International Journal of Chemistry and Pharmaceutical Sciences

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Analytical Method Development and Validation for the Simultaneous Estimation of Candesartan cilexitil and Hydrochlorothiazide by RP-HPLC Method in Bulk and Pharmaceutical Dosage Form

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A B S T R A C T

The chromatographic conditions were successfully developed for the separation of Candesartan cilexitil and Hydrochlorothiazide by using zodiac sil C_{18} Column (150mm x 4.6mm)5µm, flow rate was 1ml/min, mobile phase ratio was Methanol: Phosphate buffer P^H 3 (70:30 v/v), detection wavelength was 240 nm. The Spectroscopic method was done in solvent using methanol and the instrument lab India 3000+ with UV win software. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, UV detector, Empower-software version 2. The retention times for Candesartan cilexitil and Hydrochlorothiazide were found to be 2.170 min and 7.025 min. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Candesartan cilexitil and Hydrochlorothiazide was found in the concentration range 16 ppm-80 ppm and 25ppm -125 ppm and correlation coefficient (r^2) was found to be 0.999 and 0.999 respectively, % recovery was found to be 101.7% and 102.0% respectively. %RSD for repeatability and precision was found to be <2. LOD values for candesartan cilexitil, Hydrochlorothiazide were found to be 2.17 and 6.60 respectively and LOQ values for candesartan cilexitil, Hydrochlorothiazide were found to be 6.60 and 0.1125 respectively.

Keywords: Candesartan cilexitil, Hydrochlorothiazide, HPLC

A R T I C L E I N F O

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Article History: Received 29 March 2016, **Accepted** 31 May 2016, **Available Online** 27 June 2016

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Manuscript ID: IJCPS3071

PAPER-QR CODE

Citation: Idavalapati Chandana, *et al.* Analytical Method Development and Validation for the Simultaneous Estimation of Candesartan cilexitil and Hydrochlorothiazide by RP-HPLC Method in Bulk and Pharmaceutical Dosage Form. *Int. J. Chem, Pharm, Sci.*, 2016, 4(6): 251-256.

1. Introduction

Analytical methods: Methods are developed for new products when no official methods are available. Alternate methods for existing (non-pharmacopoeial) products are developed to reduce the cost and time for better precision and ruggedness [1, 2]. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available

Description of the Various Analytical Methods

Titrimetric and gravimetric method of analysis is suitable when the sample is present in pure form or when no interference is observed in the mixture with other materials [3]. Ultraviolet and visible spectrometric method is suitable when no Interference is observed in the mixture [4]. HPLC and GC methods are more advantageous than the above due to their capability in separating organic mixtures and quantitative estimations. AAS is used mainly for quantitative estimation in ppm and ppb levels of elements [5]. Infra-red spectroscopy though mainly used for qualitative analysis can be used for quantitative estimation also. Out of all the above methods, thin layer chromatography plays a very important role in analysis due to its adaptability, flexibility, and cost and time. It can be used both for qualitative and quantitative determination. After separation spots can be scanned with the help of a scanner and quantitative measurement can be made [6].

Chromatography:

Chromatography is a technique used in analytical chemistry to separate and identify components of mixtures. The name comes from the Greek term for "color writing" because this method was originally used to separate colored samples. The advent of high-performance liquid chromatography (HPLC).in this system pressure is applied to the column, forcing the mobile phase through at much higher rate[7]. The pressure is applied using a pumping system. The action of the pump is critical, since it must not pulsate and mix up the sample being separated in the solvent, causing it to lose resolution [8]. Development of pumps has proceeded quite quickly over the last several years, and now it is possible to achieve good resolution under the conditions required for HPLC.

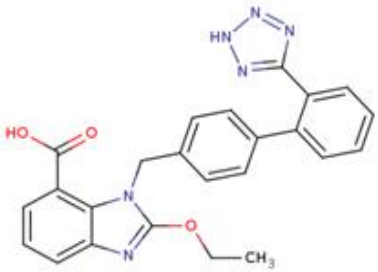

Figure 1: Candesartan cilexitil

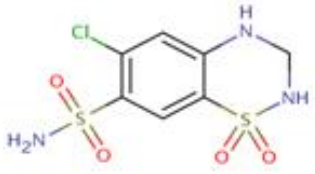

Figure 2: Hydrochlorothiazide

2. Experimental

Apparatus: The instrument used for the study was Waters HPLC Auto Sampler, Separation module 2695, PDA detector with Empower-software version-2.

Reagents and Materials

The solvents used were Methanol, Ortho phosphoric acid, Acetonitrile, Potassium dihydrogen ortho phosphate and HPLC Water.

Selection of detection wavelength:

10 mg of Candesartan cilexitil and Hydrochlorothaizide was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Candesartan cilexitil and Hydrochlorothaizide. The isobestic point was taken as detection wavelength [9].

Selection of mobile phase

- pH 3 phosphate buffer : Methanol (70 : 30% v/v)
- Buffer pH should be between 2 to 8.
- Below 2: siloxane linkages are cleaved.
- Above 8: dissolution of silica.
- pH selected: 3 ±0.05
- pH controls the elution properties by controlling the ionization characteristics.
- Reasons: To decrease the retention and improve separation. Good Response, Area, Tailing factor, Resolution [10].

Optimization Chromatographic trials for Simultaneous Estimation of Candesartan cilexitil, Hydrochlorothiazide by RP- HPLC.

Optimization Chromatographic conditions

Column : Zodiac sil C18 column (4.6×150mm)5µ

Mobile phase ratio: Methanol: pH 3 phosphate buffer (70: 30 % v/v)

Detection wavelength	:	240 nm
Flow rate	:	1.0ml/min
Injection volume	:	20µl
Column temperature	:	Ambient
Auto sampler temperature	:	Ambient
Run time	:	10min
Retention time	:	2.170 and 7.280 mins

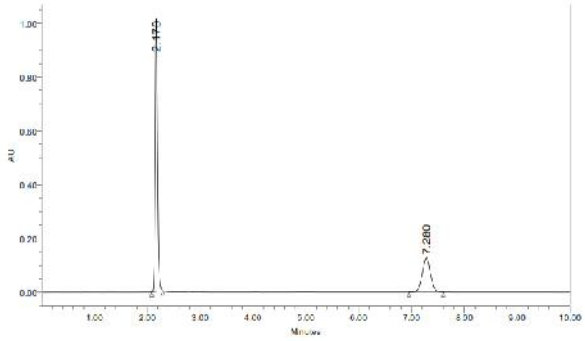

Figure 3: Optimization Chromatogram

Observation:

The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks, so it is taken as final method.

Procedure

Preparation of phosphate buffer

2.95 grams of $KH_2PO_4$and 5.45 grams of K_2HPO_4 was weighed and taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water and pH was adjusted to 3 with ortho phosphoric acid. The resulting solution was sonicated and filtered.

Preparation of mobile phase

Mix a mixture of above buffer 300 ml (30%) and 700 ml of methanol (HPLC grade-70%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

Candesartan cilexitil and Hydrochlorothiazide standard preparations: Accurately weigh and transfer 12.5 mg & 8 mg of Hydrochloro thiozide and Candesartan working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)Further pipette 0.6ml of Hydrochloro thiozide & Candesartan the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Sample solutions preparation

Accurately weigh and transfer 59.8 mg of Hydrochloro thiozide and Candesartan Tablet powder into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.6ml of Hydrochloro thiozide& Candesartan the above stock solution into a10ml volumetric flask and dilute up to the mark with diluents [11, 12].

3. Results and Discussion

Method Validation Parameters

1. Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by Injecting blank.

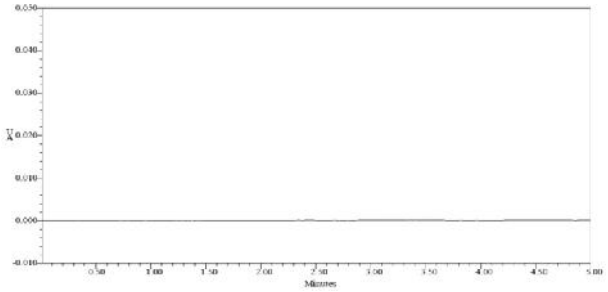

Figure 4: Chromatogram of Blank

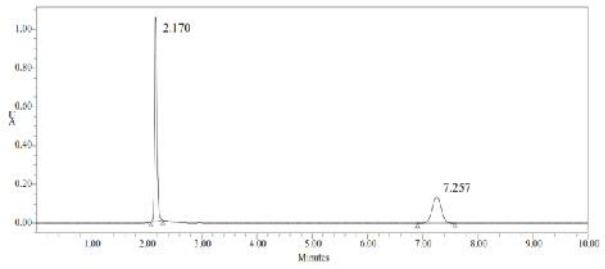

Figure 5: Chromatogram of Sample

2. Linearity

The linearity study was performed for the concentration of 25 ppm to 150 for Hydrochlorothaizide and 16ppm to 80ppm for Nebiolol. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient [13].

Acceptance criteria: Correlation coefficient should be not less than 0.999.

3. Range

Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 16µg/ml-80µg/ml and 25µg/ml to 125µg/ml of Candesartan cilexitil and Hydrochlorothaizide respectively.

4. Accuracy

Preparation of standard stock solution

Accurately weigh and transfer 12.5 mg &8 mg of Hydrochloro thiozide and Candesartan working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)Further pipette 0.6ml of Hydrochloro thiozide&Candesartan the above stock solution into a10ml volumetric flask and dilute up to the mark with diluents [14].

Preparation of sample solutions

For preparation of 50% solution (with respect to target assay concentration)

Accurately weigh and transfer 7mg of Hydrochloro thiozide and 4.25mg of Candesartan working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock Solution). Further pipette 0.6ml of Hydrochloro thiozide&Candesartan the above stock solution into a10ml volumetric flask and dilute up to the mark with diluent

For preparation of 100% solution (with respect to target assay concentration)

Accurately weigh and transfer 13.1mg of Hydrochloro thiozide and 8.25mg of Candesartan working standards into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).Further pipette 0.6ml of Hydrochloro thiozide & Candesartanthe above stock solution into a10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 150% solution (with respect to target assay concentration)

Accurately weigh and transfer18.5mg of Hydrochloro thiozide and 12.2mg of Candesartan working standards into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)

Further pipette 0.6ml of Hydrochloro thiozide & Candesartan the above stock solution into a10ml volumetric flask and dilute up to the mark with diluent

Procedure

The standard solutions of accuracy 50%, 100% and 150%were injected into chromatographic system. Calculate the amount

found and amount added for Candesartan cilexitil and Hydrochlorothaizide and calculate the individual % recovery and mean % recovery values.

Acceptance criteria

The % recovery for each level should be between 98.0 to 102.0%

5. Precision

Accurately weigh and transfer 12.5 mg &8 mg of Hydrochloro thiozide and Candesartan working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution).

Further pipette 0.6ml of Hydrochloro thiozide & Candesartan the above stock solution into a10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits..

Acceptance Criteria: The % RSD for the area of five standard injections results should not be more than 2%**.**

Validation of the Method

Linearity

The linearity study was performed for the concentration of 25 ppm to 150 for Hydrochlorothaizide and 16ppm to 80ppm for Nebiolol and chromatograms are shown below.

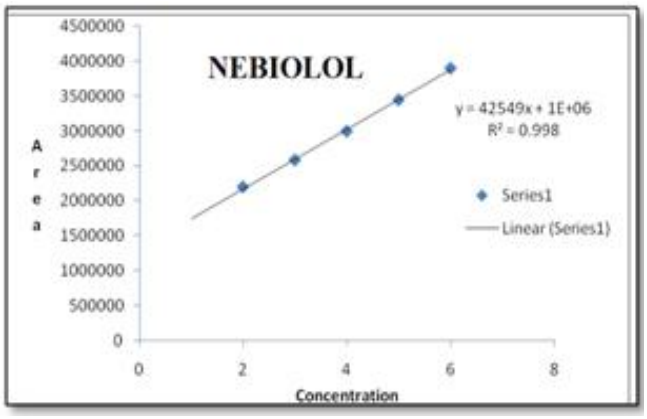

Figure 6: Calibration graph of Candesartan cilexitil

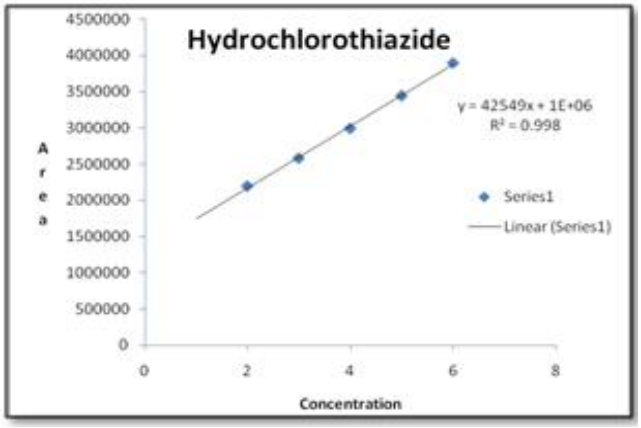

Figure 7: Calibration graph of Hydrochlorothiazide

Table 1: Calibration data of Candesartan cilexitil

S.No	Linearity Level	Concentration	Area
1	I	16ppm	1027461
2	II	32ppm	1233566
3	III	48ppm	1437030
4	IV	64ppm	1644336
5	V	80ppm	1880590
Correlation Coefficient			0.999

Table 2: Calibration data of Hydrochlorothiazide

S.No	Linearity Level	Concentration	Area
1	I	25ppm	2201022
2	II	50ppm	2585033
3	III	75ppm	2996553
4	IV	100ppm	3446224
5	V	125ppm	3897922
Correlation Coefficient			0.999

Robustness:

Table 3: System Suitability Results for Candesartan cilexitil.

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	7515.5	0.9
2	1.0	10026.7	1.0
3	1.1	5948.0	1.0

Table 4: System Suitability Results for Hydrochlorothiazide

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	8573.5	1.0
2	1.0	12458.5	1.2
3	1.1	6114.5	1.1

Table 5: Precision 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 6: Precision of Hydrochlorothiazide

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

Detection limit

The LOD was performed for Candesartan cilexitil and Hydrochlorothaizide was found to be 2.17and 0.0372 respectively.

Quantitation limit

The LOQ was performed for Candesartan cilexitil and Hydrochlorothaizide was found to be 6.60 and 0.112 respectively.

Recovery studies

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Table 7: Showing accuracy results for Candesartan cilexitil

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

Table 8: Showing accuracy results for Hydrochlorothiazide

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

4. Conclusion

A new method was established for simultaneous estimation of Candesartan cilexitil and Hydrochlorothaizide by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Candesartan cilexitil and Hydrochlorothaizide by using Zodiac sil C18 column (4.6×150mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: phosphate buffer (KH_2PO_4 & K_2HPO_4) phosphate pH3 (pH was adjusted with ortho phosphoric acid), detection wavelength was 240 nm. The Instrument used was WATERS HPLC auto sampler, separation module 2695 and photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.170 mins and 7.025 mins. The % purity of Candesartan cilexitil and Hydrochlorothaizide was found to be 99.1% and 98.2% respectively. The system suitability parameters for Candesartan cilexitil, Hydrochlorothaizide such as theoretical plates and tailing factor were found to be 12294, 1.27 and 10491 and 1.03, the resolution was found to be 8.67. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Candesartan cilexitil and Hydrochlorothaizide was found in concentration range of 16µg-80µg and 25µg-125µg and correlation coefficient (r^2) was found to be 0.999 and 0.998, % recovery was found to be 101.7% and 102.0%, %RSD for repeatability was 0.8and 0.5, % RSD for intermediate precision was 1.99 and 1.82 respectively. The precision study was precision, robustness and repeatabilty.LOD value was 2.17 and 0.0372 and LOQ value was 6.60 and 0.1125 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Candesartan cilexitil and Hydrochlorothaizide in API and Pharmaceutical dosage form.

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