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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS DETERMINATION OF CANDESARTAN CILEXITIL AND HYDROCHLOROTHAIZIDE

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Abstract

A simple, rapid, precise, and stability-indicating reverse phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the simultaneous estimation of Candesartan cilexetil and Hydrochlorothiazide in pharmaceutical dosage forms. Chromatographic separation was achieved using a Symmetry C18 (150 × 4.6 mm, 3.5 µm) column with an isocratic mobile phase comprising Methanol: pH 3 phosphate buffer (70:30 % v/v) at a flow rate of 1 mL/min and detection at 240 nm. The method was validated according to ICH guidelines for system suitability, linearity, precision, accuracy, robustness, LOD, and LOQ. The calibration curves were linear over the concentration ranges of 16–80 ppm for Candesartan cilexetil and 25–150 ppm for Hydrochlorothiazide with satisfactory correlation coefficients. The %RSD values were below 2%, and recovery studies showed results within acceptable limits, confirming accuracy and precision. The developed method was found to be sensitive, reliable, cost-effective, and suitable for routine quality control analysis.

INTRODUCTION

Candesartan Cilexetil is chemically known as (±)-1-Hydroxyethyl2-ethoxy-1-[p-(o-1H-tetrazol-5ylphenyl)benzyl]-7-benzimidazolecarboxylate, cyclohexyl carbonate (ester), It is a prodrug and is hydrolysed to Candesartan during absorption from the gastrointestinal tract. Candesartan is a selective AT1 subtype angiotensin II receptor antagonist [1,2]. Hydrochlorothiazide is chemically known as 6-chloro-1,1-dioxo-3,4-dihydro-2H-1λ,2,4-benzothiadiazine-7-sulfonamide.It is diuretic medication used to treat high blood pressure and swelling due to fluid buildup. Other uses include diabetes insipid us, renal tubular acidosis, and to decrease the risk of kidney stones in those with high calcium level in the urine [3, 4].

The existing literature review indicates a gap in analytical methods for the simultaneous estimation of Candesartan cilexitil and Hydrochlorothiazide using RP-HPLC. Although spectrophotometry, HPLC, and HPTLC methods have been reported for these compounds individually or in combination with other dosage forms, there is a lack of simultaneous analysis methods. This underscores the need for a new analytical method to fulfill this specific requirement. The objective of the present work is to develop a novel, straightforward, rapid, accurate, efficient, and reproducible RP-HPLC method for the simultaneous determination of Candesartan cilexitil and Hydrochlorothiazide. The intention was to address the absence of a suitable method in the literature and provide a practical solution for analyzing these two compounds together.

MATERIALS AND METHOD

Chemicals and materials

Candesartan cilexitil and Hydrochlorothiazide working standard were received as gift sample from Spectrum Pharma Research Centre Hyderabad. HPLC grade Acetonitrile Methanol (Merck) and AR grade Potassium Hydrogen Phosphate, Orthophosphoric acid, Hydrochloric acid, Sodium Hydroxide, Hydrogen Peroxide (RANKEM) was used. Mili Q water used in mobile phase and diluent preparation.

Equipment

Candesartan and Hydrochlorothiazide were isolated using a Waters alliance e2695 model HPLC with a PDA (photodiode array) detector and the chromatographic program Empower 2.0 [5].

Chromatographic Conditions

Using a symmetry C18 (150x4.6mm, 3.5) column, chromatographic separation was performed in an isocratic mode at room temperature [6]. The mobile phase is an isocratic mixture of Methanol: pH 3 phosphate buffer (70: 30 % v/v) with a flow rate of 1 ml/min with a detection wavelength of 240nm. The injection volume was 10 µl, with a 6- minute run time.

Preparation of standard solution

Working standards of 12.5 mg Hydrochlorothiazide and 8 mg Candesartan cilexitil was correctly weighed. These standards were put in a 10 mL volumetric flask, filled with diluents, and sonicated for 10 minutes to dissolve the contents before being made up to the mark with the same diluents. Using the diluents, dilute 0.6 mL of the above solution to 10 mL.

Preparation of sample stock solution

In a 10 ml volumetric flask, measure correctly the 59.8 mg equivalent weight of Hydrochlorothiazide and Candesartan cilexitil sample. Add about 7 mL of diluents, sonicate for 30 minutes to fully dissolve the contents, and makeup up the mark with diluents. Using a 0.45 syringe filter, filter the solution.

Validation of the Proposed Method

The developed method for Hydrochlorothiazide and Candesartan cilexitil was validated according to ICH guidelines of the parameters like System suitability, linearity, precision, accuracy, robustness, repeatability [7, 8].

i. System suitability

System suitability was studied under each validation parameter by injecting six replicates of the standard solution. The system suitability parameters were shown in Table 1.

Table 1. System suitability parameters for Hydrochlorothiazide and Candesartan cilexitil

S.No	Drug	Flow Rate(ml/min)	System Suitability Results	
			USP Plate Count	USP Tailing
1	Candesartan cilexitil	0.9	7515.5	0.9
2		1.0	10026.7	1.0
3		1.1	5948.0	1.0
4	Hydrochlorothiazide	0.9	8573.5	1.0
5		1.0	12458.5	1.2
6		1.1	6114.5	1.1

ii. Precision

Repeatability

The precision study was performed for five injections of Candesartan cilexitil and Hydrochlorothaizide. Each standard injection was injected into chromatographic system. The area of each Standard injection was used for calculation of % RSD.

Table 2. Precision table of Candesartan cilexitil

Injection	Area
Injection-1	1475698
Injection-2	1461561
Injection-3	1481379
Injection-4	1467049
Injection-5	1472628
Average	1471663
Standard Deviation	7664.08
%RSD	0.52

Table 3. Precision table of Hydrochlorothaizide

Injection	Area
Injection-1	3045768
Injection-2	3030853
Injection-3	3063519
Injection-4	3065127
Injection-5	3099001
Average	3060854
Standard Deviation	25535.28
%RSD	0.83

iii. Linearity

The linearity study aimed to establish the linear relationship between concentration and response for both compounds. Concentrations ranging from 25 ppm to 150 ppm were employed for Hydrochlorothiazide, while concentrations ranging from 16 ppm to 80 ppm were used for Candesartan cilexitil. Each concentration level was injected into the chromatographic system, and the resulting areas were utilized to calculate the correlation coefficients.

The calibration graphs visually illustrating the linear relationship are displayed in Figure. These findings attest to the suitability of the method for accurately quantifying Candesartan cilexitil and Hydrochlorothiazide over a range of concentrations in the specified pharmaceutical formulations.

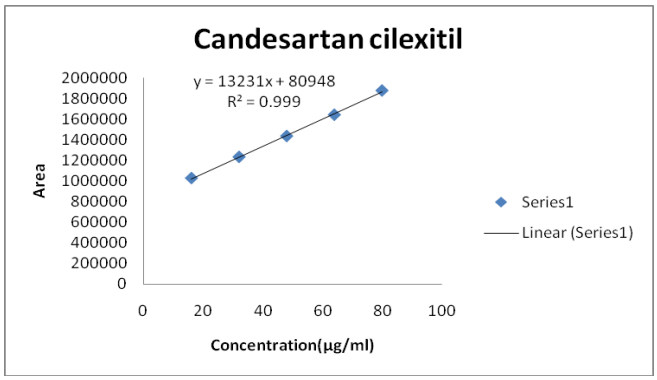

Fig. 1 Calibration graph for Candesartan cilexitil

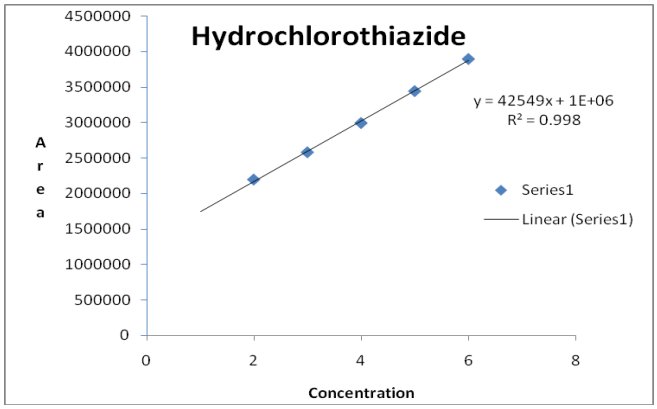

Fig. 2 Calibration graph for Hydrochlorothiazide

iv. Robustness

The robustness was performed for the flow rate variations from 0.4ml/min to 0.6ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Candesartan cilexitil and Hydrochlorothaizide. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%.

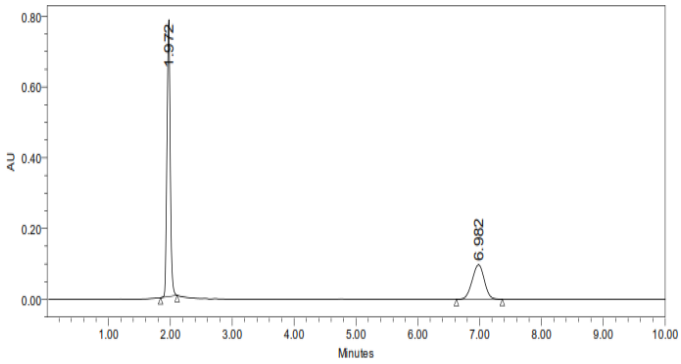

Fig. 3 Chromatogram showing more flow rate 0.8ml/min

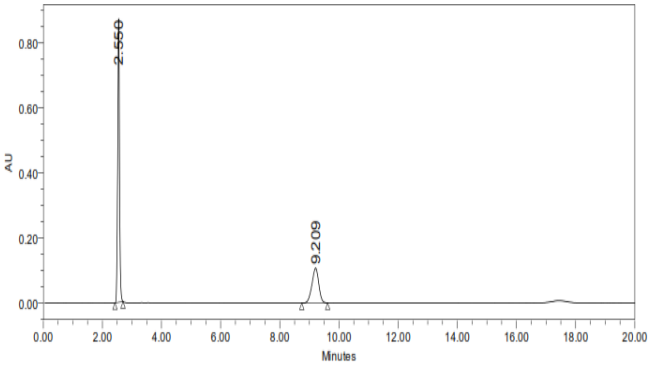

Fig. 4 Chromatogram showing less flow rate 1.2ml/min

v. Accuracy

In the accuracy study, three concentration levels of 50%, 100%, and 150% were employed for both Candesartan cilexitil and Hydrochlorothiazide. Each concentration level was injected in triplicate into the chromatographic system. The resulting areas of the peaks for each concentration level were utilized to calculate the percentage recovery. These accuracy assessments provide valuable insights into the method's ability to accurately and reliably determine the concentration of Candesartan cilexitil and Hydrochlorothiazide within the specified ranges, further confirming its suitability for pharmaceutical analysis.

Table 4. Accuracy results of Candesartan cilexitil

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	%Recovery	Mean Recover
50%	765624	4.25	4.30	101.2%	
100%	1508055	8.25	8.48	101.5%	
150%	2204983	12.2	12.39	101.6%	101.4%

Table 5. Accuracy results for Hydrochlorothiazide

% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1726242	7.05	7.1	101.9%	
100%	3187170	13.1	13.2	101.3%	
150%	4521881	18.5	18.8	101.8%	101.7%

vii. LOD and LOQ

The LOD and LOQ values for Candesartan cilexitil and Hydrochlorothiazide meet the acceptance criteria. This demonstrates that the analytical method is highly sensitive and reliable for detecting and quantifying Candesartan cilexitil and Hydrochlorothiazide at low concentrations.

Table 6. Sensitivity parameters (LOD & LOQ)

Name of drug	LOD(µg/ml)	LOQ(µg/ml)
Candesartan cilexitil	2.17	6.60
Hydrochlorothiazide	0.0372	0.112

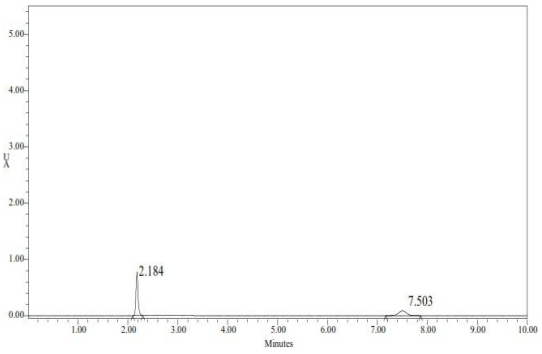

Fig 5. Chromatogram for LOD

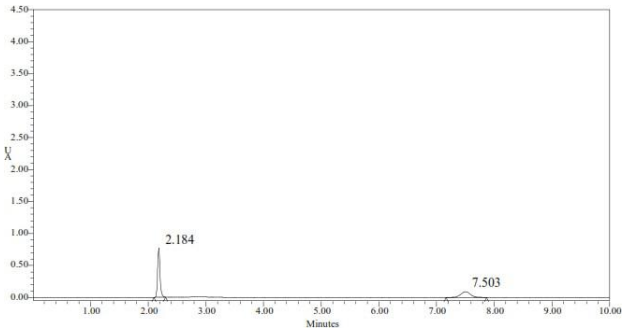

Fig 6. Chromatogram for LOQ

CONCLUSION

The HPLC approach that has been devised for the measurement of certain medications is quick, easy, precise, accurate, reliable, and cost-effective. The solvents and mobile phase are cheap, easy to make, dependable, sensitive, and quick to prepare. The sample recoveries were consistent with the promises made on the labels, which means that the formulation receivers did not interfere with the estimate. This means that the medications may be routinely tested in labs. It has been concluded that the suggested methods, which are both simple and brief, would be the most useful for analysis because the system validation parameters of the HPLC method have demonstrated satisfactory, accurate, and repeatable results (without recipient interference, of course). Results showed that the RP-HPLC stability indicating assay technique was free of interference with degradation products and placebos, and it was also easy to use, accurate, exact, and specific. You may use them for your regular Hydrochlorothiazide analyses.

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Nil

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

INFORM CONSENT AND ETHICAL CNSIDERATIONS

Not applicable

AUTHOR CONTRIBUTIONS

Both are contributed equally.

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