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Original Article

SIMPLE ANALYTICAL METHOD FOR THE SIMULTANEOUS ESTIMATION OF HYDROCHLOROTHIAZIDE AND CANDESARTAN BY RP-HPLC

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ABSTRACT

Objective: To develop a simple, rapid, economic, accurate and precise reverse phase-high performance liquid chromatographic (RP-HPLC) method for the determination of hydrochlorothiazide and candesartan in the pharmaceutical dosage form and to validate as per international conference on harmonization (ICH) guidelines.

Methods: The chromatographic separation was performed on Silanol BDS C_{18} column (250 x 4.6 mm, 5 µm), a mobile phase consisting of water (p^H adjusted to 2.8 with orthophosphoric acid): acetonitrile (30:70 % v/v), with a flow rate 1 ml/min and the detection wavelength of 210 nm using photodiode array (PDA) detector.

Results: The developed method resulted in elution of hydrochlorothiazide at 2.28 min and candesartan at 4.28 min. The calibration curves were linear (r^2=0.999) in the concentration range of 6.25-18.75 µg/ml and 8-24 µg/ml for hydrochlorothiazide and candesartan respectively. The percentage recoveries were found to be 99.78-100.39 for hydrochlorothiazide and 99.87-100.64 for candesartan. The limit of detection (LOD) was found to be 0.410 µg/ml and 0.699 µg/ml for hydrochlorothiazide and candesartan respectively. The limit of quantitation (LOQ) was found to be 1.367 µg/ml and 2.330 µg/ml for hydrochlorothiazide and candesartan respectively.

Conclusion: A simple, economic, accurate, precise, linear and rapid RP-HPLC method was developed for simultaneous quantitative estimation of hydrochlorothiazide and candesartan in bulk and pharmaceutical formulation and the method was validated as per ICH guidelines. Hence, the method holds good for the routine analysis of hydrochlorothiazide and candesartan in various pharmaceutical industries as well as in academics.

Keywords: Hydrochlorothiazide, Candesartan, RP-HPLC, Method development, Validation

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INTRODUCTION

Hydrochlorothiazide [fig. 1] is chemically 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide, belongs to the thiazide class of diuretics. The drug reduces extra fluid in the body caused by conditions such as heart failure and kidney disease.

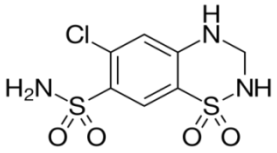

Fig. 1: Chemical structure of hydrochlorothiazide

Candesartan [fig. 2] is chemically 1-((2'-(1H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)-2-ethoxy-1H-benzo[d]imidazole-7-carboxylic acid, belongs to the class of angiotensin II receptor blockers. It is used for treating high blood pressure (hypertension).

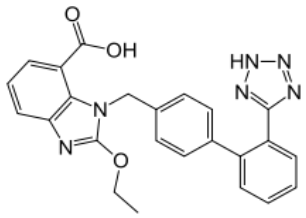

Fig. 2: Chemical structure of candesartan

A detailed literature survey revealed that there were analytical methods for the estimation of specified drugs with other combinations by ultraviolet spectroscopy (UV) [1-7], high-performance thin layer chromatography (HPTLC) [8], liquid chromatography/mass spectrometry/mass spectrometry (LCMS/MS) [9] and HPLC [10-12]. There were few RP-HPLC methods for the estimation of hydrochlorothiazide and candesartan[13-17] but the developed methods involve use of buffers as mobile phases which decrease the life of the column and has longer retention times, leading to more analysis time. Hence, an RP-HPLC method has been developed using simple mobile phase with less runtime.

In the present work an easy, rapid, accurate and specific method was developed by using low-cost solvent water and acetonitrile for the simultaneous estimation of hydrochlorothiazide and candesartan in bulk and pharmaceutical dosage form. The developed method was validated as per validation of analytical procedures i.e. ICH guidelines: Q2 [R1] Validation of Analytical Procedures: Text and Methodology.

MATERIALS AND METHODS

Chemicals and reagents

Pharmaceutical grade hydrochlorothiazide and candesartan were provided as a gift sample by Spectrum Pharma Labs, Hyderabad and the marketed formulation (Candesar-H, candesartan-16 mg, hydrochlorothiazide-12.5 mg) was purchased from local market, acetonitrile, orthophosphoric acid and HPLC grade water purchased from Merck.

Instrument

Chromatography was performed on Waters HPLC 2695 equipped with quaternary pumps with PDA detector. The chromatographic separation was performed using Silanol C_{18} column (4.5 x 250 mmx5 µm). The data acquisition and integration were performed using Empower 2 software.

Chromatographic conditions

The developed method used a reverse phase C_{18} column, Silanol C_{18} column (4.5 x 250 mm x 5 µm), a mobile phase consisting of water (p^H adjusted to 2.8 with orthophosphoric acid): acetonitrile (30:70 %v/v), flow rate of 1.0 ml/min and a detection wavelength of 210 nm using PDA detector.

Preparation of standard solutions

Standard stock solutions of hydrochlorothiazide and candesartan were prepared by dissolving 12.5 mg of hydrochlorothiazide and 16 mg of candesartan insufficient amount of mobile phase and sonicated for 5 min. After that, the solution was filtered and diluted to 10 ml with mobile phase. Further dilutions in five replicates were made to get the final concentration of 12.5 µg/ml and 16 µg/ml for hydrochlorothiazide and candesartan respectively. This has been treated as 100 % target concentration [18].

Preparation of sample solutions

20 tablets were weighed, crushed and quantity of powder equivalent to 12.5 mg of hydrochlorothiazide and 16 mg of candesartan were weighed and transferred into a 10 ml volumetric flask, sufficient amount of mobile phase was added and sonicated for 5 min. After that, the solution was filtered and the volume was made up to 10 ml with mobile phase. Further dilutions in five replicates were made to get the final concentration of 12.5 µg/ml and 16 µg/ml for hydrochlorothiazide and candesartan respectively.

Validation of the developed method

The proposed analytical method was validated for system suitability, linearity and range, precision, LOD, LOQ and accuracy in accordance with ICH guidelines for analytical procedures Q2 [R1] [19].

System suitability

System suitability parameters were studied to verify the system performance. Six replicate samples containing hydrochlorothiazide (12.5 µg/ml) and candesartan (16 µg/ml) were analyzed using the developed method. Factors such as theoretical plate count, tailing factor, percent relative standard deviation of peak area and retention time were taken into consideration for testing system suitability.

Linearity and range

The linearity was evaluated at six concentration levels in the range between 6.25-18.75 µg/ml and 8-24 µg/ml for hydrochlorothiazide and candesartan respectively. A calibration curve was obtained by plotting concentration against corresponding peak area and linearity was determined using least square regression analysis. The analytical range was established by the highest and lowest concentrations of analyte and the acceptable linearity was obtained with the specified analytical range.

Precision

The precision of the developed analytical method was carried out for same concentration level. Six determinations were performed and were expressed in terms of percent relative standard deviation [% RSD].

LOD and LOQ

Limit of detection and quantitation of the developed method were calculated from the standard deviation of the y-intercept and slope of the calibration curve of hydrochlorothiazide and candesartan using the following formula:

Limit of detection = 3.3 α/s

Limit of quantitation = 10 α/s

Where α is the standard deviation of the y-intercept and 's' is the slope of the calibration curve.

Accuracy

Accuracy of the method has been studied by recovery experiment by applying the standard addition method. A known quantity of drug substance corresponding to 50%, 100%, and 150% of the label claim of drug were added, to determine if there are positive or negative interferences from excipients present in the formulation. Each set of addition were repeated three times. The accuracy was expressed as the percentage of analytes recovered by the assay.

Application of validated method for assay of hydrochlorothiazide and candesartan in pharmaceutical dosage form

Tablet powder equivalent to 12.5 mg of hydrochlorothiazide and 16 mg of candesartan were weighed and transferred into a 10 ml volumetric flask, 7 ml of diluent was added and sonicated for 5 min. After that, the solution was filtered and the volume was made up to the mark with diluent. From this solution, further dilution was made to get the final concentration of 12.5 µg/ml of hydrochlorothiazide and 16 µg/ml of candesartan. The prepared solutions were injected into the HPLC system, chromatograms were recorded and from the peak areas of hydrochlorothiazide and candesartan, the amount of drug present in the sample was computed.

RESULTS AND DISCUSSION

Method development

Different chromatographic conditions were tried for better separation and resolution. Silanol BDS C_{18} column (250×4.6 mm, 5 µm) was found satisfactory. Detection of analytes was carried out using PDA detector and 210 nm was considered satisfactory for detecting both the drugs with adequate sensitivity. A number of trails were performed with different solvents in the different ratios over a wide range of p^H, with different flow rates and column temperatures.

But, either peak shape was broad or resolution was not good. Repeated trials to obtain good, sharp peaks with better retention times and efficient resolution between hydrochlorothiazide and candesartan were performed on Silanol BDS C_{18} column. The runtime was good in isocratic trial with a flow rate of 1 ml/min using mobile phase containing water (p^H adjusted to 2.8 with orthophosphoric acid):acetonitrile (30:70 % v/v) and the detection wavelength of 210 nm using PDA detector gave satisfactory results in terms of retention time, resolution, symmetry and sensitivity. A typical RP-HPLC chromatogram for simultaneous determination of hydrochlorothiazide and candesartan from standard preparation and sample preparation was shown in fig. 3 and 4.

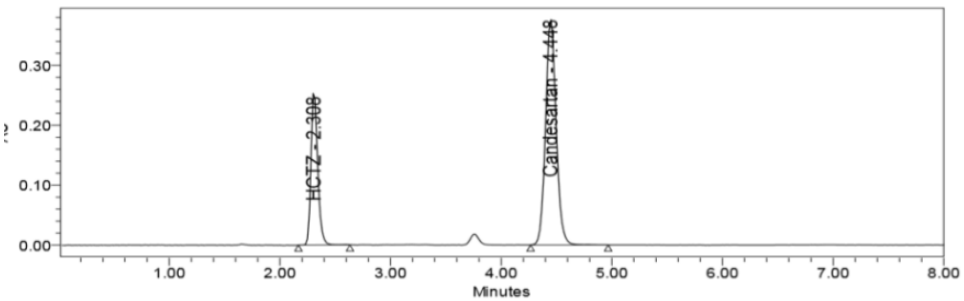

Fig. 3: Standard chromatogram of hydrochlorothiazide and candesartan

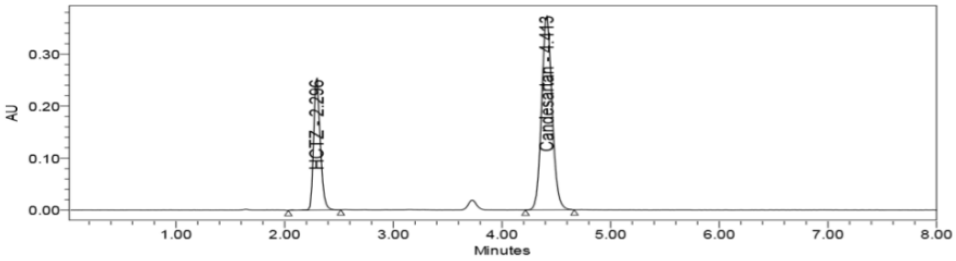

Fig. 4: Sample chromatogram of hydrochlorothiazide and candesartan

On comparison with literature, it is found that the mobile phase used by Rahul *et al.* [13] was phosphate buffer and acetonitrile. Quatab *et al.* [14] and Anand Rao *et al.* [15] used phosphate buffer and methanol. Whereas Mathrusri *et al.* [16] and Balamuralikrishna *et al.* [17] used 0.01 M tetrabutylammonium hydrogen sulphate: methanol and 0.02% triethylamine: acetonitrile respectively. All the methods used buffers in their mobile phases takes more preparation time and also the usage of buffers reduces the life of column. The retention time of drugs in the method developed by Rahul *et al.* [13] was 4.3 min and 16.1 min, Anand Rao *et al.* [15] was 2.1 min and 7.2 min and Mathrusri *et al.* [16] was 2.7 min 8.1 min thereby increasing the analysis time. In the present study, a simple mobile phase consisting of water (p^H adjusted with OPA) and acetonitrile was used which elute the hydrochlorothiazide and candesartan with lower retention time.

Method validation

System suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and tailing factor were evaluated. The system suitability parameters were summarized in table 1. All the parameters were found to be within the limits.

Table 1: Results of system suitability study

Parameters	Acceptance limits	Hydrochlorothiazide	Candesartan
Retention time* (min)	-	2.30±0.16	4.40±0.18
Resolution*	NLT 2	-	14.2±0.03
Theoretical plates*	NLT 2000	6493±2	10170±4
Tailing factor *	NMT 2	1.23±0.02	1.05±0.03

*=results of six determinations

Linearity and range

The linearity of the test solutions for the assay method was prepared from hydrochlorothiazide and candesartan standard stock solution at five concentration levels from 50% to 150% of assay concentration. The peak area versus concentration data was treated by least-square linear regression analysis. The results showed an excellent correlation between peak areas and concentration within the concentration range of 6.25-18.75 µg/ml and 8-24 µg/ml for hydrochlorothiazide and candesartan respectively. The results of linearity of hydrochlorothiazide and candesartan were summarized in table 2. The correlation coefficients were found to be 0.999 for both hydrochlorothiazide and candesartan, which meet the method validation acceptance criteria and hence the method was said to be linear for both the drugs. The results showed that a linear relationship between peak area and concentration of the drug in the calibration curve.

Table 2: Linearity data for hydrochlorothiazide and candesartan

% level	Hydrochlorothiazide concentration (µg/ml)	Hydrochlorothiazide peak area	Candesartan concentration (µg/ml)	Candesartan peak area
50	6.25	292718	8	626266
75	9.37	408707	12	909947
100	12.50	544937	16	1213266
125	15.62	681175	20	1516578
150	18.75	812002	24	1881404
Correlation coefficient	0.999		0.999	
Slope	43031		77306	

Accuracy

The accuracy of the method was determined by recovery studies by the determination of % mean recovery of both the drugs at three different levels (50 %, 100 % and 150%). At each level, three determinations were performed. The percentage recovery and mean percentage recovery were calculated for the drugs and presented in table 3. The observed data were within the specified range. Hence good recovery values indicate that the developed method is accurate for the determination of specified drugs.

Precision

Method precision

The precision of the method was verified by precision method studies. The sample solution was prepared at working concentration and analysis was carried out at replicates. The sample solutions of hydrochlorothiazide and candesartan were prepared as per the test method and injected six times into the column. The results of precision were tabulated in table 4. The mean of peak area was calculated, % RSD was calculated and reported. Method precision %

RSD values lower than 2% clearly assured that the developed method was found to be fairly precise and reproducible.

Table 3: Results of accuracy

Level (%)	Hydrochlorothiazide		Candesartan	
	% recovery	% mean recovery	% recovery	% mean recovery*
50	99.59	99.78	99.89	99.87
50	99.75		99.78	
50	100.01		99.96	
100	99.50	99.57	100.87	101.08
100	99.99		101.63	
100	99.23		100.76	
150	100.10	100.39	101.45	100.64
150	101.00		100.66	
150	100.08		99.83	

*Average of triplicate determinations; Acceptence criteria: % recovery must be 98%-102%

Table 4: Method precision data for hydrochlorothiazide and candesartan

No. of injections	Hydrochlorothiazide		Candesartan	
	Rt	peak area	Rt	peak area
Injection 1	2.286	544936	4.462	1213265
Injection 2	2.287	544933	4.485	1213266
Injection 3	2.291	544940	4.491	1213267
Injection 4	2.301	544936	4.488	1213259
Injection 5	2.302	544939	4.487	1213258
Injection 6	2.299	544938	4.480	1213262
Mean*±SD	544937±2.52	0.4	1213263±3.76	0.3
% RSD #				

*The value is represented as a mean±SD of 6 observations (n=6), SD: Standard Deviation, #RSD: Relative Standard Deviation, Acceptance criteria:<2

Limit of detection and limit of quantitation

LOD and LOQ were estimated from the standard deviation of the y-intercept and slope of the calibration curve of hydrochlorothiazide and candesartan.

The LOD and LOQ were found to be 0.410 µg/ml and 1.367 µg/ml for hydrochlorothiazide and 0.699 µg/ml and 2.330 µg/ml for candesartan. The results of LOD and LOQ specified the sensitivity of the developed method.

Robustness

Robustness of the developed method was determined by deliberately altering the experimental conditions and the system suitability parameters were evaluated. The solutions prepared as per the test method and injected at different variable conditions like flow rate (0.8, 1.2 ml/min) and wavelength (208 nm, 212 nm), system suitability parameters were compared with that of method precision.

The results were tabulated in table 5. Even though, small changes were made in the conditions there was no significant effect on tailing, plate count and retention time of specified drugs. Hence, the developed method was found to be robust.

Application of validated method for assay of hydrochlorothiazide and candesartan in pharmaceutical dosage form

The developed method was successfully implemented in the assay of hydrochlorothiazide and candesartan in pharmaceutical dosage form. Assay of hydrochlorothiazide and candesartan was found to be 99.16% and 99.60% respectively. The results of the assay were summarized in table 6.

Table 5: Results of robustness

Parameter	Hydrochlorothiazide		Candesartan	
	Plate count#	Tailing*	Plate count#	Tailing*
Less flow rate (0.8 ml/min)	5416	1.21	9606	1.11
More flow rate (1.2 ml/min)	6692	1.19	8807	1.04
Less wavelength (208 nm)	3284	1.09	9875	1.12
More wavelength (212 nm)	6217	1.17	9294	1.09

Acceptance criteria (Limits): #Peak Asymmetry: >2000, *Tailing: <2

Table 6: Assay of hydrochlorothiazide and candesartan in pharmaceutical dosage form

Name	Standard average area*	Sample average area*	% Assay
Hydrochlorothiazide	544837	534811	99.16
Candesartan	1213262	1208408	99.60

*Mean of six determinations

CONCLUSION

In the present study, a simple and efficient RP-HPLC method was developed for the simultaneous analysis of hydrochlorothiazide and candesartan in bulk and in pharmaceutical dosage form. The method was validated as per ICH guidelines and found to be applicable for routine quality control analysis. The results of analysis of pharmaceutical formulation by the proposed method were highly reproducible and reliable. Hence, it can be concluded that the proposed RP-HPLC method was simple, cost-effective, specific, accurate, precise, robust and rapid.

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CONFLICT OF INTERESTS

Declared none

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