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Development and validation of high performance liquid chromatographic method for the Simultaneous Estimation of Candesartan cilexetil and Hydrochlorothiazide in combined tablet dosage form

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ABSTRACT

A simple, sensitive and reproducible reverse phase high performance liquid chromatographic method has been developed for simultaneous estimation of Candesartan cilexetil and Hydrochlorothiazide in combined tablet dosage form. Chromatography was performed on a 250 mm x 4.6 mm, 5-µm particle size, C_8 Hypersil BDS column with a 60:40 (v/v) mixture of acetonitrile and Triethylamine (0.02 %) as a mobile phase and the pH was adjusted to 5.5 with dilute o-phosphoric acid. The detection of the combined dosage form was carried out at 262 nm and a flow rate employed was 1.0 mL/min. The retention times were 2.449 and 4.895 min for Candesartan and Hydrochlorothiazide respectively. Linearity was obtained in the concentration range 50 to 150 µg/mL for Candesartan cilexetil and 75-225 µg/mL for Hydrochlorothiazide, with a correlation coefficient of 0.9999 and 0.9999. The results of the analysis were validated statistically and recovery studies confirmed the accuracy and precision of the proposed method.

Keywords: Candesartan cilexetil, Hydrochlorothiazide, RP-HPLC, Simultaneous estimation.

INTRODUCTION

Candesartan cilexetil (CAN) [1] belongs to angiotensin II receptor blocker effective in lowering blood pressure in hypertensive patients. Chemically [Fig. 1] 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) [2] 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 [Fig. 2] 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 [3-39], spectrophotometry [40-47] and high performance thin layer chromatography [48, 49] 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) [50] for the simultaneous determination of CAN and HCTZ in bulk and in tablet dosage form.

MATERIALS AND METHODS

Experimental

Chemicals and Reagents

HPLC-grade Acetonitrile, o-Phosphoric acid and Triethyl amine analytical grade were procured from Rankem Chemicals (Mumbai, India) and pure standards of CAN (99.26%) and HCTZ (99.89%) were obtained as gift samples from Aurobindo laboratories Pvt. Ltd. (Hyderabad, India). HPLC grade water was procured from Rankem Chemicals (Mumbai, India).

Instrumentation and Chromatographic Conditions

Chromatography was performed with a shimadzu isocratic pump, variable wavelength detector and a rheodyne 9013 injector with 20-µL loop, C_8 Hypersil BDS column (250 x 4.6nm., 5-µm particles) was used for chromatographic separation under suitable condition. Detection was carried out at 262 nm and the software used was spinchrome.

The mobile phase was a 60:40 (*v/v*) mixture of freshly prepared Triethyl amine and Acetonitrile. The mobile phase was filtered through 0.45 µ membrane filter and sonicated before use. The flow rate of mobile phase was maintained at 1.0 mL/min.The column temperature was maintained at 40°C. The detection wavelength was 262 nm for both drugs. The injection volume was 20 µL and total run time was 10 min. The peaks were identified by retention time; a typical chromatogram is shown in Fig. 2.

Preparation of standard solution for calibration Plots

About 100 mg of CAN and 150mg of HCTZ were weighed and transferred into a 100 mL volumetric flask containing 25 mL of methanol. The solution was sonicated for 5 min and then volume was made up with a further quantity of the mobile phase to get 1mg/mL and 1.5 mg/mL of CAN and HCTZ respectively. 10 mL of this solution was further diluted to 100 mL with mobile phase to get a concentration of 100µg/mL and 150 µg/mL CAN and HCTZ respectively. Stock solution was diluted with mobile phase to give working standard solution containing 50 to 150 µg /mL of CAN and 75 to 225 µg /mL HCTZ. These standard solutions were injected for construction of calibration plots by plotting drug peak-area ratio (*y*) for each of the drug against concentration (*x*). Analysis was performed at ambient temperature. The retention times of Candesartan and hydrochlorothiazide under these conditions were 2.449 and 4.895 min respectively.

Assay procedure

Twenty tablets were weighed and powdered into uniform size in a mortar. From this the average weight of a tablet was calculated. An accurately weighed portion from this powder equivalent to 160 mg of Candesartan cilexetil and 125 mg of Hydrochlorothiazide was transferred to a 100mL volumetric flask containing 20 mL of the methanol. The contents of the flask were sonicated for about 20 min for complete solubility of the drug and the volume was made up to 100 mL with

mobile phase. Then the mixture was filtered through 0.45µ membrane filter. From the above solution a five mL of aliquot was taken into a separate 100 mL volumetric flask and made up to the volume with mobile phase and mixed well. The above solution (20 µL) was then injected eight times into the column. The mean peak areas of the drugs were calculated and the drug content in the formulation was calculated by the regression equation of the method.

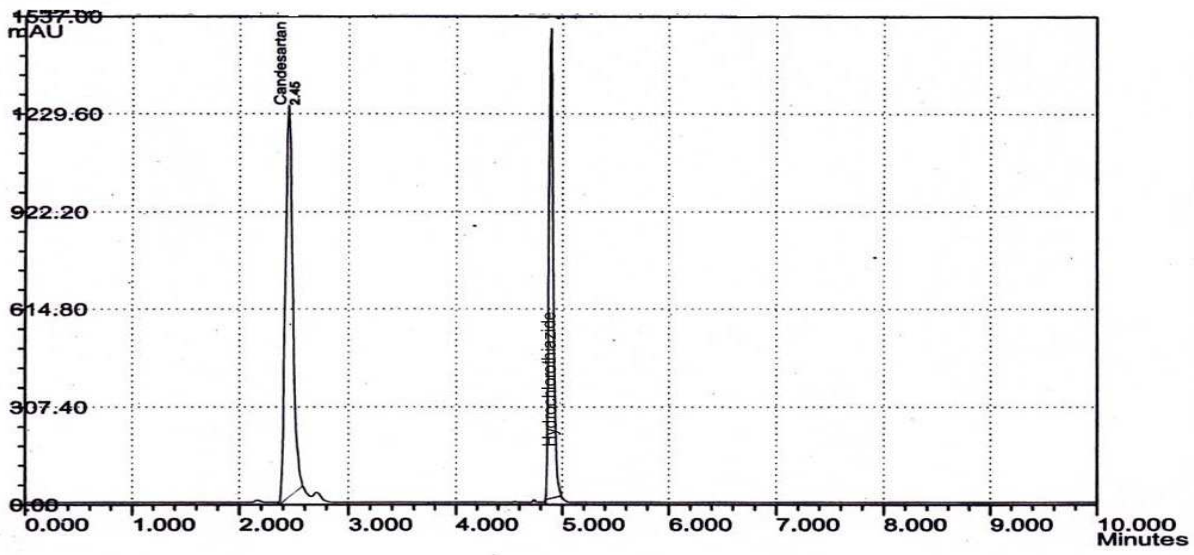

Fig. 3 Typical chromatograms obtained from Candesartan cilexetil and hydrochlorothiazide standard

RESULTS AND DISCUSSION

Method development

The objective of this study was to develop simultaneous estimation of two components under isocratic conditions. The mobile phase used was the mixture of acetonitrile with buffer in different ratios. Finally a mixture of acetonitrile and Triethylamine (pH 5.5) in the ratio of 60:40 (*v/v*), proved to be effective mixture than the other mixture used for the separation. Then the flow rate tested includes 0.4, 0.8, 1.0, 1.2 and 1.5 ml. Among the flow rates 1.0 ml was selected for the assay because of better resolution of the peaks. The mentioned chromatographic conditions revealed to provide better resolution between CAN and HCTZ in a reasonable time of 2.449 and 4.895 min, respectively. The optimum wave length for detection was 262 nm, no indigenous interfering compounds eluted at the retention times of the drugs.

Table.1 Results from validation and system-suitability studies

Method characteristic	Candesartan cilexetil	Hydrochlorothiazide
Theoretical plates	5235	53024
Linearity range (µg /mL)	50-150	75-225
Percentage Recovery (%) *	99.88-99.95	99.79-99.91
Symmetry factor	1.46	1.48
Correlation coefficient	0.9999	0.9999
Accuracy (RSD (%))* *	0.18	0.21
Intra-day precision (RSD (%))*	0.45	0.56
Inter-day precision (RSD (%))*	0.38	0.45

* *Results are mean five replications*

* * *Results are mean three replications with three different drug concentrations.*

Validation of the Method

The method was validated, in accordance with ICH guidelines, for linearity, accuracy, precision, specificity, sensitivity, ruggedness and robustness.

Linearity

Linearity was assessed with the aid of serially diluted calibration solutions as mentioned above. The standards were injected separately. Calibration graphs were plotted on the basis of triplicate analysis of each calibration solutions. Linear correlations were obtained over the range studied, with correlation coefficients of 0. 9999 for both the drugs. In case of tablets, the regression equation was Y=11787 X+14.43 (r^2=0.9999) for CAN and Y=11095.7 X–508.3 (r^2=0.9999) for HCTZ.

Precision

The precision of the method was done by replicate (n=5) analysis of tablet preparations. The precision was also studied in terms of intra-day changes in peak area of drug solution on the same day and on three different days over a period of one week. The intra-day and inter-day variation was calculated in terms of percentage relative standard deviation and the results are given in Table. 1.

Accuracy

Accuracy was determined by the method of standard additions at three different levels, by multiple level recovery studies. Solution containing 100 µg /mL CAN and 150 µg /mL HCTZ for tablets was prepared from the stock solutions and was spiked with amounts of the standard drugs equivalent to 50, 100 and 150% of the amounts present in the original solution. These solutions were then analyzed for recovery studies and consistent values by replicated injections cum analysis. Results for determination of precision and accuracy are presented in Table. 1.

Specificity

Specificity was tested against standard compounds and against potential interferences in the presence of placebo. No interference was detected at the retention time of CAN and HCTZ in sample solution. Peak purity for CAN and HCTZ was tested by comparing spectra acquired at the start (S), apex (A), and end (E) of the peaks.

Stability

To demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 h at room temperature. The results showed that for both solutions, the retention times and peak areas of CAN and HCTZ remained almost unchanged (RSD<2.0%) indicating that no significant degradation occurred within this period, i.e. both solutions were stable for at least 24 h, which was sufficient to complete the whole analytical process. Sample solution were then stored at 4 and 25° C and checked after three days of storage. When results were compared with those from freshly prepared sample in each case no significant degradation occurred within the indicated period.

Ruggedness and Robustness

The ruggedness of the method was determined by using different instrument (Waters 2489) and different column (Symmetry C_8) of similar type. The robustness of the method was determined by making slight changes in the chromatographic conditions (buffer pH ± 0.1, flow rate±0.2 min). Again there was no marked change in the chromatograms. These results indicated that the method was rugged and robust with regard to these conditions. When mobile phase composition was changed by ± 10%, however, proper resolution could not be achieved; separation of the drugs was very sensitive to mobile phase ratio.System suitability tests are an integral part of

chromatographic method. They were used to verify that the reproducibility of the chromatographic system is adequate for the analysis. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared standard stock solution of CAN and HCTZ. In addition, standard deviation of CAN and HCTZ standards were evaluated by injecting mixed standard of both CAN and HCTZ (100 and 150 µg/mL). All the parameters are shown in Table. 2

Table 2: Results from validation and system-suitability studies

Sample	Labeled amount (mg)	Amount found*	% Recovery*
Candesar-H	C-16mg H-12.5 mg	C-15.7mg H-12.9 mg	C-98.12 H- 103.2

**Average of eight determinations* *C – Candesartan* *H-Hydrochlorothiazide*

CONCLUSION

The proposed RP-HPLC method for simultaneous assay of CAN and HCTZ in combined tablets dosage forms is simple, precise, specific and highly accurate and less time consumption for analysis could be recorded. So, it can definitely be employed for the routine analysis. Hence this RP-HPLC method is suitable for quality control of raw materials and formulations and also for dissolution studies.

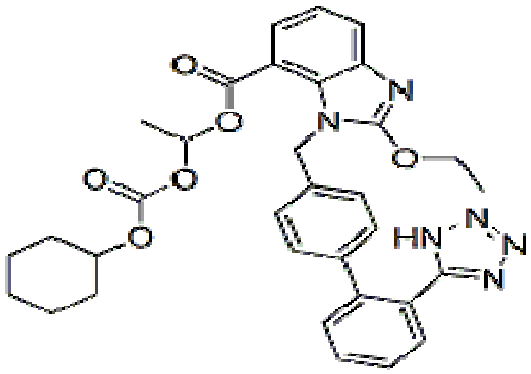

Fig 1: Molecular structure of Candesartan cilexetil

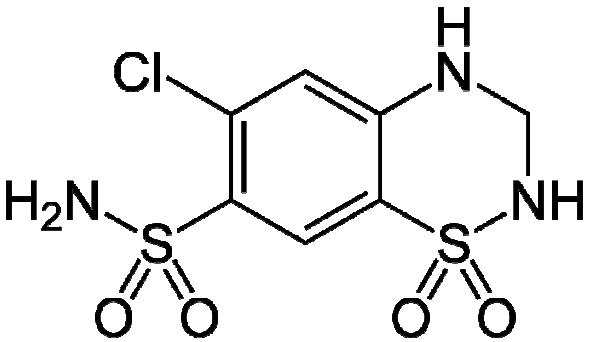

Fig 2: Molecular structure of Hydrochlorothiazide

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