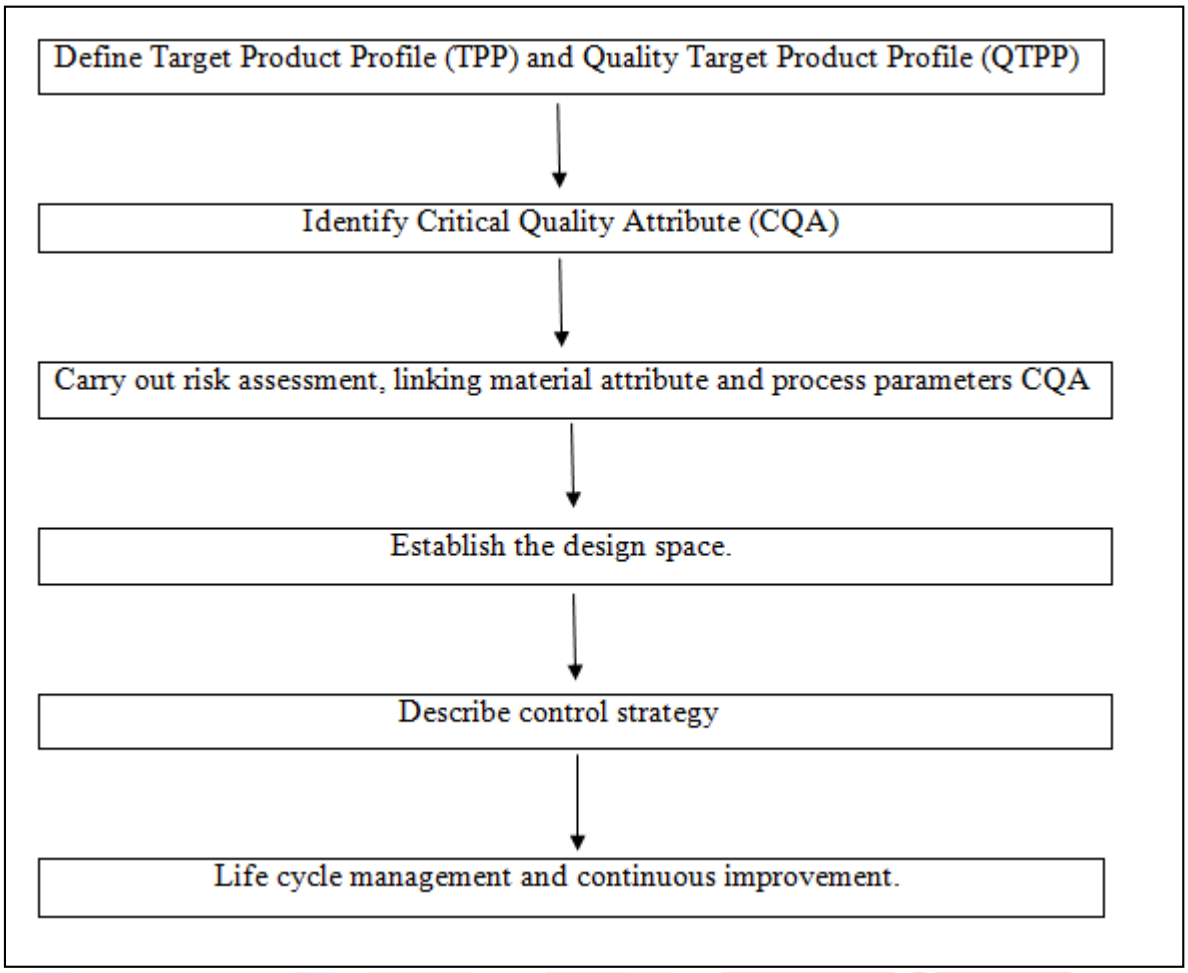

Figure No.1: Flow chart of QbD [9].

Force Degradation:

Forced degradation is a procedure in which drug products and drug substances are degraded under conditions that are more severe than accelerated settings, resulting in degradation products that may be examined to assess the molecule's stability. According to the ICH recommendation, stress testing is used to identify probable degradation products, which aids in determining the molecule's inherent stability and defining degradation routes, as well as to validate the stability indicating techniques utilized [10].

Drug Profile:

Candesartan cilexetil [11,12]:

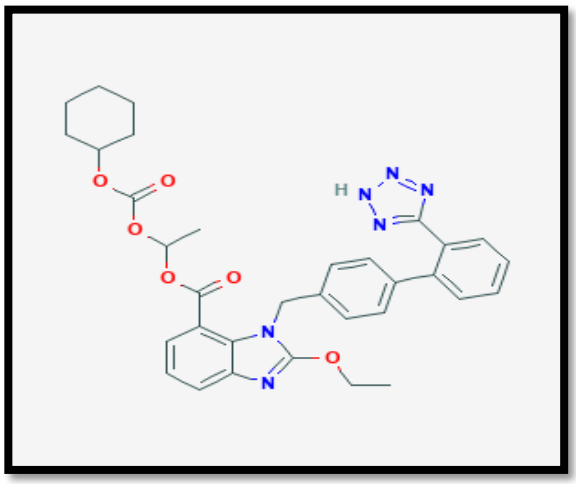

Figure no. 2: Chemical structure of Candesartan cilexetil.

Main structure activity:

IUPAC Name : 1-cyclohexyloxycarbonyloxyethyl 2-ethoxy-3-[[4-[2-(2*H*-tetrazol-5-yl) phenyl] phenyl] methyl] benzimidazole-4-carboxylate

Therapeutic : Angiotensin II receptor inhibitor.

Molecular weight : 610.7g/mol

Chemical formula : $C_{33}H_{34}N_6O_6$

Pka : 6

Melting point : 163-165° C

Solubility : Sparingly soluble in methanol, insoluble in water

Hydrochlorothiazide [13, 14]:

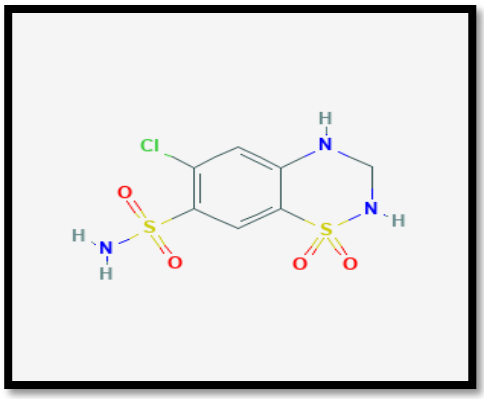

Figure no 3: Chemical Structure of Hydrochlorothiazide

Molecular Formula	: $C_7H_8ClN_3O_4S_2$
IUPAC Name	: 6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1λ^6,2,4 Benzothiadiazine-7-sulfonamide
Molecular Weight	: 297.7 g/mol
Melting Point	: 272-73 ° C
Solubility	: Slightly or very slightly soluble in water; sparingly soluble in alcohol; soluble in acetone; freely soluble in dimethylformamide.
Plasma half-life	: 5.6 - 14.8 hours

Material and Methods:

Chemicals, reagents & Instruments

Candesartan and Hydrochlorothiazide were obtained from R.S.I.T.C. Jalgaon. Orthophosphoric acid of HPLC grade from Avantor performance material India Ltd. Thane. Acetonitrile, Methanol and Water of HPLC grade from Merck speciality Ltd. Mumbai. Other chemicals used were of analytical grade. The analysis of the drug was carried out on Agilent (S.K.) Gradient System DAD Detector. Equipped with Reverse Phase (Agilent) C18 column (4.6mm x 250mm; 2.5µm), a SP930Dpump, a 20µl injection loop and UV730D (DAD) Absorbance detector and running Chemstation software.

Methods:

Preparation of standard solution:-

- **Preparation of std. Candesartan cilexetil solution: (Stock I)**

An accurately weighed quantity, 16 mg of Candesartan (CND) was dissolved in methanol in a 10 ml volumetric flask and volume made up to 10.0 ml to produce a solution of 1600 ug/ml. From the freshly prepared standard stock solution (1600ug/ml), 0.1ml stock solution was pipetted out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration of 16ug/ml.

- **Preparation of std. Hydrochlorothiazide solution: (Stock II)**

An accurately weighed quantity, 12.5 mg of Hydrochlorothiazide (HCZ) was dissolved in methanol in 10 ml volumetric flask and volume made up to 10.0 ml to produce a solution of 1250 ug/ml From the freshly prepared standard stock solution (1250 ug/ml), 0.1 ml stock solution was pipetted out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 12.5 ug/ml.

- **Preparation of std. Candesartan cilexetil and Hydrochlorothiazide solution: (Stock III)**

From the freshly prepared standard stock solution (1600 & 1250ug/ml), 0.1 ml stock solution was pipetted out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 16 -80& 12.5 – 62.5 ug/ml.

Results and Discussion:

Mobile phase optimization

After the selection of suitable mobile phase, it was then optimized for its reproducibility, sensitivity & accuracy. The optimized parameters for selected method are as below.

The final chromatographic conditions selected were as follow:

DAD Detector Agilent (S.K) Gradient System

- Analytical column : (Agilent) C18 column (4.6mm x 250mm)
- Injection volume : 20µl
- Flow rate : 0.7 ml/min
- Mobile phase : ACN +0.1%OPA(25+75 % v/v)
- Detection : 262 nm
- Run Time : 15 min

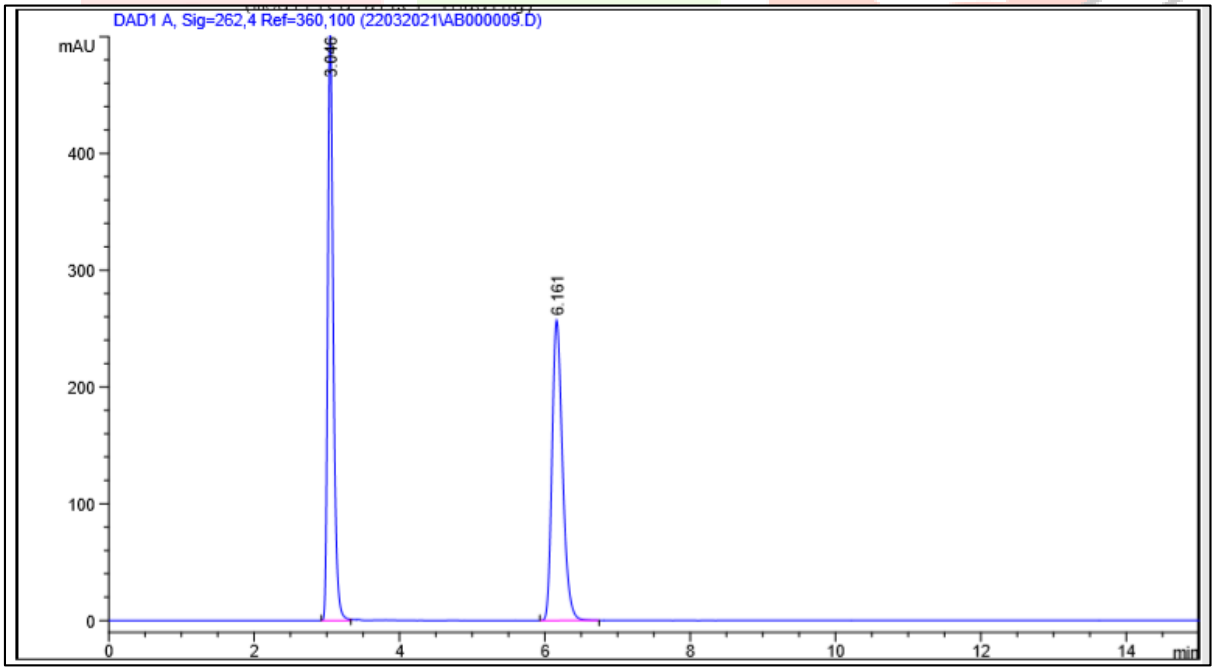

Figure No. 4: Chromatogram of standard Combination of Candesartan cilexetil and Hydrochlorothiazide

Design of Experiments

Preliminary Screening and Optimization Data Analysis Preliminary experiments were performed by using Taguchi screening Method to identify the critical factors and to set their levels (maximum and minimum) for the experimental design. In this step The following parameters were investigated: selection of a chromatographic Column (C8 and C18), column temperature, mobile phase (ratio of ACN : MEOH :buffer), concentration of the buffer if present In the mobile phase, buffer pH, injection volume, mode of flow (isocratic/gradient) as well as determining the ideal flow rate. Based on the results obtained from the Taguchi screening, multiple linear regression analysis (MLRA) was applied for the studied Design using Design Expert® software version 9.0.02 to fit the full second-order polynomial equations with added Interaction terms. The method chosen to optimize separation of Candesartan cilexetil and Hydrochlorothiazide with the shortest analysis time was Box–Behnken design (BBD) with three replicates at the centre point (middle level). The independent variables were investigated and their low, Medium, and high levels described in Table below the QbD trials. The evaluated Responses (dependent variables) were the no. of theoretical plates (Y1), assay (Y2), and tailing factor (Y3). Prediction of the optimum Composition was carried out using overlay plotting, brute Force method, and numeric approach of desirability function. Overlay Plot (i.e., combined contour plot) option in the software was Also embarked upon to locate the optimum composition. Within This optimal area, an optimum chromatographic condition was located by trading off different responses. The prognosis of the Optimum analytical condition was also conducted using numerical Optimization technique/with help of Design Expert software. The Box-Behnken Design Validation Fifteen runs were done, selected from grid search data, prepared as per the chosen composition(s), and evaluated for the critical Quality attributes (CQA), viz. Number of theoretical Plates (TP), Assay, and tailing factor (TF). The predicted and observed Responses were compared, and linear correlation plots were constructed Percent bias (error) was calculated with respect to the Observed responses and the residual plots were also constructed For TP, assay, and TF.

Method Validation:

Linearity:

Linearity of Candesartan cilexetil and Hydrochlorothiazide was observed in both methods the range of 16-80 &12.5-62.5ug/ml. Detection wavelength used was 262 nm.

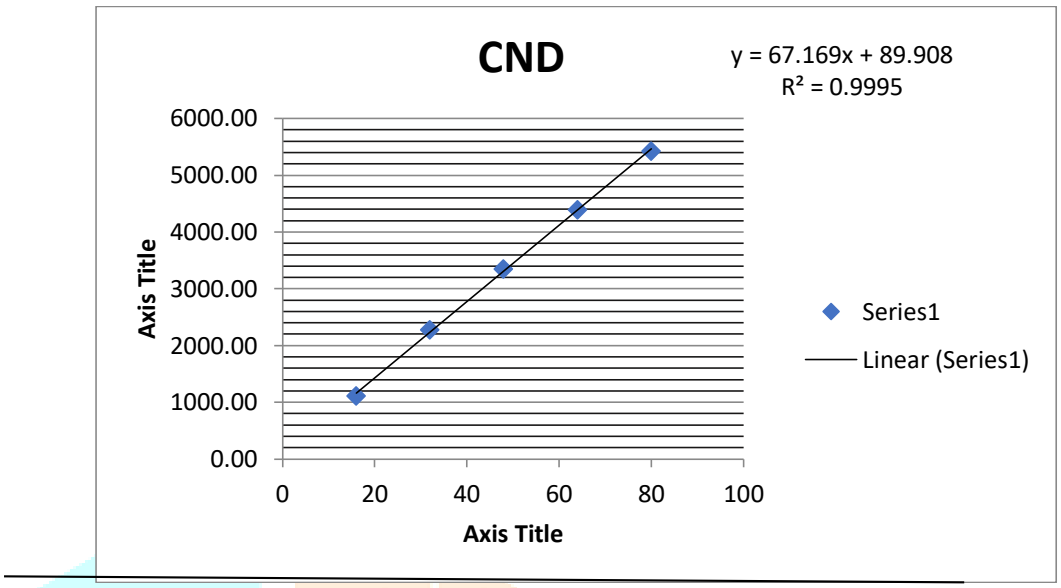

Figure no. 5: Calibration curve of Candesartan cilexetil

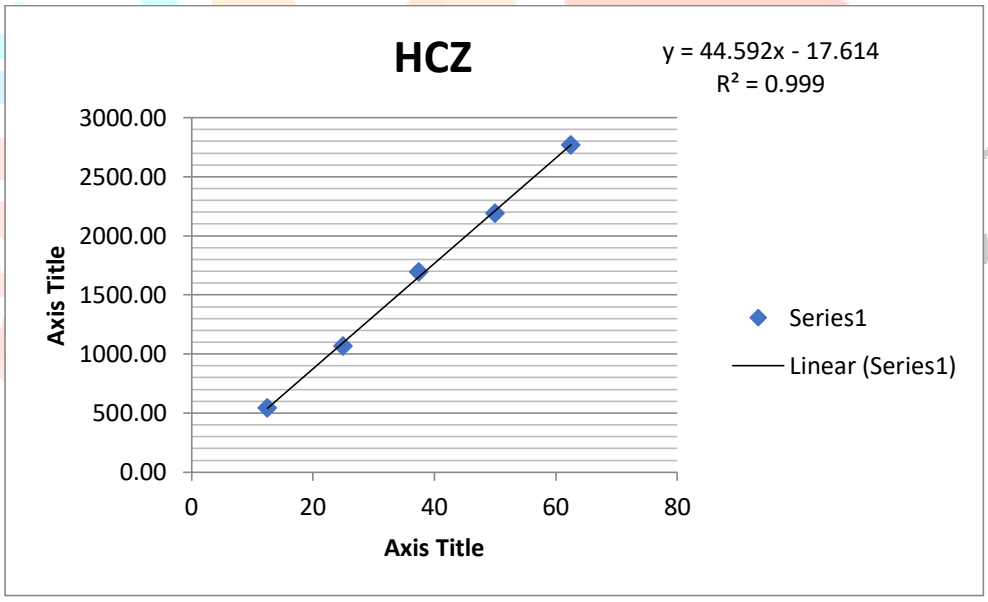

Figure no. 6: Calibration curve of Hydrochlorothiazide

Accuracy (recovery):

Recovery studies were performed to validate the accuracy of developed method. To pre analyzed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Table no. 1: Result of recovery data for Candesartan and Hydrochlorothiazide

METHOD	Drug	Level (%)	Amt. taken (ug/ml	Amt. Added (ug/ml	Absorbance Mean* ± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean *± S.D.
RP-HPLC Method	CND	80%	32	25.6	28.76±0.029	12.76±0.029	99.66±0.23
		100%	32	32	32.33±0.102	16.33±0.102	102.08±0.64
		120%	32	38.4	35.45±0.114	19.45±0.114	101.29±0.59
	HCTZ	80%	25	20	22.33±0.067	9.83±0.067	98.34±0.67
		100%	25	25	25.16±0022	12.66±0.022	101.28±0.17
		120%	25	30	27.18±0.042	14.68±0.042	97.90±0.28

Accuracy of RP-HPLC method and UV Spectrophotometric method is ascertained by recovery studies performed at different levels of concentrations (80%, 100% and 120%). The % recovery was found to be within 98-101%.

System suitability parameters: (Repeatability)

To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of Candesartan and Hydrochlorothiazide system suitability parameters were studied. The results are shown below.

Table No. 2: Repeatability studies on RP-HPLC for Candesartan and Hydrochlorothiazide

METHOD	Concentration of Candesartan and Hydrochlorothiazide (mg/ml)	Peak area	Amount found (mg)	% Amount found
HPLC CND	64	4371.86	63.65	99.45
METHOD	64	4356.96		
	Mean	4364.42		
	SD	10.53		
	%RSD	0.24		
	50	4197.77	50.34	100.69
HPLC HCTZ	50	4176.54		
METHOD	**Mean**	4187.16		
	SD	15.01		
	%RSD	0.36		

Repeatability studies on RP-HPLC method for Candesartan and Hydrochlorothiazide was found to be, the %RSD was less than 2%, which shows high percentage amount found in between 98% to 102% indicates the analytical method that concluded.

Precision:-

The method was established by analyzing various replicates standards of Candesartan and hydrochlorothiazide. All the solution was analyzed thrice in order to record any intra-day & inter-day variation in the result that concluded.

Table no.3: Result of Intraday and Inter day Precision studies on RP-HPLC and UV method for Candesartan and Hydrochlorothiazide

METHOD	Drug	Conc. (µg/ml)	Interday Precision		Intraday Precision	
			Mean± SD	% Amt Found	Mean± SD	% Amt Found
Rp-HPLC METHOD	CND	32	32.39±1.42	101.23	32.36±0.26	101.14
		48	48.17±11.73	100.36	48.33±11.53	100.69
		64	65.14±6.29	101.78	64.59±76.73	100.92
	HTZ	25	24.99±22.66	99.96	25.06±34.44	100.25
		37.5	37.94±6.26	101.16	38.02±11.53	101.40
		50	51.24±7.09	102.48	50.96±59.88	101.93

*Mean of each 3 reading for RP-HPLC method

Intraday and Inter day Precision studies on RP-HPLC and UV method for Candesartan and Hydrochlorothiazide which shows the high precision % amount in between 97% to 101% indicates to analytical method that concluded.

Robustness:

Robustness Study of Candesartan cilexetil:

The changes were did flow rate (±1 ml/ min^{-1}), PH of mobile phase composition (±1 ml/ min), and Wavelength (±1 ml/ min). %RSD for peak area was calculated which should be less than 2%.the result shown in analytical method that concluded.

Robustness Study of Hydrochlorothiazide:

The changes were did flow rate (±1 ml/ min), PH of mobile phase composition (±1 ml/ min), and Wavelength (±1 ml/ min). %RSD for peak area was calculated which should be less than 2%. The result shown in analytical method that concluded**.**

LOD AND LOQ

The LOD of Candesartan cilexetil and Hydrochlorothiazide was found to be 0.2037 (ug/mL) and 0.5654 (ug/mL), analytical method that concluded

The LOQ of Candesartan cilexetil and Hydrochlorothiazide was found to be 0.6173 (ug/mL) and 1.71 (ug/mL), analytical method that concluded.

Analysis of tablet formulation:

Table no. 4. Analysis of marketed formulation

Assay	Drug	Amt. Found	%Label Claim	SD	%RSD
RP-HPLC Method	CND	48.07	100.14	6.648	0.200
	HCTZ	37.86	100.96	5.290	0.167

Analysis of marketed formulation were also % Label Claim was found to be 99-100% Satisfactory are concluded.

Degradation study

Table no. 5. Result of Degradation Study of Candesartan and Hydrochlorothiazide

Parameters	Drug name	Area	% assay	% degradation
Acid	HCTZ	3695.46	91.62	8.38
	CND	2682.07	89.01	10.99
Base	HCTZ	3432.52	95.95	4.05
	CND	3279.06	97.88	2.15
Oxidative	HCTZ	3660.74	84.19	15.81
	CND	2567.58	81.28	18.72
Neutral	HCTZ	3801.97	98.15	1.85
	CND	2717.61	99.03	0.97

Conclusions:

Simple, rapid, accurate and precise RP-HPLC have been developed and validated for the routine analysis of Candesartan cilexetil and Hydrochlorothiazide in API and tablet dosage forms. Both methods are suitable for the simultaneous determination of Candesartan cilexetil and Hydrochlorothiazide in Single-component formulations without interference of each other. The developed methods are recommended for routine and quality control analysis of the investigated drugs in two component pharmaceutical preparations. The amount found from the proposed methods was in good agreement with the label claim of the formulation. Also the value of standard deviation and coefficient of variation calculated were satisfactorily low, indicating the suitability of the proposed methods for the routine estimation of tablet dosage forms.

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