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DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR ESTIMATION OF HYDROCHLOROTHIAZIDE AND CANDESARTAN CILEXETIL IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the simultaneous determination of hydrochlorothiazide and candesartan cilexetil in pharmaceutical dosage form. The column used was Zorbax C_8 (150×4.6 mm, 3.5µm) in isocratic mode, with mobile phase containing phosphate buffer-methanol (30:70) adjusted to pH 3.0 using ortho phosphoric acid was used. The flow rate was 1.0 mL/ min and effluents were monitored at 230 nm. The retention times of hydrochlorothiazide and candesartan cilexetil were 2.170 min and 7.280 min, respectively. The linearity for Hydrochlorothiazide and Candesartan cilexetil were in the range of 25-125 mg/mL and 16-80 mg/mL respectively. The recoveries of Hydrochlorothiazide and Candesartan-cilexetil were found to be 101.5% and 100.9%, respectively. The proposed method was validated and successfully applied to the estimation of Hydrochlorothiazide and Candesartan cilexetil in combined tablet dosage forms.

Keywords: Validation, RP-HPLC, Hydrochlorothiazide, Candesartan cilexetil

INTRODUCTION

Candesartancilexetil (CAN) belongs to angiotensin II receptor blocker effective in lowering blood pressure in hypertensive patients. Chemically it is known as 2, 3-dihydroxy-2-butenyl 4-[1-hydroxy-1-methylethy] -2-propyl-1- [p (o-1H-tetrazol-5-ylphenyl) benzylimida -zole-5-carboxylate, cyclic-2, 3-carbonate. Hydrochlorothiazide (HCTZ) is a diuretic of the class of benzothiadiazine widely used in antihypertensive pharmaceutical formulations, alone or combination with other drugs, which decreases active sodium reabsorption and reduced peripheral vascular resistance. It is chemically 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide, and was successfully used as one content in association with other drugs in the treatment of hypertension. Literature survey revealed that a various analytical methods have been reported for the determination of Candesartan cilexetil and Hydrochlorothiazide in pure drug, pharmaceutical dosage forms and in biological samples using liquid chromatography either in single or in combined forms**.** Confirmation of the applicability of the developed method was validated according to the International Conference on Harmonization (ICH) for the simultaneous determination of CAN and HCTZ in bulk and in tablet dosage form.

MATERIALS AND METHODS

A Waters HPLC system consisting of a Water 2695 binary gradient pump, an inbuilt auto sampler, a column oven and Water 2487 dual wavelength absorbance detector (DAD) was employed throughout the analysis. The data was acquired using Empower 2 software. The column used was Zorbax C_8 (150×4.6 mm, 3.5µm) A Bandline sonerex sonicator was used for enhancing dissolution of the compounds. An Adwa digital pH meter was used for pH adjustment. Analytically pure HCTZ and CAN were obtained as gift samples from M/s Blue Cross Ltd., (Mumbai, India) and M/s Mercury Laboratories Ltd., (Vadodara, India), respectively. Acetonitrile, methanol, water (E. Merck, Mumbai, India) were of HPLC grade, while ortho-phosphoric acid and

potassium dihydrogen phosphate (S. D. Fine Chemicals, Mumbai, India) were of Analytical grade used for the preparation of mobile phase.

Preparation of mobile phase and stock solutions: Potassium dihydrogen phosphate was weighed (7.0 g) and dissolved in 1000 ml of water. Finally the pH was adjusted to 3.0 with ortho phosphoric acid (0.1 M). The solution was sonicated for 10 minutes and filtered using Whatman filter paper (No.1) and used. (CAN) and (HCTZ) were weighed (8mg and12.5mg each) and transferred to two separate 10 ml volumetric flasks and dissolved in mobile phase, which gives 1000 µg/mL of (CAN) and (HCTZ).

Chromatographic conditions: A reverse phase C8 column equilibrated with mobile phase phosphate buffer-methanol (30:70) adjusted to pH 3.0 was used. Mobile phase flow rate was maintained at 1.0 mL/min and effluents were monitored at 230 nm.

The sample was injected using a 20 µL fixed loop, and the total run time was 10 min. Appropriate aliquots of (HCTZ) and (CAN) stock solutions were taken in different 10 ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 25,50,75,100,125 µg/mL of (HCTZ) and 16,32,48,64,80 µg/mL of (CAN). The solutions were injected using a 20 µl fixed loop system and chromatograms were recorded. Calibration curves were constructed by plotting average peak area versus concentrations and regression equations were computed for (HCTZ) and (CAN).

Determination of (**HCTZ) and (CAN**) in ***their combined dosage forms:*** The content of twenty tablets were taken and weighed. Powder equivalent to 59.8mg (12.5mg and 8mg each) was accurately weighed and transferred to a 10 ml volumetric flask and 6 ml of mobile phase was added to the same and flask was sonicated for 5 min. The flask was shaken, and the volume was diluted to the mark with the same mixture.

The above solution was filtered using Whatman filter paper No.1,Further pipetteout 0.6ml of above stock solution into a 10ml of volumetric flaskand dilute upto the mark with diluent to obtain 75 µg/ mL of HCTZ and 48µg/mL of CAN. The solution was injected at above chromatographic conditions and peak areas were measured. The quantification was carried out by keeping these values to the straight line equation of calibration curve. The method was validated for accuracy, precision, specificity, detection limit, quantitation limit and robustness.

Accuracy: The accuracy of the method was determined by calculating recoveries of HCTZ and CAN by method of standard additions. Known amount of HCTZ and CAN were added to a pre quantified sample solution, and the amount of HCTZ and CAN were estimated by measuring the peak areas and by fitting these values to the straight-line equation of calibration curve.

Precision: The intraday and inter day precision study of HCTZ and CAN was carried out by estimating the corresponding responses 5 times on the same day and on different days. The results are reported in terms of relative standard deviation. The Repeatability studies were carried out by estimating response of 5 different concentrations of HCTZ and CAN and results are reported in terms of relative standard deviation (RSD).

Specificity: Commonly used excipients were spiked into a pre weighed quantity of drugs. The chromatogram was taken by appropriate dilutions and the quantities of drugs were determined.

Detection limit and quantitation limit: *Baseline noise obtained from blank injection is 52 µV.* Signal to Noise ratio for the determination of detection limit for HCTZ is 3 and quantitation limit is 10 and for the determination of detection limit for CAN is 3 and quantitation limit is 10.

Robustness: Robustness of the method was studied by changing the composition of organic phase by ± % 10 and the flow is by ±0.1ml/min.

RESULTS AND DISCUSSION

Optimization of mobile phase was performed based on resolution, asymmetric factor and peak area obtained for both HCTZ and CAN. The mobile phase phosphate buffer-methanol (30:70) adjusted to pH 3.0 using ortho phosphoric acid was found to be satisfactory and gave two symmetric and well-resolved peaks for HCTZ and CAN. The resolution between HCTZ and CAN was found to be 27.7, which indicates good separation of both the compounds. The retention time for HCTZ and CAN were 2.17 min and 7.28 min, respectively (Figure 1).

The calibration curve for HCTZ was obtained by plotting the peak area of HCTZ versus the concentration of HCTZ over the range of 25-125 µg/mL, and it was found to be linear with r^2 = 0.9993. Similarly, the calibration curve for CAN was

obtained over the range of 16-48 µg/mL and was found to be linear with r^2 = 0.9995. The data of regression analysis of the calibration curves are shown in (Table-1).

The detection limit for for HCTZ and CAN were 0.005µg/mL and 0.03µg/mL, respectively. The quantitation limit for HCTZ and CAN were 0.02µg/mL and 0.09µg/ml, respectively, which suggest that a nanogram quantity of both the compounds can be estimated accurately. The validation parameters are summarized in (Table-1). The recoveries of for HCTZ and CAN were found to be 101.5% and 100.9%, respectively. The system suitability test parameters are shown in (Table-1). The liquid chromatographic method was applied to the determination of for HCTZ and CAN in their combined dosage forms. The results for HCTZ and CAN were comparable with the corresponding labeled amounts

CONCLUSION

Proposed study describes a new RP-HPLC method for the estimation of for HCTZ and CAN combination in mixture using simple mobile phase with low buffer concentration compared to the reported method. The method gives good resolution between both the compounds with a short analysis time (<10 min). The method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the proposed method can be used for routine analysis of for HCTZ and CAN in their combined dosage form.

Table 1: Validation parameters and data for proposed method

Validation parameter	**Results**	
	HCTZ	***CAN***
Linearity	25-125 µg/mL	16-80 µg/mL
Regression coefficient (r^2)	0.9993	0.9995
Limit of detection (µg/mL)	0.005	0.03
Limit of quantitation (µg/mL)	0.02	0.09
Accuracy (% recovery)	101.5	100.9
Precision		
Repeatability of injection (%RSD)	0.83	0.52
Intermediate precision (%RSD)	1.46	1.99
Assay value (%)	100.8	98.3
System suitability parameter		
Tailing factor	1.2	1.0
Number of theoretical plates	12458	10026
Resolution	27.7	

** Replicates of three concentration levels (in three determinations); ** Ten repetitive injections of same homogeneous sample*

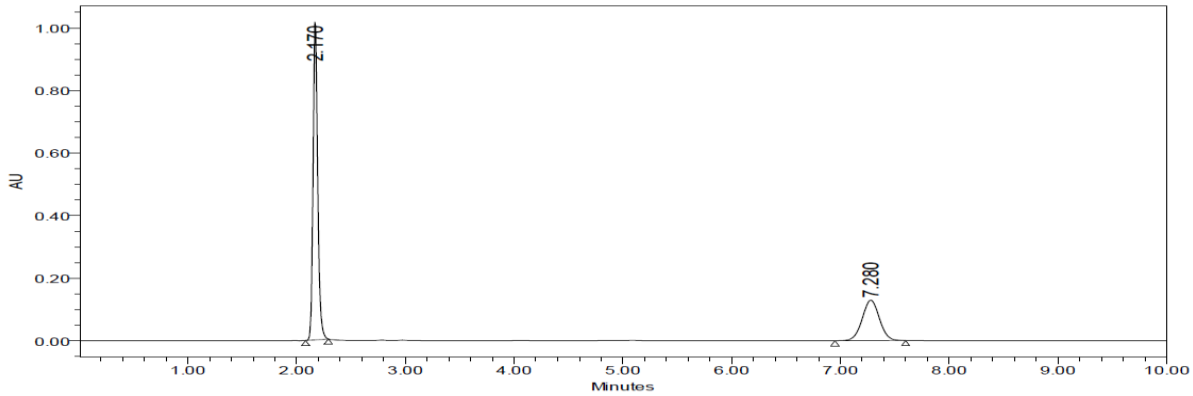

Figure 1: HPLC chromatogram of Hydrochlorothiazide and Candesartan in optimized chromatographic conditions

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